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SUMMARY

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VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

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FRUIT GROWING

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RESEARCH REGARDING THE INFLUENCE OF LOCAL CONDITIONS ON SEVERAL QUALITY INDICATORS FOR APPLES

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Abstract

Apple is one of the most important fruit growing species from the northern hemisphere. Fruit quality is important for capitalization, which is why technologists are trying to ensure, using technology, the best possible conditions to produce qualitative apple fruits. The current research presents a comparison of fruit quality for four varieties: 'Florina', 'Generos', 'Red Topaz' and 'Redix', cultivated in three areas: Pitesti, Bucharest and Moara Domnească. Fruit quality was influenced by the culture area, but also largely by the climatic year. Fruit size had values within the limits of each variety for all three areas, but the location had a different influence on this parameter. Dry soluble substance had higher values recorded for the varieties 'Florina' and 'Redix' cultivated in Bucharest area, while the varieties 'Generos' and 'Red Topaz' for Pitesti area. Fruit firmness was better for the fruit obtained in Pitești area (hilly area), compared to the other two areas, Bucharest and Moara Domnească (lower areas), while the content in anthocyanins and polyphenols was influenced more by the culture conditions than by the varieties' characteristics.

Key words*: fruit quality, fruit weight, biochemical characteristics.*

INTRODUCTION

Apple (*Malus domestica* L.) is one of the dominant fruit growing species in the northern hemisphere, the area and obtained production place the species amongst the top ones (Stănică et al., 2003). The quality of apple fruit is important in order to ensure the best possible capitalization and satisfying economical results. Fruit quality is a variety characteristic, influenced by the genetic traits, but it largely depends on the culture technology applied and the pedo-climatic conditions from the culture area (Chira and Beceanu, 2003). The plant management system (Tustin et al., 2022) and the degree of fruit exposure to light influence the coloring and accumulation of active principles through pigment synthesis (Hoza, 2000). The pigment accumulation at epicar level is conditioned by the illumination degree (Delgato, 2014) and especially by the thermal amplitude between day and night from the culture area before harvest (Curry, 1997; Lakatos et al., 2012). Recent research related to pigment synthesis at epicar level also highlighted a series of genetic mechanisms involved in this process (Chen et al., 2021; Ding et al., 2021). Soil maintenance system can influence the quality, the soil maintained grassy ensures more colorful fruits compared to the worked soil (Bărăscu et al., 2018; Ilie et al., 2017; Pantea 2012). Weather conditions, especially times with small amounts of rainfall but on longer periods, affect the fruit production, influencing the development of diseases with negative effects on fruit quality (Bui et al., 2021). The grafting combination and the rootstock used influence both tree precocity and fruit quality (Bărăscu et al., 2016; Hoza et al., 2020; Macedo et al., 2012). Ensuring minerals at soil level within the normal quantity and distribution during the vegetation period ensures a normal fruit growth and reaching the biological potential at a variety level (Ilie et al., 2018).

MATERIALS AND METHODS

Research was conducted during 2015-2017, on fruits obtained from 3 different locations: the teaching field of the Faculty of Horticulture Bucharest, Moara Domnească farm and ICDP Pitești - Mărăcineni. Four varieties with genetic resistance were used: Florina, Generos, Redix and Red Topaz, using for measurements 10 fruit of each variety, while the measurements were made at the same moment of each year. Biometric measurements were made related to fruit dimensions using the caliper (height, large and small diameter), and using them the shape index If = H/D (H= fruit height; D = large fruit diameter) and size index Im = (H+D+d)/3 (d = small fruit diameter) and average weight were calculated. The firmness of the apples was determined using a piston of 1.1 cm diameter (Bessemans, 2016; Both et al., 2017; Rizzolo, 2010) of an electronic penetrometer Turoni TR and the results were expressed in kg/cm^2. The total soluble solids content of the apples juice was obtained with refractive device Kruss DR301-95 (% Brix) (Muresan et al., 2014).

RESULTS AND DISCUSSIONS

Fruit dimensions fluctuated within rather low limits for the same variety between the 3 locations, which showed a quite good stability of the characteristics and that the conditions of the area were favorable for the apple (Table 1). For the variety Red Topaz, the fruit obtained in Pitești area were slightly bigger in terms of large diameter, which led to obtaining a shape index with a value lower than for the other locations. The small diameter had fluctuating values for the varieties Florina and Red Topaz, aspect that determined higher values for the size index for these varieties.

Table 1. Morphological indexes of fruit for some apple varieties, cultivated within 3 different locations

Variety	Location	Large diameter (mm)	Small diameter (mm)	Height (mm)	Shape index	Size index
Florina	USAMV Bucharest	73.65	63.39	69.40	0.94	68.81
	ICDP Pitești	75.62	72.55	62.27	0.82	70.14
	Moara Domnească	74.15	64.35	63.40	0.85	67.30
	Average	74.47	66.76	65.02	0.87	68.75
Generos	USAMV Bucharest	76.22	71.85	59.21	0.78	69.09
	ICDP Pitești	75.30	71.35	57.32	0.76	67.99
	Moara Domnească	75.21	70.23	58.32	0.77	67.92
	Average	75.58	71.14	58.28	0.77	68.33
Red Topaz	USAMV Bucharest	65.52	64.12	51.16	0.78	60.27
	ICDP Pitești	77.20	73.61	50.10	0.64	66.97
	Moara Domnească	66.22	64.12	50.65	0.76	60.33
	Average	69.65	67.28	50.64	0.73	62.52
Redix	USAMV Bucharest	70.30	67.29	68.45	0.97	68.68
	ICDP Pitești	70.53	67.62	71.15	1.00	69.77
	Moara Domnească	70.44	67.32	69.25	0.98	69.00
	Average	70.42	67.41	69.62	0.98	69.15

In what concerns average fruit weight during the 3 analyzed years, differences were recorded both between the locations and especially between the years when the measurements were made (Table 2). Thus, for the 3 varieties, Florina, Red Topaz and Redix, the year 2016 was better, the obtained fruit being larger than the ones obtained in 2015, when the fruit were the smallest. For the variety Generos, a slight increase from one year to another during research period.

The location had no uniform influence on the 4 varieties; the fruits obtained for Florina and Red Topaz were larger in the Pitești area, the Generos variety reacted better in the Moara Domnească area, while Redix scored better in Bucharest area. For all locations, fruit size had values within the variation limits of the varieties, the influence of the climatic year being obvious.

Fruit quality expressed through total dry substance content and firmness had different values both amongst the varieties, as it should have, but also amongst the same variety between the culture area (Table 3). Thus, regarding the content in soluble dry substance, for the Florina variety the highest value was recorded for the fruit obtained in Bucharest area, while the lowest value was recorded for Pitești area; in the case of Generos, fruit accumulated more substance in Pitești and less in Bucharest. Redix accumulated more dry substance in Pitești location and less in Moara Domnească, while Red Topaz reacted better to the conditions from Moara Domnească.

Table 2. Average fruit weight (g) depending on the production area

Variety	Locations	2015	2016	2017	Average	± St. Dev.
Florina	USAMV Bucharest	139.50	201.00	161.00	167.17	31.2103
	ICDP Pitești	162.00	194.00	170.00	175.33	16.6533
	Moara Domnească	172.00	180.00	157.00	169.67	11.6761
	Average	157.83	191.67	162.67	170.72	18.2987
Generos	USAMV Bucharest	145.00	179.00	168.00	164.00	17.3493
	ICDP Pitești	151.00	145.00	171.00	155.67	13.6137
	Moara Domnească	176.00	175.00	168.00	173.00	4.3588
	Average	157.33	166.33	169.00	164.22	6.1131
Red Topaz	USAMV Bucharest	182.00	154.00	137.00	157.67	22.7229
	ICDP Pitești	145.50	199.00	158.00	167.50	27.9866
	Moara Domnească	122.00	176.00	153.00	150.33	27.0985
	Average	149.83	176.33	149.33	158.50	15.4461
Redix	USAMV Bucharest	144.00	172.00	167.00	161.00	14.9331
	ICDP Pitești	158.00	170.00	132.00	153.33	19.4250
	Moara Domnească	138.00	172.00	162.00	157.33	17.4737
	Average	146.67	171.33	153.67	157.22	12.7119

Fruit firmness was better for the fruits obtained from 3 out of the 4 studied varieties in the Pitești area, which confirmed that this area ensures proper conditions for apple culture.

Table 3. Content in soluble dry substance and fruit firmness for some apple varieties depending on the production area

Variety	Location	Dried substance (%)	±St. Dev.	Firmness (kgf/cm^2)	±St. Dev.
Florina	USAMV Bucharest	13.9	2.7222	5.17	2.1501
	ICDP Pitești	11.53	2.2538	5.90	2.0663
	Moara Domnească	13.55	0.3535	5.10	1.5556
	Average	12.88	0.4284	5.68	2.0166
Generos	USAMV Bucharest	12.07	1.3219	5.23	1.3576
	ICDP Pitești	13.30	0.2000	5.60	1.2489
	Moara Domnească	13.20	0.0700	3.60	0.0800
	Average	12.79	0.6646	5.17	1.0016
Red Topaz	USAMV Bucharest	12.70	1.9000	4.80	1.4730
	ICDP Pitești	12.63	0.5773	5.33	1.6802
	Moara Domnească	13.90	1.2513	4.00	0.0070
	Average	12.71	0.984	4.94	1.5607
Redix	USAMV Bucharest	12.77	1.3051	4.77	3.0237
	ICDP Pitești	12.93	1.5373	4.70	1.8681
	Moara Domnească	11.15	1.9091	4.60	0.1414
	Average	12.58	1.0605	4.98	2.0678

The content in polyphenols and anthocyanins was influenced by the apple production area, amongst the same variety there were large differences recorded between the locations (Table 4). The highest average value for polyphenols content was obtained for the variety Florina, 1.14 mg/100 g f.p., with a maximum recorded for the fruit obtained in Pitești, 1.38 mg/100 g f.p. and a minimum in Moara Domnească 0.90 mg/100 g f.p. A similar situation was observed also for the variety Redix. In the case of Red Topaz and Generos, Bucharest area was better from this perspective, the values being obviously higher for Red Topaz with approx. 45% compared to the other two locations. The content in anthocyanins was higher for the fruits obtained in Pitești area, except for Generos for which the values were slightly higher for the fruit obtained in Bucharest and Redix for Moara Domnească area.

Table 4. Content in polyphenols and anthocyanins for some apple varieties cultivated in different areas

Variety	Location	Polyphenols		Anthocyanins	
		mg/100 g p.p.	± St. Dev.	mg/100 g p.p.	± St. Dev.
Florina	USAMV Bucharest	1.15	0.0006	0.22	0.1035
	ICDP Pitești	1.38	0.0001	0.40	0.1033
	Moara Domnească	0.90	0.1140	0.20	0.1140
	Average	1.14	0.0382	0.27	0.1100
Generos	USAMV Bucharest	0.92	0.0732	0.16	0.1100
	ICDP Pitești	0.43	0.0374	0.08	0.1226
	Moara Domnească	0.51	0.0412	0.12	0.0074
	Average	0.62	0.0506	0.12	0.0800
Red Topaz	USAMV Bucharest	1.44	0.1705	0.42	0.1114
	ICDP Pitești	0.80	0.1855	0.42	0.1042
	Moara Domnească	0.75	0.1211	0.41	0.1105
	Average	1.00	0.1590	0.42	0.1087
Redix	USAMV Bucharest	0.84	0.0071	0.30	0.0102
	ICDP Pitești	0.90	0.0045	0.30	0.1030
	Moara Domnească	0.75	0.2780	0.40	0.0278
	Average	0,83	0.0006	0.33	0.1035

CONCLUSIONS

Apple quality was influenced both by the variety and also by the culture area and climatic year. Fruit size had values within the biological limits of the variety, but for 3 out of the 4 studied varieties, the conditions in the Pitești area led to obtaining larger fruit.

Average fruit weight was influenced by the culture area, each variety having an area where it manifested better its biological characteristics.

Fruits obtained in the lower culture area generally had a higher content in soluble dry substance, while fruit obtained in Pitesti had a better firmness.

The content in polyphenols and anthocyanins was slightly higher for the fruit obtained in the hilly area.

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CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN THE LEAVES OF 'WILLAMETTE' AND 'MEEKER' RASPBERRY CULTIVARS

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Abstract

The present study observed the content of biologically active compounds in raspberry leaves in different phenophases (full blossoming, fruit harvesting and after fruit harvesting) and two stages of agrotechnics (0.50 m, 0.30 m). The study was conducted during the period 2019-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan with two introduced raspberry cultivars 'Willamette' and 'Meeker'. The field experiment is based at intra-row spacings between plants (0.50 m and 0.30 m) and inter-row spacings of 3.00 m. The content of biologically active compounds in the leaves was found during the phenophases: full blossoming, fruit harvesting and after fruit harvesting. The results show that the highest content of the studied indicators chlorophyll "a", chlorophyll "b" and β-carotene in the leaf samples is the cultivar Willamette, with a variant of 0.50 m, respectively (2.56 mg/g FW, 1.62 mg/g FW, 1.44 mg/g FW), from 2019 in the phenophase of full blossoming of plants. In the Meeker cultivar, the highest content of chlorophyll "a" and β-carotene was found from the variant of 0.50 m in 2019 in the phenophase of full blossoming (1.80 mg/g FW, 1.10 mg/g FW) and chlorophyll "b" from the same variant after fruit harvest for 2020-1.04 mg/g FW.

Key words: *raspberries, cultivars, agricultural techniques, biologically active compounds in the leaves.*

INTRODUCTION

Red raspberry (*Rubus idaeus* L.) is one of the most common berry crops in the world (Williams, 1960; Kazakov, 2001). The research and consumer interest in it is determined by its attractive appearance, gorgeous aroma and excellent nutritional and healing properties, which have a beneficial effect on human health. Recent studies have shown that the leaves of berry plants, such as strawberries, raspberries, blueberries and blackcurrants, are a potential source of biologically active compounds with strong healing, anti-cancer and anti-inflammatory properties (Oszmiański, et al., 2011; Costea, et al., 2016; Grochowski, 2016; Veljković, 2019). Red raspberry leaves contain biologically active polyphenols that can be used to supplement the daily intake of valuable natural antioxidants (Durgo et al., 2012). The main bioactive compounds in the leaves are pigments (chlorophylls and carotenoids). In living plants, chlorophyll plays an important role as the primary photosynthetic pigment for capturing light energy from the sun. Composed together with carotenoids (additional photosynthetic pigments) in pigment-protein complexes, it exhibits a color specific to each leaf and is even used as an indicator for maturity, quality and freshness of food crops. The beneficial effects of natural pigments on human health have led to increased interest in the study of these substances.

The aim of the present study is to observe the content of biologically active compounds in raspberry leaves in various agrotechnics of planting.

MATERIALS AND METHODS

The experiment was conducted during the period (2018-2020) in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan. The objective of the study are the widely distributed raspberry cultivars, such as 'Willamette' and 'Meeker', characterized by high fruitfulness. The area is maintained in black fallow in the intra-row space and has naturally grassed inter-row spacing. Fertilizing was performed in the intra-row area to achieve optimal values of the individual nutrients for the raspberry crop. The plants were grown under irrigated conditions with drip irrigation. The experimental variants are:

I var. - planting at 0.50 m in the intra-row area;

II var. - planting at 0.30 m in the intra-row area;

In both variants the inter-row spacing is 3.00 m. The content of chlorophyll "a", chlorophyll "b" and ß-carotene was determined spectrophotometrically after extraction of the pigments with an organic solvent. Leaf samples were taken during the phenophases of full blossoming, fruit harvesting and after fruit harvesting.

Cut the fresh leaves of the plant -100 g. The sample is ground in a mortar with a little quartz sand and 1-2 drops of acetone. With the help of 10 ml of acetone in 2-3 portions, the mop is washed from the mortar and transferred to a test tube, where it stays for 5 minutes. The resulting green extract was filtered using a funnel and filter paper. Dilute the resulting extract 5 times by adding with in a test tube (Tsvetkova et al., 2017).

The methodology for studying plant resources in fruit plants was used to report the indicators (Nedev et al., 1979). The data processing was performed by the methods of two-factor analysis of variance, correlation and regression analysis (Lidanski, 1988), using the software product.

RESULTS AND DISCUSSIONS

Carotenoids and chlorophylls (lipid-soluble pigments) are natural pigments responsible for yellow, orange, red and green colous, widely distributed in various plant organs (leaves, blossoms, stems). Chlorophylls are pigments capable of capturing light energy from sunlight and producing carbohydrates. They are responsible for the process of photosynthesis and green hues in plants. Among the several types of chlorophyll pigments, chlorophyll a and b are the most common pigments that are mainly involved in photosynthesis. Carotenoids are chemical compounds that reflect yellow, orange and red colors. They can be used as an indicator of maturity, quality and freshness of food crops (Limantara et al., 2015).

In 2019, in the phenophase of full blossoming, higher content of chlorophyll "a", chlorophyll "b" and β-carotene were reported in the leaves of both variants of 'Willamette' (Table 1).

In both genotypes studied, the pigments had higher values at longer planting distances. Variation coefficient was high with 20.57% (chlorophyll "a") and 28.09% (chlorophyll "b"). Statistical evidence between genotypes was reported only in terms of chlorophyll "a" content, and the differences between the variants are unproven.

The content of chlorophyll "a" and chlorophyll "b" during the period of fruit harvesting was less than the blossoming period. It was in the range from 1.00 to 1.69 mg/g FW (chlorophyll "a") and from 0.48 to 0.78 mg/g FW (chlorophyll "b"). In 'Willamette', the highest (in the second variant) and the lowest values of the two pigments (in the first variant) of planting were reported. The differences in chlorophyll values were minimal in both agricultural techniques used for 'Meeker'. During the fruit harvesting period, the coefficient of variation of chlorophyll "a" and "b" pigments was high at values above 20%. There is no mathematical proof between the variants and genotypes of both cultivars regarding the presence of chlorophyll.

The content of ß-carotene during fruit harvesting was in the range 0.63 - 0.83 mg/g FW, values lower than the blossoming period. The first versions of "Willamette"

(0.81 mg/g FW) and "Meeker" (0.83 mg/g FW) have a higher content.
During the next phenophase after fruit harvesting, the tendency to decrease the amount of the studied indicators continues, as again in the leaves of "Willamette" higher values of chlorophyll "a", "b" and carotene were reported compared to "Meeker". Variation coefficient was in the range 22.64-34.96%. Statistically, differences between genotypes were proven (P<0.05), and between variants were insignificant.

Table 1. Biologically active compounds in the leaves of raspberry genotypes in 2019

Cultivars/indicators	Chlorophyll "a" (mg/g FW), $X \pm S_x$	Chlorophyll "b" (mg/g FW), $X \pm S_x$	ß-carotene (mg/g FW), $X \pm S_x$
Blossoming period			
Willamette-0.50 m	2.56 ± 0.02	1.62 ± 0.14	1.44 ± 0.04
Willamette-0.30 m	2.21 ± 0.12	1.33 ± 0.19	1.24 ± 0.05
Meeker-0.50 m	1.80 ± 0.25	1.03 ± 0.27	1.10 ± 0.13
Meeker-0.30 m	1.62 ± 0.35	0.85 ± 0.30	0.96 ± 0.18
VC %	20.57	28.09	17.34
Minimum	1.62	0.85	0.96
Maximum	2.56	1.62	1.44
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	P<0.05	n.s	n.s
Fruit harvesting period			
Willamette-0.50 m	1.00 ± 0.10	0.48 ± 0.10	0.81 ± 0.02
Willamette-0.30 m	1.69 ± 0.07	0.78 ± 0.09	0.63 ± 0.07
Meeker-0.50 m	1.39 ± 0.02	0.68 ± 0.01	0.83 ± 0.02
Meeker-0.30 m	1.09 ± 0.14	0.60 ± 0.04	0.68 ± 0.04
Minimum	1.00	0.48	0.63
Maximum	1.69	0.78	0.83
VC %	24.46	20.16	13.12
Proving the differences (P) among variants	n.s	n.s	P<0.05
Proving the differences (P) among genotypes	n.s	n.s	n.s
After fruit harvesting			
Willamette-0.50 m	1.10 ± 0.24	0.56 ± 0.07	0.65 ± 0.12
Willamette-0.30 m	1.27 ± 0.07	0.58 ± 0.00	0.68 ± 0.03
Meeker-0.50 m	0.61 ± 0.02	0.35 ± 0.01	0.44 ± 0.03
Meeker-0.30 m	0.68 ± 0.15	0.40 ± 0.06	0.46 ± 0.06
Minimum	0.61	0.35	0.44
Maximum	1.27	0.58	0.68
VC %	34.96	24.42	22.64
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	P<0.05	P<0.05	P<0.05

In 2020, in the phenophase of full blossoming, the content of chlorophyll “a” in the leaves was in the range 1.56-1.88 mg/g FW (Table 2). Higher values were reported for longer planting distances, 1.88 mg/g FW for Willamette (0.50 m) and 1.72 mg/g FW for ‘Meeker’ (0.50 m), respectively. Statistical differences between variants and genotypes are unproven. The second variant of ‘Meeker’ had the highest variant of chlorophyll "b" (0.81 mg/g FW). Moreover, both variants of planting of ‘Willamette’ contain the same amount (0.75 mg/g FW). The presence of carotene in the leaves of the first variants was 0.96 mg/g FW (‘Willamette’) and 0.94 mg/g FW (‘Meeker’), respectively. Variation coefficient is low for the three studied indicators. Statistical differences between variants and genotypes are unproven.

Table 2. Biologically active compounds in leaves of raspberry genotypes in 2020

Cultivars/indicators	Chlorophil "a" (mg/g FW), $X \pm S_x$	Chlorophil "b" (mg/g FW), $X \pm S_x$	ß-carotene (mg/g FW), $X \pm S_x$
Blossoming period			
Willamette-0.50 m	1.88 ± 0.05	0.75 ± 0.02	0.96 ± 0.00
Willamette-0.30 m	1.66 ± 0.18	0.75 ± 0.06	0.88 ± 0.10
Meeker-0.50 m	1.72 ± 0.00	0.72 ± 0.03	0.94 ± 0.02
Meeker-0.30 m	1.56 ± 0.05	0.81 ± 0.06	0.88 ± 0.02
VC %	7.86	5.24	4.42
Minimum	1.56	0.72	0.88
Maximum	1.88	0.81	0.96
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	n.s	n.s	n.s
Fruit harvesting period			
Willamette-0.50 m	1.59 ± 0.04	0.71 ± 0.05	0.88 ± 0.01
Willamette-0.30 m	1.55 ± 0.11	0.70 ± 0.01	0.86 ± 0.04
Meeker-0.50 m	1.28 ± 0.14	0.56 ± 0.06	0.77 ± 0.08
Meeker-0.30 m	1.12 ± 0.10	0.52 ± 0.05	0.64 ± 0.07
Minimum	1.12	0.52	0.64
Maximum	1.59	0.71	0.88
VC %	16.13	15.25	13.76
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	$P<0.05$	$P<0.05$	n.s
After fruit harvesting			
Willamette-0.50 m	1.13 ± 0.11	0.94 ± 0.07	0.68 ± 0.05
Willamette-0.30 m	1.01 ± 0.25	0.73 ± 0.17	0.65 ± 0.18
Meeker-0.50 m	1.37 ± 0.23	1.04 ± 0.02	0.81 ± 0.12
Meeker-0.30 m	1.05 ± 0.27	0.92 ± 0.14	0.64 ± 0.11
Minimum	1.01	0.73	0.64
Maximum	1.37	1.04	0.81
VC %	34.96	24.42	22.64
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	n.s	$P<0.01$	n.s

During the phenophase fruit harvesting (2020) the highest content was found in 'Willamette' in both variants, as the longer planting distances had a predominance, although minimal. Variation coefficient is mean. Mathematically, differences were demonstrated only between genotypes ($P <0.05$).

After fruit harvesting, the highest levels of chlorophyll "a", chlorophyll "b" and ß-carotene were reported in the first variant of 'Meeker' (1.37 mg/g FW; 1.04 mg/g FW; 0.81 mg/g FW). Although the content of biologically active compounds in the leaves of 'Willamette' is lower than in 'Meeker', higher genotypes were found in both genotypes at longer planting distances. Variation coefficient is high with values, respectively for chlorophyll "a" - 34.96%; chlorophyll "b" - 24.42%; ß-carotene - 22.64%. Statistically, the differences between variants and genotypes with respect to the biologically active compounds studied were insignificant. The results of scientific research show that the content of chlorophylls and carotenoids also depends on the technology of growing raspberries (conventional and organic). In this regard, a study by Ponder et al. (2019) showed that raspberry leaves from conventional farming contained significantly more ß-carotene (1.22 mg 100 g^{-1} FW and 0.46 mg 100 g^{-1} FW), chlorophyll "a" (8.09 mg 100 g^{-1} FW and 3.96 mg 100 g^{-1} FW), chlorophyll "b (2.43 mg 100 g^{-1} FW and 1.79 mg 100 g^{-1} FW), than did the leaves from organic farming.

A correlation analysis was conducted to follow the relationships between the biologically active compounds on average for the three phenophases over the years.

In the first experimental year in the (0.50 m) variant of the variety ‘Willamette’, there is a very strong positive correlation between chlorophyll "a" and "b", as well as between chlorophyll and carotene (r = 0.94; r = 0.92; r = 0.91) (Table 3). In variant (0.30 m) a very strong correlation between chlorophyll (r = 0.96) and a strong correlation between chlorophyll "a" and "b" with carotene (r = 0.83; r = 0.88) was again reported.

Table 3. Correlation dependences between biologically active compounds of ‘Willamette’ for the period (2019-2020)

Indicators	Chlorophyll “a” (mg/g FW)	Chlorophyll “b” (mg/g FW)	ß-carotene (mg/g FW)
2019	**0.50 m**		
Chlorophyll “a”	1		
Chlorophyll “b”	0.94	1	
ß-carotene (mg/g)	0.92	0.91	1
	0.30 m		
Chlorophyll “a”	1		
Chlorophyll “b”	0.96	1	
ß-carotene (mg/g)	0.83	0.88	1
2020	**0.50 m**		
Chlorophyll “a”	1		
Chlorophyll “b”	-0.83	1	
ß-carotene (mg/g)	0.99	-0.88	1
	0.30 m		
Chlorophyll “a”	1		
Chlorophyll “b”	0.49	1	
ß-carotene (mg/g)	0.96	0.69	1

In the second year of the experiment with the two planting variants of ‘Willamette’, a very strong positive correlation was observed only between chlorophyll “a” and ß-carotene (r = 0.99; r = 0.96). In the 0.50 m variant between chlorophyll "a" and "b" and between chlorophyll "b" and carotene, a strong but negative dependence was reported (r = -0.83; r = -0.88).

In the first experimental year, in both planting variants of ‘Meeker’, there was a very strong positive correlation dependence between all studied indicators with values ranging from r = 0.93 to r = 0.99 (Table 4). In the second year, in both variants of “Meeker”, a high dependence was found only between chlorophyll “a” and ß-carotene (r = 0.98; r = 0.99).

Table 4. Correlation dependences between biologically active compounds of ‘Meeker’ for the period (2019-2020)

Indicators	Chlorophyll “a” (mg/g FW)	Chlorophyll “b” (mg/g FW)	ß-carotene (mg/g FW)
2019	**0.50 m**		
Chlorophyll “a”	1		
Chlorophyll “b”	0.96	1	
ß-carotene (mg/g)	0.99	0.96	1
	0.30 m		
Chlorophyll “a”	1		
Chlorophyll “b”	0.93	1	
ß-carotene (mg/g)	0.96	0.96	1
2020	**0.50 m**		
Chlorophyll “a”	1		
Chlorophyll “b”	0.07	1	
ß-carotene (mg/g)	0.98	0.12	1
	0.30 m		
Chlorophyll “a”	1		
Chlorophyll “b”	0.36	1	
ß-carotene (mg/g)	0.99	0.42	1

CONCLUSIONS

In both experimental years, the highest content of biologically active compounds in the leaves of raspberry cultivars ‘Willamette’ and ‘Meeker’ were registered at the phenophase of full blossoming.

In 2019, in both studied genotypes the pigments had higher values at longer planting distances at the phenophase of full blossoming.

During the phenophase of full blossoming and after fruit harvesting in 2019, a higher content of pigments in ‘Willamette’ fruit was reported, and during the phenophase harvesting of ‘Meeker’.

The correlation analyzes of the biologically active compounds chlorophyll "a", chlorophyll "b" and ß-carotene in the leaf samples determine the following dependences at over r <0.7:

In the first experimental year in both variants in the genotypes, a strong correlation between the three studied indicators was reported.

In 2020, only the first variant of ‘Willamette’ was found. In the case of the 0.30 m planting variant, a strong pattern was observed only between chlorophyll “a” and ß- carotene.

In 2019, in the phenophase of full blossoming, in both studied genotypes, the pigments had higher values at longer planting distances.

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COMPARISON OF FRUIT QUALITY ATTRIBUTES OF SOME FIG (*FICUS CARICA* L.) GENOTYPES FROM THE SOUTH-WEST REGION OF ROMANIA

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Abstract

The study aims to make a comparison between fourteen fig genotypes, grown in the South-West region of Romania, that have fruits with different quality traits and to assess which of these genotypes have elevated potential and represent valuable biological material for future propagation. Biometrical variables (weight, length, and width) were measured for the first and the main crop, stalk length and ostiole diameter were also measured. Visual observations were done and the fruits were characterized using the fig characterization given by IPGRI (The International Plant Genetic Resources Institute), 2003. The fruit weight had values between 67 g in SV2 and 11 g (S1), the fruit length varied between 5.6 cm (SV2) and 2.6 cm (S1) and the width of the fruits was between 2.81 cm (C1) and 4.68 cm (SV2) genotype. The total soluble solids/TSS (sugar) and the titratable acidity (TA) of the fruits were also assessed, the sugar content recording values of 17.41% Brix for IJ1 genotype and 27.83% Brix for C3 genotype.

Key words*: Ficus carica L., genotype, fruit quality, sugar content, titratable acidity, fruit size.*

INTRODUCTION

Fig (*Ficus carica* L.) is one of the ancient fruits known to mankind which also finds its mention in the Bible. It is reported to be under cultivation from 3000-2000 BC in the eastern Mediterranean region (Fig, 2016). Fruits are consumed fresh as well as in the dried form. Fresh figs are delicious and nutritious as they are rich in calorie, protein, calcium and iron. Fig has nutritive index of 11, as against 9 for apple and 6 for raisin (Mazmanyan V., 2022). The bulk of the fruit (about 80%) is consumed in the dried form (Fig, 2016). The fruit is also credited with laxative and medicinal properties and is being applied on boils and for other skin infections (Mazmanyan V., 2022). Studying the fruit quality of different fig cultivars in order to select and preserve valuable biological material represents the a big part of the work of many authors (Holia, 2018; Zhang et al. 2020; Pereira et al., 2017; Trad et al., 2014; Polat et al., 2017, 2008; Çalişkan and Polat, 2012, 2008; Saddoud et al., 2008 etc.).

The productivity and popularity of fig trees in the South-West region of Romania has increased in the last years due to the climate changes that have created a favourable environment for growing figs and harvesting high quality fruits with minimum of maintenance required, creating an opportunity for researchers to expand their studies on *Ficus carica* L. in this region, as well as giving a more precise and complex cultivar characterization to farmers and growers.

In the climatic conditions of Romania, when planted in the right environmental conditions, fig trees produce two crops every year, this fact giving big yields to farmers who grow figs for commercial purposes.

The study aims to make a comparison between fourteen fig genotypes, grown in the South-West region of Romania, that have fruits with different quality traits and to assess which of these genotypes have elevated potential and represent valuable biological material for future propagation. This research could offer to growers a scientific based characterization of the fig varieties in the area.

MATERIALS AND METHODS

The study was conducted in the South-West region of Romania, during the year 2020. The

genotypes were collected from Svinița village (44°32′11″N 22°05′15″E), location well known for the mild and favourable climate and numerous fig trees, and Orșova town (44°43′ 31″N 22°23′46″E) which is 48 km away from Svinița.
A total of fourteen genotypes were included in the study, four collected from Svinița (S1, SV1, SV2, SM1) and the other ten (C1, C2, C3, M0, M2, F1, F2, F3, L1 and IJ1) from different parts of Orșova town (Figures 1-6).

Figure 1. S1 genotype

Figure 2. SV1 genotype

From each genotype, 30 fruits were randomly selected from the fig trees. Pomological characteristics were determined for each genotype. The biometrical aspects (weight, length, width) were measured for both crops, first and main crop, for every genotype. Fruit characteristics such as external appearance and internal quality aspects such as sugar content and titratable acidity were recorded only for the main crop, the reason being that this is the crop which represents higher commercial value.

Figure 3. SM1 genotype

Figure 4. SV2 genotype

Figure 5. IJ1 genotype

Figure 6. F3 genotype

Fruit weight was measured with a precise kitchen scale. The ostiole width was measured with a manual clipper, as well as the fruit width, fruit length and fruit stalk. The sugar content was determined with a digital ATAGO refractometer (ATAGO Co., Tokyo, Japan). The titratable acidity (expressed as % citric acid) was determined by titrating with 0,1*N* NAOH, and using phenolphthalein as an indicator, up to an end point.
The fruit index was calculated by dividing the width by the length. The fruit shape, the petiole length, the shape of stalk, the osteole width, the fruit skin cracks, the fruit skin colour and the fruit flesh colour, were determined based on the fig descriptor developed by IPGRI and CIHEAM, 2003.
The harvest period was determined for the main crop.
The data regarding the biometrical aspects was statistically processed using variance analysis, as the experiment control being used the experience average.

RESULTS AND DISCUSSIONS

The results regarding the biometrical aspects of the fruits for the both crops (first and main), in the year 2020, are presented in Tables 1-6. The weight and the fruit size (expressed by width and length) of the majority of genotypes, varied from one crop to another; the fruits in the main crop being slightly smaller compared to the first crop. The external and some internal fruit characteristics (TSS, TA, colour of the pulp) are presented in Tables 7 and 8. It is shown that the genotypes have different coloured skin and pulp, also some present crack on the skin surface, or medium to very large ostiole width (Table 8.). The fruit shape, the length of the petiole, the shape of the stalk and the harvest period, are presented in Table 7. The sugar and the titratable acidity are shown in Table 8.

Table 1. Fruit weight values (first crop), year 2020

Genotype	Fruit weight (g)	Relative value (%)	Difference to control	Significance
C1	25.00	67.65	-11.95	000
C2	26.20	70.90	-10.75	000
C3	29.10	78.75	-7.85	00
M0	50.57	136.84	13.61	XXX
M2	24.73	66.93	-12.22	000
S1	19.07	51.60	-17.89	000
SV1	26.13	70.72	-10.82	000
SV2	60.70	164.26	23.75	XXX
SM1	60.03	162.46	23.08	XXX
F1	13.27	35.90	-23.69	000
F2	60.33	163.26	23.38	XXX
F3	34.9	94.44	-2.05	-
L1	47.23	127.81	10.28	XXX
IJ1	40.13	108.60	3.18	-
Average	36.95	100.00	0.00	Control
LD5% = 5.55 g LD1% = 7.50 g LD0.1% = 9.99 g				

In the first crop the fruit weight of the studied genotypes, had values between 13.27 g (F1) and 60.72 g (SV2) g with an experience average of 36.95 g (Table 1). Six genotypes have exceeded the mean value, five of them being very significant positive compared to the control (SV2, SM1, F2, M0 and L1) and one was not statistically assured (IJ1). Eight of the genotypes recorded values under the experience average, being very significant negative, other significant negative (C3), while F3 genotype was not statistically assured.

Table 2. Fruit weight (main crop), year 2020

Genotype	Fruit weight (g)	Relative value (%)	Difference to control	Significance
C1	21.63	76.51	-6.64	00
C2	20.70	73.21	-7.57	00
C3	22.27	78.76	-6.01	0
M0	40.37	142.77	12.09	XXX
M2	19.97	70.62	-8.31	00
S1	12.97	45.86	-15.31	000
SV1	21.83	77.22	-6.44	00
SV2	47.70	168.71	19.43	XXX
SM1	41.47	146.66	13.19	XXX
F1	10.80	38.20	-17.47	000
F2	39.3	139.00	11.02	XXX
F3	30.86	109.17	2.59	-
L1	34.96	123.673	6.69	XX
IJ1	31.03	109.76	2.76	-
Average	28.27	100.00	0.00	Control
LD5% = 4.83 g LD1% = 6.352 g LD0.1% = 8.69 g				

For the main crop the fruits weighted between 10.80 g (F1), being very significant negative and 47.40 g (SM1), being very significant positive compared to the control, with an experience average of 28.27 g (Table 2). Higher values compared to the experience average were also recorded in M0, SV2, F2 genotypes, all being very significant positive and in L1 being distinct significant positive. Lower values compared to the average were recorder for S1 genotype being very significant negative, others, C1, C2, M2, SV1 were distinct significant negative, while F3 and IJ1 were not statistically assured. Similar weight values were also recorded by Çalişkan and Polat (2012), in their research about some Turkish fig genotypes, the figs' weight values varying between 22.8-57.5 g. Koyunku et al. (2004, 1998, 1998) recorded fruit weights between 23-84 g, 9.00-38.37 g and 11.35- 58.00 g. In the first crop, the fruit width measured between 4.68 cm (SV2) and 2.81 cm (C1), with an experience average of 3.81 cm (Table 3).

Table 3. Fruit width (first crop), year 2020

Genotype	Fruit width (cm)	Relative value (%)	Difference to control	Significance
C1	2.81	73.91	-0.99	000
C2	2.91	76.53	-0.89	000
C3	3.71	97.46	-0.10	-
M0	4.46	117.25	0.66	XX
M2	2.87	75.31	-0.94	000
S1	3.38	88.88	-0.42	0
SV1	3.69	96.94	-0.12	-
SV2	4.68	122.85	0.87	XXX
SM1	4.66	122.42	0.85	XXX
F1	2.84	74.69	-0.96	000
F2	4.60	121.01	0.80	XXX
F3	4.08	107.35	0.28	-
L1	4.43	116.46	0.62	XX
IJ1	4.26	111.90	0.45	X
Average	3.81	100.00	0.00	Control
LD5% = 0.39 cm LD1% = 0.53 cm LD0.1% = 0.71 cm				

The highest width values were recorded in SV2 genotype, followed by SM1, all being very significant positive compared to the experience average. M0 and L1 were distinct significant positive, while IJ1 was significant positive. C1, C2, M2 and F1, recorded the lowest values (2.81 cm, 2.91 cm, 2.87 cm and 2.84 cm), all four being very significant negative followed by S1 genotype with a significant negative value. C3, SV1 and F3, were not statistically assured.

For the main crop, seven genotypes exceeded the experience average (3.47 cm), M0, SV2, SM1 were very significant positive, L1- distinct significant positive, while F2, F3 and IJ1 were significant positive. Five genotypes recorded the lowest width values - M2, C2, C1, F1 and S1, all being very significant negative. C3 and SV1 genotypes were not statistically assured (Table 4).

Table 4. Fruit width (main crop), year 2020

Genotype	Fruit width (cm)	Relative value (%)	Difference to control	Significance
C1	2.67	77.02	-0.80	000
C2	2.58	74.33	-0.89	000
C3	3.39	97.69	-0.08	-
M0	4.30	124.04	0.83	XXX
M2	2.49	71.92	-0.97	000
S1	2.90	83.56	-0.57	000
SV1	3.34	96.44	-0.12	-
SV2	4.43	127.88	0.97	XXX
SM1	4.26	122.79	0.79	XXX
F1	2.73	78.85	-0.73	000
F2	3.85	111.25	0.39	X
F3	3.8	109.61	0.33	X
L1	4.02	116.05	0.55	XX
IJ1	3.86	111.34	0.39	X
Average	3.47	100.00	0.00	Control
LD5% = 0.31 cm LD1% = 0.41 cm LD0.1% = 0.55 cm				

The fruit length for the first crop, varied between 5.21 cm (F2) and 2.82 cm (F1), with an experience average of 3.87 cm (Table 5).

Table 5. Fruit length (first crop), year 2020

Genotype	Fruit length (cm)	Relative value (%)	Difference to control	Significance
C1	2.94	76.06	-0.93	00
C2	2.96	76.40	-0.91	00
C3	3.00	77.52	-0.87	00
M0	4.75	122.65	0.88	XX
M2	2.59	66.84	-1.28	000
S1	3.03	78.29	-0.84	00
SV1	3.96	102.24	0.09	-
SV2	4.96	128.25	1.09	XXX
SM1	4.96	128.08	1.09	XXX
F1	2.82	72.78	-1.05	000
F2	5.21	134.79	1.34	XXX
F3	4.32	111.80	0.45	-
L1	4.45	114.98	0.58	X
IJ1	4.32	111.71	0.45	-
Average	3.87	100.00	0.00	Control
LD5% = 0.52 cm LD1% = 0.71 cm LD0.1% = 0.94 cm				

Five genotypes exceeded the experience average - SV2, SM1 and F2 being very significant positive, M0 - distinct significant positive and L1 - significant positive. The lowest values were recorded by M2 (2.59 cm) and F1 (2.82 cm) being very significant negative, followed by C1, C2, C3 and S1 with significant negative values. SV1, F3 and IJ1 genotypes were not statistically assured. The main crop fruits, had length values between 2.35 cm (C2) and 5.05 cm (SV2), with an experience average of 3.50 cm (Table 6). Six of the studied genotypes had length values that exceed the experience average, M0, SV2, SM1 and F3 all being very significant positive, while L1 and IJ1 were distinct significant positive. Other six genotypes had lower values that the experience average, C1, C2, C3, M2 and F1, being all very significant negative, S1 - distinct significant negative, while SV1 and F2 were not statistically assured. Similar results were obtained by Ali Koyuncu et al. (2004, 1998, 1998), recording values of 36-56 mm, 24.48-43.60 mm and 3.10-5.25 cm (fruit width) and 30-56 mm, 22.00-39.80 mm and 2.20-6.20 cm (fruit length).

Table 6. Fruit length (main crop), year 2020

Genotype	Fruit length (cm)	Relative value (%)	Difference to control	Significance
C1	2.60	74.38	-0.90	000
C2	2.35	67.24	-1.15	000
C3	2.42	69.05	-1.08	000
M0	4.63	132.38	1.13	XXX
M2	2.38	67.90	-1.12	000
S1	2.76	78.86	-0.74	00
SV1	3.36	96.00	-0.14	-
SV2	5.05	144.38	1.55	XXX
SM1	4.34	124.00	0.84	XXX
F1	2.66	76.00	-0.84	000
F2	3.83	109.61	0.33	-
F3	4.32	123.42	0.82	XXX
L1	4.27	122	0.77	XX
IJ1	4.10	117.238	0.60	XX
Average	3.50	100.00	0.00	Control
LD5% = 0.45 cm LD1% = 0.60 cm LD0.1% = 0.80 cm				

Table 7. External fruit characteristics for the studied genotypes and the harvest period, in 2020 (main crop)

Genotype	Fruit width/length (index)	Fruit shape	Petiole length	Shape of stalk	Harvest period
C1	1.02	Globose	Short	Short and thick	August-September
C2	1.09	Globose	Short	Short and thick	August-September
C3	1.40	Oblate	Short	Short and thick	August-September
M0	0.92	Globose	Short	Short and thick	August-September
M2	1.04	Globose	Short	Short and thick	August-September
S1	1.05	Globose	Short	Short and thick	August-September
SV1	0.99	Globose	Short	Short and thick	August-September
SV2	0.87	Oblong	Short	Short and thick	August-September
SM1	0.98	Globose	Short	Short and thick	August-September
F1	1.02	Globose	Short	Short and thick	August-September
F2	1.00	Globose	Short	Short and thick	August-September
F3	0.89	Oblong	Long	Long and slender	August-September
L1	0.94	Globose	Short	Short and thick	August-September
IJ1	0.89	Oblong	Short	Short and thick	August-September

The fruit shape index had values between 0.97 (SV2) and 1.40 (C3). The fruit shape was globose, oblate and oblong (Table 7). Similar result were recorded by Polat and Çalişkan (2008). The petiole length was short for the most genotypes with the exception of F3 with a long petiole. The shape of the stalk was short and thick, with one exception (F3) of long and slender. The harvest period for the main crop in the studied genotypes is long (41-60 days), harvesting the fruits in August-September for all genotypes.

Table 8. External and internal fruit characteristics for the studied fig genotypes, year 2020 (main crop)

Geno-type	Osteole width	Fruit skin cracks	Fruit skin colour	Fruit flesh colour	TTS %	TA citric acid %
C1	Medium	None	Purple	Pink	27.70	0.22
C2	Medium	None	Purple	Pink	25.26	0.16
C3	Medium	None	Purple	Pink	28.90	0.16
M0	Large	Scarce longitudinal cracks	Brown	Amber	21.20	0.20
M2	Medium	None	Purple	Pink	26.54	0.18
S1	Medium	None	Purple	Pink	24.33	0.21
SV1	Medium	None	Purple	Pink	21.63	0.28
SV2	Large	Scarce longitudinal cracks	Brown	Amber	25.75	0.13
SM1	Very Large	Scarce longitudinal cracks	Brown	Amber	25.33	0.23
F1	Medium	None	Purple	Pink	27.70	0.21
F2	Large	Scarce longitudinal cracks	Yellow green	Amber	24.20	0.26
F3	Large	Scarce longitudinal cracks	Yellow green	Pink	21.1	0.29
L1	Large	Scarce longitudinal cracks	Brown	Amber	22.3	0.21
IJ1	Very Large	Scarce longitudinal cracks	Brown	Amber	18.15	0.27

The ostiole width was of medium size for C1, C2, C3, M2, S1, SV1 and F1 genotype, large for M0 and SV2 genotype and very large for SM1 genotype (Table 8). The fruit skin colour was purple, brown and yellow green with scarce longitudinal cracks or no cracks at all (Table 8). The flesh colour was pink for some genotypes and amber for others (Table 8.)
The total soluble solids content recorded values between 18.15 % (IJ1) and 28.90% (C3) and the titratable acidity between 0.13% (SV2) and 0.29% (F3). Similar values of TSS were recorded in some fig cultivars from Tunisia, the lowest being 16.53% and the highest 34 % and 36.54% (Aljane et al., 2009). Koyunku et al. (2004, 1998, 1998) recorded TTS contents ranging from 12 to 21.3%, 11.9-24.30% and 16.6-20.0%. Polat and Çalişkan (2008), recorder values of the titrable acidity ranging between 0.20 and 0.38% and TTS between 22.7 and 27.2%.

CONCLUSIONS

The South-West region of Romania has a high potential for fig cultivation, the results of the study showing that the studied genotypes give good quality fruits. Regarding the external appearance of the fruits in the studied fig genotypes, the size, shape and colour varied. The purple, smaller sized fruits (C1, C2, C3, F1, S1, M2, SV1) are more suitable for eating fresh and are more resistant to transportation, and storing, whereas bigger sized fruits (M0, L1, F2, F3, SM1, SV2, IJ1), are mostly used processed in jams and for making alcoholic drinks. They are easily perishable and do not perform well when transported.

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STUDY OF SOME STRAWBERRY VARIETIES IN THE ORGANIC SYSTEM IN THE PEDO-CLIMATIC CONDITIONS OF THE VLASIA PLAIN

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Abstract

During 2019-2022 a study on organically grown strawberries (Fragaria x ananassa Duch.) was carried out, in order to find which most suitable variety for Vlasia Plain area is. The crop was established in 2019 in open-field cultivation, with four strawberry cultivars 'Asia', 'Clery', 'Roxana' and 'Thutop', mound cropping system, with 2 rows, mulched with agrotextile and drip irrigation system. The fruits were harvested and the damaged fruits were eliminated and 20 randomly selected marketable fruits were assessed. Fruit weight, diameter and firmness, sugar content and the pH, were measured. Regarding average fruit weight, 'Thutop' had the highest value (19.75 g/fruit), while the lowest 'Clery' (12.43 g/fruit). Regarding sugar content, 'Clery' had average value of 8.8% Brix and the highest firmness of 1.1363 kgf/cm^2. After three years of studies, it can be drawn the conclusion that 'Thutop' recorded the best and most stable results in terms of fruit weight. 'Thutop' has large fruits so it is recommended to be sold as fresh fruit. 'Clery' is recommended for industrialization and obtaining processed products due to the high sugar content.

Key words: *strawberry, organic, cultivars, biometric, preliminary.*

INTRODUCTION

The study aim was to determine the behaviour analysis of four strawberry cultivars with early maturation, cultivated in organic culture, targeting diversification of strawberry assortment, at the same time to capitalize the pedo-climatic conditions from the Vlăsia Plain.
The strawberries (*Fragaria x ananassa*) chosen for our research are some of the most consumed fruits in the world, appreciated especially for their flavour. Strawberry fruit quality is defined by several characteristics and is influenced by genetic and climatic factors (Maltoni et al., 2009; Temocico et al., 2008).
The strawberry (*Fragaria x ananassa*) is a plant with a very high ecological plasticity that produces fruits economically in various climatic and soil conditions. They are among the first fruits to appear on the market in early summer, and can be consumed either fresh, or processed in the form of jam, compote, gummy bears, wines, juices, yogurt, cakes and ice cream. (N. Cepoiu et al., 2006).
Strawberry is the only herbaceous fruit species and due to this biological peculiarity it can easily be cultivated in protected areas, ensuring fresh fruit crops all year long, by combining it with another characteristic biological, remontant.
In 1988, Romania produced 39,000 tons of strawberries per year, being the 15th largest strawberry producer in the world. And after due to a period of decrease of cultivated areas the production consequently decreased to 11,750 tons. Afterward, a slightly upward trend began, reaching a production of around 22,900 tons in 2020 (FAOSTAT 2020).
In order to support the small holds strawberry production cultivated by small farmers and to increase the area cultivated with strawberries by commercial farms, a study using technological input was done.
Having as main goal the production of ecological fruits, we did not use any kind of fertilizer, growth promoters, chemical or organic substance that would influence the production and or the quality of the fruits and no phytosanitary protection substances were used.
The results were directly influenced on the natural fertility of the soil, the climatic conditions specific to the area, and the natural resistance of the varieties.

MATERIALS AND METHODS

During 2019-2022 a study on organically grown strawberries (*Fragaria x ananassa* Duch.) was carried out, in order to find which is the most suitable variety for Vlasia Plain area.
In 2019, the open-field cultivation of strawberries began at Research-Development Station for Fruit Tree Growing Băneasa. The crop was established in 2019 in open-field cultivation, with four strawberry cultivars: 'Asia', 'Clery', 'Roxana' and 'Thutop', using a mound cropping system, with 2 rows, mulched with agro textile and drip irrigation system.
The plantation was located on a reddish-brown forest soil, with a loamy-clay texture, and the following chemical properties:
- in horizon A 0-20 cm: a humus content of 4.04% and a pH of 7.5, organic carbon of 6.96 (105C), Nitrogen index 3.98 and mobile phosphorus 73.45;
- in Horizon B 20-40 cm: a humus content of 3.4% and a pH of 7.48, Organic carbon of 5.45, Nitrogen index 3.43 and mobile phosphorus 72.25.

Analyzing the agrochemical fertility indicators (PH, total organic carbon, humus content, mobile phosphorus content, and nitrogen index), determined before planting, it was found that the soil was characterized by a medium to good natural fertility.
The maintenance technology applied after the establishment of the experimental crops was minimal: weeds were removed 3 times between mounds and one time from the plant alveoli, regular irrigations and mowing were performed after the end of the harvest periods.
The mound was 25 cm and 70 cm wide between rows and 30 cm between plants in a row. The mounds were covered with black 100 g/m² density agro textile foil (Figure 1). Beneath the foil, drip tubes were placed with drippers at a distance of 30 cm and a flow rate of 4 l/h.
The four strawberry cultivars used within the study originated from Italy and present the following characteristics:
'Clery' - the fruit has an average weight of 19-25 g, regular conical shape, intense red colour and a very pronounced luster. The pulp is fleshy, juicy and aromatic, with a dry matter content of 9-10% Brix and high firmness. The fruits tend to shrink towards the end of the harvest, and under abundant irrigation conditions, the plant is susceptible to rotting (Figure 2).

Figure 1. Mounds covered agrotextile

Figure 2. Mounds with Clery variety 2020

'Roxana' - variety with very large fruits, average weight of 25-30 g, conical-elongated shape, bright red colour, and a dry matter content of 5-6% Brix.

Figure 3. Mounds with Roxana variety in 2020

'Thutop' - large fruit, flattened conical shape, with the average fruit weight of 25-28 g, a very bright surface and a homogeneous red colour, exhibiting a pleasant appearance (Figure 4).

Figure 4. Thutop variety (appearance)

Asia -, the fruit is large with an average weight of 19-30 g, elongated conical shape, bright to dark red colour, with bit of a neck under the calyx, a good shelf-life and a dry matter content of 6-10% Brix. 'Asia' is tolerant against most common root-diseases.
On each cultivar an area of six consecutive average-sized plants was demarcated.
From these areas, four fruit samples were collected for measurements and analyze during the fruiting period each year, each sample consisting of 20 fruits chosen at random from the 6 demarcated plants.
A schematically presentation of the strawberry varieties used within this study is presented in Figure 5. The structural differences can be easily observed.

Figure 5. Strawbery varieties cultivated by SCDP Băneasa

The biometric and biochemical determinations performed in the laboratory during the 3 years of study were:
- the average weight of the fruit, determined by weighing a sample of 20 fruits in 4 repetitions from each cultivar, annually with an analytical balance Kern compact laboratory balance weighing range 300 g, readability 0.001 g;
- the diameter of the fruit determined with an electronic calliper, measuring range: 0-300 mm, resolution: 0.01 mm, accuracy: +/- 0.03 mm, registering the height, small diameter and large diameter;
- the firmness of the fruits determined with a manual penetrometer FT-327 expressed in kgf/ cm^2;
- the sugar content of the samples, determined by the refractometric method, with the ATAGO ATC-1E refractometer, expressed in % Brix.

RESULTS AND DISCUSSIONS

This study was conducted with the aim of growing organic strawberries crops, for family farms or small farms, without the use of treatments or nutrient additives, with minimal technological input on a multi-annual strawberry crop. Furthermore, the lifespan will be determined by crop yield
The evaluation of the varieties performed from the point of view of the quality of the fruits was a very important component. Two aspects were considered: firstly, small scale cultivation, intended for personal consumption, and secondly, large scale production, intended for sale, thus providing financial benefits to producer.
The main physical properties of the fruits refer to their size, represented by the average weight and size of the fruit. Other physical characteristics that can influence the quality of the fruits are: firmness, uniformity, colour, shine, perfume, position of the achiness on the surface of the fruit.
If the size of the fruit is easy to determine, the quality expressed by the taste of the variety is more complex and depends on many factors, including the human one. Among the most important aspects to consider is the sugar content and the pH of the fruit.
In Table 1 are presented the size determinations of the studied cultivars.
If we compare the weight of the fruits obtained in the experimental field with the reference data associated with each studied cultivar, it can be observed that our specimens are much smaller. An explanation for this discrepancy is the manner in which the crop was cultivated. It can be assessed that the resulted specimens exhibited more reduced dimensions that the

standard, due to the lack of any additional fertilizers or growth stimulators.

Table 1. Average annual fruit size

Year of study	Cultivar	Weight [g]	Height [cm]	Small diameter [cm]	Large diameter [cm]
2019	Clery	11.3346	3.555 0	2.0163	2.6713
	Roxana	19.1952	3.977 5	2.8463	3.4401
	Asia	17.2461	3.968 8	2.5250	3.2475
	Thutop	20.2308	3.962 5	2.6188	3.4675
2020	Clery	14.8934	3.586 3	2.7650	3.0000
	Roxana	21.0414	4.172 5	2.9525	3.3413
	Asia	19.0010	4.136 3	2.8238	3.1650
	Thutop	20.5951	4.001 3	2.9113	3.2800
2021	Clery	11.0770	3.398 5	2.4747	2.6904
	Roxana	17.3318	3.570 5	2.6423	2.9354
	Asia	15.3480	3.916 1	2.5566	2.8330
	Thutop	18.4149	3.651 2	2.8150	3.1517

For example, in the case of ‘Clery’ variety, which produces the smallest fruits in our study, the standard description mentions fruits weighing between 19 and 25 g. The fruits obtained within our experiment, registered a weigh between 5.9 g, obtained in the last 2019 harvest and 29.63 g, obtained in the first 2020 harvest in, with a multiannual average of 12.43 g/fruit. The ‘Thutop’ variety, which stood out in size in the organic study, is also no closer to the characteristics provided by the standard description. The smallest fruit weighing 7.7 g was harvested in the fourth harvest of 2019 and in the same year, while the largest ‘Thutop’ strawberry was produced in the second harvest of the same year, weighing more than 40.56 g. The ‘Thutop’ variety has a multiannual average of 19.75 g. Other differences are presented in (Chart 1).

The firmness analysis evidenced that a smaller fruit have a better firmness than the larger ones. ‘Clery’ variety had the firmest fruits with a multiannual average of 1.1333 kgf/cm^2, results of the other varieties being close to each other. For ‘Thutop’ a firmness of 0.9128 kgf/cm^2 was registered, for Asia variety 0.8663 kgf/cm^2 and for ‘Roxana’, 0.8492 kgf/cm^2 (Table 2).

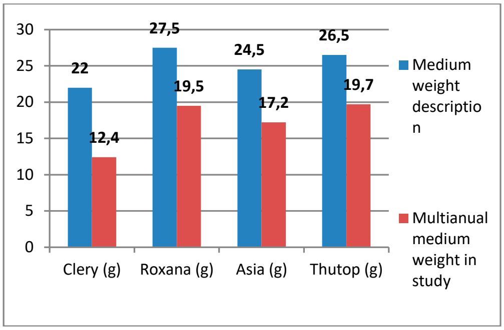

Chart 1. Weight between variety description and study

Table 2. Annual average fruit firmness

Year	Cultivar	Firmness [kgf/cm^2]
2019	Clery	1.1378
	Roxana	0.8436
	Asia	0.8606
	Thutop	0.9068
2020	Clery	1.0170
	Roxana	0.8938
	Asia	0.8925
	Thutop	0.8600
2021	Clery	1.2450
	Roxana	0.8103
	Asia	0.8458
	Thutop	0.9717

If we are going to produce organic fruit for self consumption, as is usually done on family farms, the most important aspect is not the size of the fruit but its taste, a factor influenced by the amount of sugar and its acidity.

Considering this aspect, analysis of the biochemical characteristics of the fruits, consisting of the sugar content (expressed in % BRIX) and pH, was performed using the same fruits subject to biometrically measurements Annual average results were presented in Table 3.

Table 3. Annual average sugar content and pH

Year	Cultivar	Sugar content [% Brix]	Ph
2019	Clery	9.3850	3.8700
	Roxana	8.4263	3.9150
	Asia	7.9563	3.8425
	Thutop	8.3000	3.8775
2020	Clery	8.3350	3.8537
	Roxana	6.0025	3.9200
	Asia	7.0675	3.7800
	Thutop	6.1550	3.9470
2021	Clery	8.6765	3.6386
	Roxana	7.2710	3.7500
	Asia	6.7408	3.6966
	Thutop	7.5093	3.9470

Strawberry fruit quality is defined by several characteristics and is influenced by genetic and climatic factors (Maltoni. et al., 2009; Temocico et al., 2008).

If we consider strawberry cultivars adaptability in our region, we must also mention the most important climatic factors that influenced the study in these 3 years, data presented according to the site https://rp5.ru/ In terms of climatic conditions, the 3 year period of the experiment were not ideal for strawberry crops, large fluctuations being remarked between consecutive years. The air temperatures and precipitations registered within the studied period are presented in Table 4.

Table 4. Annual meteorologiccal parameters

Meteorological parameters		Period	1-30 April	1-31 May	1-13 June
Air temperatures C°	Average of highs	2019	25.9	28.2	31.8
		2020	26.5	29.7	31
		2021	24.4	29.1	26.8
	Monthly average	2019	11	16.6	21.5
		2020	11.6	15.7	19.2
		2021	9.3	16.4	16.9
	Minimum average	2019	-1.7	5.5	12.9
		2020	-2.9	3.9	6.1
		2021	-4	2.4	9.7
Atmospheric precipitation (monthly amount mm)		2019	77	129	33
		2020	19	89	11
		2021	35	100	59

Data from sites https://rp5.ru/

In 2020, during the fruiting period the temperature was quite high and little precipitation was registered. Thus, it was necessary to supplement the water supply by irrigation. On the other hand 2021 was a year with temperatures below the normal average in April and May with abundant rainfall at the beginning of June, and temperatures higher during the night, created optimal conditions for the development of *Botritys cinerea* Pers. fungus, which caused great damage to the strawberries (Chart 2).

Holger Daugaard write in the paper Cultural methods for controlling *Botrytis cinerea* Pers. in strawberry, published in *Biological Agriculture & Horticulture* (2016): "*Botrytis cinerea* undoubtedly is the most important strawberry disease which, in conventional farming, is controlled by chemical fungicides during flowering. In organic farming, however, there is a need for the development of non-fungicide methods of control.

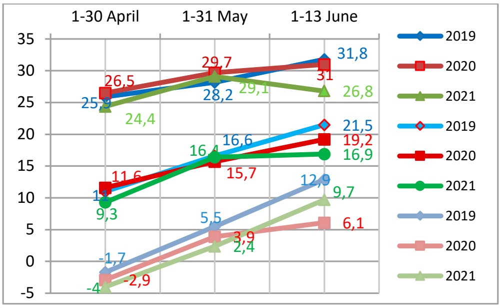

Chart 2. Evolution of annual minimum, average and maximum temperatures C° during fruit growth and harvest

Climate condition and factors could be the answer for smaller fruits obtained in 2021 according to Chart 3

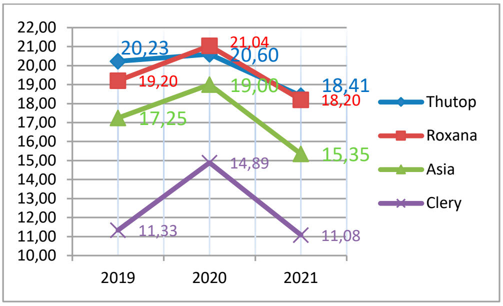

Chart 3. Annual average fruit weight

In this review cultural methods for controlling *B. cinerea* in strawberry are discussed. Although several methods have a documented effect in controlling *B. cinerea*, it is concluded that there are no methods - individually or in combination - capable of controlling this disease satisfactorily. Non-fungicide management of *B. cinerea* requires a dynamic and flexible approach to agroecosystem management."

That is why in this study we manually removed all fruits with signs of fungus contamination from the earliest stages, aiming order to reduce the sources of infection. (Figure 6)

Figure 6. *Botrytys cinerea* Pers. fungus on the 'Asia' variety, in 2021

The production of 2021 was affected in all varieties by a mixed attack the *Botritys cinerea* fungus and the *Deroceras agreste* pest (also favoured by the humid and warmer climate of 2021. The most affected cultivar was 'Asia' variety with 31% affected fruit followed by 'Roxana' and 'Thutop' with 23% and 21% respectively. The least affected variety was 'Clery', with only 17% compromised specimens.

CONCLUSIONS

Upon complex analysis of the main factors that influence the success of organic strawberry crop cultivation, it can be concluded that: 'Clery' (Figure 7) is the most suitable cultivar for being used in organic farming for family or small farms. This variety can be cropped for personal use (fresh or processed as jams, compotes and freezers) because it tastes better due to its high sugar content (8.8 Brix average and high fruit firmness).
For commercial farms that produce organic strawberry fruit for sale, following our study the 'Thutop' variety has shown the best characteristics having large fruits, with a constant production and a good resistance to the *Botritys cinerea* Pers fungus.

Figure 7. Clery fruit

The agricultural technology used within this study was minimal, easy to apply by anyone with low costs, which makes it very profitable and healthy. Further research is needed in order to establish whether the crop that was used for this 3 year study could be exploited for another years in terms of crop profitability.

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ROOT SYSTEM ARCHITECTURE IN 'VÂLCEAN' VARIETY (*PRUNUS DOMESTICA* L.) DEPENDING ON ROOTSTOCKS AND TRUNK

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Abstract

European plum (Prunus domestica) is one of the fruit tree species cultivated around the world for fresh consumption, prunes (dried fruits), smoked plums, in jams or jellies, juices, plum purée as a baby food, liqueur, distilled into a 'brandy' or spirits, having also a large potential for rural and metropolitan zones landscaping. The experiment was conducted during 2020 year to individual trees in a randomized compete block design in four replicate blocks (10 plum trees/block) within a private plum orchard in the proximity of Craiova city, Dolj county, Romania and included 'Vâlcean' plum variety and four rootstocks (Oteşani 8, Pixy, Miroval, Roşior văratic). The study assessed the architecture of root system, in thickness and variable depths, at 1 and 2 meters away from trunk. For 0-3 mm root thickness category Miroval rootstock has developed the highest roots number (104 roots) at 1m distance from the trunk, while at 2 m trunk distance the best root system development was noticed in Roşior văratic rootstock (58 roots). Among all plum rootstocks included in the experiment Miroval had the most performant root system.

***Key words**:* architecture, *Prunus domestica*, rootstock, root, plum variety.

INTRODUCTION

The European Plum (*Prunus domestica*) has been known in Europe for more than two thousand years, being originated in eastern Europe or western Asia around the Caucasus and the Caspian Sea. Nowadays, aside other spontaneous and cultivated plants and trees in temperate regions, plums are cultivated in many European countries, being suitable to be grown in light (sandy), medium (loamy) and heavy (clay) soils over a wide range of climatic conditions (Răduțoiu et al., 2012; Bonciu, 2019b; Butac et al., 2019; Cosmulescu et al., 2020; Gaši et al., 2020; Răduţoiu, 2020; Răduțoiu and Cosmulescu, 2020; Butac et al., 2021; Durău et al., 2021; Nesheva et al., 2021, Răduţoiu and Băloniu, 2021; Velea et al., 2021).
Within hexaploid European plum (*Prunus domestica*) there are many varieties and hybrids, varying from red, purple to yellow and green skin or pulp, being suitable for fresh consumption, dried prunes, smoked plums, jams or jellies, juices, plum purée, liqueur, spirts (brandy). Plums are also a great source of vitamins (vitamin C, A, K) and minerals (Ca, Mg, K, P) and other phytochemicals influencing human and preventing many diseases (heart diseases, Alzheimer`s disease, lung and oral cancer, reducing blood sugar, protecting bones, muscular degeneration, improving memory) (Arjmandi et al., 2002; Byrne et al., 2009; Stacewicz-Sapuntzakis, 2013; Birwal et al., 2017; Wallace, 2017; DiNardo et al., 2018; Gill et al., 2019; Mohammadi-Moghaddam et al., 2020 a, b). However, the nutraceutical effects of food or parts of food were underlined previously by many authors, being of great importance nowadays (Lever et al., 2015; Igwe and Charlton, 2016; Sadler, 2016; Soare et al., 2016; Chiu et al., 2017; Shamloufard et al., 2017; Mirza et al., 2018; Al-Dashti et al., 2019; Alsolmei et al., 2019; Tomić et al., 2019; Bonciu, 2020a; Khorrami et al., 2020; Dodier et al., 2021). Despite breeding progress, nutritional components in fruits, vegetables and field crops are affected by abiotic and biotic constrainers (Saleem et al., 1970; Labusca et

al., 2012; Popa et al., 2013; Bonciu, 2018; Bonciu, 2019a; Bonciu, 2019c; Bonciu, 2020b, Cotuna et al., 2020; Paraschivu et al., 2020; Bonciu et al., 2021; Paraschivu et al., 2021).

Among all European Union countries, Spain is the main supplier, Germany is the largest destination market for fresh imported plums, while France is one of the main plum-consuming ones.

Most plums imported by these countries are originated from Romania which is, accordingly to production statistics, the largest plum producer in Europe with over 500.000 tons annually and the highest yield in 2018 year (830.000 tons) (CBI, 2020).

Worldwide the rootstocks are essential in modern breeding programs due to their capability of adapting a plum variety to different environmental conditions, cultural practices and to improve the scion tolerance to biotic (pests and pathogens) and abiotic constrainers (high soil salinity, drought, heat, etc.) (Meland, 2010; Assimakopoulou et al., 2011; Font i Forcada et al., 2020).

On the other hand, the rootstocks have a great impact on plum trees vigour, canopy structure, fruit maturation period, yield and fruits quality (Beckman and Lang, 2003; Grzyb and Rozpara, 2012; Sidorova et al., 2018; Milatović et al., 2019; Bouzari et al., 2021; Gerbi et al., 2021; Hamdani et al., 2021; Stefanova et al., 2021).

In Romania the plum breeding activity was oriented towards obtaining new cultivars and rootstocks as result of specific ecological conditions (climatic change and poor soils) in various areas, beside high productivity, fruit quality, tolerance to Sharka disease (*Plum Pox Virus*). The plum rootstock assortment contains Romanian bred ones, which are more adapted to the specific soil conditions from the plum growing areas then the foreign rootstocks (Botu et al., 2012a; Botu et al., 2012b; Butac et al., 2013; Butac et al., 2019).

Rapid progress in breeding has leaded to a large number of valuable rootstocks, but still many nurseries are propagating only the most popular ones: Myrobalan type ones (several selections such as: ‘Miroval’, ‘Mirobolan C163’, ‘Mirobolan galben’, etc); ‘Roşior văratec’ and ‘Oteşani 8’ (*P. domestica*), ‘Oteşani 11’ and ‘Voineşti B’ (*P. insititia*).

Over 85% of the plum trees sold in Romania are grafted on Myrobalan. Other rootstocks like: ‘Pixy’, ‘St. Julien A’, ‘Rival’, ‘BN 4Kr’ are rarely used.

Among the factors that increase plum trees demand include new varieties and rootstocks, population growth, products that meet healthy lifestyle, food diversification and rising incomes.

The present study aim was to assess the architecture of root system of Vâlcean plum variety and four rootstocks (Oteşani 8, Pixy, Miroval, Roşior văratic) in the agro-environmental conditions of Dolj county, Romania.

MATERIALS AND METHODS

The experiment was conducted during 2018-2020 to individual plum trees in a compete block design in four replicate blocks (10 plum trees/block) within a private plum orchard in the proximity of Craiova city, Dolj county, Romania and included ‘Vâlcean’ plum variety and four rootstocks (Oteşani 8, Pixy, Miroval, Roşior văratic), resulting four biosystems graft/rootstock:Vâlcean/Oteşani8,Vâlcean/Pixy, Vâlcean/Miroval şi Vâlcean/Roşior văratic.

The orchard was grown on brown reddish silt loan soil (pH = 5.5-6.6, 0.78-2.35% organic matter, high potassium and phosphorus rate), under non-irrigation conditions, at a planting distance of 4 m between rows x 4 m between trees on row.

For the assessment of the root’s development pattern of each variety/rootstock biosystem it was used the Oscamp-Dragavţev within a soil profile of 100 x 100 m size, which was oriented at the trunk at the distance of 1m and 2 m. Depending on their thickness, the roots were divided into 3 categories: roots up to 3 mm in diameter; roots with a diameter between 3 to 5 mm; roots with a diameter of more than 5 mm.

It was quantified the number of branch roots and their ramification capacity on the depth interval of 10 to 10 cm, within 100 cm.

The primary recordings were processed through the use of biometrics indicators and synthesized in a quantified form summarizing the main characteristics of the root system.

The biometrical measurements of the tree-roots were performed by the metric frame. The assessment of development of the biosystems variety/rootstock pattern was done take into consideration: canopy size (CS), tree height (TH) and canopy volume (CV).
The experimental data were calculated and analysed, using MS Office 2019 facilities, while the structure of variability of the assessed traits were statistically processed by Duncan's multiple range test.

RESULTS AND DISCUSSIONS

Worldwide consistent research on the interaction variety/rootstock has been done previously on different fruit tree species (Cichi et al., 2008; Mazilu et al., 2018; Cichi and Cichi, 2019; Santana et al., 2020; Shahkoomahally et al., 2020; Ntanos et al., 2021; Shahkoomahally et al., 2021). The proper choice of the rootstock and scion combination is probably the most important factor of the plum cultivation success.
Thus, the experiment results showed clear that rootstock had a significant effect on tree growth traits.
The assessment of the root's development pattern for roots between 0-3 mm in diameter which were oriented at the trunk distance of 1 m, on different soil depths
Roots with a diameter between 0-3 mm were developed on all soil depths for Miroval and Roşior văratic rootstocks, while they missed on the soil depths 50-60 cm, 80-90 cm, 90-100 cm for Oteşani 8 rootstock and on 90-100 cm soil depth for Pixy rootstock (Figure 1).

Figure 1. Aspects regarding the root system (0-3 cm) at the distance of 1 m of the trunk on different soil depths (original photo Ciobanu Andi)

On the soil 0-100 cm depth interval, the roots were more developed for Miroval rootstock (104 roots), followed by those of Pixy rootstock (72 roots), Roşior văratic (64 roots) and Oteşani 8 (48 roots).
As 0-3 cm roots assessed for all experimented rootstocks, approximatively 72% were spread over the soil depth range 10-40 cm at the trunk distance of 1 m (Table 1).

Table 1. Roots between 0-3 mm in diameter oriented at the trunk distance of 1m, on different soil depths

Rootstock Soil depth range	Oteșani 8	Pixy	Miroval	Roșior văratic	Average	Percent (%)
0-10	3	6	14	6	7,25	10,06
10-20	11	14	25	14	16,00	22,22
20-30	19	10	18	12	14,75	20,49
30-40	11	13	20	12	14,00	19,46
40-50	2	12	8	6	7,00	9,72
50-60	0	8	7	5	5,00	6,94
60-70	1	4	4	2	2,75	3,82
70-80	1	3	4	3	2,75	3,82
80-90	0	2	2	2	1,50	2,08
90-100	0	0	2	2	1,00	1,39
TOTAL	48	72	104	64	72,00	100

The assessment of the root's development pattern for roots between 0-3 mm in diameter which were oriented at the trunk distance of 2 m, on different soil depths
At the trunk distance of 2 m the highest number of the roots with 0-3 mm in diameter were observed for Roşior văratic rootstock (58 roots), while the lowest roots number were developed by Pixy rootstock (49 roots).
Oteşani 8, Pixy and Miroval rootstocks developed roots on the whole soil depth interval (1-100 cm) (Figure 2).

Figure 2. Aspects regarding the root system (0-3 cm) at the distance of 2 m of the trunk on different soil depths (original photo Ciobanu Andi)

Roşior văratic rootstock developed 0-3 mm roots only to 70 cm soil depth. As 0-3 cm roots assessed for all experimented rootstocks, approximatively 77% were spread over the soil depth range 0-50 cm at the trunk distance of 2 m (Table 2).

Table 2. Roots between 0-3 mm in diameter oriented at the trunk distance of 2 m, on different soil depths

Rootstock Soil depth range	Oteşani 8	Pixy	Miroval	Roşior văratic	Average	Percent (%)
0-10	2	8	2	8	5,00	9,48
10-20	11	11	7	16	11,25	21,33
20-30	11	11	5	14	10,25	19,43
30-40	11	2	13	7	8,25	15,64
40-50	6	5	9	4	6,00	11,37
50-60	4	3	5	4	4,00	7,58
60-70	2	2	4	2	2,50	4,74
70-80	1	2	3	0	1,50	2,85
80-90	1	2	4	1	2,00	3,79
90-100	1	3	2	2	2,00	3,79
TOTAL	50	49	54	58	52,75	100

The assessment of the root's development pattern for roots between 3-5 mm in diameter which were oriented at the trunk distance of 1m, on different soil depths

At the trunk distance of 1 m the roots number (3-5 mm in diameter) was limited for all rootstocks. Thus, for Oteşani 8 and Roşior văratic rootstocks only one root was developed for each soil depth interval (0-10cm, 10-20 cm, 30-40 cm), while for Pixy it was noticed for 10-20 cm, 40-50 cm, 50-60 cm soil depths (Figure 3).

Figure 3. Aspects regarding the root system (3-5 cm) at the distance of 1 m of the trunk on different soil depths (original photo Ciobanu Andi)

For Miroval rootstock one root of 3-5 mm was developed on the 10-20 cm soil depth and other one on the 20-30 cm soil depth interval. To the four rootstocks it was observed an average of 2.75 roots developed, most of them being observed on the 10-20 cm soil depth interval (36.37%) (Table 3).

Table 3. Roots between 3-5 mm in diameter oriented at the trunk distance of 1 m, on different soil depths

Rootstock Soil depth range	Oteşani 8	Pixy	Miroval	Roşior văratic	Average	Percent (%)
0-10	1	0	0	1	0,50	18,18
10-20	1	1	1	1	1,00	36,37
20-30	0	0	1	0	0,25	9,09
30-40	1	0	0	1	0,50	18,18
40-50	0	1	0	0	0,25	9,09
50-60	0	1	0	0	0,25	9,09
60-70	0	0	0	0	0	0
70-80	0	0	0	0	0	0
80-90	0	0	0	0	0	0
90-100	0	0	0	0	0	0
TOTAL	3	3	2	3	2,75	100

The assessment of the root's development pattern for roots between 3-5 mm in diameter which were oriented at the trunk distance of 2 m, on different soil depths

At the trunk distance of 2 m, the rootstocks Oteşani 8 and Pixy developed two roots each one, as fallows: Oteşani 8 had one root for every soil depth interval of 40-50 cm and 80-90 cm, while Pixy had one root for every soil depth interval of 40-50 cm and 60-70 cm (Figure 4).

Figure 4. Aspects regarding the root system (3-5 cm) at the distance of 2 m of the trunk on different soil depths (original photo Ciobanu Andi)

At a distance of 2 m from the trunk the most roots were observed to Miroval rootstock, two roots were developed at soil depth of 30-40 cm (2 roots) and soil depth of 40 -50 cm (1 root).

For the rootstock Roşior văratic no roots were observed for the whole soil depth interval (0-100 cm).

For all rootstocks it was observed an average of 1,75 roots of 3-5 mm, most of them being developed at 40-50 cm soil depth (Table 4).

Table 4. Roots between 3-5 mm in diameter oriented at the trunk distance of 2 m, on different soil depths

Rootstock Soil depth range	Oteşani 8	Pixy	Miroval	Roşior văratic	Average	Percent (%)
0-10	0	0	0	0	0	0
10-20	0	0	0	0	0	0
20-30	0	0	0	0	0	0
30-40	0	0	2	0	0,50	28,57
40-50	1	1	1	0	0,75	42,85
50-60	0	0	0	0	0	0
60-70	0	1	0	0	0,25	14,29
70-80	0	0	0	0	0	0
80-90	1	0	0	0	0,25	14,29
90-100	0	0	0	0	0	0
TOTAL	2	2	3	0	1,75	100

The assessment of the root's development pattern for roots up to 5 mm in diameter which were oriented at the trunk distance of 1 m, on different soil depths

At a distance of 1 m from the trunk, all rootstocks included in the experiment developed at least one root up to 5 mm in diameter. A significant number of roots (9 roots) was developed by Miroval rootstock on soil depth of 0-30 cm (Figure 5).

Figure 5. Aspects regarding the root system up to 5 mm at the distance of 1 m of the trunk on different soil depths (original photo Ciobanu Andi)

For Pixy rootstock two roots were developed on soil depth of 10-20 cm and three roots on soil depth pf 40-50 cm.

For all rootstocks an average of 5 roots were developed on the soil depth interval of 10-20 cm. None of the rootstocks developed roots on depths up to 70 cm (Table 5).

Table 5. Roots up to 5 mm in diameter oriented at the trunk distance of 1 m, on different soil depths

Rootstock Soil depth range	Oteşani 8	Pixy	Miroval	Roşior văratic	Average	Percent (%)
0-10	0	0	3	0	0,75	15,00
10-20	1	2	3	2	2,00	40,00
20-30	1	0	3	0	1,00	20,00
30-40	1	0	0	0	0,25	5,00
40-50	0	3	0	0	0,75	15,00
50-60	0	0	0	0	0	0
60-70	1	0	0	0	0,25	5,00
70-80	0	0	0	0	0	0
80-90	0	0	0	0	0	0
90-100	0	0	0	0	0	0
TOTAL	4	5	9	2	5,00	100

The assessment of the root's development pattern for roots up to 5 mm in diameter which were oriented at the trunk distance of 2 m, on different soil depths

At a distance of 1 m from the trunk, only Oteşani 8 rootstock developed 3 roots, one on the soil depth of 20-30 cm and two roots on the soil depth of 30-40 cm (Figure 6 and Table 6).

Figure 6. Aspects regarding the root system up to 5 mm at the distance of 2 m of the trunk on different soil depths (original photo Ciobanu Andi)

Table 6. Roots up to 5 mm in diameter oriented at the trunk distance of 2 m, on different soil depths

Rootstock Soil depth range	Oteşani 8	Pixy	Miroval	Roşior văratic	Average	Percent (%)
0-10	0	0	3	0	0,75	15,00
10-20	1	2	3	2	2,00	40,00
20-30	1	0	3	0	1,00	20,00
30-40	1	0	0	0	0,25	5,00
40-50	0	3	0	0	0,75	15,00
50-60	0	0	0	0	0	0
60-70	1	0	0	0	0,25	5,00
70-80	0	0	0	0	0	0
80-90	0	0	0	0	0	0
90-100	0	0	0	0	0	0
TOTAL	4	5	9	2	5,00	100

The biometric parameters assessed during 2018-2020 varied significantly among the experimented biosystems rootstock/variety. Thus, the highest value of canopy diameter and canopy volume were recorded by the biosystem rootstock/variety Vâlcean/Miroval (399 cm), while Vâlcean/Oteşani 8 was less developed (297 cm), respectively 47 m^3 and 21 m^3. Also, the highest plum trees were those that used Pixy and Miroval rootstocks (457 cm) (Table 7).

Among all plum rootstocks included in the experiment Miroval had the most performant root system and together with Vâlcean plum variety emphasized the highest biometric parameters for the assessed period.

Table 7. The biometric parameters of rootstocks/ Vâlcean variety biosystem for period 2018-2020

No	Variety/ rootstock	Biometric parameters			
		Canopy diameter (cm)	Tree height (cm)	Canopy volume (m^3)	Degree of space used (%)
1.	VÂLCEAN/ OTEŞANI 8	297b	383b	21b	43,2b
2.	VÂLCEAN/ PIXY	364a	456a	40a	65,0a
3.	VÂLCEAN/ MIROVAL	399a*	457a	47a	78,1a
4.	VÂLCEAN/ ROŞIOR VĂRATIC	334b	391a	28b	54,7b
Average		349	422	34	59,7

**Mean values followed by a different letter show a statistically significant difference by Duncan's multiple range test (p<0.05).*

CONCLUSIONS

The present study was carried out to assess the architecture of root system of Vâlcean plum variety and four rootstocks (Oteşani 8, Pixy, Miroval, Roşior văratic) in the agro-environmental conditions of Dolj county, Romania. The root system and biometric parameters (canopy size, tree height, canopy volume) were strongly influenced by genetic background of rootstock and environmental conditions. Both rootstock and plum variety have been found to influence roots and tree growth. Thus, the highest roots number were developed for 0-3 mm thickness category and it was observed to Miroval rootstock (104 roots) at 1m distance from the trunk, while at 2 m trunk distance the best root system development was noticed in Roşior văratic rootstock (58 roots). The best value of canopy diameter (399 cm) and canopy volume (21m^3) were recorded by the biosystem rootstock/variety Vâlcean/Miroval. Also, the highest plum trees were those that used Pixy and Miroval rootstocks due to their best root system development.

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IMPACT OF FOLIAR FERTILIZATION ON THE QUALITY PARAMETERS OF BLUEBERRY FRUITS

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Abstract

Plant nutrition is one of the determinate factors in fruit production and fruit quality. The aim of the present study is to evaluate some biochemical parameters of blueberry fruits (Vaccinium corymbosum L.), varieties: 'Duke', 'Blueray', 'Hannah's Choice' and 'Elliott' under the influence of foliar organic and conventional fertilizers applied, along with a control treatment without fertilization. The experiment was conducted in the years 2020-2021 in a four-year plantation in the southern region of Romania. Two organic products: Algacifo 3000 (2 L/ha) and ERT 23 Plus (1 L/ha) and one chemical product: Poly-Feed 19-19-19 + ME (10 kg/ha) were used as fertilizer treatments applied to the leaves of the plants. The experimental design was completely randomized according to the indicated treatments with three replicas. Biochemical parameters of the blueberry fruits, like organic acids, total polyphenols content, total dry matter content, total sugar and anthocyanin pigments were analyzed annually. The results indicated that foliar application with organic treatments significantly stimulated the fruit quality.

Key words: *foliar fertilization, fruit quality, biochemical characteristics, blueberry.*

INTRODUCTION

The use of organic and mineral fertilizers at certain stages of plant development has proven to be an extremely important strategy for improving crop production (Mahmood et al., 2020) and fruit quality (Schoebitz et al., 2019). It is well known that nitrogen plays an important role in increasing crop yields and fruit quality. However, regular use of chemical fertilizers has a detrimental effect on the environment and agricultural production (Mostafa, 2008). Therefore, the focus of producers is on production methods with the least negative impact on the environment (Ochmian et al., 2015). In recent years, organic fruit production has gained particular interest (Neilsen et al., 2009). Fruits obtained by organic methods are considered to have a higher nutritional quality (Saba & Messina, 2003). Research confirms that the application of organic foliar fertilizers has had a positive effect on the accumulation of bioactive compounds compared to conventional fertilizers (Ochmian et al., 2015; Ciucu & Hoza, 2021).

Organic fruits and vegetables contain several polyphenols (Wojdyło et al., 2013), vitamin C (Caris-Veirat et al., 2004) and other antioxidant compounds. Wang et al. (2008); Ciucu & Hoza (2021) found a higher content of polyphenols in blueberry fruits fertilized with organic products. However, Häkkinen & Törrönen (2000) reported a similar content of phenolic substances to some blueberry varieties grown by organic or conventional techniques. Some authors suggest that organic fertilization induces oxidative stress on plants leading to an increase in antioxidant defense mechanisms, increased production of bioactive compounds and improved antioxidant capacity of berry fruits (Frías-Moreno et al., 2021). Some authors recommend the application of organic fertilizers at the same time as chemical fertilizers to obtain high quality fruit (Kilic et al., 2021). In order to be able to conclude which of the organic or conventional practices are more effective on the biochemical quality of blueberry fruit, further research is needed. Previous studies show that blueberry fruits have a high content of phenolic substances, tannins, vitamin C and other organic acids (Huang et al., 2012; Barberis et al., 2015). Kalt et al. (2020) reported a high antioxidant capacity in these fruits. Several studies are underway to investigate the role and functions of antioxidant compounds in humans (Jideani et al., 2021). Due to the rich content of phenolic substances, blueberries have an anti-inflammatory and antioxidant effect on the body, can prevent the occurrence of cardiovascular diseases, strokes, cancers, diabetes, hypertension, osteoporosis, etc. (Curtis et al., 2019; Si, 2020). Cranberry consumption has been associated with improved neural performance and memory (Bowtell et al., 2017; Miller et al., 2018). The cognitive benefits of eating blueberries in humans have been shown for all ages (Bell et al., 2021). Scientists have found that regular consumption of fruits rich in compounds with antioxidant and anti-inflammatory properties, such as blueberries, can delay the onset of brain aging and neurodegenerative disorders (Youdim & Joseph, 2001). The main objective of this research was to evaluate the impact of organic and chemical fertilizers on the quality of blueberry fruits, including the content of antioxidant compounds.

MATERIALS AND METHODS

Experiment location and plant material

The study was carried out in two consecutive years 2020 and 2021 in a plantation of a private farm, located in the Argeș meadow, southern Romania (44° 54'n, 24° 52'e) using as a study material a blueberry crop with tall shrub (*Vaccinium corymbosum* L.) for four years. The plantation covers 1 ha, the planting distances being 0.85 m per row and 3 m between rows (resulting in 3860 plants/ha density). The plants were placed on billets covered with black polyethylene. Four varieties of blueberries commonly grown in Romania ('Duke', 'Blueray', 'Hannah's Choice' and 'Elliott') were used as study material. The varieties were chosen according to popularity and ripening season.

Soil Description

The plantation is located on a flat area with a brown-clay soil with a loam-clay texture in the first 60-70 cm, and in depth the texture becomes sandy. The soil was improved by adding acid peat along the rows of plants (30 t/ha). In order to lower the pH of the soil, sulfur was administered at the beginning of the vegetation.

Plant fertilization

The treatments were distributed in a completely randomized block design, with three replications, with 5 plants per plot, with a spacing of 3 plants. Four different foliar treatments were administered, namely V1 (control variant - without fertilizer), V2 and V3 (organic variant - biostimulator and organic fertilizer) and V4 (conventional variant where chemical fertilizer was used). The experiment was bifactorial. Factor A, the highbush blueberry (*Vaccinium corymbosum* L.) had 4 levels. Factor B, the foliar fertilizer used had four fertilization treatments: V1 - control (untreated), V2 - Algacifo 3000 - brown seaweed extract *Macrocystis integrifolia* with betaine of vegetable origin (2% organic nitrogen, 10% organic carbon, 50% organic matter) (2 L/ ha), V3 - ERT 23 Plus seaweed extract (*Macrocystis integrifolia*), folic acid, glycine betaine (1.5% organic nitrogen, 11% organic carbon, 6.1% K_2O, 10% betaine) (1 L/ha) and V4 - a Poly-Feed chemical 19-19-19 + ME (10 kg/ha). The treatments were repeated four times every 10-14 days, from the formation of buds to the beginning of fruit ripening.

Fruit Biochemical Analysis

For analysis, hand-harvested fruits were used at the technical time of ripening. The following quality parameters were determined: total dry matter content, titratable acidity, vitamin C content, total sugar content and total content of polyphenols and anthocyanin pigments. The determinations were performed in three repetitions for each variety with the fertilization variants.

Total dry matter content (DM)

The total dry matter content was determined by a gravimetric method (drying 10 g of fruit tissue at 105 ° C to constant weight) according to Gergen (2004).

Total acidity

The organic acid content of blueberry fruit was determined by the titrimetric method, using 25 ml of aqueous fruit extract neutralized with a 0.1N NaOH solution in the presence of phenolphthalein as an indicator. The total acid content was expressed as malic acid (%).

Total sugar content

The total sugar content was estimated by the Fehling-Soxhlet method, 1968 (JAOAC, 1968). The principle of the method is based on the oxidation reaction between the copper in the copper alcoholate of sodium and potassium tartrate and the aldehyde and ketone grouping of the reducing sugars. This method determines the amount of reducing sugar which reduces a certain volume of Fehling's reagent. Total sugar content was expressed as a percentage (%).

Total polyphenol content (TPH)

The TPH of the fruit was evaluated by the spectrophotocolorimetric method, with the Folin-Ciocalteu reagent (Singleton et al., 1999). The principle of the method is based on the measurement of the optical density of the obtained extract which, by complexation with the Folin-Ciocalteu reagent, absorbs in the Vis domain. Methanol (70%) was used as the solvent for the extraction of polyphenols. Gallic acid was used as sandard and the results were expressed as mg GAE/kg FW.

Vitamin C content

The vitamin C (ascorbic acid) content of fruit expressed in mg/100 g FW was analyzed according to the method based on the oxidation of L-ascorbic acid to dehydroascorbic acid in an acid medium with a blue dye of 2.6-dichloroindophenol, followed by the reduction of the dye to the colorless form, which turns red at pH 4.2 (PN-A-04019: 1998).

Total anthocyanin content

The dosing of the total anthocyanin pigments from the fruit was performed by the Fuleki method (1968). The determined total anthocyanins were expressed as cyanidin 3-glucosides mg/kg FW (C3G mg/kg FW). A PG instruments T70 spectrophotometer was used.

Statistical Analysis

Statistical analysis was performed using an IBM SPSS 20 program (SPSS Inc., Chicago, IL, USA). All results were analyzed by unidirectional analysis of variance (ANOVA) and using the Duncan Multiple Range test. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The present study presents the biochemical quality of the fruits of four varieties of highbush blueberry (*Vaccinium corymbosum* L.) following fertilization with organic or conventional products.

Total dry matter content

Blueberry fruits contain on average about 84-88% water, the rest is dry matter. It consists of carbohydrates, proteins, lipids, minerals, organic acids, vitamins, phenolic compounds (Kader, 2002). In essence, DM is a reflection of the quality of fruits at harvest, the most relevant components of which are starch and soluble dry matter content (Travers, 2013).

Figure 1 shows statistically assured variations between organic or conventional foliar fertilizers and untreated control over the average DM content of the fruits of the four blueberry varieties chosen for analysis. An exception to this rule is the ‘Blueray’ variety where a single set of homogeneous values (a) is found for all fertilization options, including the control option. In the ‘Hannah’s Choice’ variety, there were no significant differences in the values of DM content in fruit fertilized with organic or conventional fertilizers.

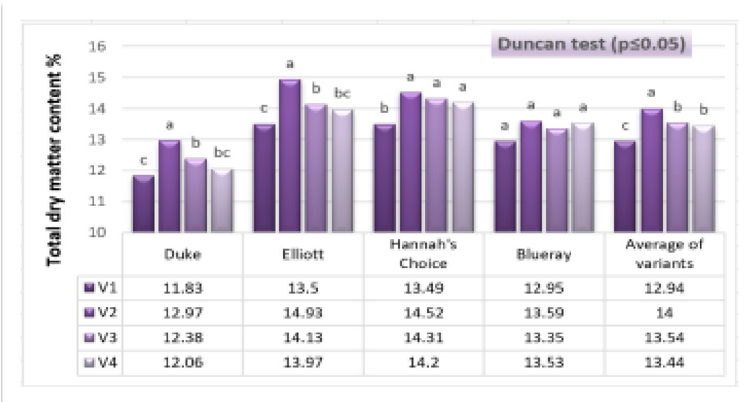


Figure 1. The influence of foliar fertilizers on the total dry matter content of fruit by variety in the years 2020-2021

The average value of DM content in fruit between 2020-2021 ranged from 11.83% ('Duke' variety, control variant - V1) to 14.93% ('Elliott' variety, variant V2 fertilized with organic fertilizer Algacifo 3000). The data of the analysis test of the Anova variance show that the total dry matter content of the fruit was very significantly influenced by the cultivar (partial eta squared = 47.9%) and the fertilization variant (19%) during the two years of study. (p = 0.000). The results are consistent with those in the literature. Studies have shown a variation in DM from 8.2% (Shevchuk et al., 2021) to 15.09% (Ostrowska & Ściążko, 1996).

Total titrable acidity expressed as malic acid

The content of organic acids in fruits can be influenced by growing and environmental conditions (Gündoğdu, 2019). In general, the organic acid content decreased significantly following the application of foliar fertilizers (Figure 2).

On average, over the two years of study, the content of organic acids was significantly higher in the control variant in all studied blueberry varieties. The highest values were recorded for the 'Elliot' variety (1.28%). The data correspond to those reported by Zenkova & Pinchykova (2019).

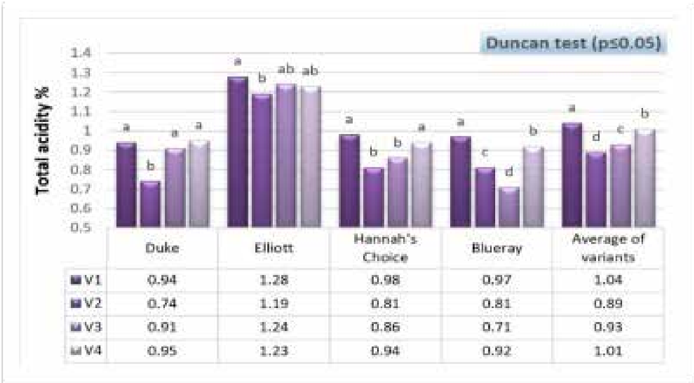
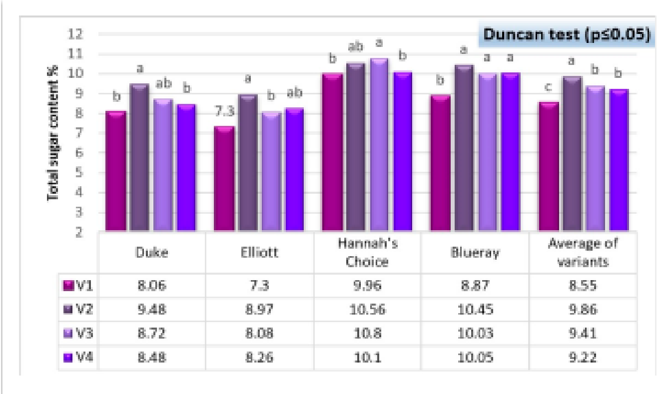


Figure 2. The influence of foliar fertilizers on the organic acid content of fruits by variety in the years 2020-2021

The titratable acidity had the lowest value in the 'Blueray' variety (0.71% - variant V3, fertilized with the biostimulant ERT 23 Plus). The average trend over the two years of the study shows a significant decrease in the titratable acidity of blueberry fruit to organic variant V2, fertilized with Algacifo 3000 fertilizer. It is largely determined by variety (partial eta squared = 82.7%). It was very significantly influenced by agronomic conditions (fertilization) by 42.8 and by meteorological conditions in 2020-2021 by 32.8% (p = 0.000). This has also been confirmed by Shevchuk et al. (2021).

Total sugar content

In the case of blueberries, the sugar content contributes to the determination of the organoleptic quality (Li et al., 2020). It can be influenced by maintenance technologies, environmental conditions, variety, soil conditions, the position of the fruit in the crown (Davidescu, 1999; Gündoğdu, 2019). On average, during the two experimental years, the total sugar content of the fruits of the studied blueberry varieties was significantly influenced by the growing conditions. All fertilization options (organic or conventional) had a beneficial effect on the value of this quality indicator compared to the control option (Figure 3). A variation was observed from 7.3% ('Elliott' variety, untreated variant) to 10.8% ('Hannah's Choice' variety, organic variant V3). The values obtained are in accordance with those reported in the literature (Kirina et al., 2020). The variety with the highest total fruit sugar content was 'Hannah's Choice' in all fertilization options followed by the 'Blueray' variety. The lowest values of the total sugar content of the fruit were obtained in the 'Elliott' variety. Among the fertilizers used, the greatest impact in terms of increasing the value of this quality indicator had the organic fertilizer Algacifo 3000 (fertilizer variant 2) as shown by the average effect of fertilizer variants on the total sugar content. In the studied highbush blueberry, the tendency of sugar accumulation was symmetrical with the dynamics of dry matter accumulation, a fact also noticed by Shevchuk et al. (2021).



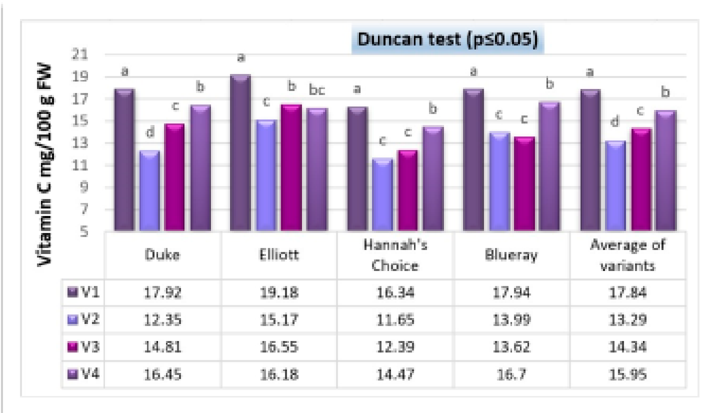
	Duke	Elliott	Hannah's Choice	Blueray	Average of variants
V1	8.06	7.3	9.96	8.87	8.55
V2	9.48	8.97	10.56	10.45	9.86
V3	8.72	8.08	10.8	10.03	9.41
V4	8.48	8.26	10.1	10.05	9.22

Figure 3. The influence of foliar fertilizers on the total sugar content of fruit by variety in the years 2020-2021

The bifactorial analysis of the variance determined a very significant variation of the influence of the genetic characteristics determined by the variety on the total sugar content of the analyzed fruits of blueberry varieties of 43.62%, while the agronomic conditions determined their sugar content by 18.2% ($p = 0.000$). The cumulative effect of these two experimental factors was not significant.

Vitamin C content

The concentration of ascorbic acid in blueberry fruits has shown great variation in the research of scientists. Correia et al., 2016, reported a variation from 6 to 162 mg/ 100 g FW. In our study, the highest content of vitamin C was accumulated in the fruits of all varieties in the control variant. With the richest ascorbic acid content, the 'Elliott' variety stands out (19.18 mg / 100 g FW). The lowest values were observed in the fruits of all four varieties fertilized with organic fertilizer - Algacifo 3000 (V2). The lowest value (11.65 mg/100 g FW) was obtained in the 'Hannah's Choice' variety (Figure 4). Ascorbic acid biosynthesis has been significantly influenced by culture technologies. According to the analysis test of the Anova variance, the agronomic practice had a very significant effect ($p = 0.000$) on the vitamin C content of blueberries of 57.9%.



	Duke	Elliott	Hannah's Choice	Blueray	Average of variants
V1	17.92	19.18	16.34	17.94	17.84
V2	12.35	15.17	11.65	13.99	13.29
V3	14.81	16.55	12.39	13.62	14.34
V4	16.45	16.18	14.47	16.7	15.95

Figure 4. The influence of foliar fertilizers on the vitamin C content of fruits by variety in the years 2020-2021

Also, the genetic background of the cultivar had a very significant effect on the content of metabolized ascorbic acid (partial eta squared = 35.4%). The climatic conditions of the two years of study determined a variation on the ascorbic acid level of 18.2% ($p = 0.000$).

Total anthocyanin content

Anthocyanin pigments are part of the flavonoid class and are responsible for the blue, purple, and red colors of the fruit depending on pH. Blueberries (*Vaccinium corymbosum* L.) are distinguished by an increased anthocyanin content: malvidin 3-galactoside, delphinidin 3-arabinoside, delphinidin 3-galactoside, petunidin 3-arabinoside, petunidin 3-galactoside, malvidin 3-c glucoside, cyanidin 3-galactoside, -arabinoside, malvidin 3-glucoside, delphinidin 3-glucoside, peonidine 3-galactoside, peonidine 3-arabinoside and peonidine 3-glucoside (Mazza, & Miniati, 2018). The level of anthocyanin compounds in blueberry fruit can be significantly influenced by the time of harvest. Connor et al. (2002) found a positive influence of anthocyanin levels in fruit due to delayed harvest time. On average, over the two years of the experiment, foliar fertilizers led to a significant increase in the total anthocyanin content of blueberry fruit up to 3121.33 mg C3G/kg FW ('Hannah's Choice' variety, variant V2 - fertilized with Algacifo 3000). In all studied varieties, the content of anthocyanin pigments was significantly lower in the unfertilized control variant (Figure 5). Among the foliar fertilizers applied, the organic fertilizer Algacifo 3000, had a stronger impact on anthocyanin pigments, in the sense of increasing their fruit content. This trend is also observed in the biosynthesis of fruit anthocyanins in variants. The exception was the 'Elliott' variety, which recorded a higher average content of anthocyanin compounds in fruit in both organic fertilizers used (variants V2 and V3), the differences between the two variants not being significant. Compared to conventional PolyFeed fertilizer, the applied organic fertilizers had a stimulating impact on the anthocyanin compounds in the fruit. The result of the analysis test of the Anova variance shows a very significant influence ($p = 0.000$) of the fertilization variant on the metabolism of anthocyanin pigments in fruits (partial eta squared = 77.5%). Also, the anthocyanin content of the fruit was very significantly influenced by the genetic background of the cultivar ($p = 0.000$) with a value of 87%. The cumulative effect of the two experimental factors on the total amount of anthocyanins in the fruit was 42.2%. And the growth conditions in the two experimental years showed a very significant influence ($p = 0.000$) on the anthocyanin pigments in the fruit (partially eta squared = 82.2%). Agronomic practices have an obvious effect on the content of anthocyanin pigments in fruits, a fact also observed by Nicola et al. (2020).

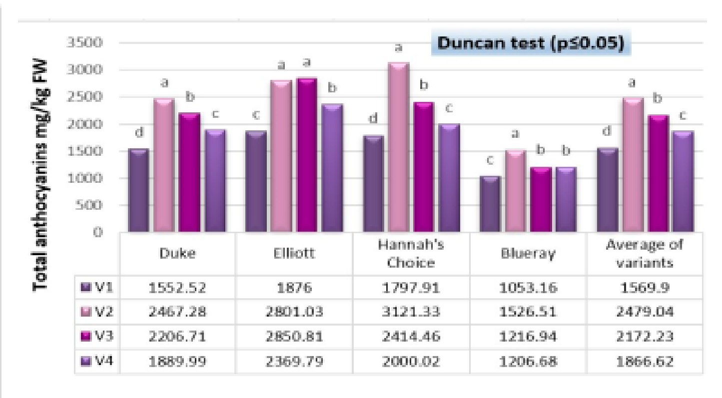
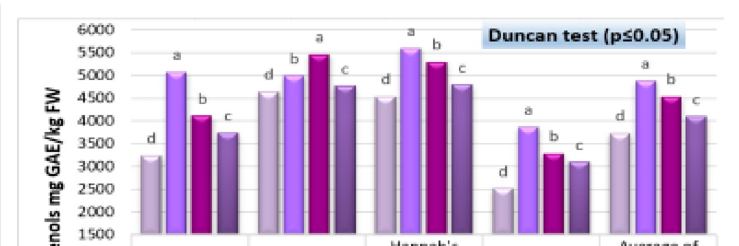


Figure 5. Influence of foliar fertilizers on the total anthocyanin content (expressed as cyanidin 3-glucosides) in fruit by variety in the years 2020-2021

Total polyphenols content

The main factor that determines the composition of blueberry polyphenols is the genetic background. However, cultivation technologies and climatic conditions can have a significant impact on the phenolic concentration of these fruits (Vittori et al., 2018). This fact was also noticed in the present study from the analysis test of the Anova variant. The data show a very significant effect (p = 0.000) of the genetic characteristics on the phenol levels in the fruits of the studied varieties of 89.7%. In addition, the organic or conventional agronomic practices used had a very significant influence on the phenolic content of blueberry fruit by 74.2%. And the environmental conditions between the years 2020-2021 had a statistically assured impact on the concentration of polyphenols in fruits (partially and squared = 80.3%). In the present study, a large variation of the total polyphenol content in the fruits of the four blueberry varieties chosen for analysis was observed. ‘Hannah’s Choice’ and ‘Elliott’ varieties have higher average levels of these compounds. The 'Elliott' variety showed an accumulation in the organic variant V3 fertilized with the ERT 23 Plus biostimulant of 5467.93 mg GAE/kg FW and the ‘Hannah's Choice’ variety obtained the highest value (5609.19 mg GAE/kg FW) in the organic variant V2 fertilized with Algacifo 3000 (Figure 6). The lowest values of the accumulated polyphenols concentration were found in the ‘Blueray’ variety (2148.4 mg GAE/kg FW in the control variant). Between the two applied organic fertilizers there are statistically assured differences on the average tendency of accumulation of phenolic compounds in fruits.



	Duke	Elliott	Hannah's Choice	Blueray	Average of variants
V1	3248.16	4645.02	4544.45	2520.04	3739.41
V2	5080.11	5002.52	5609.19	3872.02	4890.96
V3	4122.04	5467.93	5309.07	3294.83	4548.46
V4	3740.8	4772.28	4798.48	3105.75	4104.35

Figure 6. Influence of foliar fertilizers on the content of total fruit polyphenols by variety in the years 2020-2021

Table 1 shows the interdependence between fruit quality characteristics in the years 2020-2021. A distinctly significant correlation can be observed between the total dry matter content and the value of the total sugar content, the correlation coefficient being (r = 0.308**). This can be explained by the fact that sugars are also included in all dry matter. From the correlation matrix a negative correlation is observed, distinctly significant between DM and the total content of polyphenols and anthocyanins (r = 0.161 **, respectively r = 0.524**). The content of vitamin C correlates distinctly significant (negative) with the content of total polyphenols, the correlation coefficient being (r = - 0.289**). This means that during fruit ripening, the polyphenol content accumulates in the fruit to the detriment of the vitamin C content. A distinctly significant negative correlation was also obtained between the total sugar content and the amount of organic acids (r = - 0.208**), the higher the total sugar content (%), the lower the organic acid content (%). A positive correlation was obtained between the total anthocyanin content and the total polyphenol content, distinctly significant (r = 0.412**) which is normal considering that most of the phenolic compounds in blueberry fruits belong to the flavonoid class.

Table 1. Pearson correlations coefficients for the biochemical quality indicators for the studied blueberry varieties

Pearson correlations	Total dry matter %	Total acidity %	Total sugar %	Vitamin C mg/100g FW	Total anthocyanins mg/Kg FW	Total polyphenols mg GAE / Kg FW
Total dry matter %	1					
Total acidity %	0.089	1				
Total sugar %	0.308**	-0.480**	1			
Vitamin C mg/100g FW	-0.052	0.511**	-0.232**	1		
Total anthocyanins mg/Kg FW	0.524**	0.214**	0.120*	0.001	1	
Total polyphenols mg GAE / Kg FW	0.161**	0.340**	-0.056	-0.289**	0.412**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

CONCLUSIONS

It has been established that the variation in the organic matter content of highbush blueberry fruit (‘Duke’, ‘Elliott’, ‘Hannah’s Choice’ and ‘Blueray’ varieties) is dependent on genetic background and agronomic practices. Different cropping systems significantly affect the quality of blueberry fruit. Blueberries produced in organic culture have a significantly higher content of phytonutrients compared to those produced in conventional culture. Given the positive effect of organic fertilizers on fruit quality, a partial replacement of mineral fertilizers with organic fertilizers may be recommended.

Of the two organic fertilizers used, Algacifo 3000 fertilizer is recommended to increase the biochemical quality of the fruit, under the influence of which the highest values of phytonutrients in the fruit were obtained on average.

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EFFECTS OF ORGANIC AND INORGANIC FOLIAR FERTILIZERS ON THE NUTRITIONAL AND PRODUCTIVE PARAMETERS OF FOUR HIGHBUSH BLUEBERRIES CULTIVARS

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Abstract

To evaluate the effect of some organic or chemical fertilizers on the nutritional and productive parameters of blueberries (Vaccinium corymbosum L.), an experiment was carried out on a farm in the Argeş meadow in a loam-clay soil with four blueberry varieties: 'Duke', 'Blueray', 'Hannah's Choice' and 'Elliott'. Treatments with two foliar organic fertilizers were applied: Algacifo 3000 (2 L/ha) and ERT 23 Plus (1 L/ha) and one conventional treatment: Poly-Feed 19-19-19 + ME (10 kg/ha) along with a control treatment without fertilization in the years 2020-2021 in a four-year blueberry plantation. Results indicate that the nitrogen or phosphorus content of the leaves and fruits was significantly influenced by the variety and fertilization options. The highest concentration of nitrogen and phosphorus in leaves and fruits was recorded in 'Blueray' variety, the variant fertilized with Poly-Feed 19-19-19 + ME. The potassium content showed significant differences between varieties and variants of leaf fertilization. Finally, the chemical fertilizer obtained the highest values for most of the evaluated parameters in all the blueberry varieties studied.

Key words*: blueberry, organic and inorganic fertilizers, plant nutrition, yield.*

INTRODUCTION

The highbush blueberry, *Vaccinium corymbosum* L., belonging to the Ericaceae family, is native to North America and is found in the spontaneous flora of the Northeastern United States and Southeastern Canada. If until the 1900s, blueberries were only known as a North American native shrub with delicious fruit, then it became an international commercial crop (Retamales and Hancock, 2012). In Romania, in the mountainous areas, the species *Vaccinium myrtillus* and *Vaccinium vitis-idaea* grow spontaneously. The first varieties of highbush blueberry (*Vaccinium corymbosum* L.), were cultivated in our country, in 1968 at the Research-Development Institute for Pomiculture in Pitesti and at the Research Station for Pomiculture in Bilceşti (Ancu, 2014).
Soil is the most important factor for the success of blueberry cultivation. Acid soils with a pH between 4.5 and 5.5 are preferred (Alt et al., 2017), with medium texture, well drained and rich in organic matter. Lowering the pH of the soil is done by adding and incorporating into the soil substances with potential for acidification: granular sulfur, dust (approximately 250 kg/ha for the descent by one unit), acid peat (3 t/ha), resinous sawdust. To maintain the acidity of the soil, a dose of powdered sulfur of 100 kg is applied every year for sandy soils or 150 kg for clay soils (Chițu et al., 2016). In organic farms, acetic acid or citric acid and topically applied elemental sulfur are used to lower the pH of the soil in irrigated water (Sadowsky, 2010).
Compared to other fruit species, blueberries have low nutritional requirements (Zidlik et al., 2019). In intensive crops to maintain high yields, it is recommended to keep at a constant level the content of macro and micronutrients in the soil and leaves (Pormale et al., 2009). The supply of blueberries with nutrients is done on the ground, foliar or by fertigation, the doses being established following the foliar diagnosis (Ancu, 2014). Blueberry fertilization has been the focus of many studies. The dose of fertilizer varies depending on the age of the plantation, the vigor of the bushes, the location, the type of soil, the mulching of the soil (organic or inorganic), the fertility of the soil (Hanson, 2006). In the first years 20g N/plant is recommended, in the following years it can reach about 60 g N for

each plant (Virro et al., 2020). In order to obtain high quality yields for blueberry crops, the dose of fertilizer applied is important. In the precise fertilization of blueberry plants, the fertilizer must be well dosed in grams per plant (Lillerand et al., 2021). Organic fertilizers have proven to be of great importance for the implementation of sustainable agriculture techniques and for the prevention of environmental damage (Mufty et al., 2021). Research has shown that organic fertilizers have a beneficial effect on crop management, physical, chemical and biological properties of the soil, as well as being a source of macro and micro-nutrients needed for plant growth and development (Manea et al., 2019; Khan et al., 2020; Wajid et al., 2020). An increase in crop yield and quality has also been reported following the application of organic fertilizers (Habibzadeh et al., 2019). The content of micro-elements was significantly influenced by the application of mineral fertilizers (Medvecký et al., 2021). Excessive administration of chemical fertilizers can lead to a significant decrease in crop yield (Karlsons and Osvalde, 2019), can reduce soil structure and fertility as well as bacterial diversity and the activity of microorganisms (Dinesh et al., 2010). Many studies have shown a positive correlation between regular consumption of fresh fruits and vegetables and the prevention of many diseases. Blueberry fruits are increasingly appreciated by consumers due to their significant content of biologically active substances (Reque et al., 2014) but also as excellent sources of minerals (Medvecký et al., 2021). The present study aims to evaluate the effect of some organic or chemical fertilizers on the nutritional and productive parameters for blueberry highbush (*Vaccinium corymbosum* L.) in the conditions in Romania.

MATERIALS AND METHODS

Fertilizers program and plant material

The experiment took place on a farm in the Argeș meadow, in southern Romania (44° 54'n, 24° 52'e) during two consecutive seasons of vegetation in 2020 and 2021. The surface of the experimental plot was 1 ha and consisted of 20 rows. The distance between plants was 3 m between rows and 0.8 m between plants per row (4385 plants/ha). Four highbush blueberry varieties (*Vaccinium corymbosum* L.) were used as study material: 'Duke', 'Blueray', 'Hannah's Choice' and 'Elliott'. The analyzed vegetal organs were harvested as follows: the fruits at the technical stage of maturity and the leaves at the beginning of August.

The experimental design was bifactorial. Factor A, the highbidh blueberry (*Vaccinium corymbosum* L.) with 4 levels: four varieties of blueberry frequently cultivated in Romania. Factor B, foliar fertilizer with four treatments: V1 - control (untreated), V2 - Algacifo 3000 - seaweed extract *Macrocystis integrifolia* with betaine of vegetable origin (2% organic plant nitrogen, 10% organic carbon, 50% organic matter) - 2 L / ha, V3 - ERT 23 Plus seaweed extract (*Macrocystis integrifolia*), folic acid, betaine glycine (1.5% organic nitrogen, 11% organic carbon, 6.1% K_2O, 10% betaine) - 1 L/ha and V4 - a Poly-Feed 19-19-19 + ME chemical - 10 kg/ha, repeated four times every 10-14 days, from bud formation to early fruit ripening. The treatments were done in triplicate and there was a space of three plants between repetitions.

Soil Description

The plantation is located on a flat area with a brown-clay soil with a loam-clay texture in the first 60-70 cm, and in depth the texture becomes sandy. The soil was improved by adding acid peat along the rows of plants (30 t/ha).

The average nutrient content of the experimental plot in 2020 and 2021 is shown in Table 1. Soil samples were collected from the row of plants with an agrochemical probe, from a depth of 0-20 cm and 20-40 cm). To determine the concentration of macronutrients (N, P, K), $pH_{(H_2O)}$ and organic carbon content, soil samples were collected from the experimental plot, then air-dried and analyzed in the agrochemical laboratory of the Research-Development Institute for Pomiculture Pitesti - Maracineni. The pH value of the soil at fruit harvest was 5.67 in 2020, respectively 5.41 in 2021. To lower the pH of the soil, sulfur was administered at the start of vegetation.

Table 1. Macronutrient and organic carbon content of the soil in the years 2020 and 2021, at fruit harvest

Year	Depth (cm)	pH	N%	P-P_2O_5 (ppm)	K-K_2O (ppm)	C%	H%
2020	0-20	5.67	0.11	80.00	80.59	2.67	4.60
	20-40	5.97	0.09	52.14	52.35	0.77	1.33
2021	0-20	5.41	0.14	73.14	69.42	2.64	4.56
	20-40	5.80	0.09	50.43	30.72	0.80	1.38

Weather conditions

Romania is located in southeastern Central Europe in a temperate-continental climate zone with four seasons. The Argeș area is characterized by an average annual temperature of 9.9°C, with an accumulation of average annual rainfall between 450-700 mm. Precipitation is continental with a maximum in June. Irrigation of the plantation was administered from mid-May to the end of September. The plants were irrigated using two lines of polyethylene drip tubes located along the row near the base of the plants. Groundwater was about 1.5 m.

Yield and Nutritional Parameters

The production of fruit on the bush was determined by the gravimetric method, by the repeated weighing of the fruit at the time of harvest. It is known that blueberry fruits ripen in stages. In the studied varieties, the fruits were harvested in four tranches per year.

The macronutrient content was determined from previously dried and ground samples of plant material (leaves and fruits).

The total nitrogen concentration was determined by the Kjeldahl method. The principle of the method is mineralization with concentrated sulfuric acid, followed by distillation and then titration with sulfuric acid (Bataglia et al., 1983). The results were expressed as a percentage.

The available phosphorus content (P), expressed as a percentage, was estimated spectrophotocolorimetric using a PG Instruments T70 spectrometer, Uv-Vis. The principle of this method is based on the colorimetry of the yellow $[(NH_4)_3\ P(V_3O_{10})_4]$ complex formed between the vanadate anion and phosphorus in the presence of concentrated mineral acids at 420-470 nm.

The available concentrations of K in the tissues of blueberry plant material were estimated by the flammphotometric method using a Flame Photometer (Sherwood, 360, Cambridge, UK). The results were expressed as a percentage.

RESULTS AND DISCUSSIONS

The impact of foliar fertilization on the production and nutritional traits of blueberry plants (*Vaccinium corymbosum* L.) during the two years of study was significant. In terms of fertility, the soil is characterized by a poor supply of nitrogen, phosphorus and potassium as shown in Table 1. It is known that in an acidic soil reaction the mobilization of nutrients in the soil is diminished. At an acidic pH of the soil, phosphorus in the soil is found "retrograde" (inaccessible to plants) in compounds chelated with aluminum and iron. For the maintenance of vegetative growth, production and for the development of the flower buds of the next year's crop, nitrogen is the key element in blueberry nutrition (Karlsons and Osvalde, 2019). Nitrogen concentration in leaves is higher in early spring than in late autumn (Retamales & Hanson, 1990). In flowers and fruits, the concentration of nitrogen decreases from the beginning of flowering to the harvest of fruit (Banados et al., 2012).

Numerous studies have aimed to establish the optimal level of nutrients for the success of the culture of this species (Banados et al., 2012; Bryla et al., 2012; Larco et al., 2013; Ehret et al., 2014). Although the absorption of most nutrients increases throughout the growing season, it is recommended that the application of most fertilizers be administered in spring in some cases and in mid-summer (Bryla et al., 2012).

Chemical composition of blueberry leaves and fruits of the 'Duke' variety

The nitrogen content of the leaf samples taken in early August increased with the application of nitrogen fertilizers (Table 2). However, on average, after two years of study, the concentrations of nitrogen in the leaves of the 'Duke' variety were higher (in variants V2 - fertilized with Algacifo 3000 and V4 - fertilized with Polyfeed) than what is considered normal according to Hans et al., (2006) (1.76% to

2.00%) for highbush blueberry. In the control variant, where no fertilizers were applied, the nitrogen concentration in the leaves after the two years of study was 1.61%.

Table 2. The effect of foliar fertilizers on the concentration of macronutrients in the leaves and fruits of highbush blueberries, 'Duke' variety in the years 2020-2021

Foliar treatment	Macronutrients (%) of leaves			Macronutrients (%) of fruits		
	N	P	K	N	P	K
2020						
Control	$1,32 \pm 0,04^{d}$	$0,20 \pm 0,003^{c}$	$0,43 \pm 0,003^{b}$	$1,28 \pm 0,02^{b}$	$0,16 \pm 0,001^{c}$	$0,47\ 0,003^{c}$
Algacifo 3000	$2,27 \pm 0,12^{a}$	$0,21 \pm 0,003^{b}$	$0,46 \pm 0,02^{ab}$	$1,29 \pm 0,01^{b}$	$0,17 \pm 0,001^{b}$	$0,50 \pm 0,01^{b}$
Ert23Plus	$1,82 \pm 0,09^{c}$	$0,22 \pm 0,003^{a}$	$0,48 \pm 0,06^{a}$	$1,37 \pm 0,02^{a}$	$0,19 \pm 0,003^{a}$	$0,52 \pm 0,003^{a}$
PolyFeed	$1,91 \pm 0,13^{b}$	$0,21 \pm 0,003^{b}$	$0,49 \pm 0,04^{a}$	$1,09 \pm 0,02^{c}$	$0,21 \pm 0,002^{a}$	$0,50 \pm 0,01^{b}$
2021						
Control	$1,90 \pm 0,01^{d}$	$0,20 \pm 0,02^{b}$	$0,40 \pm 0,04^{c}$	$1,02 \pm 0,03^{c}$	$0,17 \pm 0,01^{c}$	$0,52 \pm 0,003^{c}$
Algacifo 3000	$1,94 \pm 0,12^{b}$	$0,21 \pm 0,01^{ab}$	$0,44 \pm 0,04^{b}$	$1,09 \pm 0,01^{b}$	$0,21 \pm 0,02^{a}$	$0,55 \pm 0,004^{b}$
Ert23Plus	$1,93 \pm 0,08^{c}$	$0,21 \pm 0,01^{ab}$	$0,46 \pm 0,04^{b}$	$1,00 \pm 0,02^{d}$	$0,19 \pm 0,01^{b}$	$0,57 \pm 0,003^{a}$
PolyFeed	$2.22 \pm 0,03^{a}$	$0,22 \pm 0,01^{a}$	$0,51 \pm 0,04^{a}$	$1,20 \pm 0,01^{a}$	$0,22 \pm 0,01^{a}$	$0,55 \pm 0,003^{b}$
2020-2021						
Control	$1,61 \pm 0,29^{d}$	$0,20 \pm 0,14^{b}$	$0,42 \pm 0,03^{c}$	$1,15 \pm 0,13^{a}$	$0,17 \pm 0,01^{c}$	$0,50 \pm 0,03^{c}$
Algacifo 3000	$2.11 \pm 0,17^{a}$	$0,21 \pm 0,01^{a}$	$0,45 \pm 0,03^{b}$	$1,19 \pm 0,10^{a}$	$0,19 \pm 0,02^{b}$	$0,53 \pm 0,03^{b}$
Ert23Plus	$1,88 \pm 0,05^{c}$	$0,22 \pm 0,14^{a}$	$0,47 \pm 0,05^{b}$	$1,18 \pm 0,19^{a}$	$0,19 \pm 0,01^{b}$	$0,55 \pm 0,02^{a}$
PolyFeed	$2.06 \pm 0,16^{b}$	$0,22 \pm 0,01^{a}$	$0,50 \pm 0,04^{a}$	$1,14 \pm 0,06^{a}$	$0,21 \pm 0,02^{a}$	$0,53 \pm 0,03^{b}$

From the data of the analysis test of the Anova variant, it appears that the fertilization variants had a very significant effect on the nitrogen content of the leaves in the 'Duke' variety, p = 0.000 (partially and squared = 99.6%). Also, the study year had a very significant influence (p = 0.000) of the nitrogen content of the leaves in the 'Duke' variety by 97.7%. The combined effect of the two factors (fertilization variant and year) on the nitrogen content was very significant, of 99.4%.

The concentration of other macronutrients in the leaves (phosphorus and potassium) increased significantly with the application of foliar fertilizers in the first year of fertilization. Regarding the phosphorus content in the leaves, in the 'Duke' variety, the appearance of two series of homogeneous values (a, b) is observed after the two experimental years. All foliar treatments had the effect of increasing the total phosphorus content compared to the unfertilized control. There were no statistically assured differences between fertilization variants with organic or conventional fertilizers regarding the accumulation of phosphorus in the leaves of this variety.

In the experiment, the concentration of potassium in the leaves of the variety 'Duke' recorded the highest value in variant V4 - fertilized with the chemical fertilizer Polyfeed (0.51%), the year 2021 and (0.50%) on average after the two years fertilization (Table 2).

In the processes of absorption of mineral components by the cells of the leaf epidermis, climatic conditions as well as various factors such as temperature and relative humidity play a significant role (Wach and Błażewicz-Woźniak, 2012).

The average values of the fertilization variants show statistically assured differences between the conventional fertilization variant V4 and the organic variants V2 and V3 on the nitrogen, phosphorus and potassium content of fruits in the 'Duke' variety. The exception was the nitrogen concentration in the fruit after the two years of study where the appearance of a single series of homogeneous values (a) between all fertilization variants is observed. Regarding the phosphorus content of fruit, 68.1% of the variation in the concentration of this nutrient in fruit could be explained by the effect of the fertilization variant.

Chemical composition of blueberry leaves and fruits of the 'Elliott' variety

The levels of mineral nutrients in blueberry leaves in the 'Elliott' variety during the years 2020-2021 are shown in Table 3.

Table 3. The effect of foliar fertilizers on the concentration of macronutrients in the leaves and fruits of highbush blueberries, variety 'Elliott' in the years 2020-2021

Foliar treatment	Macronutrients (%) of leaves			Macronutrients (%) of fruits		
	N	P	K	N	P	K
2020						
Control	$2,06 \pm 0,001^{a}$	$0,19 \pm 0,002^{b}$	$0,59 \pm 0,004^{d}$	$1,26 \pm 0,01^{d}$	$0,15 \pm 0,01^{d}$	$0,56 \pm 0,01^{a}$
Algacifo 3000	$1,86 \pm 0,005^{c}$	$0,21 \pm 0,008^{a}$	$0,63 \pm 0,005^{c}$	$1,43 \pm 0,01^{b}$	$0,18 \pm 0,002^{b}$	$0,51 \pm 0,01^{a}$
Ert23Plus	$1,61 \pm 0,001^{d}$	$0,19 \pm 0,004^{b}$	$0,65 \pm 0,001^{b}$	$1,29 \pm 0,02^{c}$	$0,16 \pm 0,001^{c}$	$0,52 \pm 0,15^{a}$
PolyFeed	$1,89 \pm 0,004^{b}$	$0,19 \pm 0,004^{b}$	$0,79 \pm 0,004^{a}$	$1,54 \pm 0,00^{a}$	$0,19 \pm 0,001^{a}$	$0,54 \pm 0,01^{a}$
2021						
Control	$1,84 \pm 0,15^{b}$	$0,16 \pm 0,01^{a}$	$0,59 \pm 0,07^{c}$	$0,75 \pm 0,01^{c}$	$0,15 \pm 0,02^{c}$	$0,55 \pm 0,002^{b}$
Algacifo 3000	$1,85 \pm 0,01^{b}$	$0,16 \pm 0,01^{a}$	$0,72 \pm 0,04^{b}$	$1,20 \pm 0,01^{a}$	$0,24 \pm 0,01^{b}$	$0,55 \pm 0,003^{b}$
Ert23Plus	$1,74 \pm 0,04^{c}$	$0,17 \pm 0,02^{a}$	$0,73 \pm 0,07^{ab}$	$1,05 \pm 0,10^{b}$	$0,16 \pm 0,01^{c}$	$0,52 \pm 0,003^{c}$
PolyFeed	$1,99 \pm 0,01^{a}$	$0,17 \pm 0,02^{a}$	$0,79 \pm 0,06^{a}$	$1,20 \pm 0,01^{a}$	$0,26 \pm 0,01^{a}$	$0,59 \pm 0,002^{a}$
2020-2021						
Control	$1,95 \pm 0,12^{a}$	$0,18 \pm 0,02^{b}$	$0,59 \pm 0,03^{c}$	$1,01 \pm 0,26^{c}$	$0,15 \pm 0,01^{b}$	$0,53 \pm 0,02^{ab}$
Algacifo 3000	$1.86 \pm 0,01^{c}$	$0,19 \pm 0,03^{a}$	$0,67 \pm 0,05^{b}$	$1,31 \pm 0,12^{a}$	$0,21 \pm 0,03^{a}$	$0,53 \pm 0,02^{ab}$
Ert23Plus	$1,67 \pm 0,07^{d}$	$0,18 \pm 0,02^{ab}$	$0,69 \pm 0,06^{b}$	$1,17 \pm 0,13^{b}$	$0,16 \pm 0,01^{b}$	$0,52 \pm 0,10^{b}$
PolyFeed	$1.94 \pm 0,05^{b}$	$0,22 \pm 0,18^{ab}$	$0,79 \pm 0,18^{a}$	$1,37 \pm 0,18^{a}$	$0,22 \pm 0,04^{a}$	$0,57 \pm 0,03^{a}$

Surprisingly, in 2020, the highest nitrogen content was recorded in unpowdered blueberry leaves, and in the second year for those treated with PolyFeed. The lowest rate of leaf nitrogen accumulation in the 'Elliott' variety was observed with the administration of the biostimulant Ert 23 plus. According to the analysis of the Anova variant, the total nitrogen content of the leaves in the 'Elliott' variety was very significant (p = 0.000) influenced by the fertilization variant (partial and squared = 98.5%). The effect of the year on the nitrogen content of the leaves in this variety was insignificant.

The phosphorus content of blueberry leaves did not vary significantly between study years. The highest levels of phosphorus were found in blueberry leaves in 2020 and the lowest in the following year. Statistical analysis showed a significant effect of foliar fertilization with Algacifo 3000 on the concentration of that element in the leaves compared to the control treatment and no significant difference compared to the variant sprayed with chemical fertilizer. From the data of the analysis test of the Anova variant, it appears that the fertilization variants had an effect on the phosphorus content of the leaves in the 'Elliott' variety of only 11.7% (p = 0.046). The combined effect of the two factors (fertilization variant and year) on the phosphorus content of the leaves in the 'Elliott' variety was significant, of 22.1% (p = 0.001).

The potassium content of blueberry leaves, the 'Elliott' variety in the years 2020-2021 is shown in Table 3. The lowest concentration of potassium was recorded in both years in the non-fertilized variant (0.59%) and the highest in both years in Polyfeed-fertilized variant study.

In fruit, the content of macronutrients (nitrogen, phosphorus and potassium) in blueberry fruit ('Elliott' variety) is shown in Table 3. Statistically assured differences were observed between fertilization variants regarding the content of macronutrients analyzed in fruit. Polyfeed-fertilized blueberry fruits and those fertilized with Algacifo 3000 organic fertilizer had a significantly higher nitrogen concentration than the control treatment (1.37% and 1.31%, respectively, after two years of fertilization). Significant differences in nitrogen accumulation in fruit were observed between the two years of fertilization. From the data of the analysis test of the Anova variant, it appears that the fertilization variants had a very significant effect on the nitrogen content of the fruit in the 'Elliott' variety, p = 0.000 (partially and squared = 99.5%). Also, the study year very significantly influenced (p = 0.000) the nitrogen content of the leaves in the 'Elliott' variety by 99.7%. The combined effect of the two factors (fertilization variant and year) on the nitrogen content was very significant, 93%. In 2020, a higher amount of nitrogen in fruits of this variety has accumulated.

Regarding the phosphorus content in the fruit, it is observed that the highest level was recorded in the fruit spraying blueberries with the chemical fertilizer PolyFeed both in 2020 and in

2021. The fertilization variant had a very significant influence on the accumulation of this nutrient in fruits of 92.3%.

The accumulation of potassium in fruits (Table 3) was also influenced by the study year ($p = 0.000$) according to the analysis data of the Anova variance (partially eta squared = 77.7%). In Table 3, analyzing the average values, can shows an insignificant level of potassium in the blueberry fruits ('Elliott' variety) influenced by the fertilization variants in the first year of fertilization, observing the existence of a single series of homogeneous values (a).

Chemical composition of 'Hannah's Choice' leaves and blueberries

Regardless of the year of study and the fertilizer administered, a statistically assured variation in the nitrogen concentration of blueberry leaves in 'Hannah's Choice' variety was obtained compared to the control treatment (Table 4). Moreover, a significant difference was observed between the chemical fertilizer and the other fertilizers used after the two consecutive years on the nitrogen concentration in the leaves.

From the data of the analysis test of the Anova variant, it appears that the fertilization variants had a very significant effect on the nitrogen content of the leaves in the 'Hannah's Choice' variety, $p = 0.000$ (partial and squared = 95.2%). Also, the study year significantly influenced ($p = 0.000$) the nitrogen content of the leaves in the 'Hannah's Choice' variety by 99.0%. The combined effect of the two factors (fertilization variant and year) on the nitrogen content was very significant, of 96.1%. The phosphorus content of leaves in the 'Hannah's Choice' blueberry variety (Table 4) recorded the highest average value (0.23%) for the chemical fertilizer sprayed in 2021. On average, after two years of experiment, the content of phosphorus accumulated in the leaves had the highest values in the plants sprayed with organic fertilizer Algacifo 3000, and the lowest in the non-fertilized version.

Table 4. The effect of foliar fertilizers on the concentration of macronutrients in the leaves and fruits of highbush blueberries, the variety 'Hannah's Choice' in the years 2020-2021

Foliar treatment	Macronutrients (%) of leaves			Macronutrients (%) of fruits		
	N	P	K	N	P	K
2020						
Control	$1,85 \pm 0,14^{d}$	$0,19 \pm 0,00^{b}$	$0,37 \pm 0,01^{d}$	$1,15 \pm 0,001^{c}$	$0,14 \pm 0,001^{c}$	$0,56 \pm 0,01^{b}$
Algacifo 3000	$2,00 \pm 0,004^{c}$	$0,21 \pm 0,01^{a}$	$0,48 \pm 0,001^{c}$	$1,29 \pm 0,001^{b}$	$0,15 \pm 0,001^{b}$	$0,54 \pm 0,01^{c}$
Ert23Plus	$2,05 \pm 0,002^{a}$	$0,19 \pm 0,01^{b}$	$0,52 \pm 0,003^{b}$	$1,13 \pm 0,002^{a}$	$0,16 \pm 0,001^{a}$	$0,58 \pm 0,00^{a}$
PolyFeed	$2,04 \pm 0,000^{b}$	$0,19 \pm 0,01^{b}$	$0,55 \pm 0,001^{a}$	$1,29 \pm 0,001^{b}$	$0,16 \pm 0,001^{a}$	$0,58 \pm 0,004^{a}$
2021						
Control	$1,74 \pm 0,12^{c}$	$0,22 \pm 0,01^{a}$	$0,49 \pm 0,07^{b}$	$0,74 \pm 0,001^{d}$	$0,11 \pm 0,02^{c}$	$0,61 \pm 0,004^{b}$
Algacifo 3000	$1,78 \pm 0,02^{b}$	$0,22 \pm 0,01^{a}$	$0,49 \pm 0,05^{b}$	$0,82 \pm 0,13^{c}$	$0,13 \pm 0,02^{b}$	$0,59 \pm 0,003^{c}$
Ert23Plus	$1,74 \pm 0,02^{c}$	$0,22 \pm 0,01^{a}$	$0,49 \pm 0,05^{b}$	$0,90 \pm 0,02^{b}$	$0,12 \pm 0,01^{c}$	$0,63 \pm 0,002^{a}$
PolyFeed	$1,81 \pm 0,01^{a}$	$0,23 \pm 0,01^{a}$	$0,64 \pm 0,04^{a}$	$0,93 \pm 0,02^{a}$	$0,15 \pm 0,01^{a}$	$0,63 \pm 0,002^{a}$
2020-2021						
Control	$1,80 \pm 0,06^{c}$	$0,20 \pm 0,02^{b}$	$0,43 \pm 0,08^{c}$	$0,94 \pm 0,04^{b}$	$0,13 \pm 0,02^{c}$	$0,58 \pm 0,03^{b}$
Algacifo 3000	$1.89 \pm 0,12^{b}$	$0,22 \pm 0,01^{a}$	$0,48 \pm 0,03^{b}$	$1,05 \pm 0,10^{ab}$	$0,14 \pm 0,02^{b}$	$0,57 \pm 0,03^{c}$
Ert23Plus	$1,90 \pm 0,16^{b}$	$0,21 \pm 0,02^{b}$	$0,50 \pm 0,04^{b}$	$1,11 \pm 0,04^{a}$	$0,13 \pm 0,02^{bc}$	$0,61 \pm 0,03^{a}$
PolyFeed	$1.93 \pm 0,12^{a}$	$0,21 \pm 0,02^{b}$	$0,59 \pm 0,05^{a}$	$1,11 \pm 0,06^{a}$	$0,15 \pm 0,01^{a}$	$0,61 \pm 0,03^{a}$

The potassium content of blueberry leaves increased significantly during the experiment. The leaves of the bushes sprayed with PolyFeed leaves had a significantly higher potassium content than the plants fertilized with organic fertilizers. Regardless of the year, the lowest level of calcium was recorded in the blueberry leaves from the control plants. Statistical analysis showed significant differences between fertilization options (72.4%; $p = 0.000$) and study years (26.7%; $p = 0.000$), and the interaction between years and fertilizer variant (38.2%; $p = 0.000$).

The accumulation of nutrients in the fruits of the highbush blueberry, the variety 'Hannah's Choice' according to the fertilization options and the years of study is presented in Table 4. In most cases, the nutrient content of the fruit had the highest values in the sprayed version with chemical fertilizer. Organic fertilizers have also had a beneficial effect on plant nutrition. However, there were a few exceptions. The potassium content of the fruit was higher in the non-fertilized version compared to the sprayed version with Algacifo 3000, which was observed in both years of testing. This can also be seen in the leaves in 2020. The average values of the other nutrients in the fruit were lower in non-fertilized plants as in the other varieties.

Chemical composition of blueberry leaves and fruits of the 'Blueray' variety

In the 'Blueray' variety, the content of macronutrients (N, P and K) in plant tissue and blueberry fruit was significantly affected by the foliar application of fertilizers. The total nitrogen content of the leaves in the 'Blueray' variety, according to the analysis of the Anova variant, was very significant (p = 0.000) influenced by the fertilization variant (partial and squared = 38.7%). The effect of the year on the nitrogen content of the leaves in this variety was also very significant (partially and squared = 63.7%). The combined effect of the two experimental factors (fertilization variant and year of study) was 52.0%.

In the 'Blueray' variety, the level of macron-trients in the leaves (Table 5) recorded the highest average values in the plants sprayed with PolyFeed fertilizer. However, after two years of experimentation, the phosphorus content in the leaves was higher in the fertilized version with organic fertilizer Algacifo 3000. As in the other varieties, the accumulation of phosphorus in the leaves was more efficient after fertilization with Algacifo 3000. In fruits, nutrition was better in chemically fertilized plants.

Table 5. The effect of foliar fertilizers on the concentration of macronutrients in the leaves and fruits of highbush blueberries, the variety 'Blueray' in the years 2020-2021

Foliar treatment	Macronutrients (%) of leaves			Macronutrients (%) of fruits		
	N	P	K	N	P	K
2020						
Control	2,20 ± 0,003^{a}	0,16 ± 0,01^{b}	0,45 ± 0,05^{c}	0,99 ± 0,003^{c}	0,15 ± 0,001^{a}	0,51 ± 0,02^{c}
Algacifo 3000	2,09 ± 0,005^{c}	0,17 ± 0,01^{b}	0,49 ± 0,01^{b}	1,05 ± 0,001^{b}	0,15 ± 0,000^{a}	0,54 ± 0,00^{a}
Ert23Plus	2,13 ± 0,004^{b}	0,17 ± 0,01^{b}	0,49 ± 0,05^{b}	0,99 ± 0,002^{c}	0,14 ± 0,001^{b}	0,52 ± 0,01^{b}
PolyFeed	2,11 ± 0,01^{b}	0,18 ± 0,01^{a}	0,55 ± 0,01^{a}	1,13 ± 0,001^{a}	0,15 ± 0,001^{a}	0,55 ± 0,01^{a}
2021						
Control	1,97 ± 0,10^{b}	0,19 ± 0,01^{c}	0,79 ± 0,08^{c}	0,92 ± 0,001^{b}	0,16 ± 0,01^{a}	0,55 ± 0,004^{c}
Algacifo 3000	1,97 ± 0,01^{b}	0,21 ± 0,01^{b}	0,86 ± 0,06^{b}	0,95 ± 0,13^{ab}	0,15 ± 0,04^{a}	0,63 ± 0,004^{a}
Ert23Plus	2,08 ± 0,01^{a}	0,22 ± 0,01^{ab}	0,87 ± 0,07^{b}	0,92 ± 0,002^{b}	0,16 ± 0,02^{a}	0,62 ± 0,003^{b}
PolyFeed	2,10 ± 0,01^{a}	0,23 ± 0,01^{a}	0,97 ± 0,10^{a}	1,01 ± 0,02^{a}	0,15 ± 0,04^{a}	0,63 ± 0,003^{a}
2020-2021						
Control	2,09 ± 0,14^{a}	0,17 ± 0,02^{c}	0,64 ± 0,17^{c}	0,95 ± 0,03^{c}	0,15 ± 0,01^{a}	0,53 ± 0,02^{b}
Algacifo 3000	2.03 ± 0,07^{b}	0,19 ± 0,03^{b}	0,66 ± 0,22^{bc}	1,00 ± 0,10^{b}	0,15 ± 0,02^{a}	0,59 ± 0,05^{a}
Ert23Plus	2,11 ± 0,03^{a}	0,19 ± 0,03^{b}	0,68 ± 0,20^{b}	0,95 ± 0,04^{c}	0,15 ± 0,02^{a}	0,57 ± 0,05^{a}
PolyFeed	2.11 ± 0,01^{a}	0,20 ± 0,02^{a}	0,76 ± 0,23^{a}	1,07 ± 0,06^{a}	0,15 ± 0,02^{a}	0,59 ± 0,04^{a}

Throughout the experiment, the results of the analysis test of the Anova variance suggest that the accumulation of nitrogen in the leaves was very significantly influenced by the variety (partially and squared = 94.2%), the fertilization variant (88.3%), the year of study with (43.9%) (p = 0.000). The cumulative effect of the three experimental factors was 93.6%. Phosphorus accumulation in plant tissue was very significantly influenced (p = 0.000) by variety (63.2%), fertilization variant (20.0) and year of study (19.7%). It was found that the level of potassium in the leaves of blueberry highbush was significantly influenced by variety (85.5%), fertilization options (59.2%) and the study year by 63.7% (p = 0.000).

The analysis of fruit macronutrients in all the varieties selected for analysis showed a very significant influence on the nitrogen concentration, due to the variety (91.8%), the applied fertilizers (84.2%) and the study year (95.9%). The cumulative effect of the three experimental factors was 77.2%. Regarding the phosphorus content in fruits, it was found that a very significant influence had the variety with 74.3%, the fertilization variant with 50.4% and the experimental year with only 9.1%. On the accumulation of potassium in fruits, the influence of experimental factors was lower: the variety by 51%, the foliar fertilizers applied by only 21.1% and the study year by 49.9% (p = 0.000).

In our study, for all treatments applied, the levels of nitrogen (N), phosphorus (P) and potassium (K) in the leaves and fruits of the highbush blueberry analyzed were relatively sufficient. It is clear that agricultural practices influence the accumulation of macronutrients in the fruits and leaves of highbush blueberry (*Vaccinium corymbosum* L.).

Crop yield

The purpose of the experiment was to examine the production of blueberries. In general, fruit yield was significantly influenced by the application of foliar fertilizers (p≤0.05). The most productive variety was 'Elliot', followed by 'Duke'. The variety with the lowest production per plant was 'Hannah's Choice'. The highest yield was obtained in plants fertilized with chemical fertilizers (V4). However, there were no significant differences in blueberry production between plants fertilized with organic fertilizers and those fertilized with chemical fertilizers after two years of study.

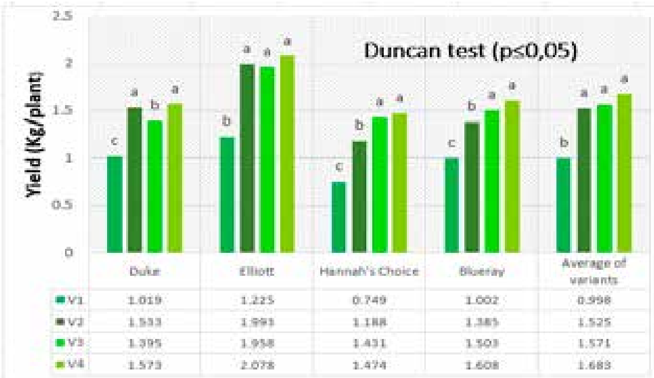

Figure 1. The influence of foliar fertilizers on plant yield by variety in the years 2020-2021

Our results are consistent with those in the literature. Starast et al. (2002), Karlsons and Osvalde (2019) reported higher yields for blueberry fertilized with foliar fertilizers. From the data of the analysis test of the Anova variance, it appears that the fruit yield was very significantly influenced by the variety (partially and squared = 45.6%) (p = 0.001). The fertilizer variant also had a very significant influence on plant yield (33.8%). The effect of the year on the production yield was 57.5% (p = 0.000).

CONCLUSIONS

The data presented suggest that different fertilization systems can significantly affect the nutrient content of plant tissue and blueberry fruit.

There are significant differences between the two cultivation practices (organic and conventional). Blueberries produced from conventional crops generally contain significantly higher amounts of macronutrients (N, P and K) than those produced in organic crops.

Foliar fertilization can become an effective method for increasing the production of blueberry crops.

Given the positive effect of organic fertilizers on plant nutrition and crop yields, partial replacement of mineral fertilizers with organic fertilizers may be recommended.

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EVALUATION OF GENETIC FIDELITY OF *IN VITRO* GROWTH PLANTS OF HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) CULTIVARS USING SCoT MOLECULAR MARKERS

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Abstract

The aim of this research was to investigate the influence of cultivar upon in vitro multiplication rate of highbush blueberry (Vaccinium corymbosum L.) and to evaluate the genetic fidelity of in vitro-propagated plants using molecular markers. Four varieties of blueberries were studied: 'Bluecrop', 'Blueray', 'Brigitta', and 'Duke'. For the in vitro multiplication, the Woody Plant Medium (WPM) basal medium was used, supplemented with 1 mg/L zeatine, 100 mg/L Sequestrene 138 and Plant agar 4 g/L, pH = 5. After ten weeks of in vitro culture, the four highbush blueberry varieties had average proliferation rates between 2.98 ± 0.25 and 9.35 ± 0.50 and the average length of the shoots varied between 2.79 ± 0.15 cm and 3.29 ± 0.13 cm. Clonal fidelity has been checked by twelve Start Codon Target Polymorphism (SCoT) primers. No polymorphism was detected, that proving that the regenerated plants showed high clonal fidelity.

Key words: *clonal fidelity; photosynthetic pigments; polymorphism; shoot proliferation; Start Codon Target Polymorphism.*

INTRODUCTION

In vitro culture or micropropagation is a method of vegetative multiplication successfully applied to highbush blueberry and the commercial use of tissue culture technology could be a profitable way to produce high-quality propagating material (Debnath & Goyali, 2020). However, maintaining *in vitro* subcultures for a long time can often lead to somaclonal variations due to chromosomal rearrangements and mutations in plant cells. Therefore, it is very important to establish the genetic uniformity of micropropagated plants in order to confirm the quality of propagated plants and their commercial utility by various methods, including morphological, biochemical, and molecular techniques (Muraseva et al., 2018). In this context, DNA-based molecular techniques could now be considered valuable tools used to evaluate the genetic fidelity of *in vitro* propagated plants (Martins et al., 2004).

A number of molecular markers, including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), arbitrary primed polymerase chain reaction (AP-PCR), DNA amplified fingerprinting (DAF), simple (short) sequence repeat (SSR), short tandem repeat (STR), sequence characterized amplified region (SCAR), sequence-tagged sites (STSs), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), expressed sequence tag (EST)-PCR and cleaved amplified polymorphic sequences (CAPS) derived from EST-PCR markers were used for genetic analysis of *in vitro* propagated plants (Debnath, 2010). In addition, the start codon targeted polymorphism (SCoT) markers are valuable PCR-based molecular markers used to analyze the genetic fidelity of *in vitro* grown plants. These molecular markers were developed based on data mining for short conserved amino acid sequences in proteins and designing polymerase chain reaction (PCR) primers based on the corresponding DNA sequence (Collard & Mackill, 2009).

The recent studies have shown that SCoT molecular markers have been used to test the clonal fidelity of *in vitro* grown plants in various species: *Bauhinia racemosa* Lam. (Sharma et al., 2019), *Annona reticulata* L.

(Kudikala et al., 2020), *Bambusa balcooa* Roxb. (Rajput et al., 2020), *Santalum album* L. (Manokari et al., 2021), *Crocus sativus* L. (Gautam & Bhattacharya., 2021), *Solanum khasianum* Clarke (Chirumamilla et al., 2021), *Prunus salicina* (Thakur et al., 2021) and *Dioscorea pentaphylla* L. (Manokari et al., 2022).

In this study, the genetic uniformity of *in vitro* regenerated plants of four varieties of highbush blueberry (*Vaccinium corymbosum* L.) was analyzed, after the 15th subculture. According to Ružić et al., 2012, this species is considered the most commercially important and biologically valuable species of the genus Vaccinium and the production of high-quality plants necessary for the establishment of highbush blueberry plantations involves the adoption of modern propagation techniques. Thus, numerous studies have focused on *in vitro* cultures of *V. corymbosum* varieties (Reed & Abdelnouresquivel, 1991; Ružic et al., 2012; Clapa et al., 2018; Wang et al., 2019; Georgieva & Kondakova, 2021), but there are only a few reports on the evaluation of clonal fidelity of *in vitro* raised plants (Gajdosova et al., 2006; Nowakowska & Pacholczak, 2017; Chen et al., 2018; Clapa et al., 2019).

Furthermore, as we know, the clonal fidelity of plants regenerated by tissue culture using ScoT molecular markers has not yet been assessed in *V. corymbosum*.

Therefore the aim of this research was to evaluate the genetic stability of micropropagated highbush blueberry plants to reveal the applicability of Start Codon Target Polymorphism (SCoT) molecular marker system.

MATERIALS AND METHODS

In vitro culture

In vitro shoot cultures of four highbush blueberry varieties (Bluecrop, Blueray, Brigitta and Duke) were established from axillary buds and maintained by regular sub-culturing after every 10 weeks, for three years, respectively fifteen sub-cultures on Woody Plant Medium (WPM) (Lloyd & McCown, 1980) supplemented with 1 $mg \cdot L^{-1}$ zeatine, 100 $mg \cdot L^{-1}$ Sequestrene 138 (FeNaEDDHA 6%) and 3% (w/v) of sugar. The culture media was solidified with 0.4 % (w/v) plant agar. The pH of the media was adjusted to 5.0 before autoclaving. The autoclave program used for sterilizing the culture media was run at 0.11 MPa at 121°C for 20 min. All the components were purchased from Duchefa Biochemie BV.

In each vessel (720 mL glass jar, 9 cm diameter, 13.5 cm high) with screw cap and ventilation holes (4 mm) 100 mL culture medium was dispensed. Ten explants per jar with a length of 1.5 to 2 cm were inoculated in such way that two-thirds to three-fourths of the basal part of the explants was immersed in the culture media.

The *in vitro* cultures were incubated in the growth room for a 16-h photoperiod with 32.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity (Cool white fluorescent lamps, 36 W; Philips, Amsterdam, The Netherlands) at 23 ± 1°C and 50% ± 2% humidity.

The length of the shoots and the rate of proliferation were recorded and calculated after fifteen subcultures. Five jars/each variety were measured for each treatment. The recorded data for proliferation rate represents the number of shoots with a length greater than one cm obtained/inoculum.

To determine the content of the photosynthetic pigment and to extract total genomic DNA, fresh leaves were collected from both *in vitro* three-year-old shoot cultures and mother plants of all varieties.

Photosynthetic pigments

Chlorophylls content. Levels of chlorophyll a (Chl a) and chlorophyll b (Chl b) were determined by spectrophotometry, using *in vitro* grown shoots. Samples of fresh vegetal material from shoots were weighed, homogenized, and extracted with 90% acetone in water using a magnetic stirrer until the residue became colorless.

The following formulas were used to quantify the chlorophyll content:

Chl a = (11.75 × A663 - 2:35 × A645) × V/g and Chl b = (18.61 × A645 – 3.96 × A663) × V/g), where A645 and A663 represent the optical density at a specific wavelength, V represents the volume of the extract (mL), and g represents the weight of the samples (mg). The recorded data were expressed as mg/g FW.

Carotenoids content. The extraction of carotenoids (Caro) was carried out with acetone. After the separation phase, the organic phase was dried over anhydrous sodium sulfate until the solvent was evaporated. Finally, the residue was dissolved in a known volume of hexane and the measurements were recorded at an absorbance of 450 nm level using the Perkin Elmer Lambda 25 spectrophotometer. The concentration of total Caro was calculated according to the following formula: X mg carotenoids = (A × V × 1000)/(A 1% 1cm × 100), where A represents absorbance at 450 nm, V represents volume (mL), and A 1% 1 cm = 2500 and expressed as mg Caro/g fresh material (Britton et al., 1995).

Genetic fidelity evaluation of in vitro grown plants using SCoT markers

In order to verify the genetic homogeneity of high bush vitroplants for each analyzed variety, DNA was extracted from both the mother plant and the vitroplants propagated after the fifteen successive subcultures. Therefore, 6 plantlets from the 15th subculture were randomly selected to harvest the leaves for DNA extraction, before passing the plant material into the acclimatization stage.

The harvested leaves were dried, ground into a fine powder (TissueLyser II, Qiagen, Germany), and kept at 4°C until ScoT genetic analysis was performed.

DNA extraction

Total genomic DNA (g DNA) was extracted using the CTAB (cetyl trimethylammonium bromide) method following the protocol reported by Lodhi et al. (1994) and improved by Pop et al. (2003) and Bodea et al. (2016). DNA purity and concentration were assessed with a NanoDrop-1000 spectrophotometer (ThermoFisher Scientific, USA). Prior to analysis with SCoT markers, DNA samples were diluted to 50 ng μL^{-1} using sterile double distilled water.

ScoT PCR - analysis

Twelve primers were used for SCoT analysis to assess the genetic uniformity of vitroplants with the mother plant as shown in Table 1.

For SCoT analysis, PCR reactions were performed with a total volume of 15 μL: 3 μL g DNA, 5.6 μL distilled H2O for the PCR reactions, 2.5 μL GoTaq Flexi Green buffer (Promega, USA), 2.5 μL MgCl2 (Promega, USA), 0.25 μL dNTP mix (Promega, USA), 1 μL SCoT primer (GeneriBiotech, Czechia), and 0.15 μL of GoTaq polymerase (Promega, USA). The PCR temperature cycling conditions were: (i) initial denaturation at 94°C for 5 min, (ii) 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 2 min, and (iii) the final elongation step of 5 min at 72°C.

Table 1. Sequences of SCoT primers used to evaluate the genetic uniformity of *in vitro* plants and mother plants

Primer Code	**Primer sequence (5'-3')**
SCOT1	CAACAATGGCTACCACCA
SCOT2	CAACAATGGCTACCACCC
SCOT3	CAACAATGGCTACCACCG
SCOT4	CAACAATGGCTACCACCT
SCOT5	CAACAATGGCTACCACGA
SCOT6	CAACAATGGCTACCACGC
SCOT7	CAACAATGGCTACCACGG
SCOT8	CAACAATGGCTACCACGT
SCOT9	CAACAATGGCTACCAGCA
SCoT10	CAACAATGGCTACCAGCC
SCoT11	AAGCAATGGCTACCACCA
SCoT12	ACGACATGGCGACCAACG

Separation of the amplified PCR products was performed by electrophoresis on 1.6% agarose gels (Promega, USA) stained with RedSafeTM Nucleic Acid staining solution (iNtRON Biotech, South Korea) in 1X TAE (Tris-acetate-EDTA buffer), at 100V and 98mA for 2.5-3 hours. DNA fingerprints were visualized in UVP Biospectrum AC Imaging System (UVP BioImaging Systems, Germany).

Data analysis

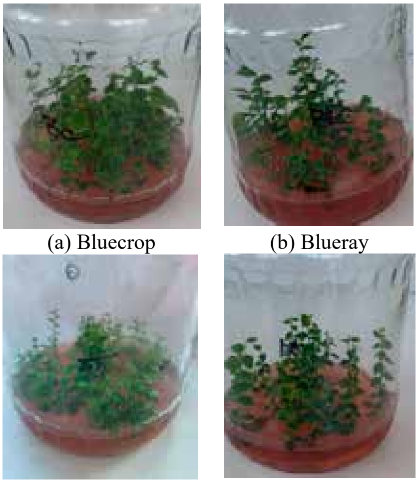
One-way ANOVA was performed to analyze the data and comparisons between the mean values of treatments were done according to Tukey's HSD test ($p<0.05$). The values shown are means ± S.E. Principal component analysis (PCA) and a dendrogram based on agglomerative hierarchical clustering (AHC) were built using the XLSTAT software. A heat map was drawn based on Pearson's correlation showing the relationships between varieties, *in vitro* growth parameters and photosynthetic pigments with OriginPro 2021 software.

ScoT gel images were analyzed using TotalLab TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) to determine the number and the range size of the amplified bands. Intensity of the bands were not considered while scoring.

RESULTS AND DISCUSSIONS

In vitro culture

The results of this study showed that the four highbush blueberry varieties analyzed reacted differently to long-term *in vitro* cultivation, especially in terms of proliferation rate (Figure 1, Figure 2).



(a) Bluecrop (b) Blueray

(c) Brigitta Blue (d) Duke

Figure 1. *In vitro* culture of *V. corymbosum*: (a) Bluecrop; (b) Blueray; (c) Brigitta Blue; (d) Duke. The *in vitro* shoot cultures of highbush blueberry were established from axillary buds and maintained by regular subculturing after every 10 weeks, for three years, respectively fifteen sub-cultures on Woody Plant Medium (WPM + 100 mg/l Se-questren 138 + 1 mg/L zeatin (Z) + 4 g/L Plant agar, pH = 5) at 22 ± 1°C, 32.4 mmol·m^{-2}·s^{-1}, 16-h photoperiod

Thus, the highest proliferation rate was obtained in Brigitta (6.76 ± 0.41) with statistically significantly higher differences than the other varieties. The lowest proliferation rates were obtained in Duke (2.98 ± 0.25) and Blueray (3.02 ± 0.27), but the differences between them were statistically insignificant (Figure 2). Contrary to our findings, Ostrolucká et al. (2004) reported that on the WPM culture medium supplemented with 2 mg/L zeatin, the Duke variety (5.28) had a higher proliferation rate than Bluecrop (3.94) and Blueray (1.71).

Consistent with our results, Sedlaka and Paprstein (2009) reported that the *in vitro* plants proliferation rate of Blueray, Bluecrop and Berkeley varieties grown on different culture media and supplemented with different concentrations of zeatin depends on the variety. Moreover, our results are in agreement with the studies of other authors (Tetsumura et al., 2008; Sedlaka and F. Paprstein, 2009), who showed that the number of newly formed shoots also depends on the variety.

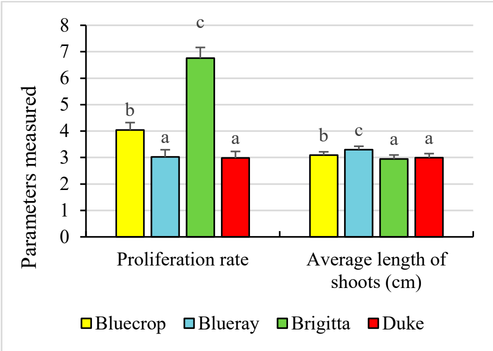


Figure 2. Proliferation rate and average lenght of shoots (Bluecrop, Blueray, Brigitta Blue and Duke) after fifteen *in vitro* sub-cultures on Woody Plant Medium (WPM + 100 mg/l Sequestren 138 + 1mg/L zeatin (Z) + 4 g/L Plant agar, pH = 5) at 22 ± 1°C, 32.4 mmol·m^{-2}·s^{-1}, 16-h photoperiod

Regarding the length of *in vitro* grown shoots, the longest ones were obtained for the Blueray variety (3.30 ± 0.13 cm) with statistically significantly larger differences compared to the other varieties. The shortest shoots were recorded in the Brigitta (2.94 ± 0.15 cm) and Duke (2.99 ± 0.16 cm) varieties, but the differences between them were statistically insignificant (Figure 2).

In a previous report, Mohamed & Alsadon (2010) stated that plantlets with higher chlorophyll content may have a better chance of survival during the acclimatization stage due to photosynthetic competence and carbon allocation. In this study, the chlorophyll content of the analyzed highbush blueberry varieties was determined due to the fact that photosynthetic pigments are important indicators of the physiological state of the plants. Brigitta was the variety with the lowest content of chlorophylls (Chla; Chlb) and carotenoids (caro). The highest content of photosynthetic pigments was determined in the shoots of the Bluecrop variety (Figure 3).

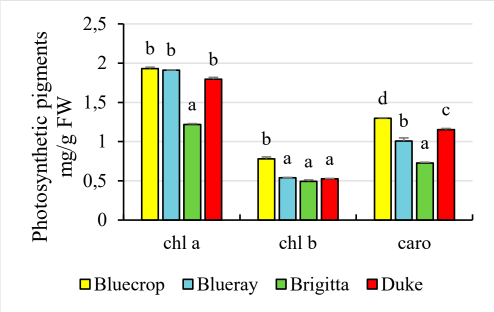


Figure 3. Photosynthetic pigments mg/g FW (Bluecrop, Blueray, Brigitta and Duke) after fifteen *in vitro* sub-cultures on Woody Plant Medium (WPM + 100 mg/l Sequestren 138 + 1mg/L zeatin (Z) + 4 g/L Plant agar, pH = 5) at 22 ± 1°C, 32.4 mmol·m^{-2}·s^{-1}, 16-h photoperiod

Principal component analysis (PCA) was performed in order to explore the relationships between the *in vitro* growth parameters and the content of photosynthetic pigments of analyzed varieties (Figure 4). The PCA biplot revealed a very clear clustering of the photosynthetic pigments (Chla, Chlb, Caro) in the third and fourth quadrant of the plot compared to the growth parameters: length of shoots and proliferation rate (LS, PR).

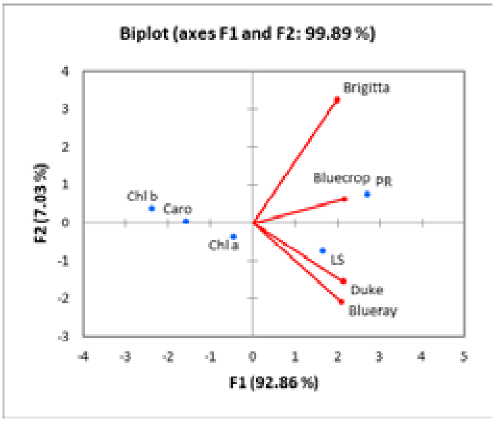


Figure 4. Principal component analysis (PCA) of all variables in four highbush blueberry cultivars (Bluecrop, Blueray, Brigitta and Duke) after fifteen *in vitro* sub-cultures on Woody Plant Medium (WPM + 100 mg/l Sequestren 138 + 1mg/L zeatin (Z) + 4 g/L Plant agar, pH = 5) at 22 ± 1°C, 32.4 mmol·m^{-2}·s^{-1}, 16-h photoperiod. The tested variables included average length of the shoots (LS), the proliferation rate (PR), chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Caro)

Evaluation of the association of growth parameters with the content of photosynthetic pigments using PCA revealed a total variability of 99.89 % explained by two components (F1 and F2). The first component (X-axis) alone explains 92.86 % of the variance. In addition, as shown in Figure 4, there was a positive correlation between Brigitta and Bluecrop varieties and the proliferation rate.

These varieties were grouped in the first quadrant with the proliferation rate, and our results show that these two varieties had the highest proliferation rates (6.75 and 4.04, respectively).

The agglomerative hierarchical grouping (AHC) using Ward's method highlighted the difference between the four blueberries varieties based on their *in vitro* growth parameters and photosynthetic pigments contents (Figure 5).

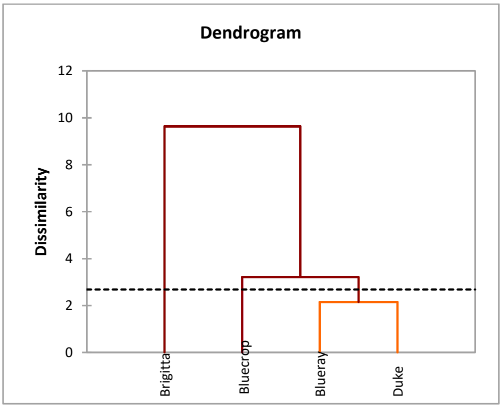


Figure 5. Dendrogram of hierarchical clustering based on *in vitro* growth parameters and photosynthetic pigments of four highbush blueberry cultivars (Bluecrop, Blueray, Brigitta and Duke) after fifteen *in vitro* sub-cultures on Woody Plant Medium (WPM + 100 mg/L Sequestren 138 + 1 mg/L zeatin (Z) + 4 g/L Plant agar, pH = 5) at 22 ± 1°C, 32.4 mmol·m^{-2}·s^{-1}, 16-h photoperiod

The AHC dendrogram grouped the varieties into three distinct classes (C1, C2 and C3). Class C1 included Bluecrop which had the highest content of photosynthetic pigments, class C2 including Duke and Blueray varieties with the lowest proliferation rates. The class C3 included the Brigitta variety with the highest proliferation rate. Similarly, the built heat map (Figure 6) grouped the four varieties into two main clusters: the first represented by Brigitta with the highest proliferation rate recorded, and the second main cluster which included two subgroups: Duke and Blueray varieties and, respectively, Bluecrop.

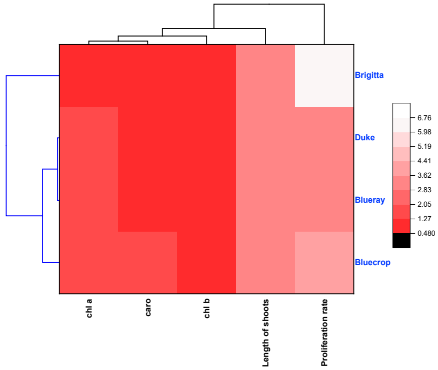


Figure 6. Heat map of Pearson correlation analysis of all variables in four highbush blueberry cultivars (Bluecrop, Blueray, Brigitta and Duke) after fifteen *in vitro* sub-cultures on Woody Plant Medium (WPM + 100 mg/l Sequestren 138 + 1mg/L zeatin (Z) + 4 g/L Plant agar, pH = 5) at 22 ± 1°C, 32.4 mmol·m^{-2}·s^{-1}, 16-h photoperiod

Genetic fidelity assessment using ScoT markers

Assessing the genetic uniformity of plants grown *in vitro* is very important and is an important objective for mass propagation. In this context, various molecular techniques have been successfully used to check genetic stability and lack of somaclonal variation in micropropagated plants (Goto et al., 1998; Tiwari et al., 2013; Butiuc-Keul et al., 2016; Thakur et al., 2016; Sharma et al., 2019; Tikendra et al., 2019).

Therefore, SCoT markers which are reproducible markers that are based on the short conserved region in plant genes surrounding the ATG translation start codon (Collard and Mackill, 2009) have been used in this study.

Table 2 evidence the number and size range of amplified fragments generated by SCoT markers in *Vaccinum corymbosum* L. varieties. As can be seen in Table 2, the lowest number of bands (6) was recorded with SCoT6 and SCoT10 primers for Blueray and Duke varieries and the highest number of bands (18) was obtained with the SCoT12 primer for Brigitta variety. In addition, Brigitta variety showed the highest total number of PCR-amplified bands (133), and the lowest number (120) was recorded for the Blueray variety. However, the size range of PCR bands varied between 250 and 2500 for all four *V. corymbosum* varieties analyzed (Table 2).

To our knowledge, this study is the first report on the application of SCoT markers for the evaluation of genetic fidelity of micropropagated plants of *Vaccinum corymbosum* L. varieties: Bluecrop, Blueray, Brigitta and Duke.

As shown in Figure 7, the results of this study confirm that micropropagated highbush blueberry plants are true-to-type with the mother plants, according to the described protocol, and authenticate that they are not suscetible to somaclonal variation.

In practice, ensuring the genetic uniformity of *in vitro* grown plants at an early stage is a very important economical goal, especially for clonal multiplication and mass production which require a high degree of genetic uniformity between regenerated plants (Clapa & Hârţa, 2021).

Table 2. Number and size range of amplified fragments generated by SCoT markers in *Vaccinum corymbosum* L. varieties

Primer Code	No. of scorable bands				Size range of bands (bp)			
	Bluecrop	Blueray	Brigitta	Duke	Bluecrop	Blueray	Brigitta	Duke
SCOT1	9	10	11	7	600-2000	250-2500	750-2500	500-2000
SCOT2	11	11	13	12	500-2500	500-2000	500-2500	250-2000
SCOT3	10	10	14	11	500-2000	500-2000	500-2000	250-2000
SCOT4	9	9	12	13	250-1800	250-1900	250-2000	250-2500
SCOT5	11	8	11	7	500-2000	500-1800	500-1500	750-1800
SCOT6	9	8	8	6	250-1500	250-1500	500-1500	750-1500
SCOT7	10	11	10	8	250-1500	250-1500	500-1500	250-1500
SCOT8	12	10	12	11	500-2000	500-2000	500-2000	500-2000
SCOT9	11	10	8	10	250-1500	250-1500	250-1500	250-1500
SCoT10	7	6	11	9	500-2000	500-2000	500-2000	500-2000
SCoT11	9	10	13	13	250-2000	250-1800	250-1800	250-2000
SCoT12	15	16	18	14	250-2500	250-2500	250-2500	250-2500
Total bands	123	120	133	121	-	-	-	-

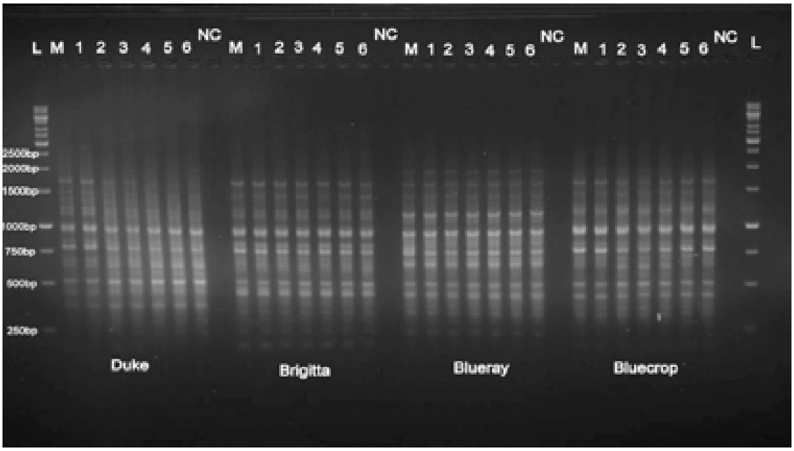


Figure 7. The assessment of genetic uniformity of micropropagated plants with their mother plants by SCoT markers. DNA fingerprinting profile obtained with SCoT-12 primer of *in vitro* plants. Lane L: 1 kb DNA Ladder (Fermentas, Leon-Rot, Germany); Lane M: PCR banding patterns of mother plants for each variety; Lane 1–6: PCR banding patterns of *in vitro* propagated plants for each analyzed variety

CONCLUSIONS

The experiments of this study confirmed that the length of the shoots, the proliferation rate and the photosynthetic pigment contents of the four highbush blueberry varieties grown *in vitro* depend not only on the culture medium but also on the response of the individual genotype. As far as we know, this is the first report to establish the clonal fidelity of micropropagated plants of *V. corymbosum* L. using SCoT markers.

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CONSIDERATIONS REGARDING THE INFLUENCE OF CLIMATE ON THE PLUM IN THE CULTIVATION CONDITIONS IN ROMANIA

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Abstract

As result of two years of research this paper presents the phenological and fruit quality parameters of five plums (Prunus domestica L.) cultivars. Observations and determinations were performed in regards to the phenological stages and the physical and chemical traits of the fruit. The climatic differences between the two years of study had an impact on the plum varieties. Temperature was one of the important factors in triggering the phenological stages. The swelling of the buds started with March 07th (`Centenar`) in 2020 and with March 25th (`President`) in 2021. Also, together with the precipitations accumulated in the studied period, significant differences of the characteristics and qualities of the fruits were observed. Fruit weight ranged from 24.23 g to 61.82 g, with higher values obtained by the cultivar `Minerva` in both years. The aim of the research is to suggest the introduction in the culture of some plum varieties with good ecological adaptability to the conditions in NE of Romania and with a good combination of qualitative and quantitative parameters of the fruits.

Key words: *plums, cultivar, climate conditions, phenological stages, fruit quality.*

INTRODUCTION

Plum culture in Romania has special importance due to the dominance of this species as well as for the use of fruit (Butac, 2020). The assortment of plums is diverse, especially in the characteristics of the fruit, such as size, shape, color, texture, aroma, and quality. Some cultivars are especially suitable for consumption in the fresh state, and others are grown as raw materials for various processed products: compote, jam, prunes, etc (Glišić et al., 2015). In the synthesis work "Genetic Resources of Temperate Fruit and Nut Crops", D.W. Ramming and V. Cociu presented the average data on the chemical and mineral components of fresh plums compared to dehydrated ones. Fruits are appreciated by consumers as sources of health for the high content of minerals and the diversity of vitamins (Ramming & Cociu, 1991). The importance of the species is also due to the aspects related to the adaptability to the environmental conditions, having high ecological plasticity (Grădinaru, 2002; Sottile et al., 2022). The favorable climatic conditions in Romania made the plum occupy an area of 69.600 ha, which represents approximately 47.42% of the total of 137.300 ha of plantations with fruit trees (MADR, 2021). According to FAO data, plum cultivation offers an annual output of 842.132 tons (FAOSTAT, 2021), thus placing our country in second place in the world top of plum producing countries, along with China, Serbia, Chile, and others. The plum cultivation area in Romania has a higher share in the southern sub-Carpathian area and in the western part of the country, but it is cultivated almost everywhere due to the low requirements due to environmental factors (Coman et al., 2012). In the period 1971-1993 at RSFG Iași observations and determinations were made regarding the vigor of the trees, the productivity, and the quality of the fruits in some varieties and selections of plum (Cârdei & Bodi, 1987).

The purpose of this study was to assess the relationship between climate and phenology of stone fruit in NE of Romania and to analyze the pomological aspects of five plum genotypes that have been created in various breeding programs, both in the country and abroad.

MATERIALS AND METHODS

The experimental research was conducted for two years (2020-2021) at the plum plantation within Research and Development Station for

Fruit Growing (RSFG) Iaşi using as biological material five plum cultivars. Four of them were created in Romania at Research Institute for Fruit Growing Piteşti: ‘Centenar’, ‘Carpatin’, ‘Silvia’, ‘Minerva’, and the cultivar ‘President’, originally from the U.K. and introduced into the culture by Rivers Nursery in 1901.

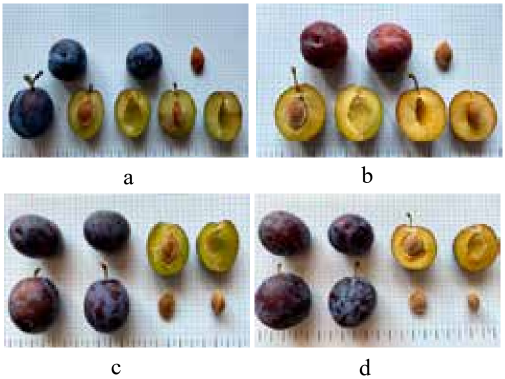

Figure 1. The fruits of the studied plum cultivars
a. ‘Centenar’ b. ‘Carpatin’ c. ‘President’ d. x (photo original)

The selected cultivars have been grafted on ‘Roşior’ as seedling rootstock. The plum trees were planted in 1988 in an experimental design of randomized blocks with three replicates. Planting distance was 4 x 3.5 m and training as free palmette, without sustaining or irrigation system. During the vegetation period, general maintenance works were carried out on the plantation, aiming at annual pruning, soil mobilization, and control of diseases and pests.
Phenological data were determined through the BBCH scale (Meier et al., 1994): bud development (01) - beginning of bud swelling (leaf buds); beginning of flowering (61) - about 10 % of flowers open; ripening time (87) - fruit ripe for picking. Based on this, the growing degree-days (GDD) from bud swelling to the beginning of flowering and after that until the start of ripening were calculated according to the equation (McMaster & Wilhelm, 1997; Aydin et al., 2019):

$$GDD=(T_{max}+T_{min})/2- T_{base},$$

where $\mathbf{T_{max}}$ and $\mathbf{T_{min}}$ are daily max and minim temperatures, $\mathbf{T_{base}}$ is base temperature. The plum base temperature for European temperate climate zones used is 6°C (Darbyshire et al., 2012; Woznicki et al., 2019; Fadón et al., 2020).

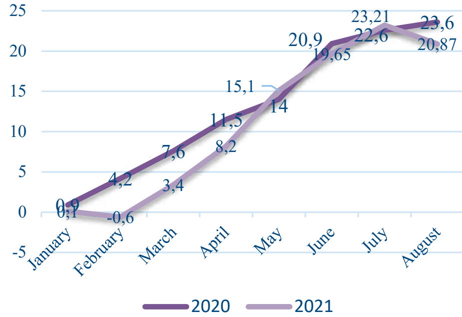

Figure 2. Average temperature in the studied period (RSFG Iași-Romania, 2020-2021)

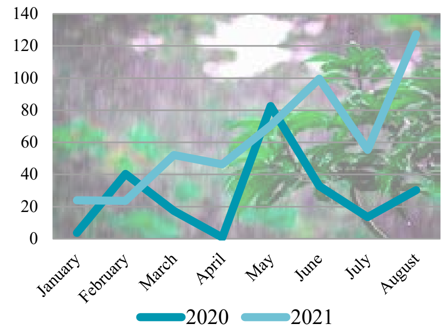

Figure 3. Accumulated rainfall in the studied period (RSFG Iași-Romania, 2020-2021)

The required active temperature for the beginning of flowering is on average about 321°C depending on the cultivar, and for fruit ripening between 2200°C and 3500°C (Grădinaru, 2002). To determine the pomological qualitative parameters were collected fruits at the full maturity stage. For each cultivar were collected 30 fruits of similar size and without visible external damage. Biometric measurements and determinations were performed: equatorial diameter of the fruit (mm), thickness (mm), and length (mm) using the Lumytools sliding tool. The fruit weight was measured using an analytical balance Radwag. The biochemical analysis consisted in determining the soluble dry solids (SDS %) using a Zeiss refractometer (Cociu & Oprea, 1989). The research results were synthesized and interpreted statistically by Microsoft Excel Starter using the Duncan test ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

The climatic conditions of the growing area have strong effects on many aspects of plant life, such as phenology and productivity (Lambers et al., 2008). During the two years of study, the evolution of the phenophases of growth and fruiting in the climatic conditions in northeast Romania was followed. To evaluate the relationship between weather temperature and temperature required for the different stages of development, the amounts of heat for the relevant periods in Growing Degree-Days (GDD) terms were used.

Temperature is a key factor in the timing of biological processes, and hence the growth and development of plants (Parthasarathi et al., 2013). Growing degree days are used to assess the suitability of production of studied plum cultivars in NE of Romania.

According to the meteorological data registered at the station from RSFG Iași-Romania, was calculated firstly GDD from bud development (01) to the beginning of flowering (61). Thus, in the conditions of spring 2020, the bud development starts between at 7th of March ('Centenar' and 'President') and the 11th of March ('Carpatin' and 'Silvia') with an average temperature of 12.1°C. In the 2021 year, the bud development started much later at a difference of about 18 days, due to the low temperatures recorded in the first part of March. This phenological stage took place between 25th March ('President') and 4th April ('Carpatin').

Based on the registered observations on the plum cultivars in the first year, it was established the stage from the bud development to the beginning of flowering.

Plums have a hiatus between the phenophases and GDD is sometimes used to estimate growing season length (Castillo et al., 2021). It is observed that within the same cultivar, in the two years of study, different GDD were accumulated for each of the phenophases. Based on this fact, it can be seen that ecological factors (especially temperature) have a greater impact on the flowering phenophase than the genetic characteristics of the variety. This is in line with the conclusions of Milatović (2019). The difference in the flowering phenophase of the plum in the two years of study can be explained by the differences in the environmental conditions in the region in the spring season. in 2020, the average duration from bud swelling to the start of flowering varied between 25 days ('Silvia') and 29 days ('President'), respectively 79.3 GDD and 118.05 GDD. In the spring season of 2021, the number of days between stages 01 and 61 varied between 20 days ('Minerva') and 32 days ('Minerva'). Thus, because the average temperatures of the spring months in 2021 were lower by 4.2°C (March) and respectively 3.3°C (April), compared to the previous year, it determined a different behavior of the cultivar. They required 13 to 27 extra GDD for 61 BBCH stage.

To start ripening, the fruits needed the highest amount of GDD depending on the precocity and the temperature. Romanian cultivars are notable for the early ripening of the fruits, since the end of July (Butac et al., 2014). The cultivars 'Minerva' and 'Centenar' had an early ripening in 2020 (20th July, 24th July), while the 'President' showed the latest ripening (10th August). The last column of Table 1 shows that the varieties needed less GDD in 2021 compared to 2020. This is confirmed in Figure no. 2, where it is observed that the temperatures in the summer months were higher than in the same period of the year. Thus, 'Centenar' needed 952.05 GDD in 2020, but in 2021 only 901.2 GDD. A significant difference in GDD was registered in the case of the 'Carpatin' cultivar, which in 2020 accumulated 1023.37 GDD until the ripening time, while in 2021 it needed only 901.2 GDD. It recorded a difference of 114 GDD between the two consecutive years.

For the ripening stage, the lowest value in 2021 was 901.2 GDD for 'Centenar', but 'President' was a cultivar that needed a sum of 1023.37 GDD, in the climatic conditions of this year.

Regarding the degree of adaptability of the plum cultivars studied to the ecological conditions in Iasi, the biometric measurements of physical and chemical characteristics of the fruit were performed such as weight, diameter, thickness, height, and soluble dry matter content. The centralized averages were statistically interpreted and presented in Tables 2 and 3.

Table 1. The growing degree-days (GDD) to the beginning of flowering and fruit ripening of plum cultivars (RSFG Iași, 2020-2021, n = 3)

Cultivars		Bud development (01 BBCH)	Start of flowering (61 BBCH)	No. days from 01 to 61	GDD (days)	Start of flowering (62 BBCH)	Ripening time (87 BBCH)	No. days from 62 to 87	GDD (days)
CENTENAR	**2020**	07.03	04.04	29	**96**	04.04	24.07	112	**952.05**
	2021	26.03	24.04	30	**112.05**	24.04	30.07	98	**901.2**
CARPATIN	**2020**	11.03	07.04	28	**118.05**	07.04	30.07	114	**1015.25**
	2021	04.04	24.04	21	**78.45**	24.04	30.07	98	**901.2**
PRESIDENT	**2020**	07.03	06.04	29	**105.45**	06.04	31.07	117	**1030.9**
	2021	25.03	25.04	32	**119.35**	25.04	10.08	108	**1023.37**
SILVIA	**2020**	11.03	04.04	25	**79.3**	04.04	30.07	118	**1029.85**
	2021	25.03	22.04	29	**106.2**	22.04	01.08	102	**937.1**
MINERVA	**2020**	10.03	05.04	26	**88.4**	05.04	20.07	107	**903.1**
	2021	05.04	24.04	20	**75.75**	24.04	31.07	99	**904**

*BBCH scale - Biologische Bundesantalt, Bundessortenamt und Chemische Industrie (Meier et al., 1994)
*GDD - The growing degree-days

Table 2. Physical and chemical characteristic of the fruit in the investigated plums cultivars (RSFG Iași, 2020, n = 3)

Cultivar	Fruit`s weight (g)	Fruit`s equatorial diameter (mm)	Fruit`s thickness (mm)	Fruit`s length (mm)	Chemical characteristics
					SDS%
Centenar	29.70^{b}	34.17^{b}	42.50^{b}	42.10^{b}	13.20^{c}
Carpatin	24.34^{c}	31.83^{b}	30.02^{d}	39.06^{c}	16.51^{a}
President	26.11^{c}	31.67^{b}	29.99^{d}	41.83^{b}	14.66^{b}
Silvia	30.74^{b}	33.28^{b}	34.51^{c}	41.50^{b}	16.60^{a}
Minerva	44.13^{a}	46.32^{a}	47.43^{a}	46.82^{a}	14.54^{b}

Different letters after the numbering within a column corresponds with statistically signifiant differences for p≤0.05 according to Duncan's multiple range test.

Table 3. Physical and chemical characteristic of the fruit in the investigated plums cultivars (RSFG Iași, 2021, n=3)

Cultivar	Fruit`s weight (g)	Fruit`s equatorial diameter (mm)	Fruit`s thickness (mm)	Fruit`s length (mm)	Chemical characteristics
					SDS%
Centenar	62.21^{a}	45.89^{a}	53.43^{a}	53.57^{a}	14.15^{b}
Carpatin	46.12^{b}	41.86^{b}	42.42^{c}	49.44^{bc}	12.15^{c}
Prezident	48.90^{b}	41.27^{b}	39.57^{c}	53.36^{a}	18.16^{a}
Silvia	50.62^{b}	41.86^{b}	42.09^{c}	49.78^{b}	12.51^{c}
Minerva	61.10^{a}	46.32^{a}	47.43^{b}	46.74^{c}	14.41^{b}

Different letters after the numbering within a column corresponds with statistically significant differences for p≤0.05 according to Duncan's multiple range test.

One of the important factors in the marketing of plum cultivars designated for fresh consumption is the fruit size (Altunas et al., 2020).

Regarding the weight of the fruit, were significant differences between all cultivars. On the fruit`s equatorial diameter, the differences were significant just between the cultivar 'Minerva' and the rest of them.

Interpreted statistically, there were significant differences between the plum cultivars. Insignificant differences were between 'Carpatin' and 'Silvia' respectively, 'Prezident' and 'Minerva'. In the climatic conditions of 2021, the water regime from March to July favorably influenced the size of the fruits. Thus, the rainfall accumulated in this period (319.2 mm) determined small differences regarding the weight of the fruit of the five cultivars, the values varying between 46.12 g at 'Carpatin' and 62.21 g for 'Centenar' cultivar, but differences significant compared to the fruits obtained the previous year when the sum of the accumulated precipitations was 201.4 mm. Also, regarding the soluble dry solids (SDS%), was observed that the 'Prezident' variety has the highest value in 2021, approximately 18.16%.

CONCLUSIONS

Following the study carried out during the two years, the selected cultivars: 'Centenar', 'Carpatin', 'Silvia', 'Minerva' and 'President' have high ecological plasticity, good adaptability to the conditions in North-Eastern Romania.

Phenological periods of the same plum cultivars are variable, depending on the climate year conditions and the cultivation area.

Regarding the pomological qualitative parameters of fruits, the rainfall accumulated during the observation period of the two years of study had a favorable influence on the weight of the fruits and the equatorial diameter. The selected varieties ‘Centenar’, ‘Carpatin’, ‘Silvia’, ‘Minerva’, and ‘President’ represent cultivars with a good combination of positive features and are recommended for the establishment of plantations and expansion in the area of culture in NE of Romania.

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PRELIMINARY RESULTS REGARDING THE BEHAVIOR OF SOME CHOKEBERRY CULTIVARS (*ARONIA MELANOCARPA*) IN ORGANIC SYSTEM

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Abstract

The paper presents partial data related to the evolution of three chokeberry Aronia varieties, grown organically. Aronia melanocarpa, is a crop that is very suitable for organic system, and its fruits have special nutraceutical qualities, containing very high levels of anthocyanins and flavonoids, as well other important substances that can reduce the risk of serious illness. The experiment was performed on plantation established in 2020 in a farm belonging to Fruit Research Station in Ilfov County. The chokeberry cultivars observed were: 'Melrom', 'Nero' and 'Galicjanka'. Planting distances 1.5 m/3 m, in 2 variants, plants canopy conducted as bush. In 2021, number of shoots and inflorescences per plant, shoots length and the number of fruits per inflorescence were done. Also, fruit weight, size, sugar content, and pH for each cultivar were determined. The observations showed for shoots growth values between 70-117 cm with and average number of fruits per inflorescence between 15-22. Regarding fruit weight varied between 0.54 and 1.63 grams. The study will continue in order to gather more data for the organic cropping system of Aronia

***Key words**: aronia, organic technology, cultivars, canopy, growth dynamic.*

INTRODUCTION

Black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) is a deciduous shrub native to North America, botanically classified as belonging to the family *Rosaceae*. Shrub of chokeberry grows 1.2 to 2.4 m producing beautiful white flowers and navy-blue berries of tart-sweet taste and aromatic flavour (Jeppsson, 2000; Brand, 2010). Chokeberry plantation can be established in any climate condition. It is a cold-hardy plant tolerant of very low temperatures even below –35°C and it is not sensitive to the spring frosts (Bussières et al., 2008). Chokeberries are noted for their modest requirements and adaptability to different soil types (sandy, acid and humid soils) and soil management systems.
According Ioan Viorel Raţi (2016), increased population demands for healthy food has led to the expansion of shrubs cultures by: the emergence of new growing areas; new systems of cultivation (crop protected in tunnels, greenhouses, growing without soil, organic production). A major issue related to shrub cultivation is given by the fact that the ripening season is overlapping with the harvesting time of the spontaneous flora. Also, few varieties of the global selection suit the pedoclimatic conditions in Romania (Ligia Ion, 2007, the National Program for Rural Development 2014-2020).
Romanian traditional agriculture relies on the use of clean technologies; it is possible to delineate green, non-polluting perimeters for the application of environmentally friendly farming practices; the demand for organic products is growing; organic farming has become a source of income for the rural population (Sumedrea D. et al., 2014; Tănăsescu N., 2005; Rati I. V, 2008).
This paper presents preliminary results of the study conducted on 3 *Aronia melanocarpa* cultivars (Melrom, Nero and Galicjanka) variety) plants of the future with high tolerance to environmental biotic and abiotic factors. The aronia berries are rich in nutrients, polyphenols and anthocyanins, with numerous beneficial effects on the health of the human body, being sought both on the domestic and international

market. Due to these characteristics, aronia has become much demanded specie for cropping in organic system.

MATERIALS AND METHODS

The research was conducted in 2021 at the Fruit Research and Development Station Băneasa, Farm Moara Domnească, county Ilfov, Romania. The farm is located N-E of Bucharest in Afumați, Ilfov County, in the Vlasiei Plain, a subunit of the Roman Plain. The site is located at 44°50’ Northern latitude and 26°24’ Eastern longitude and 70 m above the sea level.

Continental temperate climate regime is specific for the area, with hot summers, frequent droughts and cold winters. The annual mean temperature is 12°C and the total annual amount of precipitation is ranging between 550 and 600 mm, the maximum occurring between May and July, torrential rains being common. The dominant air circulation is from the East and North-East in winter and from the West in the rest of the year, with a maximum wind speed of 12.6-14.4 km/h. The zonal soil type is reddish luvisol. In the depressed areas and in the crevices there are reddish luvisols and stagnosols

The organic aronia plantation was established on spring of 2020 and the experiment was designed with 2 experimental factors: Factor A - cultivars: a1 Melrom, a2 Nero, a3 Galicjanka, Factor B - mulching of plant rows with 2 graduations: b1 - bear soil, b2 - mulched with wooden chips in a layer of 15 cm (material resulted from the previous fruit tree plantation) (Figure 1).

Figure 1. Aronia plants mulched with wooden chips

The plantation scheme consists in spacing of 3 m between rows and 1.5 m in the row. Between the rows, the soil was kept tilled and without grass. The experiment was set up as a randomized block design with three replications, giving a total of 60 black chokeberry bushes (Figure 2). During the trial, standard cultural, training and pruning practices were used, including drip irrigation.

Figure 2. Aronia plants in organic experimental field

Generative potential parameters (number of shots and inflorescences per plant, number of berries per cluster) were observed by counting.

The measurements of shoot length were made in 2021, at the end of the growth cycle, with roulette.

For the biometric measurements of fruits 60 fruits for each cultivar and variant were used.

Fruit height and diameter were measured with electronic caliper, and the fruit weight was determined using the electronic balance with an accuracy of 0.01 g

Sugar content expressed as % Brix, was determined by the electronic refractometer (Hanna instruments HI 96800) and the pH value was measured by pH Meter Hanna HI 700630.

The collection of information resulted in a series of variations were statistically analyzed using the MS Excel program “Data analysis” addon. Given the fact that in the study there were 2 variants and data was collected in 2021 for each cultivar, the samples were tested for the statistical hypothesis on differences between variants, using the Duncan – T - Student concordance test (t) with a probability 0.05.

RESULTS AND DISCUSSIONS

Regarding generative potential the analysis of the data showed differences among treatments. Mulching with wood chips affected the average number of shoots per plant so the mulched variant has showed a smaller average number of shoots per plant (13.56) compared with the

variant with bare soil (18.00). On the other hand, the average shoot length has not been highly influenced by the treatment (93.26 cm in V1 and 93.56 cm in V2). The same applies also for the average number of inflorescences per plant: 15.67 in V1 and 15.89 in V2.
Regarding number of berries per cluster the mulched variant had better results (19.15 berries) compared with the bare soil variant where the average number of berries per cluster were smaller, respectively 17.48 berries. Figures are presented in Table 1.

Table 1. Generative potential of aronia plants in 2021

Treatment	Cultivar	Average no. shoots/ plant	Average shoot length (cm)	Inflorescences no./ plant (pieces)	Number of berries/cluster
V1 - mulched	GALICJANKA	11.00	91.11	17.33	18.56
	MELROM	14.33	102.00	10.33	17.44
	NERO	15.33	86.67	19.33	21.44
	AVG	***13.56***	**93.26**	**15.67**	**19.15**
	STDEV	*2.27*	*7.89*	*4.73*	*2.06*
	VAR	*16.74*	*8.46*	*30.16*	*10.78*
V2 - bare soil	GALICJANKA	18.00	92.67	16.33	25.11
	MELROM	19.33	91.33	8.67	13.22
	NERO	16.67	96.67	22.67	14.11
	AVG	***18.00***	**93.56**	**15.89**	**17.48**
	STDEV	*1.33*	*2.78*	*7.01*	*6.62*
	VAR	*7.41*	*2.97*	*44.12*	*37.88*

Comparing the variances of average number of shoots per plant in both variants the highest value was observed in mulched variant with 16.74 % compared with the bare soil variant were the variance has reached the value of 7.41%.
In the Figure 3, can be observed that during the year of 2021 the highest average number of shoots per plant was obtained by cultivar ‘Melrom’ in V2 (19.00 shoots/plant) and the smallest by ‘Galicjanka’in V1 with 11 shoots per plant.

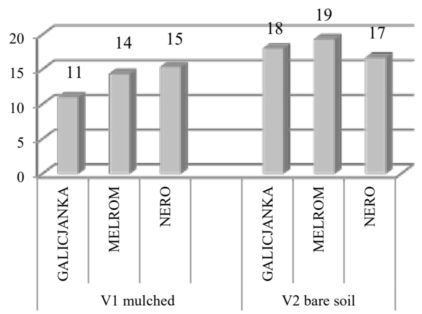

Figure 3. Average number of shoots per cultivar and per variant

Number of fruits/cluster

Statistical analysis of data on the number of berries per cluster, using Duncan’s multiple range test ($P \leq 0.05$) shows that between cultivars were no significant difference.
The highest average number of fruits per cluster, was recorded by ‘Galicjanka’ cultivar in V2 (25.00 fruits/cluster), and the lowest average number of fruits was recorded by ‘Melrom’ also in V2 (13.00 fruits/cluster). Besides the cultivar ‘Galicjanka’, a large number of fruit per cluster was recorded by Nero in V1 with 21 berries/cluster - Figure 4. On average, in the two treatments, the best results were obtained by V1 mulched variant (19.15 berries/cluster) and the lowest bare soil V2 - 17.48 berries/cluster. (fruits/plant).

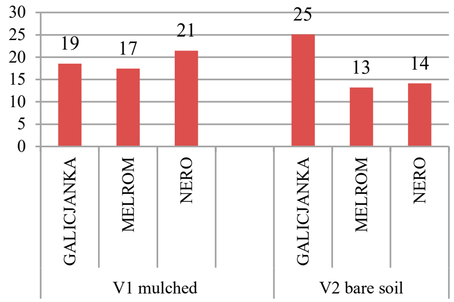

Figure 4. Average number of fruits per cluster

Fruit analysis

On the basis of the results obtained, different sizes of chokeberry fruit cultivars were observed (Table 2). Fruits of ‘Galicjanka’ variety had the highest weight of fruits, followed by fruits picked up from ‘Nero’ and ‘Melrom’. The average weight of ‘Galicjanka’ fruit amounted to 1.09 g, ‘Nero’ 1.08 g and ‘Melrom’ with 1.03 g. If we extrapolate the weight of ‘Galicjanka’ fruit to 100 fruits, the weight will be 109 g, value is higher than the one considered by Jeppsson (2000 b and a) as large fruits. Also, Jeppsson (2000 b and a) stated that the fruits of ‘Nero’ cultivar is to be considered large fruits. These cultivars weighed from 65 to almost 95 g, so the average fruit weight obtained during our experiment is confirming from the first year of crop, the conclusion of Jeppsson.
Sugar content along with other fruit characteristics determines the fruit quality. Regarding the taste of aronia fruit, it is well

known that it can be described as a complex flavor incorporating components of dryness, tartness, earthy undertones, and astringency (from the high tannin content, like a dry red wine). In order to be edible, the aronia fruit has to have high sugar content.

From the Figure 5, it can be observed that maximum average value of brix % in V1 has been reached by 'Galicjanka' cultivar (23.00) followed by 'Melrom' and 'Nero' with the same value (21.4) Brix units.

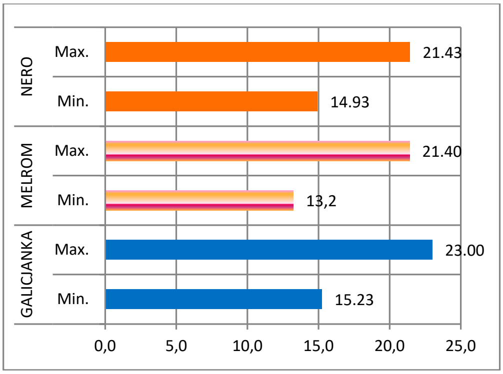

Figure 5. Maximum and minimum Brix % value in aronia fruits in V1

Regarding maximum brix value in V2, the highest brix has been reached by 'Nero' cultivar with a value of 21.43 (Figure 6).

The maximum determined values are quite similar for both treatments and for all cultivars, showing that the treatments have had no significant effect on the fruit characteristics but it has to be taken in to account that the observations were made just in the year 2021, second year after plantation was established.

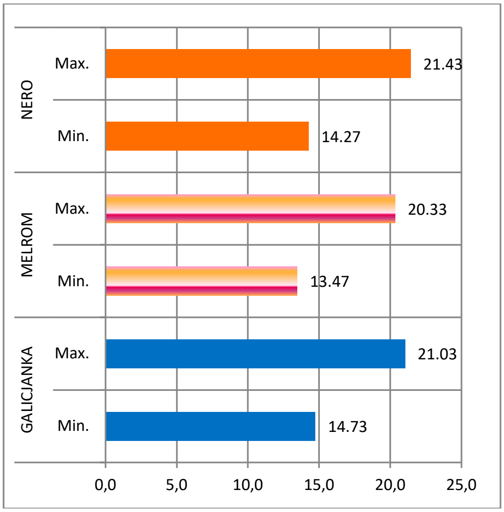

Figure 6. Maximum and minimum Brix % value in aronia fruits in V2

In the Table 2. values determined for each harvest are listed. Also, mean, standard deviation, coefficient of variation and variance for brix value are calculated.

Table 3 including all relevant data regarding fruit quality, regarding average fruit weight, the minimum and maximum fruit size.

The fruit pH is considered on normal values with the highest value determined for 'Nero' fruits (4.03), followed closely by 'Galicjanka' (4.01) and 'Melrom' with 3.98. there is no significant statistical difference between the pH cultivars values. A Duncan-T-Student concordance test (t) with a probability 0.05 has been performed and the P(T<=t) two-tail value was 1,23259E-06. between the two sets of data (pH for V1 fruits and V2).

Table 2. Brix values calculation by harvest - aronia fruits

Treatments	**Cultivar**	Values for min./max.	Harvest 1 13 Aug 2021	Harvest 2 18 Aug 2021	Harvest 3 24 Aug 2021	**Mean**	**St. dv. σ**	**CV%**	**Var**
V1 - mulched	GALICJANKA	Minim	14.20	16.20	15.30	15.23	1.00	15.21	1.00
		Maxim	22.80	25.10	21.10	**23.00**	2.01	11.46	4.03
	MELROM	Minim	11.20	16.40	12.10	13.23	2.78	4.76	7.72
		Maxim	21.20	22.60	20.40	21.40	1.11	19.22	1.24
	NERO	Minim	13.30	15.50	16.00	14.93	1.44	10.40	2.06
		Maxim	18.60	22.40	23.30	**21.43**	2.49	8.59	6.22
V2 - Bare soil	GALICJANKA	Minim	12.30	16.80	15.10	14.73	2.27	6.48	5.16
		Maxim	20.30	22.40	20.40	21.03	1.18	17.76	1.40
	MELROM	Minim	11.60	14.10	14.70	13.47	1.64	8.19	2.70
		Maxim	19.50	19.80	21.70	20.33	1.19	17.04	1.42
	NERO	Minim	13.20	16.10	13.50	14.27	1.59	8.95	2.54
		Maxim	20.20	23.50	20.60	21.43	1.80	11.90	3.24

Table 3. Overall aronia fruit characteristic for 2021

Fruit characteristics	Cultivars		
	‘Galicjanka’	‘Melrom’	‘Nero’
Average weight of fruit (g)	1.09a	1.03a	1.08a
Fruit size (mm) (min-max)	9.55-14.40	9.45-14.61	9.66-14.35
Mean	11.98a	12.03a	12.01a
Sugar content (% Brix) (min-max)	12.30-25.10	11.20-22.60	13.20-23.30
Mean (% Brix)	18.70a	16.90a	18.35a
pH mean	4.01a	3.98a	4.03a

Having in mind that the study was done just for the year 2021 and the aronia plantation has been planted in 2020, it should be considered that in this paper, the results are preliminary results.

CONCLUSIONS

The study was aiming to state the actual status of the aronia plantation and its characteristic for cultivars grown.

In order to define better the results and to be able to form a statistically based conclusion the research will continue.

From the partial results of 2021, we can conclude that *Aronia melanocarpa* is a very vigorous specie that can adapt to the climatic and pedologic conditions of south-east Romania area.

The best results has been shoed by the cultivar ‘Galicjanka’ followed by ‘Melrom’ and ‘Nero’.

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RESULTS REGARDING THE EFFICACY OF SOME FUNGICIDES IN CONTROLLING *VENTURIA INAEQUALIS* IN MĂRĂCINENI AREA IN 2016

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Abstract

This paper presents the results recorded following the application in apple culture of different treatment programs with fungicide in order to control Venturia inaequalis, a pathogen recognized for the quantitative and qualitative damages caused. In order to control this pathogen, the chemical measures are necessary in addition to the agro-phytotechnical ones. The efficacy of the new active substance fluopyram, present in Luna Experience, with effect in blocking the pathogen's respiration. The apple variety on which the experiments were carried out is Idared, chosen especially for its sensitivity to the apple scab. The observations took place in 2016 at I.C.D.P. Mărăcineni. The evolution of the meteorological parameters was noted using Watchdog station, the dedicated software and IRFAN VIEW (Freeware). With its help, the dynamics of the infections were graphically highlighted and warnings were elaborated. In the field, observations were made to determine the frequency (F%) and intensity (I%) of the attack, in order to calculate the degree attack (DA%). In the end, the effectiveness of the treatment programs was calculated.

***Key words**: fungicide, apple scab, treatments, efficacy, results.*

INTRODUCTION

The apple culture occupies the largest surfaces of fruit species both in the temperate area and Romania. Food and economic value are recognized worldwide, ranking first in world fruit production. The production potential and fruits quality can be affected by the attack of pathogens, *Venturia inaequalis* being one of the most present. The pathogen *Venturia inaequalis* causes the disease known as apple scab. The resistance of the pathogen to different fungicide active substances is one of the most approached topics, this being in continuous change, numerous data being found in research papers like (Amzăr, 1997; Bengtsson et al., 2008; 2006a; 2006b; Braun 1994; Hildebrand et al., 1988; Köller et al., 1997; Köller et al., 2004; Smith et al., 1991).

MATERIALS AND METHODS

The experimental plot was established in the Research Institute for Fruit Growing Piteşti-Mărăcineni (44°89′91″N 24°86′08″E). Climatic conditions of Mărăcineni are characterized by the average annual temperature of 9.7°C and the average annual rainfall of 663.3 mm (Butac et al., 2015). The observation took place in an 22 years old apple orchard, 3.5 x 2.5 (1842 trees/ha), sprinkler irrigation and fertilization with 90 kg/ha 16:16:16 and ammonium nitrate 30 kg/ha active substances. The evolution of meteorological parameters in 2016 was noticed using the Watchdog station, the dedicated software and IRFAN View (Freeware). The apple variety on which the experiments were performed is 'Idared', chosen especially for its sensitivity to apple scab attack. For the analysis of some fungicide efficacy in the control of *Venturia inaequalis*, two treatments schemes were proposed, presented in Table 1 and the untreated variant was used as control (blank). For the monitoring of *Venturia inaequalis* the synthesis of three observation models was taken into account Mills, WSU and Cornel NY, for the graphical elaboration of the infection dynamics and the elaboration of warnings for the application of phytosanitary. In the field observation was made to establish the frequency (F%) and intensity (I%) of the attack, in order to calculate the degree attack (DA%). At the end the efficiency of the treatments program was calculated.

Table 1. The treatment variants used

Treatment number	Date	BBCH	Products applied and rate	
			V_1	V_2
1	4.04	55	Antracol 70 WP 4.5 kg/ha	Antracol 70 WP 4.5 kg/ha
2	14.04	57-60	Dithane M-45 2 kg/ha +Shavit 72 WDG 2 kg/ha	Luna Experience 400 SC 0.75 l/ha
3	6.05	69	Flint Plus 64 WG 1.875 kg/ha	Flint Plus 64 WG 1.875 kg/ha
4	13.05	71	Topsin 70 WDG 1.05 kg/ha	Luna Experience 400 SC 0.75 l/ha
5	20.05	72-73	Folicur Solo 250 EC 0.75 l/ha	Folicur Solo 250 EC 0.75 l/ha
6	28.05	73-74	Shavit 72 WDG 2 kg/ha	Luna Experience 400 SC 0.75 l/ha
7	3.06	75	Flint Plus 64 WG 1.875 kg/ha	Flint Plus 64 WG 1.875 kg/ha
8	29.06	77	Antracol 70 WP 4.5 kg/ha	Antracol 70 WP 4.5 kg/ha
9	12.07	78	Flint Plus 64 WG 1.875 kg/ha	Flint Plus 64 WG 1.875 kg/ha
10	21.07	79	Antracol 70 WP 4.5 kg/ha	Antracol 70 WP 4.5 kg/ha
11	5.08	81	Flint Plus 64 WG 1.875 kg/ha	Flint Plus 64 WG 1.875 kg/ha
12	26.08	83	Dithane M-45 2 kg/ha	Luna Experience 400 SC 0.75 l/ha

RESULTS AND DISCUSSIONS

The efficacy of some fungicides on apple scab attack, 'Idared' variety, was monitored in 2016.The climatic conditions of 2016 (year with early spring, which followed a rather mild winter 2015-2016) were favorable for the evolution attack of *Venturia inaequalis*.
The evolution of average temperature, daily values of wind speed, gusts and foliage wetting time are presented in figures 1-3. The average monthly temperature was 6.7°C in March (high of 23.2°C and low of 4.2°C), 13.5°C in April (high of 29.4°C and low of -0.1°C), 14.6°C in May (high of 29.6°C and low of 2.6°C), 20.7 in June (high of 35.4°C and low of 9.9°C), 22.4 in July (high of 33.3°C and low of 11.1°C) and 21.6 in August (high of 35.9 and low of 8.6°C) (Figure 1).
In terms of rainfall days, it was 13 in March, 15 in April, 20 in May, 14 in June, 4 in July and 9 in August. Regarding the amount of precipitation in mm, it was 90.1 in March, 76.7 in April, 134.1 in May, 119.6 in June, 27.9 in July and 40 in August (Figure 2).
The sum of the wetting hours of the foliage was 2.5 h in March, 74.5 h in April, 136.0 h in May, 124.3 h in June, 67.8 h in July and 92 h in August. As a result of the evolution of meteorological parameters, as can be seen in figure 1, the risk of primary infections appeared since the beginning of March, but the temperatures during the night (below 6°C) and humectation period did not allow the attack evolution, the infection throw ascospores not being possible.

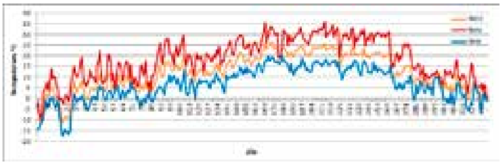

Figure 1. The daily values of temperature (source: Watchdog station)

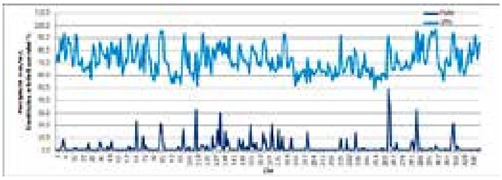

Figure 2. The daily values of precipitation and air relative humidity (source: Watchdog station)

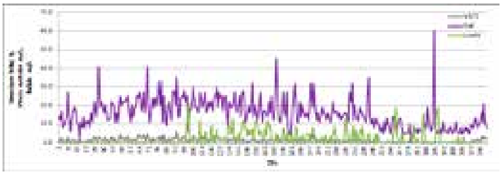

Figure 3. The daily values of wind speed, flurries and leaves time of moistening (source: Watchdog station)

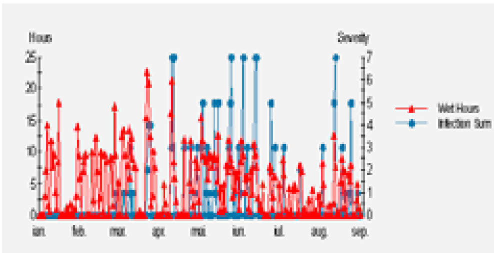

Figure 4. The risk infection with *Venturia inaequalis* between 1.01.-9.09.2016 (source: Watchdog station)

The primary infections set in the first half of April and the second half of the same month conditions were met for the secondary infections, when the foliage wetting time sometimes exceeded 20 hours/day, at the end of the month the severity attack becoming maximum (Figure 4). The risk of secondary infections was moderate in May, but increased sharply in late May and early June remaining so throughout June. The pressure of infections was lower in July but increased again in August, when the amount of foliage wetting hours exceeded 17 hours/day (Figure 4).

Field determinations found that this year the frequency attack of *Venturia inaequalis* was between 27.3% and 65.1% on leaves and between 9.1% and 44.2% on fruits, depending on the determination date. Data regarding the intensity attack of apple scab on leaves and fruits show a strong attack, the maximum values reaching 40.8% respectively 32.9%, at the last determination (Figure 5). The degree of attack recorded high values on both leaves and fruits. Thus, the value calculated for the leaf attack was between 5.4% and 31.4%, the largest differences from one determination to another being recorded in August because of the climatic conditions. The attack on fruits registered the value of 14.5% on September 10, although in the first two determinations it was weak, of 0.5% on 5 May and 1.4% on 30 May respectively. The dynamics of apple scab attack on leaves and fruits is presented in Figure 5.

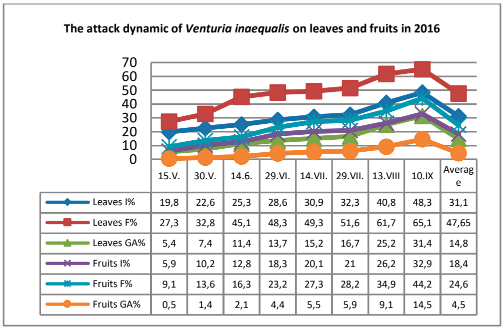

Figure 5. The attack dynamics of *V. inaequalis* on untreated leaves and fruits

In V2 variant was applied the fungicide named Luna Experience 400 SC, 0.75 l/ha, in optimal conditions for the realization of secondary infections with apple scab. Regarding the primary infections with apple scab, following the application of fungicide products from the first treatment option, the degree of attack on the leaves was 1.14% and on the fruit was 0.19% (Figure 6). In variant V2, where two treatments with the Luna Experience 400 SC fungicide were applied, the registered attack rate was only 0.19% on the leaves and the fruit attack was not present. In comparison, in the untreated variant, the degree of attack was 11.4% on leaves and 2.1% on fruits (figure 6). In the case of secondary infections, due to the application fungicides from V1, the DA% on the leaves was limited to 0.33% and on the fruit to 0.065% (Figure 6). Following the administration of products of variant V2, which included two other applications of the Luna Experience 400 SC fungicide, the degree of attack on the leaves was 0.022% and on the fruit the attack was completely absent. In the untreated variant the degree attack was 31.4% on leaves and 14.5% on fruit. It is worth noting the very good effectiveness of the Luna Experience fungicide in controlling blight, the fruit attack being absent this year, following the application of four treatments with this product (Figure 6).

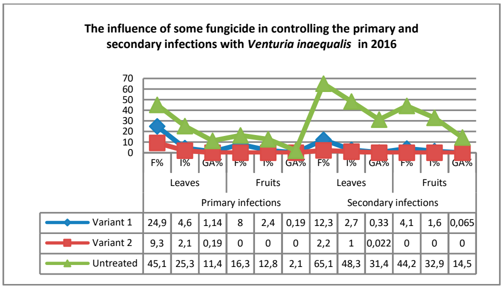

Figure 6. The influence of some fungicides on primary and secondary infections

In the control of the fungal attack on the leaves, the calculated efficacy was 90% in V1 variant and 98.33% for V2 variant, for the control of primary infections, three treatments with the product Luna Experience were applied (Table 6). For secondary infections, the calculated values were 98.94% in the case of V1 variant and 99.92% in V2, a variant in which at the last treatment (26.08), the fungicide Luna Experience 400 SC was applied again (Table 2). The calculated value of efficacy in the control of primary fruit infections was 90.95% for variant V1 and 100% for variant V2, and for the control of secondary infections the efficacy was 99.55% for V1 and 100% for V2 (Table 2).

Table 2. The efficiency of treatment program against apple scab in 2016

Va	Primary infections				Secondary infections			
	Leaves		Fruits		Leaves		Fruits	
	DA%	E%	DA%	E%	DA%	E%	DA%	E%
V_1	1.14	90%	0.19	90.95%	0.33	98.94 %	0.065	99.55 %
V_2	0.19	98.3 3%	0	100%	0.022	99.92 %	0	100%
blank	11.4	-	2.1	-	31.4	-	14.5	-

CONCLUSIONS

1. Year 2016 was a favorable year for the infections with apple scab both on leaves and fruits.
2. The fungicide products applied in first variant to control *Venturia inaequalis* significantly reduced the attack level on the leaves, both in the case of primary infections (from 11.4% to 1.14%) and in the case of secondary infections (from 31.4% to 0.33%), and in the case of fruit the degree of attack was reduced from 2.1% to 0.19% for primary infections and from 14.5% to 0.065% for secondary infections.
3. In the V2 variant, the products ensured a total protection at the level of the fruits, both in the case of primary and secondary infections, Luna Experience fungicide proving its superior efficacy. At the same time, this product is recommended as a last treatment due to the break time of only 7 days, compared to other competing products, where it is 14-28 days.

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RESULTS REGARDING THE EFFICACY OF SOME INSECTICIDES IN CONTROLLING *PHYLLONORYCTER BLANCARDELLA* IN APPLE CULTURE IN MĂRĂCINENI AREA IN 2016

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Abstract

This paper highlights the results obtained following the application of different treatments to control Phyllonorycter blancardella. The weather parameters values were correlated with the attack dynamics, establishing precisely both the moments of maximum vulnerability and insecticide application, for the highest possible effectiveness. Data were collected using the Watchdog station and also AtraBlanc-type pheromone traps were used in the field to monitor the adult's flight. In order to analyze the effectiveness, two treatment schemes were proposed, one of which included the active substance spirotetramate (V_2). In the case of the three generations (G_1-G_3) of pest larvae, the calculated efficacy of the two treatment variants recorded the following values: for G_1 the efficacy was 79.41% (V_1) and 98.52% for V_2 variant; for G_2 generation the efficiency was 78.38% for V_1 and 97.3% for V_2, and for G_3 generation, the V_1 efficiency was 76.92%, and in the variant where spirotetramate was applied at three different times the efficiency increased to 97.43%.

Key words: *insecticide, spotted teniform leafminer, treatments.*

INTRODUCTION

Apple is one of the most widespread fruit species in the world and in our country due to its food and economic value. The apple crop has a number of pests, one of the most common being spotted tentiform leafminer, *Phyllonorycter blancardella*, (Gracillariidae: Lepidoptera), which causes significant damage, researchers dedicating many studies to this pest (Proctor et al., 1982; Blommers & Vaal, 1995). The presence of the pest is mentioned in Romania since 1963. Mining moths is common in seed plantations to which abusive non-selective insecticides that reduce the activity of natural enemies are abusively applied.

The active substance spirotetramate is not harmful to insects, but when it reaches the plant, it turns into spirotetramate-enol, thus harmful only to the insects that attack the plant (Mohapatra et al., 2012). The elimination of harmful insects occurs due to the blockade of lipid synthesis. Pyrethroid insecticides effect on *P. blancardella* have been reported since 1986 in Pree et al. (1986). This paper aimed to analyze the effectiveness of insecticides in the control of *Phyllonorycter blancardella*, in apple crop, in Mărăcineni area, Argeș.

MATERIALS AND METHODS

The experimental plot was established in the Research Institute for Fruit Growing Pitesti-Mărăcineni (44°89′91″N24°86′08″E), in a 22-year-old apple orchard, where the trees are planted at a distance of 3.5 x 2.5 meters, irrigated by sprinkling and fertilized with complex fertilizers (16-16-16 dose 90 kg sa/ha and ammonium nitrate 30 kg sa/ha). Climatic conditions of Mărăcineni are characterized by the average annual temperature of 9.7°C and the average annual rainfall of 663.3 mm (Butac et al., 2015). The evolution of meteorological parameters in 2016 was done with the help of the Watchdog station, the dedicated software and IRFAN VIEW (Freeware). In the field, pest monitoring was done by using Atra-Blanc type pheromone traps, the data being correlated with those provided by the monitoring station. The dynamics of the development stages and the elaboration of warnings for the application of treatments were followed. The apple variety on which the experiments were carried out was 'Idared'. For the analysis of the efficacy of some pesticides in the control of *Phyllonorycter blancardella* in apple, two treatment schemes were proposed, presented in Table 1, and the untreated variant was used as a control.

Table 1.The treatment scheme used

Treatment number	Date	BBCH	Active substances applied and rate	
			V_1	V_2
1	4.04	55	Deltamethrin 0.225 l/ha	Deltamethrin 0.225 l/ha
2	14.04	57-60	Thiacloprid 0.3 l/ha	Thiacloprid 0.3 l/ha
3	28.04	66-67	Lambda-cyhalothrin 0.225 l/ha	Spirotetramate 1.875 l/ha
4	6.05	69	Thiacloprid 0.3 l/ha	Thiacloprid 0.3 l/ha
5	13.05	71	Deltamethrin 0.225 l/ha	Deltamethrin 0.225 l/ha
6	20.05	72-73	Thiacloprid+ Deltamethrin 0.75 l/ha	Thiacloprid+ Deltamethrin 0.75 l/ha
7	28.05	73-74	Deltamethrin 0.225 l/ha	Deltamethrin 0.225 l/ha
8	3.06	75	Lambda-cyhalothrin 0.225 l/ha	Spirotetramate 1.875 l/ha
9	29.06	77	Thiacloprid 0.3 l/ha	Thiacloprid 0.3 l/ha
10	12.07	78	Lambda-cyhalothrin 0.225 l/ha	Spirotetramate 1.875 l/ha
11	5.08	81	Deltamethrin 225 l/ha	Deltamethrin 0.225 l/ha
12	26.08	83	Deltamethrin 0.225 l/ha	Deltamethrin 0.225 l/ha

RESULTS AND DISCUSSIONS

The results of the dynamics of the evolution of the pest *Phyllonorycter blancardella* are presented in Table 2.

Table 2. Eco-biology of *Phyllonorycter blancardella* in 2016 (According Specware 9.0)

Date	Necessary days degrees	Calculated days degrees	Event
January 1st	0	0	
March 20th	97	97	First capture
April 7th	183	184	Maximum of G_1 flight
May 13th	434	438	End of G_1 flight
May 30th	590	601	Beginning of G_2 flight
June 21st	891	904	Maximum of G_2 flight
July 14th	1224	1239	End of G_2 flight
July 23rd	1370	1381	Beginning of G_3 flight
August 3rd	1571	1575	Maximum of G_3 flight
August 22nd	1854	1869	End of G_3 flight

The analysis of the results shows that the first catch was recorded on 20th of March. The maximum flight of the first generation took place on 7th of April and the end of the flight was 13th of May. For the second generation, the flight began on 30th of May, the maximum on 21st of June and the end on 14th of July. The third generation began its flight on 23rd of July, the maximum was on 3rd of August and the end on 22nd of August (Table 2).

The graphical representation of the differences between the number of days * degree required and calculated for each biological stage is shown in Figure 1.

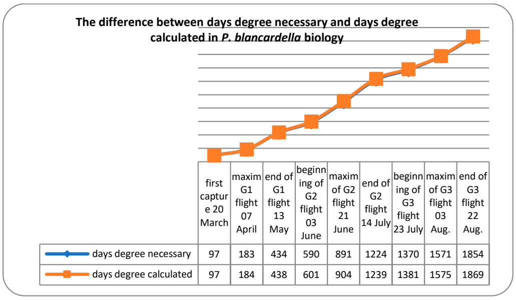

Figure1. Difference between day degree value necessary and day degree value calculated in completing the biological stages of *P. blancardella*

The dynamic flight of *P. blancardella* in untreated variant in 2016 is represented in Figure 2. As shown in the figure, three maximum values in the amount of catches were identified in weeks 18 (over 1200 specimens), 26 (approximately 1300 specimens) and 36 (approximately 1100 specimens). In the same weeks, the average catch was 620, 650 and 550, respectively.

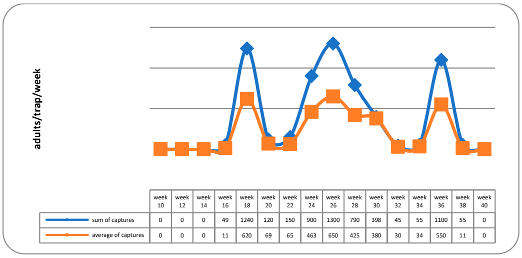

Figure 2. The dynamic flight of *P. blancardella* in untreated variant

The influence of some treatment programs against P. blancaredlla

In 2016, in order to control the main harmful organisms in the apple crop, 12 treatments were administered, the moments of application, the way of complexing the products within the two treatment variants (V_1 and V_2) being presented in table 1. When the flower buds were visible

(BBCH 55), in both variants, a treatment with deltamethrin 0.225 l/ha was applied. The treatment was given at the warning, three days after the registration of the maximum flight G_1 of the pest *P. blancardella*. In the pink bud phenophase - opening of the first flower (BBCH 57-60), thiacloprid was applied for pest control, practically one day after the registration of the end of flight G_1 in the case of the pest *Phyllonorycter blancardella*. After the first petals fading (April 28) (BBCH 66-67), an insecticide treatment was applied with the lambda-cyhalothrin active substance in the first variant, respectively spirotetramate in the second variant, 21 days after the registration of the maximum flight G_1 of the pest *P. blancardella*. On 06.05 (BBCH 69) thiacloprid was applied and at 7 days deltamethrin, followed at another week by both active substances (thiacloprid + deltamethrin) (BBCH 72-73). In variant 2, the second and third treatment with spirotetramate were applied in key phenophases, namely BBCH 75 and BBCH 78, respectively generations. Applying the treatment scheme presented in table 1, it was found that the degree of attack on the leaves of the larvae of the G_1 generation had the value of 7% in the V_1 variant and 0.5% in the V_2 variant (34% in the untreated control). In the case of G_2 larvae, the degree of attack was 8% in the V_1 variant and 1% in the spirotetramate variant, compared to the control (37%) and for the G_3 generation an attack rate of 9% in the V_1 variant and 1% in V_2, in the variant with spirotetramate (39% in untreated).

The efficiency of treatment program applied against P. blancardella

In the case of the three generations of *P. blancardella* larvae, the calculated efficacy of the two treatment variants recorded the following values: for the first generation, the efficacy was 79.41% (V_1) and 98.52% for the V_2 variant; for the G_2 generation the efficiency was 78.38% for the V_1 variant and 97.3% for the V_2 variant, and for the G_3 generation, the effectiveness of the V_1 variant was 76.92%, and in the variant where the spirotetramate was applied at three different times the efficiency of increased to 97.43% (Table 3). Spirotetramate has also proven effective in combating *P. ulmi* in an apple orchard, ensuring very good control with an effectiveness of up to 100% in 1 to 3 weeks after treatment (Labanowska & Piotrowski, 2017). Experiments conducted by Schoevaerts et al. (2011) by applying spirotetramat showed that the precise positioning of the active substance controls several pests simultaneously and can drastically reduce the number of insecticide sprays required in an orchard, and thus the total load of crop residues is reduced. The application of V_1 scheme has reduced the degree of attack of the mining moth, in the case of generation G_1, to 7%, in the case of generation G_2 from 8% and in the case of generation G_3 to 9% while the use of insecticides in variant V_2 decreased the attack rate to 0.5% for G_1 generation larvae and to 1% for G_2 and G_3 generation larvae. In the last years has been noticed an increasing resistance of *Phyllonorycter blancardella* to the contact insecticides. Replacing 2-3 treatments based on contact insecticides per year with a new active systemic substance like spirotetramate increased the efficacy of treatment programs. In the present case, the efficacy increased from 76.92-79.41% up to 97.3-98.52%.

Table 3. The efficiency of treatment program against *P. blancardella*

The variant	G_1		G_2		G_3	
	Leaves		Leaves		Leaves	
	GA%	E%	GA%	E%	GA%	E%
V_1 variant	7	79.41	8	78.38	9	76.92
V_2 variant	0.5	98.52	1	97.3	1	97.43
Untreated variant	34	-	37	-	39	-

CONCLUSIONS

The effectiveness of insecticides applied in variant V_1 for the control of *Phyllonorycter blancardella* pest was 81.08% in the case of G_2 larvae and 76.92% (in 2016) in the case of generation G_3, while the effectiveness of treatment variant V_2 in the control of the mining moth was 98.52 in the case of G_1 generation larvae, 97.3% (in 2016), in the case of G_2 generation larvae and 97.43%, in the case of G_3 generation larvae, the spirotetramate active substance proving its superior effectiveness.

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THE EVIDENCE OF THE PRESENCE OF APPLE SCAB RESISTANCE *VF* GENE OF SOME LOCAL APPLE CULTIVARS FROM TRANSYLVANIA

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Abstract

Apple is one of the most important crops in temperate areas, and it is situated on the second place, after plum, in Romania. The scab caused by Venturia inaequalis is a widespread disease in apple crop, which produces considerable economic losses. The aim of the study was to identify the presence of the Vf gene in four local apple cultivars ('Goldprim', 'Dany', 'Doina' and 'Alex'), obtained by the FRDS Bistrita. The investigated cultivars are known to be resistant to scab, but their selection was made by classical methods which do not guarantee the presence of Vf gene. To get an accurate confirmation of the Vf resistance mechanism in the genome of the cultivars, a molecular tool was used. Thus, the mentioned apple cultivars were tested by using the Marker Assisted Selection (MAS) method, with three pairs of specific markers: AL07, which allows the producing two bands corresponding to Vf-dominant, and vf-recessive respectively; the other two markers are AM19 and $U1_{400}$ as dominants (Vf). According to the results obtained, the Vf resistance genes were identified as heterozygous (Vfvf) in all four local apple cultivars tested.

Key words*: apple, disease, molecular marker, scab, Vf gene.*

INTRODUCTION

Apple cultivation is one of the most widespread crops in the temperate areas of the world. The variability of the genus *Malus* allows adaptation of the species to different geographical areas. Being a perennial species, apple is long-term crop and requires a number of specific cultivation technologies (Kellerhals et al., 2009; Muneer et al., 2017). In apple crops, the list of diseases caused by fungi, bacteria, viruses and phytoplasmas, include about 80 such diseases and physiological imbalances, to which must be added 64 species of insects and 8 species of nematodes (Köller & Parker, 1989). Certain pests and diseases are constantly dynamic, with an increasing trend, but not all are present in the same growing areas (Köller and Parker, 1989). One of the most widespread and damaging diseases in apple species is scab caused by the ascomycete fungus *Venturia inaequalis* (Luo et al., 2020). For this reason, breeding programs are aiming to obtain disease resistant/tolerant cultivars (Cordea, 2014). Thus, through them, the number of crop protection treatments can be significantly reduced. There are two types of apple resistance to scab: monogenic and polygenic (Durel et al., 2003). An important sources of monogenic apple resistance to scab is given by the *Vf* gene (named after the new nomenclature *Rvi*6), derived from *Malus floribunda* clone 821 (Bus et al., 2009). There are plenty of apple cultivars obtained in the past from controlled crosses based on empirical selection, and considered resistant to disease, because one of the genitors was known to have the *Vf* gene in the genome.
In the field of plant breeding, new molecular methods allow on the one hand the identification of genes of interest at the seedling stage of plants, and on the other hand reduced costs and shorten selection process (Soriano, 2020). In this context, selection efficiency is a key issue in the proper choice of cultivars to be used as parents in breeding

programs (Kellerhals et al., 2009). Tartarini et al. (2000) concluded that classical selection does not prove sufficient accuracy in plant resistance of trees to scab compared to the Marker Assisted Selection. The aim of the present study was to verify the presence of the *Vf* gene in four local apple cultivars breeded at FRDS Bistrita supposed to carry the *Vf* gene.

MATERIALS AND METHODS

The material used for the experiment consists of four local apple cultivars ('Goldprim', 'Dany', 'Doina' and 'Alex') registered by Fruit Research & Development Station Bistrita (Table 1).
In this study, the Florina cultivar (*Vfvf* - Pătrașcu, et al., 2006) was used as a positive control and the Golden delicious (*vfvf* - Sestraș, 2004) variety as a negative control.
Following a classic selection process, which was initially carried out empirically, the cultivars mentioned are known to be resistant to scab (Braniște et al., 2007), but this is not a guarantee of the presence of the *Vf* gene.

Table 1. List of cultivars used in experience

Cultivar	Genitors	Year of approval
'Goldprim'	♀Golden delicious x ♂Prima	2003
'Dany'	♀Jonathan irradiated with P33 x ♂Prima	2003
'Doina'	♀Jonathan x ♂Prima	2003
'Alex'	♀Golden delicious x ♂BN 33/39	2004

Therefore, to accurately determine the presence or absence of the *Vf* gene conferring scab resistance, PCR (Polymerase Chain Reaction) analyses were performed by using specific markers: two pairs of dominant primers (AM19, $U1_{400}$) and another codominant primer (AL07) to distinguish between homozygous and heterozygous genotypes (Table 2).

Table 2. List of primers used

No.	Marker name	Primer sequences 5' to 3'	No. nucleotide	Primer type	Fragment size (bp)	References
1	AL07	F-TGGAAGAGAGATCCAGAAAGTG	22	Codominant	570 (*Vf*)	Khajuria et al., 2014
		R-CATCCCTCCACAAATGCC	18		823 (*vf*)	Tartarini et al., 1999
2	AM19	F-CGTAGAACGGAATTTGACAGTG	22	Dominant	526 (*Vf*)	Khajuria et al., 2014
		R-GACAAAGGGCTTAAGTGCTCC	21			Tartarini et al., 1999
3	$U1_{400}$	F-GTAAAGCAAGCACTTCAACG	20	Dominant	338 (*Vf*)	Hemmat et al., 1998
		R-GTAAAATAGATGTGTGGGTAGC	22			

DNA extraction protocol

DNA extraction was performed from lyophilized leaves in calcium chloride stored at 4°C. Bioline's Plant DNA Extraction Kit was used to isolate DNA according to the protocol indicated by the manufacturer. Finally, 50 µl of pure DNA was obtained for each sample. Measurements of DNA concentration and purity were performed using the NANODROP 2000c spectrophotometer.

DNA amplification

The preparation of DNA amplification mix was performed using MyTaq Red Mix from Bioline, obtained a final volume of 25 µl/tube for each sample. DNA amplification was performed in an Eppendorf Mastercycler. For primers AL07 and AM19, the cycling parameters used were as follows: 1 min at 95°C - initial denaturation; 35 cycles, each consisting of: 1 min at 94°C - denaturation, 1 min at 60°C - annealing, 2 min at 72°C - extension, and 10 min at 72°C - final extension. For primer $U1_{400}$ the cycling parameters used were as follows: 1 min at 95°C - initial denaturation, 2 min at 94°C - denaturation, 2 min at 69°C - annealing, 2 min at 72°C - extension. The cycle was repeated each time 1 min. at 94°C with a reduction of 1°C for annealing temperature per cycle. The cycle was repeated 30 times when annealing temperature reached 62°C. The final extension cycle was applied for 10 minutes at 72°C. The final concentration of primers in the reaction was 1 µM.

Migration of amplified products

The amplified fragments were fractionated in 1,5% agarose gel in a 1X TAE buffer for 50 min, stained by RedSafe Nucleic Acid Staining Solution. 100 bp DNA Ladder RTU was used as a size marker. Bands were visualised using Quantity One 1-D Analysis Software system under UV light.

RESULTS AND DISCUSSIONS

As expected, due to the fact that the male genitors of each apple cultivar studied (Table 1) carry the *Vf* gene (Braniște et al., 2007), it was also transmitted to the progenies.
Thus, according to the molecular tests performed, all four tested cultivars ('Goldprim', 'Dany', 'Doina', and 'Alex') had the scab resistance gene. The specific fragments of *Vf* gene (*Rvi6*) were amplified by all three molecular markers. Thus, both the 570 bp fragment of dominant gene and the 823 bp of recessive gene were amplified by the codominant primer AL07.
Using the other two sets of dominant primers, PCR reaction amplified fragments of 526 bp with the AM19 marker, and of 338 bp with the $U1_{400}$ marker, respectively, identical with molecular size of positive controls (Figure 1).

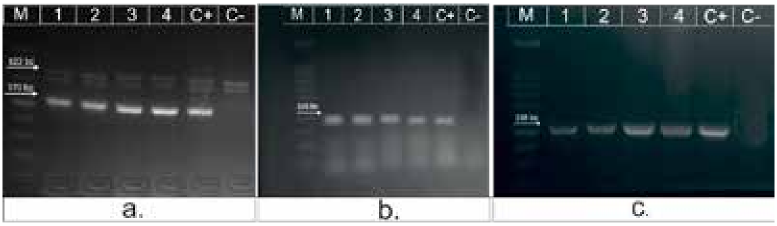

Figure 1. Electrophoresis profile of the genotypes identified at *Vf* locus in cultivar with AL07 primer set (a), AM19 primer set (b) and $U1_{400}$ primer set (c)
M – Marker; 1 - 'Goldprim'; 2 - 'Dany'; 3 - 'Doina'; 4 - 'Alex'; 'Florina' (C+); 'Golden delicious' (C-)

The bands obtained on gels were represented in the form of binary data, being noted by "+" for the presence and "-" for the absence of the *Vf* gene for each cultivar depending on the marker used (Table 3). According to the results obtained, all the studied cultivars are heterozygous for the resistance gene *Vf*.

Table 3. Cultivars investigated for scab resistance

Cultivar	AL07		AM19	$U1_{400}$	Detected genotype
	Vf	*vf*	*Vf*	*Vf*	
'Goldprim'	+	+	+	+	*Vfvf*
'Dany'	+	+	+	+	*Vfvf*
'Doina'	+	+	+	+	*Vfvf*
'Alex'	+	+	+	+	*Vfvf*
'Florina' (C+)	+	+	+	+	*Vfvf*
'Golden delicious' (C-)	-	+	-	-	*vfvf*

Similar results in confirming the presence of the *Vf* gene in other local varieties of old apples or apple hybrids have been reported in other studies. Militaru et al. (2020) studied the presence of the *Vf* gene in some local varieties that are known to be resistant to crust. Bivolariu et al. (2021) used molecular selection techniques on a sample of seedling apple hybrids. In the mentioned studies, the same sets of specific markers (AL07, AM19 and U1400) were used to identify the *Vf* apple resistance gene in different apple varieties or hybrids.

CONCLUSIONS

The studies confirmed the presence of the *Vf* gene, which confers scab resistance (monogenic resistance) to all analyzed varieties, using the MAS technique. The *Rvi6* gene was amplified by the three markers used. Therefore, these cultivars can be used as donors of *Vf* resistance gene in further apple breeding programs.

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RESEARCH REGARDING THE RESPONSE OF SOME BLUEBERRY VARIETIES IN GARDITSA AREA, GREECE

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Abstract

Blueberry culture has become more attractive to many pomiculturists due to the special quality of fruits and of their nutritive and nutraceutical value. The comparative research of 4 blueberry culture varieties: Duke, Draper, Patriot and Brigitta and 2 pruning variants proved different responses in similar culture conditions. The biological characteristics of the varieties selected manifested through different capacity of producing shoots in the collet area, through different reaction to pruning and through the capacity of forming inflorescences. Pruning influenced the growth in height of plants, the ramification capacity of the bush and the size of the fruits. Heavy pruning determined a bigger growth, larger fruits and eventually a higher capacity of production. From the studied varieties Duke was the earliest one but with a small production capacity, Brigitta was late and the most productive and Draper and Patriot registered mediate values.

Key words*: growth, fructification, pruning.*

INTRODUCTION

Blueberry (*Vaccinium* ssp.) is know since ancient times and the fruits are consumed due to a very appreciated organoleptic quality (20) and to some features highly beneficial to human health (2, 9, 13). The plant is traditionally used as tea plant, especially when collected from spontaneous flora where it grows as self-sown (4; 10). Blueberries can be consumed fresh, they can be stored for a given time in deposits, depending on variety and storage conditions (3, 6, 8), they can be processed and turned into comfiture, syrup, candied fruits, dry fruits, etc. (18). Blueberry fruits are very appreciated for their gustative features, for the high antioxidant action, for the high content of flavonoids and vitamins C and E (5, 12, 13, 16, 19). Anthocyanins, substances extremely useful to human body, are present in large quantities in blueberries and their accumulation can be enhanced through the application of abscisic acid during the fruit maturation process (15). Moreover, it had been observed that during maturation the biochemical composition changes, the total content of phenols, flavonoids and gibberellic acid decreases and the content of anthocyanin and abscisic acid increases (1). Due to the extraordinary quality of fruits blueberry culture has become more appealing to small scale fruit growers but also for farmers, reaching second place after strawberry (7). In culture, the varieties used are from the American blueberry (*Vaccinium corymbosum*) because the fruits are larger and the plant more productive. Although the species seems to be quite rustic in reality is particular to soil reaction, preferring a soil with an acid reaction and a good water penetrability (4). Choosing the right area for blueberry culture is influenced also by the minimum temperature limit in winter and moreover by the late frosts in spring which can cause great damage (10, 11, 14, 18).

The present study presents the response of some blueberry variants in field culture using two pruning variants.

MATERIALS AND METHODS

The experiment was conducted during 2018-2019 on four blueberry varieties: Duke, Draper, Patriot and Brigitta in a plantation set up in 2014, with the planting distance of 3 m between rows and 1 m on the row. Planting was made on billons on Polish acid peat with pH of 5.2 and the billon was covered (mulched) with agro textile.

Irrigation was done through a drip system with 2 lines located on each side of the plants. Fertilization was managed through irrigation water with 50 kg/ha nitrogen applied three times every 40 days during the intense growth of shoots and in order to maintain the acid reaction the product used was Nutex, with a pH of 4, 1.5 l/plant, applied three times.

Pruning was made in two stages in order to observe the reaction of the four varieties:

V1 - normal pruning;

V2 - heavy pruning.

Phenological observations have been taken and measurements have been done regarding growth, ramification and the fructification capacity of plants.

The results obtained have been statistically interpreted through analysis of variance.

RESULTS AND DISCUSSIONS

The reaction of the four blueberry varieties used was good and the development of the phenological process was slightly staggered (Table 1). The shoots started opening in the second part of February at Patriot variety and continued through middle March at Duke and Draper varieties. Small differences have been noted between the two pruning variants, dependent of the climatic year as well, heavy pruning caused a slightly faster start, as year 2017 was rather early.

Table 1. Development of reproductive phenological phases of some blueberry varieties, Garditsa, Greece, 2016

Variety	Variant	Shoots opening	Beginning of efflorescence	Beginning of maturation	End of maturation
Duke	V1	5-11.03	15-20.03	10-25.05	9.06
	V2	1-5.03	10-12.03	10-22.05	6.06
Draper	V1	5-11.03	20-22.03	15.05-2.06	20.06
	V2	1-7.03	12-15.03	2-4.06	15.06
Patriot	V1	18-20.02	25-27.02	10-22.05	10.06
	V2	18-20.02	25-27.02	10-22.05	10.06
Brigitta	V1	25-30.02	5-10.03	25.05-2.06	23.06
	V2	25-30.02	5-10.03	25.05-2.06	23.06

Patriot variety effloresced faster, at the end of February and continued until the second decade of March. Fruit maturation was early at Duke variety, at the beginning of the third decade of May, this being the earliest of all, and the late one was Brigitta where fruits started maturing at the beginning of June. Maturation time ended between 6-23 of June, for Duke the last fruits were harvested on June 9th, and for Brigitta on June 23rd.

Plant growth capacity was influenced by the variety and the intensity of pruning (Table 2). Thus, plant height was higher at Duke and Brigitta at the heavy pruning variant and smaller numbers were registered at Draper, for both variants. Plant reaction to pruning was not the same, Duke and Brigitta reacted strongly and the growth gain caused by heavy pruning was between 19 and 35% for these varieties. Differences between varieties, variants and the average value of the experiment were statistically assured.

Bush capacity to ramify was also influenced by the variety and the pruning variant. Generally, pruning determined a better bush ramification at Duke and Brigitta, whereas for Patriot the variant with the lighter cut provided better results. For Draper there were no differences registered at neither pruning variants. As numbers, most ramifications were observed at Patriot variety, variant V1, with 90 ramifications, and the least at Draper variety, variant V2, with only 40 ramifications.

Ramification capacity from collet was also different and influenced by the variety but not by the pruning variant. The best ramification was seen at Draper variety with roughly 10 new ramifications from collet and the weakest was registered at Patriot and Brigitta with 1.5 ramifications from collet.

Table 2. Growth and ramification capacity of the bush

Variety	Variant	Height of the bush (cm)	Number of ramifications in the bush	Average number of annual branches formed from parcel	Average lenght of annual branches (cm)	Sum of annual growth (cm)
Duke	V1	103.3^{ooo}	45	5.5**	16.70^{ooo}	750.5^{oo}
	V2	141.2***	67	4.5n	17.07^{oo}	1145.5^{oo}
Draper	V1	112^{ooo}	46	10.5***	22.12n	1225.5n
	V2	111^{ooo}	40	9.0***	34.42***	1031.0n
Patriot	V1	113^{ooo}	90	1.5^{ooo}	22.12n	1864.0**
	V2	128***	73	1.5^{ooo}	30.77***	2236.5***
Brigitta	V1	118^{o}	54	1.7^{ooo}	21.63n	1163.0n
	V2	141.6***	77	1.3^{ooo}	22.39n	1638.5n
Average	Control	121.01	61.5	4.44	23.40	1340.02
LSD 5%		2.90	3.26	0.64	3.20	354.5
LSD 1%		4.03	4.54	0.89	4.56	492.6
LSD 0.1%		5.61	6.31	1.24	6.35	685.3

The average length of annual branches was different depending on the variety and the intensity of the pruning made. For these indicators, at all varieties, heavier pruning determined bigger growth numbers. The average length of annual branches was between 34.4 cm at Draper variety, V2, as maximum value and 16.7 Duke variety, less intense cut, as the minimum value. The differences in comparison to the average value of the experiment were statistically assured.

The sum of annual growth, indicator that highlights vigour of plants, was very fluctuant. The maximum value was obtained by Patriot variety, variant with heavy pruning of 2236 cm and the minimum value at Duke variety, variant less intensely cut, of 750 cm. The other variants had mediate values. The differences in comparison to the average value of the experiment were statistically assured, at least for the 5% probability.

The blueberry production capacity is influenced by the number of inflorescences left on plant while pruning, by the number of fruits from the inflorescence and the average weight of the fruit.

The average number of inflorescences per plant fluctuated very much and spanned from 468 inflorescences at Patriot variety V2 and 212 inflorescences at Duke variety V1 (Table 3).

Table 3. **Fructification capacity of some blueberry varieties**

Variety	Variant	Number of inflorescences per plant (piece)	Number of fruits in inflorescence (piece)	Average weight of fruit (g)	Production per plant (kg)	Production per unit area (t)
Duke	V1	212^{ooo}	9.1	1.6n	3.64^{ooo}	12,13
	V2	369n	9.0	1.9***	6.32***	21.86***
Draper	V1	392**	10.0	1.3^{o}	5.10n	16.99***
	V2	322^{oo}	10.0	1.5n	4.84^{oo}	16.13***
Patriot	V1	416***	10.1	0.96^{ooo}	3.99^{ooo}	13.29n
	V2	468***	10.2	1.16^{ooo}	5.53n	18.43***
Brigitta	V1	268^{ooo}	10.0	1.85**	4.66^{ooo}	15.53***
	V2	420***	10.1	2.0***	8.61***	28.69***
Average	Control	358		1.53	5.34	17.88
LSD 5%		23.05		0.18	0.27	1.21
LSD 1%		32.03		0.25	0.38	1.68
LSD 0.1%		44.57		0.36	0.53	2.34

The number of fruits in inflorescence was relatively equal except for Duke variety which had fewer fruits. The average fruit weight was influenced by variety and also by pruning. Heavy pruning determined the formation of larger fruits. Thus, the size of fruits at the variant with a lighter pruning was between 0.96 g at Patriot variety and 1.85 g at Brigitta variety, while for the heavy pruning the size limits were registered at the same varieties and had values of 1.16 g and 2 g, respectively.
Production per plant oscillated between 3.64 kg at Duke variety V1 and 8.61 kg at Brigitta variety V2. In comparison to the average value of the experiment the differences of varieties were statistically assured. Potential production per unit area was between 12.3 t/ha at Duke variety, variant V1 and 28.69 t/ha at Brigitta variety, variant V2.

CONCLUSIONS

The following conclusions can be drawn from this study:
The behaviour of blueberries in the studied area was good, the differences between varieties are given by their biological characteristics. The development of the flowering phenophases was determined by the variety and the climatic year, the year 2017 was slightly earlier.
The intensity of pruning influenced the height of the plants, the branching capacity, the size of the fruit and the production capacity.
The production potential was between 12 and 28 t/ha, depending on the variety and the intensity of the cutting.

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CORRELATION AND REGRESSION DEPENDENCES BETWEEN VEGETATIVE AND REPRODUCTIVE INDICATORS IN TEGERA AND ELENA CULTIVARS (*PRUNUS DOMESTICA* L.) AS A RESULT OF DIFFERENT AGROTECHNICAL SISTEMS

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Abstract

During the period 2016-2018 at the Research Institute of Mountain Stockbreeding and Agriculture in Troyan a study was conducted to analyze different agrotechnical systems (organic and conventional fertilizing technology) in plum plantations. As a result of the fertilization, the vegetative and reproductive manifestations of plum cultivars with different ripening periods (Tegera and Elena) were monitored. Correlation and regression analysis established the relationship between the studied indicators. Depending on the applied system, genotypic similarity but also cultivar difference was analyzed. The biological fertilization of the Tegera cultivar determines a positive correlation between the average fruit weight and the average yield kg/tree at a correlation coefficient (r = 1.000) and regression equation y = 11.422 x - 341.62 with a coefficient of determination R^2 = 1.0. In contrast to organic production, in the conventional technology in both cultivars a correlation was found between the average mass of the stone and the average yield kg/tree; (Tegera r = 0.866; Elena r = 0.714), with a coefficient of determination (Tegera R^2 = 0.750; Elena R^2 = 0.510).

Key words: *Prunus domestica L., fertilizers, vegetative and reproductive indicators, correlation and regression dependences.*

INTRODUCTION

The agrotechnics that is used today in the cultivation of stone fruit species must be environmentally and economically sustainable. This means that the production systems that are used, in addition to improving fruit yield and quality, are important to maintain natural resources (Rombolà et al., 2012). Fertilizing and the choice of fertilizer is one of the main cultural practices in the orchards, contributing to the efficiency and intensity of fruit production. (Pešaković et al., 2020). The growing interest in organic food provokes many comparisons of products grown in different (organic and conventional) production systems, and the differences may be due to different technologies of organic and conventional fertilizing (Mditshwa et al., 2017; Rahman et al., 2021). The analysis of existing relationships between the studied indicators is extremely important due to the possibility to choose a combination of several effective characteristics (Tripon et al., 2016). The aim of the present study is to study the correlations between vegetative and reproductive indicators in Tegara and Elena (*Prunus domestica* L.) cultivars as a result of different agrotechnical systems.

MATERIALS AND METHODS

The experiment was conducted in 2016-2018 at the Research Institute of Mountain Stockbreeding and Agriculture - Troyan at a plum plantation of cultivars with different ripening periods, such as Tegera (early ripening) and Elena (late ripening), with a planting scheme 4 x 2.5 m.

In both plum cultivars, organic and conventional fertilizing (soil and foliar) was applied, analyzed in parallel with the untreated control variant.

Agriful (soil) fertilizers - 5 l/da, with content of Total Humic Extract - 306 g/l were used in the biological fertilizing; Fulvic acids-306 g/l; Nitrogen (N) -55 g/l; Phosphorus (P_20_5) - 13 g/l; Potassium (KjO) -13 g/l; Total organic

matter – 551 g/l; pH - 4.7, applied five times since the beginning of the vegetation, over a period of 15-20 days.
Tekamin Flower (foliar) - 0.3%, with a composition of seaweed extract-51 g/l; Free "L" amino acids - 38 g/l; Nitrogen (N) - 38 g/l; Phosphorus (P_2O_5) - 127 g/l; Boron (B) - 13 g/l; Molybdenum (Mo) - 6.5 g/l; pH - 2, applied twice, before blossoming and during fruit setting.
Teknokel Amino Ca (foliar) - 0.4%, with content Calcium oxide (CaO) water-soluble-148 g/l; Boron (B) water-soluble-3 g/l; Free "L" amino acids - 89 g/l; pH - 4.0-4.5, applied after blossoming and a month before harvesting.
Yara Mila Complex fertilizers (soil) - 0.500 kg/tree, with nitrogen composition (N) -12% were used in the conventional fertilization; Potassium (K) -18%; Magnesium (MgO) - 2.7%; Boron (B) - 0.015%; Manganese (Mn) - 0.02%; Phosphorus (P) -11%; Sulfur (SO_3) - 20%; Iron (Fe) - 0.2%; Zinc (Zn) - 0.02%, applied once before vegetation in 2016 and 2018; **YaraVita Frutrel** (foliar) - 0.500 ml/da, with content Calcium oxide (CaO) - 280 g/l; Phosphorus (P) - 104 g/l; Nitrogen (N) - 69 g/l; Magnesium (MgO) - 100 g/l; Zinc (Zn) - 40 g/l; Boron (B) -20 g/l, applied four times. First in the winter bud phase, in the white bud phase, during fruit setting and one month before harvest; **Yara Vita Universal Bio** (foliar) - 0.500 ml/da, with composition Nitrogen (N) - 100 g/l; Phosphorus (P2O5) -40 g/l; Potassium (K2O) - 70 g/l; Manganese (Mn) -1.3 g/l; Copper (Cu) - 1.0 g/l; Zinc (Zn) - 0.7 g/l; Boron (B) - 0.2 g/l; Molybdenum (Mo) - 0.03 g/l, applied three times - before and after blossoming and after harvest.
The reported vegetative and reproductive characteristics of fruit trees were performed according to the methodology for studying plant resources (Nedev et al., 1979).
Vegetative indicators:
• trunk circumference (cm) - measured at a height of 40 cm from the soil surface, after the end of vegetation;
• crown height (m) - measured from the level of the first skeletal branch to the top of the tree;
• width of the crowns (m) - is reported in two directions - between the rows and in the row;
• crown volume (m^3) - calculated by the formula $V = \pi * d^2 * h/12$, including: π - 3.14; d - circumference, on average from the two mutually perpendicular directions, excluding individual protruding branches; h - crown height (m) (excluding the stem), measured from the level of the first skeletal branch to the top of the tree;
• Annual shoot length growth - the indicator includes, average length (cm) of branches over 5 cm and total annual shoot length growth on marked branches, after the end of vegetation;

Reproductive manifestations:
• percentage of useful fruit-set, reported thirty days after full blossoming, on marked branches;
• average weight of fruit and stone (g) - determined by measuring 25 randomly selected fruits and their stones by technical scales;
• average yield kg/tree.
Statistical processing
The obtained experimental data were statistically processed using the software products Analysis Toolpak for Microsoft Excel 2010, at a level of statistical significance, p-value <0.05.
Correlation and regression analysis were applied to determine the impact of individual indicators in different agro-technical systems.

RESULTS AND DISCUSSIONS

The correlation dependences of the studied vegetative and reproductive indicators presented in Table 1 in early and late ripening plum cultivar with biological agrotechnical system show genotypic similarity, but also a difference. In contrast to the Elena cultivar, the trunk circumference of Tegera cultivar correlates positively with the percentage of useful fruit-setting and the average fruit weight ($r = 0.867$). It was found that the reported reproductive manifestations in Elena cultivar as average fruit weight, stone weight and average tree yield are positively correlated with the vegetative indicators, such as height, width of crowns in the row and their volume. The reported dependencies are absent in the early maturing cultivar Tegera. Similarity in both tested cultivars was found between the indicators useful fruit-settings percentage and the average fruit weight with correlation coefficient (Tegera $r = 1.000$) and (Elena r =

0.820). The percentage of useful fruit-setting in the Tegera cultivar also showed a positive correlation with the average stone weight (r = 0.866) and the average yield kg/tree (r = 1.00). The average fruit weight of both cultivars is positively correlated with the average stone weight (Tegera r = 0.866) and (Elena r = 0.952). Only in the Tegera cultivar, the average fruit weight has a correlation coefficient (r = 1.000) compared to the average yield kg/tree.

Table 1. Correlation dependences between vegetative and reproductive indicators of plum cultivars obtained in biological cultivation system

	Trunk circumference (cm)	Crown height (m)	Crown width in the row (m)	Crown width in the row spacing (m)	Crown volume (m^3)	Annual shoot length growth (cm)	Total annual shoot length growth (cm)	Percentage of useful fruit-set (%)	Average fruit weight (g)	Average stone weight (g)	Average yield kg/tree
Tegera											
Trunk circumference	1.000										
Crown height	-0.865	1.000									
Crown width in the row	-0.952	0.978	1.000								
Crown width in the row spacing	-0.992	0.795	0.905	1.000							
Crown volume	-0.994	0.915	0.980	0.972	1.000						
Annual shoot length growth	-0.090	0.577	0.392	-0.037	0.199	1.000					
Total annual shoot length growth	-0.090	0.577	0.392	-0.036	0.199	1.000	1.000				
Percentage of useful fruit-set	0.867	-0.500	-0.671	-0.923	-0.807	0.419	0.418	1.000			
Average fruit weight	0.867	-0.500	-0.671	-0.923	-0.806	0.419	0.419	1.000	1.000		
Average stone weight	0.501	0.000	-0.211	-0.606	-0.403	0.817	0.817	0.866	0.866	1.000	
Average yield kg / tree	0.867	-0.500	-0.671	-0.923	-0.807	0.419	0.418	1.000	1.000	0.566	1.000
Elena											
Trunk circumference	1.000										
Crown height	-0.786	1.000									
Crown width in the row	-0.938	0.951	1.000								
Crown width in the row spacing	0.638	-0.025	-0.332	1.000							
Crown volume	-0.855	0.993	0.982	-0.146	1.000						
Annual shoot length growth	-0.998	0.750	0.917	-0.680	0.825	1.000					
Total annual shoot length growth	-0.998	0.748	0.916	-0.683	0.823	1.000	1.000				
Percentage of useful fruit-set	0.089	0.546	0.262	0.823	0.441	-0.144	-0.147	1.000			
Average fruit weight	-0.498	0.928	0.768	0.350	0.876	0.449	0.446	0.820	1.000		
Average stone weight	-0.739	0.997	0.927	0.047	0.981	0.700	0.698	0.605	0.952	1.000	
Average yield kg / tree	-0.989	0.687	0.878	-0.743	0.770	0.996	0.996	-0.233	0.366	0.633	1.000

The theoretical regression line and the equation y = 11.422 x – 341.62 of the regression dependence between the average fruit weight and the average yield are presented in Figure 1, with a determination coefficient R^2 = 1.0. Such a functional dependence was not observed for cultivars with a conventional fertilizing system.

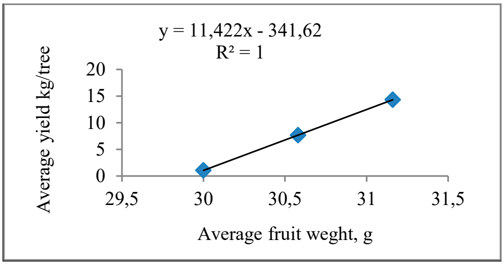


Figure 1. Graphical model of regression dependence for Tegera cultivar between the average fruit weight and average yield kg/tree with applied biological cultivation system

In their study of biological fertilization in plum varieties Butac & Chivu (2020) found a negative correlation between the average fruit weight and average yield kg/tree (r = -0.444), in the regression equation y = -0.3254 x + 46.709 and a coefficient of determination (R^2 = 0.1972).

Table 2 presents the correlations between vegetative and reproductive indicators of plum cultivars obtained by applying a conventional cultivation system.

In contrast to biological fertilizing, in the conventional system, the trunk circumference of the Tegera cultivar is reported to correlate positively with the length of the annual (r = 0.838) and total annual shoot length growth (r = 0.837).

Table 2. Correlation dependences between vegetative and reproductive indicators of plum cultivars obtained under conventional cultivation system

	Trunk circumference (cm)	Crown height (m)	Crown width in the row (m)	Crown width in the row spacing (m)	Crown volume (m^3)	Annual shoot length growth (cm)	Total annual shoot length growth (cm)	Percentage of useful fruit-set (%)	Average fruit weight (g)	Average stone weight (g)	Average yield kg / tree
					Tegera						
Trunk circumference	1.000										
Crown height	-0.915	1.000									
Crown width in the row	-1.000	0.909	1.000								
Crown width in the rowspacing	-0.954	0.994	0.950	1.000							
Crown volume	-0.975	0.981	0.972	0.997	1.000						
Annual shoot length growth	0.838	-0.546	-0.845	-0.638	-0.697	1.000					
Total annual shoot length growth	0.837	-0.544	-0.844	-0.635	-0.695	1.000	1.000				
Percentage of useful fruit-set	0.284	0.128	-0.296	0.015	-0.065	0.760	0.763	1.000			
Average fruit weight	-0.283	-0.129	0.296	-0.016	0.065	-0.760	-0.762	-1.000	1.000		
Average stone weight	-0.234	0.607	0.221	0.513	0.443	0.334	0.337	0.866	-0.866	1.000	
Average yield kg / tree	0.284	0.128	-0.296	0.015	-0.065	0.760	0.763	1.000	-1.000	0.866	1.000
					Elena						
Trunk circumference	1.000										
Crown height	-0.334	1.000									
Crown width in the row	-0.465	0.990	1.000								
Crown width in the row spacing	-0.561	0.968	0.994	1.000							
Crown volume	-0.303	0.999	0.985	0.959	1.000						
Annual shoot length growth	-0.998	0.391	0.518	0.611	0.360	1.000					
Total annual shoot length growth	-0.998	0.390	0.517	0.610	0.359	1.000	1.000				
Percentage of useful fruit-set	0.020	0.936	0.876	0.816	0.947	0.041	0.040	1.000			
Average fruit weight	-0.630	0.942	0.981	0.996	0.931	0.676	0.675	0.764	1.000		
Average stone weight	-0.756	0.869	0.931	0.966	0.853	0.795	0.794	0.639	0.985	1.000	
Average yield kg / tree	-0.998	0.275	0.409	0.509	0.243	0.992	0.993	-0.082	0.581	0.714	1.000

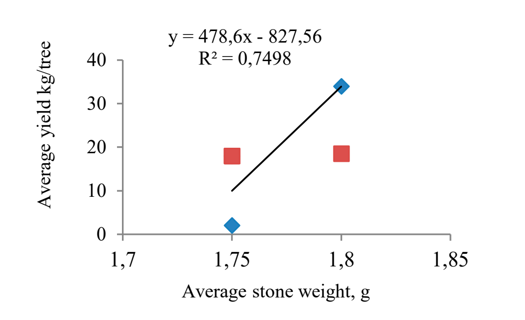
Identical to the biological agrotechnical system, in the cultivar Elena a high correlation was found between the vegetative (height, width and volume of the crowns) and reproductive indicators (average fruit weight and average stone weight).

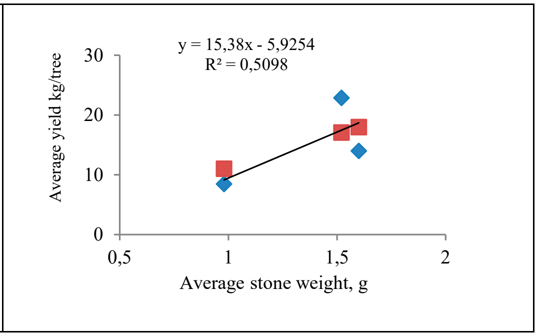
As a result of the conventional fertilizing technology applied for Tegera cultivar, the percentage of useful fruit-settings is in a high positive correlation with the length of the annual (r = 0.760) and total annual shoot length (r = 0.763).

The length of the annual and total annual shoot length in both tested cultivars has a high correlation coefficient against the average yield kg/tree Tegera r = 0.760; r = 0.763) and (Elena r = 0.992; r = 0.993), as in the cultivar Elena the vegetative indicators are in correlation with the average stone weight (r = 0.795; r = 0.794).

Both in the biological agrotechnical system and in the conventional, the percentage of useful fruit-setting in Tegera cultivar has a correlation coefficient r = 0.866, relative to the weight of the stone and r = 1.000, compared to the average yield kg/tree. In the case of Tegera, the percentage of useful fruit-setting is in negative correlation dependence with the average fruit weight (r = -1.000), but with a positive correlation coefficient between the indicators in the cultivar Elena (r = 0.764).

In contrast to biological fertilizing, in the conventional system in both cultivars a correlation was found between the stone weight and the average yield kg/tree (Tegera r = 0.866; Elena r = 0.714). The regression equations between the indicators are presented in Figure 2, as for Tegera it is y = 478.6 x - 827.56 at a coefficient of determination R^2 = 0.749 and Elena y = 15.38 x - 5.9254 at a coefficient of determination R^2 = 0.509.





Tegera cultivar Elena cultivar

Figure 2. Graphical model of regression dependence in Tegera and Elena cultivars between the average stone weight and the average yield kg/tree in the applied conventional cultivation system

CONCLUSIONS

Depending on the applied fertilizing systems (biological and conventional), genotypic similarity was found, but also a difference between the cultivars in some of the indicators.

In the biological fertilizing of Tegera, the trunk circumference correlates positively with the percentage of useful fruit-settings and the average fruit weight (r = 0.867).

The positive correlation was analyzed between the average fruit weight and the average yield kg/tree at a correlation coefficient (r = 1.000) and regression equation of y = 11.422 x - 341.62 with a coefficient of determination R^2 = 1.00.

In contrast to organic production, in conventional technology the trunk circumference of Tegera cultivar correlates strongly positively with the length of annual growth (r = 0.838) and total annual growth (r = 0.837).

In both cultivars, a correlation was found between the average stone weight and the average yield kg/tree (Tegera r = 0.866; Elena r = 0.714), with a coefficient of determination (Tegera R^2 = 0.750; Elena R^2 = 0.510).

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GENETIC RESOURCES OF SOUR AND BITTER CHERRY IN THE SPONTANEOUS AND CULTIVATED FLORA FROM NORTHEAST AREA OF ROMANIA

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Abstract

The aim of the paper is to describe the valuable traits of bitter and sour cherry genotypes selected from the spontaneous and cultivated flora (in the Moldova region), approved as new cultivars between 1990-2016. That improve the domestic assortment of sour and bitter cherry with new cultivars that shows resistance to disease and frost, have qualitative fruits, adapted to the particular conditions of the Northeastern region of Romania. In terms of the weight of the fruits (g) cultivars that were highlighted are the cherry cultivar 'Amaris' (5.5 g) with very significant positive differences and the sour cherry cultivar 'De Botoşani' (6.0 g) with significant positive differences in comparison with the control (4.2 g for bitter and 5.7 g for sour cherry). In terms of stone size, the values for the cherry cultivars were between 0.25-0.33 g, while the values for the sour cherry cultivars were between 0.30-0.34 g, being classified as small to middle size according to the UPOV guideline. The studied bitter and sour cherry cultivars present good resistance to fruit cracking with 0.0-3.7% cracked fruits.

Key words: *fruit, biometry, size, traits.*

INTRODUCTION

In the Moldova region (Northeast area of Romania), the cherry and sour cherry tree species have been cultivated since ancient times (Grădinariu & Istrate, 2003). Evidence of this fact is represented by numerous old local cultivars and populations as: 'Boambe de Cotnari', 'Vârtoase de Comarna', 'Crăieşti de Comarna' (for cherry) and 'Mocăneşti', 'Crişane' (for sour cherry) (Istrate, 2007; Sestraş, 2004; Budan and Grădinariu, 2000; Dumitrescu, 1981).

Considering the high interest for cherry and sour cherry crops expansion, many recent studies were initiated to identify and select biotypes and valuable clones (Radičević et al., 2012; Petre et al., 1994). The genotypes collected were adapted to the climate conditions specific to the area, that can fit efficiently with the modern crop technologies from all points of view and their fruits can fit to various ways of capitalization both in fresh or processing way (Petre et al., 2007).

The aim of this paper is to describe the valuable traits of bitter and sour cherry genotypes selected from the spontaneous and cultivated flora adapted to the specific conditions of the Northeast area of Romania.

MATERIALS AND METHODS

Aiming to highlight the biologic fund of genotypes that exist in the spontaneous and cultivated flora in the NE of Romania, positive selection tasks on valuable sour and bitter cherry biotypes were performed. For the selected genotypes, a minimum of 3 years of observations were recorded at their place of origin about particular agronomical traits of the trees and organoleptical traits of the fruits before they were grafted in the nursery and planted in trial field next to other local cultivars.

The studies were performed between 2013-2020, using three bitter cherry cultivars ('Amar Galata', 'Amar Maxut' and 'Amaris') and two sour cherry cultivars ('Timpuriu de Osoi' and 'De Botoşani') as research material.

The trees are found in experimental lots, grafted on mahaleb. The cherry tree gets planted at a distance of 5 × 4 m and the sour cherry tree at a distance of 3.5 × 4 m, with the crown guided as free flattened palmette on the direction of the row with trees, without a supporting or irrigation system. On the row of trees, the soil was prepared with the rotary orchard tiller and between the rows, the soil was grassed.

In the experimental plantation, observations and measurements have been performed regarding the trees' vigour, their resistance to frost and anthracnose (Cociu & Oprea, 1989) and the main growing and fructification phenophases (Meier, 2001) along with measuring the cultivars' self-fertility, according to the UPOV TG/35/7 questionnaire for cherry and TG/230/1 for sour cherry, measurements and determinations about the physical, chemical and quality traits of the fruits were also performed.

The productivity was determined based on the fruits yield (kg/tree) and fertility index, representing the percentage of fruits resulted 25-30 days after the petals' fall and the cultivars with values above 30-35% are considered highly productive (Cociu & Oprea, 1989).

The experimental data was statistically analysed by the variance test.

RESULTS AND DISCUSSIONS

In terms of growing vigour of the trees, the cherry cultivar 'Amaris' was classified to be of low vigour, 'Amar Maxut' and 'Amar Galata' were of average vigour, while the two sour cherry cultivars ('Timpuriu de Osoi' and 'De Botoșani') were of average vigour (Table 1). Low vigour of the tree is an important parameter for establish high density of the orchards, the new trends in the fruit tree growing being to increase constant the productivity (Stănică, 2019).

In terms of the studied cultivars' resistance to frost, during 2013 (when the cherry was out of dormant stage), 2017, 2019 and 2020, at the end of March to beginning of April when the cherry was in bloom, the recorded temperatures were between -2.5°C and -10.8°C. Under these conditions, the flower buds are more sensitive, getting the gynoecium damaged inside the flower bud and the recently fertilized ovary damaged in the opened flowers, compromising at the same time an important amount of the fruits production (these results are in accordance with other research in cherry cultivars (Asănică et al., 2014; Long, 2013; Rodrigo, 2000).

Table 1. Tree's features in cherry and sour cherry cultivars (FGRS Iasi, 2013-2020)

Cultivar	Tree's vigour	Resistance to: Frost			
		Frost	Anthracnose (*Coccomyces hiemalis* Higg.)		
		Damaged flower buds (%)	F[1] (%)	I[2] (%)	AD[3] (%)
Bitter cherry					
Amar Galata	average	2	3.3	8	0.26
Amar Maxut	average	3	2.9	11	0.32
Amaris	weak	8	2.7	13	0.35
Sour cherry					
Timpuriu de Osoi	average	0	0.0	0	0.0
De Botoșani	average	0	0.0	0	0.0

[1]- attack frequency; [2]- attack intensity mark on a scale of 1-6: 1 = 1-3% attacked surface; 2 = 4-10%; 3 = 11-25%; 4 = 26- 50%; 5 = 51-75%; 6 = 76-100%; AD[3]- attack degree (Cociu & Oprea, 1989).

In our study, the degree of damage of the flower buds in cherry tree varied in rather small limits, recording 2% in 'Amar Galata', 3% in 'Amar Maxut' and 8% in 'Amaris'.

The sour cherry cultivars had a very good resistance (Table 1). With regard to resistance to diseases, the years 2013, 2016, 2017 and 2018 were rainy years (with surplus of rainfall compared with normal period), favourable to pathogens evolution as *Monilia* sp. or anthracnose.

During these years, the bitter cherry cultivars manifested a slight sensitivity to anthracnose, the frequency of the attack being between 2.7-3.3%, while the sour cherry cultivars presented a very high tolerance (Table 1).

The flowering period for the two species (bitter and sour cherry) took place between the 3rd and the 30th of April (Table 2).

The values recorded for the natural fertility in the three cherry cultivars was between 35.1% (‘Amaris’) and 45.6% (‘Amar Galata’) and for the sour cherry cultivars, ‘Timpuriu de Osoi’ recorded 23.8% and ‘De Botoşani’ 41.5%, all of them being classified as highly productive cultivars, because their fertility index recorded values above 30%, except for the sour cherry cultivar ‘Timpuriu de Osoi’ (23.8%) (Table 2).

The results recorded for self-pollination throughout the eight years shows that the cherry cultivars are partially self-fertile, recording a low percentage of fruits (0.9-7.1%) and the sour cherry are self-sterile (table 2). For these cultivars, it is mandatory to plant them together with the suitable pollinators.

The harvesting maturity was recorded over the three decades of June both for the cherry and sour cherry cultivars. The number of days between the end of flowering to maturation was 42-68 days for cherry and 51-62 days for sour cherry (Table 2). The phenological periods for the same cherry genotypes are variable depending on the climatic conditions of each year (Darbyshire et al., 2012).

Table 2. The main fructification phases in bitter and sour cherry cultivars (FGRS Iasi, 2013-2020)

Cultivar	Beginning of flowering (BBCH 61)	End of flowering (BBCH 69)	Natural fertility (%)	Self-fertility (%)	Fruits’ maturation date (BBCH 87)	Period from end of flowering to maturation (days)
Limit dates (earliest - latest):						
Bitter cherry						
Amar Galata	05 - 25.04	11 - 30.04	45.6	7.1	17 - 24.06	55 - 68
Amar Maxut	05 - 25.04	12 - 29.04	36.7	0.9	05 -18.06	45 - 51
Amaris	03 - 19.04	09 - 27.04	35.1	3.7	27.05 - 07.06	42 - 49
Sour cherry						
Timpuriu de Osoi	04 - 22.04	13 – 28.04	23.8	0.0	02 -20.06	51 - 54
De Botoşani	08 – 25.04	14 – 30.04	41.5	0.0	11 - 30.06	59 - 62

The average yield (kg/tree), the physical, chemical and quality traits of the fruit are presented in tables 3 and 4. Analyzing the average yields over eight years, from a statistical point of view, it is found that the bitter cherry varieties Amar Galata (34.6 kg/tree), Amar Maxut (31.1 kg/tree) and sour cherry variety Timpuriu de Osoi (25.4 kg/tree) recorded a significantly positive difference in production compared to the average of the all studied varieties taken of control.

In terms of fruits’ weight (g) and equatorial diameter (mm), the ‘Amaris’ cherry cultivar got highlighted (5.5 g and 21.1 mm) with very positive significant differences and the ‘De Botoşani’ cultivar (6.0 g and 21.1 mm) with positive significant differences in comparison with the variants average (in cherry: 4.2 g and 18.9 mm; in sour cherry: 5.7 g and 21.8 mm) (Table 3).

With regards to the stone’s size, the values for the cherry cultivars were between 0.25-0.33 g and for the sour cherry cultivars were between 0.30-0.34 g, classifying them as small to middle size according to the UPOV questionnaire.

The fruit/stone ratio for the cherry cultivars was between 12.0 (‘Amar Maxut’) and 19.6 (‘Amaris’), recording distinctly negative significant differences in comparison to the control (14.4) and for the sour cherry cultivars, it was between 17.6 and 18.0 being non-significant in comparison to the control (17.8).

In cherry, the percentage of stone from the weight of the fruit recorded values between 5.09% (‘Amaris’) and 8.33% (‘Amar Maxut’) but in sour cherry, the values were between 5.55% (‘Timpuriu de Osoi’) and 5.66% (‘De Botoşani’). Statistically, the cherry cultivar ‘Amaris’ recorded negative significant differences in comparison with the control (6.90%), while ‘Amar Maxut’ and ‘Amar Galata’ recorded very significant and respectively distinctly positive significant differences. In sour cherry, the values were non-significant in comparison with the control (5.61%).

In terms of soluble dry substance, the values in cherry were between 17.5% for ‘Amar Galata’ and 20.1% for ‘Amar Maxut’ and the values in sour cherry were between 14% for ‘De Botoșani’ and 14.2% for ‘Timpuriu de Osoi’, the differences being non-significant in comparison with the variants average for both species (Table 3).

Table 3. The physical-chemical traits in cherry and sour cherry cultivars (RSFG Iași, 2013-2020 average; n = 8)

Species	Cultivar	Average fruit yield (kg / tree)	Fruit’s weight (g)	Stone’s weight (g)	Fruit/stone ratio	Stone in the fruit’s weight (%)	Equatorial diameter of the fruit (mm)	SDS[1] (%)
Bitter cherry	Amar Galata	34.6^{+++}	4.2	0.33^{++}	12.7^{00}	7.85^{++}	18.4	17.5
	Amar Maxut	31.1^{+++}	3.0^{000}	0.25^{00}	12.0^{00}	8.33^{+++}	17.3^{00}	20.1
	X (Control)	26.9	4.2	0.29	14.4	6.90	18.9	18.6
	Amaris	15.0^{000}	5.5^{+++}	0.28	19.6^{+++}	5.09^{000}	21.1^{+++}	18.3
	LD 5%	2.1	0.51	0.03	1.43	0.61	1.00	2.2
	LD 1%	2.9	0.71	0.04	1.98	0.85	1.38	3.0
	LD 0.1%	4.1	0.99	0.06	2.75	1.18	1.92	4.2
Sour cherry	Timpuriu de Osoi	25.4^{+}	5.4^{0}	0.30	18.0	5.55	21.1	14.2
	X (control)	23.0	5.7	0.32	17.8	5.61	21.8	14.1
	De Botoșani	20.6^{0}	6.0^{+}	0.34	17.6	5.66	22.6+	14.0
	LD 5%	2.1	0.28	0.05	2.0	0.74	0.8	1.6
	LD 1%	3.1	0.41	0.07	2.6	1.10	1.2	2.4
	LD 0.1%	4.7	0.64	0.11	4.7	1.71	1.8	3.7

[1]SDS - soluble dry solids; [2]Control = the average of variants.

The fruits’ colour in cherry was from yellow-reddish (‘Amar Galata’), dark red (‘Amaris’) to black (‘Amar Maxut’) (Figures 1-3) and in sour cherry, both cultivars taken into study were dark red (Table 4; Figures 4-5).

Table 4. Fruits’ physical and quality traits in cherry and sour cherry cultivars (RSFG Iasi, 2013-2020)

Cultivar	Epidermis colour	Pulp firmness	Fruit shape	Stone adherence to pulp	Cracked fruits (%)	Fruit’s destination
Bitter cherry						
Amar Galata	yellow-reddish	semi-firm	heart-shaped	semi-adherent	3.7	jam
Amar Maxut	black	average	kidney-shaped	semi-adherent	0.4	jam, liqueur
Amaris	dark red	average	heart-shaped	non-adherent	0.2	jam
Sour cherry						
Timpuriu de Osoi	dark red	average	flattened sphere	semi-adherent	0.2	jam, compote
De Botoșani	dark red	average	flattened sphere	semi-adherent	0.0	jam, compote

Figure 1. ‘Amaris’ bitter cherry cultivar (original)

Figure 2. ‘Amar Galata’ bitter cherry cultivar (original)

Figure 3. 'Amar Maxut' bitter cherry cultivar (origina

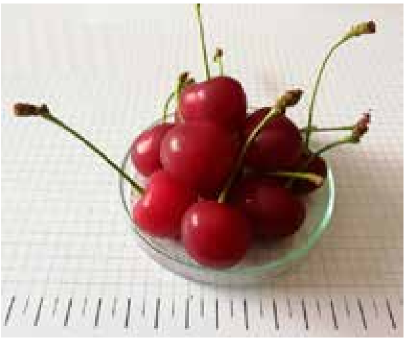

Figure 4. 'De Botoșani' sour cherry cultivar (original)

Figure 5. 'Timpuriu de Osoi' sour cherry cultivar (original)

In terms of stone's adherence to pulp and its firmness, both the cherry and sour cherry cultivars have semi-firm pulp and are semi-adherent, except for the 'Amari's genotype for which the stone does not have any adherence to the pulp.

The shape of the fruit was heart-shaped for 'Amar Galata' and 'Amaris', kidney-shaped for 'Amar Maxut' and flattened sphere for the two sour cherry cultivars.

The studied bitter and sour cherry genotypes present very good resistance to the phenomenon of fruit cracking, the values being between 0.0 - 3.7% cracked fruits (table 4).

The fruits of the sour and bitter cherry genotypes are targeted only for processing.

CONCLUSIONS

'Amaris' and 'Timpuriu de Osoi' are harvested in early season and 'Amar Galata' has a late harvest season being varieties with high economic potential.

The studied varieties were highlighted by the tree's low vigour, good resistance to frost and fruit cracking, quality of the fruits targeted for processing as jams, compote or liqueurs.

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RESEARCH ON EFFECTIVENESS OF SULPHUR BASED – ON FUNGICIDES TREATMENTS AGAINST *PODOSPHAERA LEUCOTRICHA* IN APPLE IN VOINESTI AREA, DAMBOVITA COUNTY

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Abstract

Apple powdery mildew caused by Podosphaera leucotricha is one of the most common and severe diseases of this plant in all production areas. In years under favorable conditions for the disease, especially for susceptible varieties, it causes significant damage to apple orchards of up to 80% of the harvest. The research was carried out between 2019 and 2021 within the Research and Development Station for Fuit Growing Voinești and aimed at the effect of Polisulf product type MIF and Sulfomat 80 PU on the attack of the monitored pathogen on Jonathan and Golden Delicious varieties. The highest degree of attack was calculated for the Jonathan variety of 60.8% in 2021. The effectiveness of the treatments was 80.5% - 84.8% for the Jonathan variety and 88.2% - 96.4% for the Golden Delicious variety. Sulfur-based products have provided good protection against powdery mildew and are recommended in integrated apple protection systems.

***Key words**: apple orchard, degree of attack, effectiveness, powdery mildew, sulphur based - on product.*

INTRODUCTION

Caring for the trees, making them produce more, better, with tastier fruits, has preoccupied man since ancient times and will continue to be an everlasting activity. Fruits are essential in human nutrition because they are an indispensable source of vitamins and energy.

Apple growing has occurred since oldest times and apple has become one of the most important fruits that are fit for human consumption. Anatolia, Caucasia, Turkistan, and Europe were centres of origin for the domestic apple (*Malus domestica* Borkh.), now grown in continental climates in the Northern and Southern Hemispheres (Pirlak et al., 2003). Apples are among the world`s fruits crops, figuring in both the Bible and the tales of Homer (Jackson, 2003). They are considered to be one of the oldest fruit species and ancient Roman and Greek historians have offered extensive descriptions (Jaloba et al., 2019).

The apple is very well adapted to the temperate climate whose extremes endures much better than other fruit tree species (Hoza, 2000). Our country has favorable climatic and pedological conditions for a large number of fruit species such as stone fruits, pome fruits, fruit bushes, which are found from the sea to the mountains. Within these, apple has a great ecological resilience in Romania where it could find very favourable pedo - climatic conditions to obtain good quality yields (Ghena & Branişte, 2003).

By the other hand, in the apple growing areas, a significant number of pathogens may cause large qualitative and quantitative losses in apple orchards and warehouses. Under the pedoclimatic conditions of the Voinești orchard traditionally consacrated to apples cultivation since ancient times, there is a high biological reserve of diseases and pests, a high risk of infection, which allows in favorable years the successive or simultaneous attack of specific or non-specific pathogens and pests, which can cause very serious damage.

In the Voinești area, the most harmful are scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*), which makes it mandatory to apply phytosanitary treatments.

A strong infection of powdery mildew, especially on susceptible varieties, can defoliate the tree and affect the growing shoots with serious consequences in the following year by weakening the trees. Briefly, the apple requires a large number of phytosanitary treatments that make fruit growers to allocate about 40-50% of total production costs (Petre et al., 2006).

Its importance emerges from the fact that it can start very early, from the bud burst and can manifest throughout the growing season (Gheorghieș & Geamăn, 2003; Dulugeac, 2011). The attack can be manifested on leaves, shoots, flowers and less often on fruits. The young leaves are most susceptible to attack.

A fine mycelium is noted on the shoots grown from infected buds (Gheorghieş & Geamăn, 2003), which shortly can reach the entire shoot, but also the leaves and flowers.

Severely infected shoots have shortened internodes and are covered with a silver-gray mycelium. White-gray mycelial felt turns brown, numerous dark brown fruits appear - chasmothecia (Hickey & Yoder, 1990; Gheorghieș & Geamăn 2003). From shoots with primary infection, the fungus spreads to other organs, causing secondary infections.

On the leaf surface, symptoms of powdery mildew appear as irregular chlorosis of gray-white with white powder and also may include whitish lesions on curled or longitudinally folded leaves, stunted whitish gray twig growth and fruit russeting. Due to attack, the leaves do not fully open, remain small and thicken, curled and distorted and twist to the top, taking on the appearance of a "little boat" (Paraschivu, 2010).

Most economic damages occur in the form of aborted blossoms, reduced fruit finish quality, reduced vigor and yield of the bearing trees and stunting and poor form of young, nonbearing trees. Powdery mildew can cause considerable loss due to nutrient removal, reduced photosynthesis, increased respiration and transpiration, and impaired growth (Retrieved from:https://www.saillog.co/PowderyMildewOfApples.html).

P. leucotricha may overwinter as chasmothecia (cleistothecia) but is tipically observed as mycelium developing from within buds produced by the apple host during the previous growing season (Strickland et al, 2021). Perennating in buds, it makes it difficult to control and, although it can cause fruit russet, it primarily causes losses due to its chronic effect on tree vigor and yield (Biggs et al., 2009). Infection and the evolution of the attack are favored by temperatures of 18-22°C and 80% humidity. Powdery mildew can spread to lower humidity (70%), for infection being sufficient moisture formed by perspiration, on the surface of green organs (Gheorghieș & Geamăn, 2003).

Elemental sulphur is known, undeniably, the oldest pesticide ever to control powdery mildew. Ancient Greeks were aware of its pesticide properties as early as 1000 B.C. In the scientific published literature, Forsyth (1802) cited by Tweedy (1969) was the first to suggest the application of sulphur for disease control. Unfortunately, the fungicidal properties of sulphur were apparently forgotten during the middle age and were not rediscovered until the beginning of the nineteenth century. Ever after, sulphur has been used in various forms for disease and pest control, especially against powdery mildew and mites on plant (Jalobă & Grădilă, 2019). On the other hand, sulphur is an element essential for plant growth and development. It is the building block for the synthesis of amino acids (cysteine, methionine), proteins, coenzymes, sulpholipids and polysaccharides (Bělíková et al., 2019). It is generally accepted that the efficacy of sulphur for powdery mildew control is related to contact and vapour activity. Contact activity appears to have a minor role in the efficacy of sulphur products. Vapour activity has a major role but is temperature dependent. Below 15ºC, sulphur activity is confined to contact activity because vapour activity is negligible (Retrieved from https://www.saillog.co/PowderyMildew OfApples.html). The aim of this paper was to monitor the behaviour towards the powdery mildew of two apple varieties and the effectiveness of the sulphur based – on fungicides treatments applied against the monitored pathogen.

MATERIALS AND METHODS

A three years trial (2019-2021) was established on April 2019 at the Research and Development Station for Fruit Growing Voineşti (Middle North Muntenia, Romania).

The climatic conditions of the area are favorable for apple growing, with an average annual temperature of 8.8°C and an average annual rainfall exceeding 750 mm. The typical soil in the area is brown-eumezobasic, weakly pseudogleized, loamy texture, weakly acidic pH (5.6-6). The humus content is medium at the surface: 2.15-3.25. Jonathan and Golden Delicious were the two classic varieties tested, within a 18-year-old orchard. The planting distance for Jonathan and Golden Delicious varieties is 4 meters between rows and 3 meters between trees within a row. (833 trees/ha) The shape of the crown is palmette with oblique arms. Trees in the orchard were on M9 rootstock grafted. During the period of vegetative rest, autumn tillage was carried out by ploughing on the intervals between rows. Pruning works for growth and bearing control were carried out early spring, before the growing season began. The trial was conducted using the randomized complete block method in four repetitions which included four untreated plots. The application equipment was a mist blower Grùnman 3 WF-3 knapsack mist blower, suitable for fungicide treatments in orchards. 100 leaves per tree were assessed before each spray and after ten days until the latest treatment by visually rating. Assessments were made on the frequency (F, %, or Pest Incidence) and the intensity (I, % or Pest Severity) of pathogen attack and the degree of attack (DA, %) and efficacy (E, %) were calculated. The degree of attack was calculated using the formula: F% x I%/100. The efficacy of fungicides was calculated according to Abbott`s formula: (degree of attack in untreated control - degree of attack in treated plot)/ degree of attack in untreated control x 100. All data were subjected to statistical analysis provided by ARM-8 sofware (Jalobă et al., 2021). At harvest time, the yield/tree was also recorded by weighing all the fruits per tree.

Three treatments with Polisulf and Kumulus DF and five treatments with Sulfomat 80 PU and Kumulus DF were proceeded in the experimental orchard. The tested products are shown in the Table 1. Treatments were scheduled at the following stages:

Polisulf and Kumulus DF:
- 1st treatment (A): mouse ear (BBCH 10);
- 2nd treatment (B): shoots about 40% of final length (BBCH 34);
- 3rd treatment (C): at the end of flowering (BBCH 69).

Table 1. Fungicides used to control powdery mildew in the trials

Product	Active ingredient	Rate (kg, l/ha)	Water volume (l)
Polisulf	thiosulphuric sulphur 3% + polisulfhydric sulphur 12%	30	1500
Sulfomat 80 PU	sulphur 800 g/kg	4.5	1500
Kumulus DF	sulphur 800 g/kg	4.5	1500

Sulfomat 80 PU and Kumulus DF:
- 1st treatment (A): mouse ear (BBCH 10);
- 2nd treatment (B): shoots about 40% of final length (BBCH 34);
- 3rd treatment (C): red bud stage (BBCH 57);
- 4th treatment (D): end of flowering (BBCH 69);
- 5th treatment (E): second fruit fall (BBCH 73).

Polisulf is a fungicide that acts directly by the decomposition of elemental sulphur as a protective fungicide. Thus, sulphur interrupts the transfer of electrons, causing sulphur reduction in hydrogen sulphide (H_2S). Sulphur also forms a protective layer, which inhibits germination of spores.

Sulfomat 80 PU is a dust free, flowable micronised sulphur granules, containing 80% sulphur as active ingredient and balance adjuvants, easy of measuring and handling. It has a lot of advantages as follow: it has instant dispersion and high suspensability in water, therefore it don't cause scorching; it has triple action as fungicide, micronutrient (Sulphur) and miticide and it has sustained action for longer effect; there are no stains on fruits and leaves after spraying, nor do leaves get burnt.

RESULTS AND DISCUSSIONS

While the Jonathan variety are known to be very sensitive to powdery mildew and Golden Delicious is supposed to be middle sensitive, the applied treatments considerably reduced the attack of *Podosphaera leucotricha* in the treated plots (Table 2). Under the weather conditions of Voinești area, the pathogen attack was noted every year, being detecteted even with the begining of the spring. Assessments made on the Pest Incidence (Pesinc %) and Pest Severity (Pessev %) of the pathogen attack were recorded in the database Tables 2, 3, 6 and 7. As a result, the degree of attack (DA%) was calculated for each of the four repetitions in untreated checks, non-polluting products and standard product applied, for both Jonathan variety and Golden Delicious variety. Also, photographs and samples were taken to be studied.

Table 2. Development of *P. leucotricha* in Polisulf trial (Jonathan variety)

Assessment time	Assessment	2019			2020			2021		
		Untreated	Polisulf	Kumulus	Untreated	Polisulf	Kumulus	Untreated	Polisulf	Kumulus
0 DA-A (0 day after first treatment)	Pesinc % Pessev %	34 25	34 25	34 25	7.5 15	7.5 15	7.5 15	22 20	22 20	22 20
10 DA-A (10 days after treatment A)	Pesinc % Pessev %	41 30	32 25	31.4 25	17.5 20	12.5 15	12 15	36 25	18.7 15	22.5 14
10 DA-B (10 days after treatment B)	Pesinc % Pessev %	65.5 40	40 25	35 25	35 25	18 15	16.5 15	45 35	25.5 15	27 15
10 DA-C (10 days after treatment C)	Pesinc % Pessev %	76 50	37 20	32.5 20	55 35	24 15	19.7 15	64 40	26 15	28 16

Polisulf trial

Infections on leaves appeared first on the lower surface. These lesions may spread to the upper surface and cover the entire leaf with a white spores and mycelium. Infections noted along the leaf margin often resulted in leaf curling and crinkling.

The degree of attack (DA%) evolution for each study is shown in Figure 1 and Figure 2. As it can be seen, the degree of attack at untreated check has had an ascending development and has increased with each assessment of untreated controls due to weather conditions and lack of phytosanitary treatments.

Table 3. Development of *P. leucotricha* in Polisulf trial (Golden Delicious variety)

Assessment time	Assessment	2019			2020			2021		
		Untreated	Polisulf	Kumulus	Untreated	Polisulf	Kumulus	Untreated	Polisulf	Kumulus
0 DA-A (0 day after first treatment)	Pesinc % Pessev %	16 10	16 10	16 10	12 10	12 10	12 10	11 15	11 15	11 15
10 DA-A (10 days after treatment A)	Pesinc % Pessev %	26 20	25 15	24 15	18 15	13 10	12 10	30 25	25 15	25.5 12.25
10 DA-B (10 days after treatment B)	Pesinc % Pessev %	33 30	25 12.25	23 12.5	24 30	18 10.25	20 10	32 30	22 10	27 10
10 DA-C (10 days after treatment C)	Pesinc % Pessev %	36 40	11 12.5	8 10	32 35	8.5 7.5	14 7.25	40 35	5 10	9 10

As it can be seen in Figures 1 and 2, at the experiment with Polisulf, the degree of attack ranged from 8.5% to 38% (in 2019), from 1.13% to 19.25% (in 2020) and from 4.4% to 25.6% (in 2021) for the Jonathan variety. In the Golden delicious variety, DA% evolved from 1.6% to 14.4% (in 2019), from 1.2% to 11.2% (in 2020) and from 1.65% to 14% (in 2021).

Regarding the samples treated with fungicides against powdery mildew, in Figures 1 and 2 are shown the values between which the degrees of attack ranged in this trial where the non-polluting product Polisulf and the standard product Kumulus DF were applied. Thus, for the Jonathan variety, the samples where the product Polisulf type MIF was applied registered a degree of attack registered between 1.88% (in 2020, 10 days after treatment A) and 9.85% (in 2019, at 10 days after treatment B). In the Golden Delicious variety, Polisuf type MIF proved to be even more effective, registering a lower degree of attack, risen from between 1.3% (in 2020, 10 days after treatment A) and 3.75% (in 2021, 10 days after treatment A). These results were almost similar to those of the standard product Kumulus DF in efficacy and there were no statistically significant differences between the two fungicides.

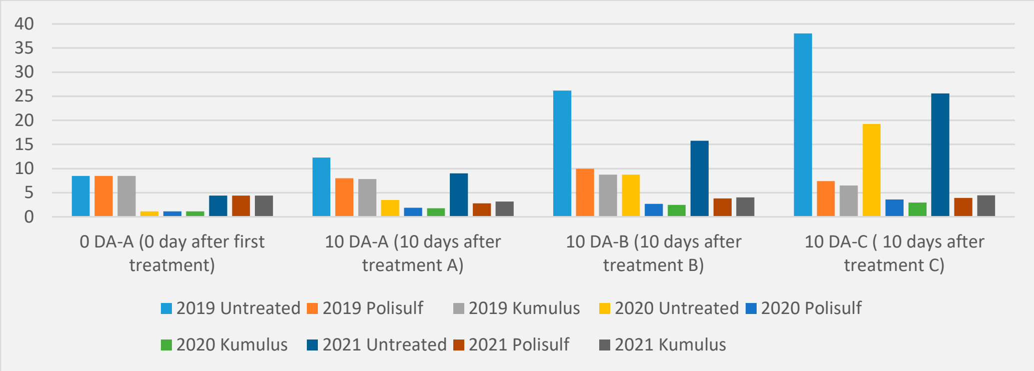

Figure 1. Degree of attack in Polisulf trial (Jonathan variety)

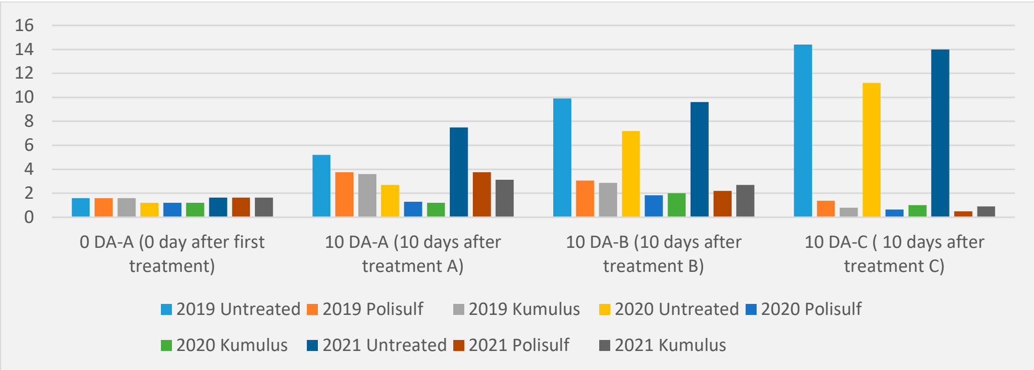

Figure 2. Degree of attack in Polisulf trial (Golden Delicious variety)

Under such conditions, the non-polluting product applied performed very well in both apple varieties compared to that of the standard product, their effectiveness being shown in Tables 4 and 5. As one can be seen, the product was effective in those three years of experimentation, the efficacy after the last treatment ranging between 80.6% and 84.8% for the Jonathan variety and between 90.5% and 96.4% for the Golden delicious variety.

Table 4. Efficacy% of products applied to control powdery mildew in the Jonathan variety

Year	2019		2020		2021	
Assessment time	Polisulf	Kumulus DF	Polisulf	Kumulus DF	Polisulf	Kumulus DF
10 days after A	35.4	36.3	46.4	49.1	68.8	65.3
10 days after B	62.4	66.8	69.2	71.8	75.8	74.2
10 days after C	80.6	82.8	81.4	84.7	84.8	82.9

Table 5. Efficacy% of products applied to control powdery mildew in the Golden Delicious variety

Year	2019		2020		2021	
Assessment time	Polisulf	Kumulus DF	Polisulf	Kumulus DF	Polisulf	Kumulus DF
10 days after A	28.4	31.6	52.4	55.5	50.00	58.5
10 days after B	68.6	70.8	74.5	72.2	77.4	72.2
10 days after C	90.5	94.5	94.3	90.9	96.4	93.5

Sulfomat 80 PU trial

Simultaneously with the testing of the Polisulf, the effectiveness of the Sulfomat 80 PU product in controlling powdery mildew in both varieties was also monitored.

Table 6. Development of *P. leucotricha* in Sulfomat 80 PU trial (Jonathan variety)

Assessment time	Assessment	2019			2020			2021		
		Untreated	Sulfomat	Kumulus	Untreated	Sulfomat	Kumulus	Untreated	Sulfomat	Kumulus
0 DA-A (0 day after first treatment)	Pesinc % Pessev %	35.0 20.0	35 20	35 20	10 15	10 15	10 15	20 25	20 25	20 25
10 DA-A (10 days after treatment A)	Pesinc % Pessev %	40.0 25.0	24.5 20.25	28 17.5	20 25	16.75 15	16 15	32 25	25.25 15	17.75 20
10 DA-B (10 days after treatment B)	Pesinc % Pessev %	62.5 40.0	49 20	48.5 20	38.0 25.0	19.25 17.5	24.25 13	48 30	29.5 20	29.5 20
10 DA-C (10 days after treatment C)	Pesinc % Pessev %	65.0 45.0	42 20	43 20	44.0 30.0	24.25 15	22.5 15	50 40	31 20	29.25 20
10 DA-D (10 days after treatment D)	Pesinc % Pessev %	72.0 60.0	47.5 20	42.5 20	65.0 50.0	33.75 20	32 20	74 60	43.5 25	43 20
10 DA-E (10 days after treatment E)	Pesinc % Pessev %	84 70	48 22.5	44 22	72.0 60.0	38 20	36.5 17.5	76.0 80.0	47.5 25	48.5 20

In the experiment where Sulfomat 80 PU and Kumulus DF were applied, the symptoms on the leaves were identical to those of Polisulf trial. First, in the primary infection, the leaves were colonized as they emerged from the buds, while in the secondary infections windborne spores landed on young leaves as they unfurled and expanded. Fungal colonies composed of mycelium and spores appeared as white, felt-like patches. As it was expected, secondary infections appeared first on the lower leaves surface, and was detectable on the upper leaf surface as chlorotic spots.

Table 7. Development of *P. leucotricha* in Sulfomat 80 PU trial (Golden Delicious variety)

Assessment time	Assessment	2019			2020			2021		
		Untreated	Sulfomat	Kumulus	Untreated	Sulfomat	Kumulus	Untreated	Sulfomat	Kumulus
0 DA-A (0 day after first treatment)	Pesinc % Pessev %	12 10	12 10	12 10	8 10	8 10	8 10	14 15	14 15	15 15
10 DA-A (10 days after treatment A)	Pesinc % Pessev %	28 20	8 5	7 5	16 15	10.6 7.5	7.2 10	30 20	15.3 15	14.5 15
10 DA-B (10 days after treatment B)	Pesinc % Pessev %	34 25	18 7.5	16 7.5	22 20	10.8 10	9.9 10	28 30	15.4 17.5	19.25 15
10 DA-C (10 days after treatment C)	Pesinc % Pessev %	38 40	30.5 10	28.1 10	30 35	12.6 10	10 10	36 40	19 18	18.5 17.5
10 DA-D (10 days after treatment D)	Pesinc % Pessev %	45 50	27 12.5	30.4 10	34 40	14.6 7	12.1 7.5	46 45	20.6 20	18.1 20
10 DA-E (10 days after treatment E)	Pesinc % Pessev %	48 50	22.6 10	20.4 10	38 40	13 8.5	10.5 7.5	52 45	18.4 15	13 15

In the Sulfomat experiment, the assessments were staggered over a longer period of time due to the higher number of treatments (up to the second fall of the fruit), so that the degree of attack after the last treatment on the untreated control was higher than that recorded in the experiment with Polisulf, implicitly the incidence and severity recorded in Tables 6 and 7. Thus, the highest degree of attack reached 60.88% in 2021. As we can see, the degree of attack evolved from 7.0% to 58.8% (in 2019), from 1.5% to 43.2% (in 2020) and from 5.0% to 60.8% (in 2021) for the Ionathan variety (Figure 3). In the Golden delicious variety trial, the degree of attack increased from 1.20% to 24.0% (in 2019), from 0.8% to 15.2% (in 2020) and in 2021 from 2.1% to 23.40% (Figure 4). Under this infection pressure, the product Sulfomat 80 PU proved to be very effective, comparable to the standard product Kumulus DF. Analyzing the results obtained after the last treatment, efficacy ranged from 80.5% to 82.4% for the Ionathan variety and from 88.2% to 92.7% for the Golden delicious variety, quite similar to standard product Kumulus DF (Tables 8 and 9). Such good results were also obtained by Mitre et al., 2018, using wettable sulphur to control powdery mildew in Cluj Napoca, Romania.

Other researches (Cristea et al., 2017; Buzatu et al., 2018; Alexandru et al., 2019; Chitulescu et al., 2019; Chiriac et al., 2021; Mandru et al., 2021) also obtained favourable results testing unpolluted products which are suitable to be used in ecological production.

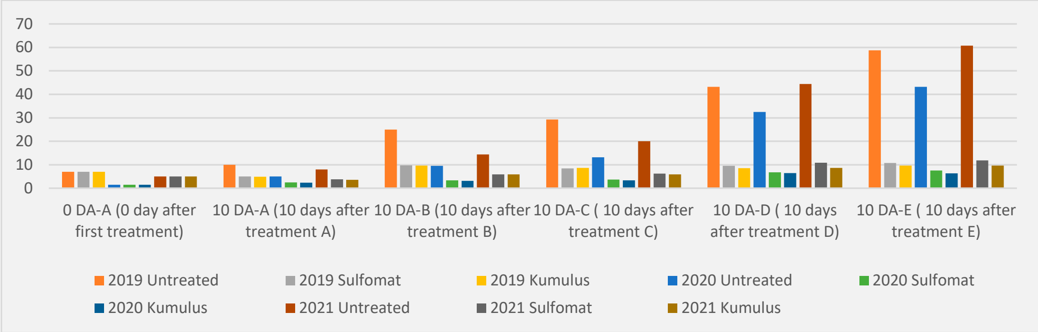

Figure 3. Degree of attack in Sulfomat trial (Jonathan variety)

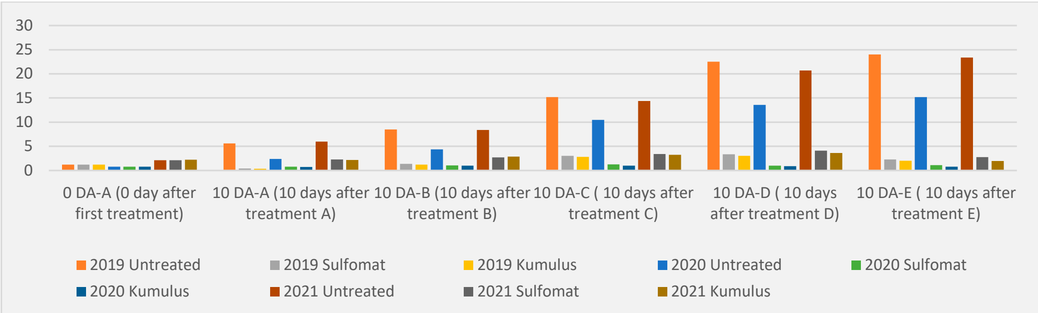

Figure 4. Degree of attack in Sulfomat trial (Golden Delicious variety)

Table 8. Efficacy% of products applied to control powdery mildew in the Jonathan variety

Year	2019		2020		2021	
Assessment time	Sulfomat 80 PU	Kumulus DF	Sulfomat 80 PU	Kumulus DF	Sulfomat 80 PU	Kumulus DF
10 days after A	50.4	51.2	49.8	52.3	52.7	55.6
10 days after B	60.8	61.3	64.6	66.8	59.4	59.1
10 days after C	71.2	70.6	72.4	74.5	69.1	70.7
10 days after D	78.1	80.3	79.1	80.3	75.5	80.6
10 days after E	81.6	83.5	82.4	85.2	80.5	84.1

Table 9. Efficacy% of products applied to control powdery mildew in the Golden Delicious variety

Year	2019		2020		2021	
Assessment time	Sulfomat 80 PU	Kumulus DF	Sulfomat 80 PU	Kumulus DF	Sulfomat 80 PU	Kumulus DF
10 days after A	66.4	69.8	68.2	70.1	61.9	63.9
10 days after B	75.7	78.2	75.4	77.6	67.8	65.6
10 days after C	79.9	81.5	88.0	90.5	76.3	77.4
10 days after D	84.9	86.5	92.5	93.3	80.1	82.5
10 days after E	90.6	91.5	92.7	94.8	88.2	91.7

CONCLUSIONS

Sulphur is a low cost, multi-site fungicide that is widely used to control powdery mildew all over the world. Good coverage of sulphur sprays on apple trees foliage is required for effective powdery mildew as it was proved in this paper.

Spray programs in which sulphur - based on products are included continously are also important to prevent development of resistance to newer fungicides and guarantee an optimum control of powdery mildew. They are mandatory to be included in each management control program of apple diseases.

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PRELIMINARY RESULTS ON EARLY CROP LOAD AND GROWTH RESPONSES OF 'LAPINS' SWEET CHERRY CULTIVAR (*PRUNUS AVIUM* L.) GRAFTED ON 'GISELA 5' AND 'GISELA 6' ROOTSTOCKS IN A DRIP IRRIGATED FIELD TRIAL

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Abstract

The growth responses of 'Lapins' sweet cherry cultivar (Prunus avium L.) grafted on 'Gisela 5' and 'Gisela 6' rootstocks was evaluated in drip irrigation conditions in Bistrita Fruit Region, in Northern Transylvania Romania, during 2020-2021, in a high density field trial, trees were trained as spindle bush with 1250 trees/ha density. During the study the following parameters were evaluated, trunk cross section area, volume of the tree crown, leaf area, length of shoots, number of shoots, height of trees, leaf area/fruit, crop load, yield, fruit number/tree, average fruit size and main quality characteristics of the fruits. Preliminary data showed that trees grafted on 'Gisela 6' when compared with 'Gisela 5' proved to be more vigorous when considering trunk cross sectional area and canopy volume indicators. Data showed that generally in both rootstock combinations a high fruit number per tree was observed at 'Lapins' cultivar and fruit size was largely a function of crop load and leaf area: fruit ratio. In order to obtain a good fruit size most probably, crop load of self fertile 'Lapins' cherries on Gisela rootstocks must be regulated by different pruning treatments. Drip irrigation had a crucial influence on the vegetative development of the trees and completed the physiological need of plants mainly in critical summer drought periods.

Key words: *crop, cherry, rootstocks, cultivar, crop load, fruits.*

INTRODUCTION

Sweet cherry cultivation worldwide is focusing lately on using special training systems and specific rootstock combinations which effectively control tree size and maximizes yield. Among several rootstocks available in modern fruit growing in many countries Gisela rootstock is used and from thousands of *Prunus cerasus* and *Prunus canascens* selections, `Gisela 5` and `Gisela 6` and others were patented. After many years of testing, worldwide it was proved that induces tree size control, precocious bearing and it is very productive (Franken Bambenek, 1998). In order to remain economically viable, beside complex orchard management techniques of fertigation (Neilsen, 2004,2010) and other techniques in the fruit growing world the reduction of tree size is crucial, it is highly needed to achieve higher yields, increased labor efficiency and productivity (Lang, 2000; Webster, 1995; A.S.A. Santos et al., 2007; A. Kankaya et al., 2008, Robinson, 2008). Rootstocks influences not just growth characteristics but also fruit quality (Valter Martins, 2021). `Gisela 5` performed well worldwide in different soil and climate conditions producing trees about 50 % weaker than *Prunus avium* and in Germany half of the newly established intensive orchards are planted on `Gisela 5` with different grafted cultivars. `Gisela 5` also needs good soil and site conditions and recent results showed that sweet cherry in dry seasons necessitates irrigation system (Blaya Ros et al., 2021; Victor Blanco, 2020) to complete the physiological processes of trees. `Gisela 6` rootstock is a more vigorous rootstock than the previous rootstock mentioned earlier, being between *Prunus avium* and `Gisella 5` regarding the tree vigor. Researchers from Consortium Deutschen Baumschulen state that trees grafted on `Gisela 6` crop early and as rootstock is less demanding on soil quality and site conditions and could work without irrigation system. Worth mentioning is that `Gisela 6` is intensely used in the USA as a new size controlling rootstock. The objective of the present research was to study the early bearing capacity of `Lapins` cultivar grafted on `Gisela 5` and `Gisela 6` rootstocks and the vegetative growth and development of trees in drip irrigation conditions in Bistrita Fruit Region, Northern Transylvania, Romania.

MATERIALS AND METHODS

The study was carried out during 2020-2021 in a 3 years old, drip irrigated sweet cherry experimental orchard (*Prunus avium* L.) planted with 'Lapins' cultivar grafted on 'Gisela 5' and 'Gisela 6' rootstocks (knip trees), in a high density plot, planted at 4 x 2m distance, the adopted training system was the spindle bush. The research plot is located in Bistrita, Northern Transylvania, Romania. The soil is a deep eutricambosoil with medium NPK and organic matter content. During the study standard orchard management techniques were applied regarding weed management with herbicide sprays and appropriate fertigation (Solfert, macroelements 20% N; 20% P; 20% K and microelements B (0.01%); Cu-EDTA (0.0055%); Fe-EDTA (0.05%); Mn-EDTA (0.025%), Mo-EDTA (0.035%); Zn-EDTA (0.0075%) and phytosanitary treatments. Biometrical measurements were made on: the height of tree, trunk cross-sectional area 15 cm above grafting point, volume of trees, length of shoot growth, and there were calculated the yield per tree (kg/tree). Fruit maturation occurred between 13-17 th July 2020 and harvest day in 21.07.2020. In the following year in 2021 fruit maturation occurred a week earlier between 05-10 th July 2021 and harvest day in 13.07.2021. The measurements were done in September at the final of vegetation period, on 14 trees of the `Lapins` cultivar grafted on the two different rootstocks, in the 2th year after planting. The trees were subjected to two different irrigation treatments (T1- standard irrigation, T2- standard+25 %) with different amounts of water in 2020 (T1-209 mm;T2-261mm) and in 2021 (T1-164 mm;T2-205mm) based on adjusted coefficients of Class A pan evapotraspiration. Drippers 2 L h^{-1} flow rate, were placed in line, 1 m apart.

The trunk diameter was measured with a digital caliper and the height of trees and length of the branches was measured using a tape measure. The pruning was at minimum level applied, since the experimental orchard is very young, mainly the too developed branches were thinned and some other branches stub cut to equilibrate the growing and fruiting processes. Canopy volume calculations were effectuated with specific software based on calibrated digital images taken from the cherry trees. The software called Tree analyser, version 1.20b was elaborated by researchers from Institute Nationale de la Recherche Agronomique, France. The software calculates the geometrical parameters of tree crown starting with black& white photographs executed from different angles but precisely determined toward cardinal angles. For every tree it was needed 6 successive photographs around the tree. Photographs were taken on digital cameras placed on tripod at 1 m height and from 4 m distance toward the tree, with a vertical angle of 10°. The background of trees was shielded with a white panel in order to produce black& white photos. The color photos with a resolution of 1600 x 1200 were transformed in black & white photos and loaded in the Tree Analyser software. The statistical analyses were performed using XLSTAT software version 2018.1 (Addinsoft, Paris, France).

RESULTS AND DISCUSSIONS

Drip irrigation (Figure 1) generally had a great impact on the development of trees in both rootstock combinations affecting both vegetative and generative physiological processes. Especially the increased T2 irrigation (standard + 25%) had a distinguished effect on the development of main stem, canopy, shoot number, shoot length.

Figure 1. Irrigation coordination center

Research results showed that the highest vegetative development (Figure 2) was recorded at `Lapins` cultivar grafted on `Gisela 6` in T2 irrigation condition in both the experimental years when analysing the trunk cross sectional area parameter (Table 1). Data showed in T2 irrigation condition a TCSA value of 8.48 cm^2 in 2020 and 17.50 cm^2 in 2021. In 2020 the other research variants at `Gisela 5` combinations had close values being in the same statistical class.

Figure 2. Canopy measurement of 'Lapins' sweet cherry cultivar grafted on 'Gisela 5' rootstock

In 2021 after the best recorded TCSA value of `Lapins`/`Gisela 6`- T2-combination (17.50 cm2) followed the normal irrigation variant T1 (15.04 cm^2) at the same `Gisela 6` rootstock combination. The other variants grafted on `Gisela 5` had lower TCSA values.

Figure 3. Measurement of TCSA at 'Lapins' sweet cherry cultivar

It seems that the `Gisela 6` rootstock induced higher vigour (Figure 3) and the root system had the possibility to use the applied irrigation water more efficiently in terms of TCSA development (Table 1).

Table 1. Measurement data on TCSA at 'Lapins' sweet cherry cv grafted on 'Gisela 5' and '6'

Treatment variant	TCSA (cm^2)	
	2020	2021
T1-Lapins /Gisela 5	3.39 **b**	11.52 **b**
T2-Lapins /Gisela 5	5.43 **b**	11.58 **b**
T1-Lapins /Gisela 6	5.76 **b**	15.04 **ab**
T2-Lapins /Gisela 6	8.48 **a**	17.50 **a**

Regarding the number of shoots (Table 2), results showed that `Gisela 6` rootstock in both in 2020 induced more shoot growth than `Gisela 5` (average values of 6.28-10.28).

Figure 4. Shoot growth measurement of 'Lapins' sweet cherry cultivar grafted on 'Gisela 6' rootstock

In 2021 interestingly the increased irrigation level (normal T1 + 25 %) influenced also in `Gisela 5` a higher number of shoots, thus the two irrigation levels induced in both rootstock combinations a good vegetative growth (Fig 4).

Table 2. Measurement data on number of shoots at 'Lapins' sweet cherry cv grafted on 'Gisela 5' and '6'

Treatment variant	Number of shoots	
	2020	2021
T1-Lapins /Gisela 5	5.16 **b**	8.16 **c**
T2-Lapins /Gisela 5	7.28 **b**	8.28 **b**
T1-Lapins /Gisela 6	6.71 **b**	6.28 **c**
T2-Lapins /Gisela 6	10.00 **a**	10.28 **a**

The shoot length growth (64-65 cm) in 2020 was intensive in `Gisela 6` rootstock combination in both irrigation levels (Table 3), followed by `Gisela 5` combination (42-49 cm).

Table 3. Measurement data on length of shoots at ‘Lapins’ sweet cherry cv grafted on ‘Gisela 5’ and ‘6’

Treatment variant	Length of shoots (cm)	
	2020	**2021**
T1-Lapins /Gisela 5	42.70 **b**	52.09 **c**
T2-Lapins /Gisela 5	49.15 **b**	66.41 **b**
T1-Lapins /Gisela 6	64.00 **a**	67.61 **b**
T2-Lapins /Gisela 6	65.59 **a**	85.77 **a**

In 2021 as the trees developed, higher measurements were recorded in T2-`Gisela 6` category (85.77 cm) followed by normal T1 category in `Gisela 6` and the two `Gisela 5` rootstock combinations. Elevated irrigation thus had an effect also on the length of shoots (Table 3).

Table 4. Measurement data on tree height at ‘Lapins’ sweet cherry cv grafted on ‘Gisela 5’ and ‘6’

Treatment variant	Height of trees (cm)	
	2020	**2021**
T1-Lapins /Gisela 5	2.02 **b**	1.96 **b**
T2-Lapins /Gisela 5	2.39 **ab**	2.04 **b**
T1-Lapins /Gisela 6	2.53 **a**	2.36 **a**
T2-Lapins /Gisela 6	2.58 **a**	2.53 **a**

In 2020 the recorded height of trees showed similar growth characteristics (2.53-2.58) in `Gisela 6` combination and slight lower values at `Gisela 5`. In 2021 the higher vigor rootstock produced quite the same height values as in 2020 despite the irrigation effect, `Gisela 5` produced trees with lower height than Gisela 6 (Table 4).
The canopy enlargement can be observed at the more vigorous `Gisela 6` cultivar in T2 irrigation variant (Table 5) in both experimental years showing values ranging 0.61-1.16 m^3.

Table 5. Measurement data on canopy volume at ‘Lapins’ sweet cherry cv grafted on ‘Gisela 5’ and ‘6’

Treatment variant	Canopy volume (m^3)	
	2020	**2021**
T1-Lapins /Gisela 5	0.36 **b**	0.73 **c**
T2-Lapins /Gisela 5	0.48 **ab**	0.81 **b**
T1-Lapins /Gisela 6	0.49 **ab**	0.87 **b**
T2-Lapins /Gisela 6	0.61 **a**	1.16 **a**

In 2020 the increased irrigation level in `Gisela 5` combination and the standard T1 irrigation level at `Gisela 6` canopy showed very close values (0.48-0.49). In early development stages it seems that the rootstock effect it is not so pronounced. In 2021 we can observe that the growing and expanding tendency remains, less canopy value is recorded at T1 standard irrigation level in `Gisela 5` rootstock combination, whereas in `Gisela 6`the canopy has a greater volume.
One of the most important technical-economical indicator is the yield per tree (kg/tree) value and in our experiment results data showed that it is possible to achieve early production of the young trees in the 2-3rd leaf stage (Table 6) with knip trees.

Table 6. Measurement data on yield per tree (kg/tree) at ‘Lapins’ sweet cherry cv grafted on ‘Gisela 5’ and ‘6’

Treatment variant	Yield per tree (kg/tree)	
	2020	**2021**
T1-Lapins /Gisela 5	0.18 **c**	0.91 **b**
T2-Lapins /Gisela 5	0.32 **bc**	0.92 **b**
T1-Lapins /Gisela 6	0.36 **b**	1.13 **ab**
T2-Lapins /Gisela 6	0.53 **a**	1.42 **a**

Results showed that the higher vigor `Gisela 6` rootstock induces in both experimental years higher yield (0.53-1.42 kg/tree) than `Gisela 5` grafted trees, due to more intensive tree development in elevated T2 conditions.
In normal irrigation conditions the yield per tree was also good at `Gisela 6` with close values to T2 but the watering contributed well to production. In Gisela 5 in both the irrigation variants the recorded yield had similar values. Early observations showed that a high number of fruits/branches seems to be characteristic for the `Lapins` sweet cherry cultivar (Figure 5). Further researches will elucidate if there is a need for more specific pruning techniques in order to regulate the crop load.

Figure 5. Yield of ‘Lapins’ sweet cherry cultivar grafted on ‘Gisela 6’ rootstocks

Table 7. Fruit weight (g) of ‘Lapins’ sweet cherry cv grafted on ‘Gisela 5’ and ‘6’

Treatment variant	Fruit weight (g)	
	2020	2021
T1-Lapins /Gisela 5	8.47 **a**	7.96 **c**
T2-Lapins /Gisela 5	8.28 **a**	8.17 **bc**
T1-Lapins /Gisela 6	8.02 **a**	8.34 **b**
T2-Lapins /Gisela 6	8.64 **a**	8.73 **a**

Figure 6. Weighing the ‘Lapins’ cultivar fruits

Regarding fruit weight (Table 7) we can observe that the `Lapins` cultivar has medium sized fruits (8.64-8.73 g), maybe due to the intensive crop load, thus in 2020 there were no statistical differences between variants, trees being in the first production year.

In 2021 the T2 drip irrigated `Lapins`/`Gisela 6` trees presented fruits weighing 8.73 g followed by the T1 variant with 8.34 g fruits (Figure 6). Quite similar fruit weight values were observed in T2 irrigated `Gisela 5` grafted trees (8.17) followed by the T1 irrigated trees on `Gisela 5` (7.96 g).

CONCLUSIONS

Under our experimental conditions tree establishment of `Lapins` sweet cherry cultivar was very effective on both rootstocks, `Gisela` 6 was more vigorous when compared with `Gisela 5`, in terms of TCSA development, shoot number, shoot length, height of trees, canopy volume. However, during the first production year (2020), yield was smaller, irrigation completed the physiological need of plants and in the elevated T2 irrigation variant the root system of `Gisela` rootstocks used very effectively the applied water amount. In the second experimental year (2021) the recorded yield was higher at `Gisela 6` rootstock variant, but `Gisela 5` behaved also well. Similar results have obtained also by other researchers (Cordeiro et.al, 2007) in the 3rd leaf stage, the recorded yield was 2.53 kg/tree, at tree height of 2.80 m and 26.5 cm^2 TCSA. Bujdoso et al. (2007) obtained in early years 1.5 kg/tree at cultivar `Germersdorfi 3` grafted on `Gisela 5` rootstock in an experiment established in Hungary. Preliminary researches showed that `Gisela 5` and `Gisela 6` are precocious, size controlling and early productive rootstocks for sweet cherry cultivation but optimal specific soil and orchard management conditions must exist in order to fully provide the physiological and technological elements for optimal tree development.

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THE DIVERSITY OF SOME PHENOLOGICAL FEATURES IN BLUEBERRY CULTIVARS (*VACCINIUM CORYMBOSUM* L.) GROWN IN BANAT AREA, ROMANIA

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Abstract

This paper's aim was to investigate the adaptation to the environmental conditions of south-western Romania, Banat region (45°25'48"N 21°34'55"E) in terms of flowering and ripening seasons, of 3 commercial blueberry (V. corymbosum) cultivars of North American origin in order to examine and determine the levels of genetic difference. This study was carried out during the growing season in a blueberry plantation, for 'Duke', 'Hannah's Choice' and 'Elliott' varieties. In the study, phenological traits were recorded using the BBCH phenological scale for blueberry and the observation of phenotypic data was recorded as the number of days from January 1st for statistical analyses. Cultivars were found to have quite different characteristics in terms of phenological traits. The highest coefficient of variation belonged to early pink bud (57 BBCH) (CV = 14.08%), while the lowest CV was given by bud break (53 BBCH) (CV = 5.70%). It was found that the differences in phenology recorded between cultivars are due to the different accumulation of the amount of temperature required for the development of a certain phenophase, depending on the cultivar's requirements, knowing that the temperature factor is crucial for exiting from dormancy, flowering and fruit ripening.

***Key words**: blueberry, diversity, phenology.*

INTRODUCTION

Cultivated blueberry (*Vaccinum corymbosum*), native to North America, was introduced to Romania in the late 1960s and the fast upward trend of blueberry establishment in Romania is motivated by the growing market demand for fresh fruit consumption and the profit incentive compared with other fruit produced in the orchards (Asanica et al., 2017). Blueberry cultivation is of growing interest due to the nutritional qualities of fruits and the therapeutic virtues of both fruits and leaves (Moze et al., 2011; Kostenko et al., 2020; Tundis et al., 2021). One of the most important aspects for a successful expansion of the blueberry crop is the selection of cultivar, which basically depends on knowledge about their adaptation to local conditions (Campa & Ferreira, 2018; Bădescu et al., 2016; Peticila et al., 2009; Merca & Cosmulescu, 2020). Environmental factors, different from one year to another, or from one area to another, do require the different development of vegetation phenophases in fruit plants (Cosmulescu and Ionescu, 2018, 2021; Cosmulescu et al., 2010; Asanica et al., 2020). The registration of the landmark stages in the fruit plants is necessary in order to have useful data regarding the periods of their development, and also for the establishment of assortments adapted to the cultivation areas. Vegetation stages of fruit trees are of high economic importance because they have a direct impact on fruit production (Chmielewski, 2004), which shows the importance of knowledge of fruit trees phenology. Findings of Zverko et al. (2014) indicate that the increase in temperature has a significant influence on the onset of spring phenophases in some fruit species. Systematic long-term monitoring of phenophases provides an opportunity to estimate changes in the onset or end of phenophases, which allows the assessment of the influence of climate change on nature (Bauer et al., 2014). Monitoring of environmental conditions can be crucial for farmers wishing to implement management practices at specific stages of crop development (Kirk & Isaacs, 2012). The aim of this paper was to investigate the adaptation to the environmental conditions of south-western Romania, Banat region (45°25′48″N 21°34′55″E) in terms of flowering and ripening seasons, of 3 commercial blueberry cultivars of North American origin, in order to examine the levels of genetic difference.

MATERIALS AND METHODS

This study was conducted during the vegetation period 2020 and 2021 in a blueberry plantation (*V. corymbosum*). Three blueberry cultivars were taken under study: 'Duke' (USA origin), 'Hannah's Choice' (USA origin), 'Elliott' (USA origin). 'Duke' blueberry is an early-season cultivar with attractive fruit of medium to large size, light blue coloured.

'Hannah's Choice' is a vertical shrub with pink flowers, blooms early and may be susceptible to late frost damage (Ehlenfeldt et al., 2005). 'Elliott' cultivar blooms late to avoid late spring frosts, it is cold-resistant, ripens in August (Ehlenfeldt, 2003).

In order to determine the genetic diversity, phenological traits were identified on a total of 75 plants for each cultivar. The experiment was organized in Gherteniş (45°25′48″N 21°34′55″E), Caraş-Severin County, Banat region, located in south-western Romania. In the study, phenological traits were recorded using the BBCH phenological scale for blueberry as described by Longstroth (2008). Twelve main growth stages have been described (Table 1, Figure 1). The observations were recorded and converted to Julian Dates (number of days after January 1) with the aim to conduct a quantitative analysis (Cosmulescu et al., 2022). The data were statistically analyzed using descriptive statistics and correlation by using Statistical Package Programme - Data Analysis.

Table 1. Definitions used in the determination of phenological traits - flower bud*

BBCH scale/Traits	Description
51/Bud swell	First sign of growth as plant growth begins in the spring. Visible swelling of the flower buds; outer bud scales begin to separate at the tip revealing paler interior bud scales. This bud stage can usually tolerate cold temperatures of -12 to -9°C.
53/Bud break	Flower buds open and the individual flowers can be seen between the bud scales. Can tolerate cold temperatures of about -7°C.
55/Tight cluster	Individual flowers are distinguishable in the flower cluster. This bud stage can tolerate -7 to -5°C.
57/Early pink bud	Expanding flowers are readily visible and have separated. The pink corolla tubes (petals) are short and closed. This bud stage can tolerate -5 to -4°C.
59/Late pink bud	Individual flowers fully developed. Expanded corollas are now white but still closed. This bud stage can tolerate -4.4 to -2.8°C.
65/Full bloom	Most of the flowers on the bush have opened. The bloom stages can tolerate -2.2°C.
67/Petal fall	The corolla tubes are falling off the flowers, revealing small green fruit. This is the most vulnerable stage to freeze injury. Damage can occur at 0°C.
71/Early green fruit	Small green berries are expanding. Fruit in the cluster varies from large to small pea-sized. Early fruit growth is by cell division.
78/Late green fruit	Growth of large fruit slows. Fruit becomes pale green. Exposed fruit may develop a red blush.
81/Fruit colouring	Oldest, largest fruit in the cluster begin to change colour from green to pink to blue. Fruit begins to soften. Cell division has stopped and fruit growth is by cell expansion.
85/25% Blue	25 percent of the berries are ripe. 25% blue often coincides with first hand harvest of ripe berries.
87/75% Blue	Blueberries are picked several times as the fruit ripens with 2 to 5 pickings. Often the first harvest is by hand and then later by machines that shake berries off the bush. 75% blue often coincides with the first of 2 machine harvests in the field.

*Source: https://www.canr.msu.edu/blueberries/growing_blueberries/growth-stages

Figure 1. Blueberry phenological stages according to the BBCH scale (original pictures)

RESULTS AND DISCUSSIONS

Statistical data on phenological features of the analysed blueberry varieties are presented in the Table 2. We determined a large phenological diversity in our blueberry cultivars. Cultivars were found to have quite different characteristics in terms of phenological traits.

The highest coefficient of variation belonged to early pink bud (57 BBCH) (CV=14.08%), while the lowest CV was given by bud break (53 BBCH) (CV= 5.70%) (Table 1).

The summary findings of bud swell (51) bud break (53), tight cluster (55), early pink bud (57), late pink bud (59), full bloom (65), petal fall (67), early green fruit (71), late green fruit (78), fruit colouring (81), 25% fruit blue (85) and 75% fruit blue (87) dates of genotypes are presented in Table 2.

According to the values obtained for the coefficient of variability, it is found that the phenological changes in the population were homogeneous (CV<10%) and relatively homogeneous (10%<CV<20%).

Table 2. Descriptive statistics of the number of days (Julian date) necessary for the development of the main spring phenological traits in blueberries

TRAITS	UNIT	MIN	MAX	MEAN	SD	CV%
51/Bud swell	Day	39	60	53	3.20	6.03
53/Bud break	Day	60	88	70.33	4.01	5.70
55/Tight cluster	Day	70	101	82.5	5.39	6.53
57/Early pink bud	Day	79	117	97.66	13.76	14.08
59/Late pink bud	Day	94	121	106	10.21	9.63
65/Full bloom	Day	104	134	119.83	11.49	9.58
67/Petal fall	Day	114	140	127.33	10.32	8.10
71/Early green fruit	Day	121	152	134.5	12.14	9.02
78/Late green fruit	Day	130	166	145.33	13.50	9.28
81/Fruit colouring	Day	139	181	154.66	16.59	10.72
85/25% Blue	Day	160	205	179.16	19.27	10.75
87/75% Blue	Day	175	222	190.83	20.62	10.80

The lowest coefficient of variation was obtained for phenophase 53 BBCH/Bud break (CV%=5.70) while the largest for 57 BBCH/Early pink bud phenophase (CV%=14.08).

Analyzing the limits of variation for each phenophase, it is found that the differences between the years were quite large, ranging between 21 days for bud swell phenophase (BBCH 51) and 47 days for / 75% blue phenophase (BBCH 87) (Table 1).

Lower temperatures, with values around 0° C or lower, both in the air and on the soil, which favour hoar-frosts or late frosts, after March 20, represent a major risk for all crops. In the present case study, both over the spring of 2020 and over the year 2021, after successive days with normal values for the end of March and the beginning of April, days followed with temperatures below 0°C both in the air and on the soil. The high risk was the fact that there were consecutive days with negative values. From a calendar point of view, the early pink bud phenophase (57 BBCH) occurred between March 20 and April 29, i.e. 79-117 days from January 1 (Table 1). Over this period, the minimum air temperatures had values between -2.7ºC (March 20, 2021) and 2ºC (March 22, 2020), and the soil temperatures between -4.2ºC (March 20, 2021) and 3ºC (April 6, 2020). The negative effect of these temperatures was observed in the 'Hanna's Choice' cultivar, when 7.6% of buds were affected, with repercussions on crops. In this bud stage (57 BBCH), according to Longstroth (2008), it can tolerate temperature values of -5 to -4°C. Late pink bud (59) was evaluated on April 5 for ‘Hannah’s Choice’ cultivars in 2020 year, and on May 3 for ‘Elliott’ cultivars. The difference between January 1 and the late pink bud (59) was from 94 to 122 days (Table 2). From a calendar standpoint, the full bloom phenophase (65 BBCH) ran from April 15 ('Hannah's Choice', 2020) to May 16 ('Elliott', 2021). The flowering is a very important parameter because of the possible damage to flowers by late spring frost (Sterne & Liepniece, 2010). The full bloom (65 BBCH) stages can tolerate -2.2°C according to Longstroth (2008). In the research area, in this phenophase, the minimum recorded temperatures were between 5.5 and 20.8ºC, which did not create problems, from a climatic point of view, for this phenophase. The phenophase of early green fruit (71 BBCH) was noticed starting with May 2 ('Hannah's Choice', 2020) and a month later ('Elliott', 2021) and occurs at 121-152 days from January 1 and depends on the cultivar, weather and the plant’s vigour. Fruits continue to grow even after the fruits turn blue by accumulating water in the tissues. Since at the end of fruit ripening the water needs of the plant increase, in order to obtain quality fruits, it is very important to properly irrigate the crop during this critical period. The time of fruit ripening (25% blue, 85 BBCH) fluctuated from 160 to 205 days, by cultivar and climatic year. This phenophase occurred first in the 'Duke' cultivar (June 10, 2020) and in the latest in 'Elliott' (July 25, 2020). With regard to phenophase 87 BBCH (75% blue), the results obtained indicated that the length of the period was from 1 year to this one (87 BBCH) in cultivars of so it ranged from 175 to 222 days (Table 1). 'Hannah's Choice' ripens at approximately the same time as 'Duke', when 'Eliott' is in the fruit colouring phenophase (81 BBCH). According to Sterne & Liepniece (2010), the best blueberry yields are usually obtained by fruit ripening very early or very late in the season. The choice of the 3 cultivars, from this point of view, is an advantage. Correlation coefficient was used to determine the associations between the phenological traits (Table 3). Values of the correlation coefficient for trait pairs were significant and positive in most cases. We determined a very significant correlation between early green fruit (71 BBCH) and late pink bud (59 BBCH) ($r = 0.994$), between early green fruit (71 BBCH) and fruit colouring (81 BBCH) ($r = 0,977$), between late pink bud (59 BBCH) and fruit colouring (81 BBCH) ($r = 0.977$), between 65 / full bloom and 71 and early green fruit (71 BBCH), late green fruit (78 BBCH) ($r = 0.967$), between 25% blue (85 BBCH) and 75% blue (87 BBCH) ($r = 0.986$) etc. Our data indicated that the first phenophases of spring (late pink buds - 59 BBCH; early green fruits - 71 BBCH) were highly correlated with later BBCH stages (fruit coloring - 81 BBCH; 25% blue - 85 BBCH; 75% blue - 87 BBCH), which suggests a potential relationship between physiological resting state and apparent morphological changes in blueberry flower buds. Significant correlations between phenological traits were determined on blueberry cultivars by Campa & Ferreira (2018). Negative significant correlations were observed between flowering season and days to flowering, flowering season and days to the end of flowering, and harvesting season and days to flowering in the same study by Campa & Ferreira (2018).

Table 3. Correlation coefficient between the phenological traits of blueberry varieties*

	57 BBCH	59 BBCH	65 BBCH	71 BBCH	78 BBCH	81 BBCH	85 BBCH	87 BBCH
57 BBCH	1							
59 BBCH	0.972***	1						
65 BBCH	0895	0.951***	1					
71 BBCH	0.943***	0.994***	0.967***	1				
78 BBCH	0.893	0.955***	0.966***	0.969***	1			
81 BBCH	0.937***	0.977***	0.916***	0.977***	0.969***	1		
85 BBCH	0.818	0.896	0.817	0.906***	0.803	0.855	1	
87 BBCH	0.855	0.909***	0.791	0.905***	0.806	0.880	0.986***	1

***p<0,001

CONCLUSIONS

In conclusion, cultivars were found to have quite different characteristics in terms of phenological traits. The highest coefficient of variation belonged to early pink bud (57 BBCH) (CV = 14.08%), while the lowest CV was given by bud break (53 BBCH) (CV = 5.70%). It was found that the differences in phenology recorded between cultivars are due to the different accumulation of the amount of temperature required for the development of a certain phenophase, depending on the cultivar's requirements, knowing that the temperature factor is crucial for exiting from dormancy, flowering and fruit ripening. Our results indicate that blueberry varieties have shown a high degree of phenotypic plasticity to respond to gradual changes in environmental conditions.

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GENETIC RESOURCES OF *PRUNUS* SUBGENUS *CERASUS* (GRAY) IN THE TROYAN REGION, BULGARIA

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Abstract

Local plant genetic resources of Prunus sp. in the Forebalkans region of the Troyan municipality. The present study was conducted during the spring-summer period of 2019-2020. A number of expeditionary studies were conducted to search for local genotypes and varieties of the genus Prunus subgenus Cerasus (Gray) in the Forebalkans region. Three local genotypes were selected and described in the present study, and the Oblachinska variety was accepted as a standard.
Their main morphological characteristics have been studied: height, diameter, stem length, color, biochemical analysis, the taste of the fruits.
Their main morphological characteristics have been studied: height, diameter, stem length, color, biochemical analysis, of the fruit taste.
Cherry SCHT has a higher dry matter content of 16.50% and total sugars of 4.70%. Larger amounts of tannins contain the fruits of the SCHB 0.104%. The highest content of total polyphenols was obtained at cherry SCHT 163.14 mg/g. Anthocyanins have a higher content in cherries SCHB 41.94mg%. All three local genotypes are suitable for fresh consumption and processing

Key words: *Prunus subgenus cerasus (Gray), sour cheries, genetic resources, biochaemical compounds*

INTRODUCTION

There has been a growing interest in the recent years in the conservation and research of the gene pool of cultivated and wild fruit species, as well as products from them. The study of genotypes of local origin allows the selection of species in order to preserve, preserve and increase the biodiversity of local flora (Ivanova et al., 2009 Vieira et al., 2009; Kikindonov et al., 2017). In response to the increased demand for organic fruit, there is an alternative to the rich fund of local genetic resources. (Bozovic et al., 2013; Ferreira et al., 2016; Pereira-Lorenzo et al., 2018; Kumar et al., 2019; ; Minkov et al. Mihova, 2020). Many forms and genotypes of the local gene pool of fruit varieties have increased resistance to a number of economically important diseases. Therefore, the management of plant genetic resources is a priority management in selection programs (Bozhkova et al., 2006; Dzhuvinov et al., 2016; Minkov et al. Mihova, 2020).
The cherries and the sour cherries are among the most widely consumed fruits in temperate latitudes. They are an economically important fruit species in Bulgaria. The total area of their plantation amounts to 11,443 ha and 1,672 ha, respectively. The production of these fruits is growing annually. In recent years, many new plantations have been created in different regions of the country (Malchev et Zhivondov, 2016; Kamenova et al., 2019; Antognoni et al., 2020). The cherries are valuable fruits with specific nutritional and medicinal properties and are widely used in folk medicine and culinary cuisine of Bulgarians from ancient times to the present day. They are extremely rich in organic acids (malic, citric oxalic, etc.), mineral salts, various macro-and micronutrients, B vitamins, with the highest content of vitamins. With and especially on vitamin. P, the value, especially for black-red colored fruits reaches 2000-2500 mg%. Their sugars are mainly glucose and fructose, the latter being in minimal quantities, which allows their consumption by diabetics. The presence of tannins in them gives a pronounced astringent tart taste. The presence of pectin substances in fruits facilitates the excretion and excretion of toxins from the human body, lowers blood cholesterol. The species grown in our country

can be divided into two groups - for processing and fresh consumption (Dinkova, 2009; Bandi et al., 2010). Sour cherry (*Prunus cerasus* L.) is an autochthonous and heterogeneous species. Their sugars are mainly glucose and fructose, the latter being in minimal quantities, which allows their consumption by diabetics. The tannins in these fruits gives a pronounced astringent tart taste. The presence of pectin substances in fruits facilitates the excretion and excretion of toxins from the human body, lowers blood cholesterol. The species grown in our country can be divided into two groups - for processing and fresh consumption (Dinkova, 2009). Sour cherry (*Prunus cerasus* L.) is an autochthonous and heterogeneous species. Long-term cultivation in varieties of agri-environmental conditions and the use of different types of propagation (both by shoots and seeds) has led to numerous genotypes. In some cases, indigenous varieties are at high risk of extinction due to the introduction of foreign varieties that may have higher yields or are better known on world markets (Hjalmarsson and Ortiz, 2000; Rodrigues et al., 2008; Rakonjac et al., 2010). All these factors create the need for a detailed description and evaluation of the gene pool of cherries, which is the purpose of this study. The complete characteristic, as well as the protection of the autochthonous varieties is considered to be of great importance in order to avoid the loss of sustainable, adapted to the specific conditions of mountainous regions in Bulgaria).

The aim of the present research is to mark, describe, study and preserve the gene pool of indigenous forms of cherries with valuable biological and economic qualities, distributed in the Fore-Balkan region in the Middle Central Balkan Mountains.

MATHERIALS AND METHODS

The current study was conducted during the summer period 2020-2021. A number of expeditionary studies were conducted to search for valuable varieties and forms in the region of Troyan and its adjacent villages and neighborhoods. The trees are grown at an altitude of 400-750 m, in non-irrigated conditions and without plant protection measures. The soils are gray and dark gray forest. The average annual precipitation for the study period was 772.03 mm. The biological and morphological features of the fruits have been established according to the methodology for studying plant resources in fruit growing (Nedev et al., 1979). Their dimensions (mm) are determined; mass (g); fruit stalk length (mm); coloring of the fruit skin; taste qualities; Their main biochemical composition was studied: dry matter (%) - refractometric; total, invert sugar and sucrose - by the method of Shoorl. The organic acid content was determined by titration with 0.1 N NaOH solution; Ascorbic acid (mg/%); Pectin (mg/%) - According to Melitz; The sugar-acid index was calculated by Stanchev et al. (1958); Sensory analysis (Malchev and Zhivondov, 2016).

The obtained results were subjected to mathematical analysis using the methods of correlation and regression analysis, coefficient of variation (CV%) (Lidanski, 1998). The software products used during the study was "MS Excel Analysis ToolPak Add-Ins".

RESULTS AND DISCUSSIONS

During the expeditionary study of the genepool of genus *Cerasus* in the region of the town of Troyan was found a great variety of old cultivars and genotypes of sour cherries. Three representatives' genotypes of local cherries were identified, marked and described, with valuable biological and economic qualities, common in the region of Troyan and the surrounding villages and neighborhoods. Their fruits reached ripening stage from mid- the end of June. It has been stablished that in the reproduction is usually carried out by shoots.The morphological characteristics of selected forms and cultivars of fruits and their qualities were determined.The fruit size is a hereditary feature of fruit species, cultivars, forms (Stoichkov et al., 1958). The size and color of fruit in the present study vary widely.

SCHT - the most common cherry, spread on rivers, capes, roads. The crown is spherically elongated, than the Oblachinska variety, with larger leaves. The average fruit weight is of 3.2 g, with light fruit flesh and juice. More pleasant and sweet than the Oblachinska variety. They ripen in the last ten days of June. Resistant to economically important diseases.

SCHB - forms a relatively large globular crown. The fruits are 5.04 g, with dark red fruit flesh and juice, sweeter and more pleasant than Oblachinska. The fruits reach harvest maturity at the end of the second, beginning of the third ten days of June. It is found exclusively in the area of the village of Balkanets. In 2020, this form of cherry showed a strong sensitivity to late brown rot, as the period of growth and ripening coincided with unfavorable climatic conditions - rainfall and high humidity. Polish resistance to cylindrosporiosis, fungal powdery mildew and powdery mildew syndrome, which adversely affects fruit quality, has been observed (Stoyanova et al., 2014).

Table 1. Biometric indicators of fruits of local cherry genotypes

Genotype	Year		Fruit weight (g)	Stone wejght (g)	Fruit length (mm)	Average width (mm)	Fruit stalk length (mm)	Fruit shape index (mm^3)
Oblachinska	2020	average	**3.10**	**0.26**	**15.33**	**16.31**	**24.17**	**17.19**
		STDEV	0.57	0.05	0.42	0.84	2.01	1.74
		CV	18.31	19.86	2.71	5.16	8.30	10.11
	2021	average	**3.72**	**0.33**	**16.11**	**17.70**	**26.76**	**21.29**
		STDEV	0.29	0.07	0.41	0.75	2.86	1.88
		CV	7.69	20.45	2.53	4.24	10.70	8.82
SCHB	2020	average	**6.00**	**0.28**	**17.60**	**20.83**	**32.31**	**32.53**
		STDEV	0.82	0.08	1.19	0.95	2.55	4.48
		CV	13.61	28.17	6.74	4.50	7.89	13.79
	2021	average	**4.07**	**0.38**	**16.26**	**18.85**	**28.60**	**24.45**
		STDEV	0.26	0.06	0.73	0.52	3.63	1.65
		CV	6.35	16.64	4.51	2.78	12.70	6.75
SCHT	2020	average	**2.80**	**0.20**	**13.88**	**16.41**	**43.28**	**15.91**
		STDEV	0.63	0.07	0.68	0.78	5.36	1.99
		CV	22.59	33.33	4.93	4.73	12.39	12.56
	2021	average	**3.55**	**0.28**	**15.30**	**17.40**	**36.68**	**19.67**
		STDEV	0.47	0.04	0.66	0.99	5.52	2.75
		CV	13.23	15.06	4.33	5.68	15.05	13.97

The main biometric indicators of the marked and described local genotypes of cherries were taken into account during the study. In terms of fruit weight, with small variations, it is highest measured in the Balkanets form (average about 5 g). For the rest, it is in the range of 3-4 g. The lowest bone weight was measured in SCHT (0.24 g) with almost the same coefficient of variation in the two years (CV = 19.86 and 20.45, respectively), and the highest form Balkanets (0.33 g). The height of the fetus in the studied autochthonous genotypes is in the range of 13.88 mm at SCHT in 2020 to 17.60 mm at the SCHB in the same year. The coefficient of variation in relation to this indicator in all genotypes is low. The SCHB(19.84 mm) has the largest average diameter. The fruits of the SCHT form are the highest 19.26, the smallest and with the longest stalk 25.80mm are the fruits of Oblachinska with a diameter of 18.62/ 16.37. A significant difference is observed in the length of the handle in SCHT from (43.28 mm) in 2020 to (24.17 mm) in Oblachinska in the same year, which is determined by the average coefficient of variation. The SCHT genotype described in our study is characterized by low fetal weight and volume, but a very long stalk (Table 1). In the case of cherry varieties, the SCHB Genotype has a higher fruit weight (5.35 g) on average for the two years and the lowest on the stone (0.29 g). (Table 1).

The highest dry matter content of the studied genotypes was in SCHT (from 16 to 17.6% in the two years of study), and with the lowest - 12.00-12.5% in SCHB. Oblachinska has very close values to SCHT.). The amount of total sugars varies from 12.36 mg% in the SCHB

(2020) to 4.35 mg% in 2021. Smaller amplitudes are observed in Oblachinska (7.5-8.2 mg%) in the two years of study. In the ratio of total sugars, the share of invert sugar predominates. The highest value in relation to this indicator was reported at Oblachinska (7.5%), followed by the SCHB(6.5%). In the Cherry genotypes studied, sucrose ranged from 0 to 5.8% during the individual years of study. The highest amount of organic acids is in the Oblachinskaya genotype (1.08%), followed by SCHT (0.87%) in 2020. The fruits of the Oblachinskaya and SCHT genotypes are slightly richer in polyphenolic compounds with just over 331 mg%. Regarding the acidimetric coefficient for cherries, the highest value was reported for SCHT (13.23%) and the lowest for Oblachinska (9.59%).

Table 2. Biochemical analysis of fresh sour cherry fruits

Variety	Year	DM by Re (%)	Total sugars (%)	Inverted sugar (%)	Sucrose (%)	Acids (%)	Total polyphenols (mg/g)	Tannins (%)	Anthocyanins (mg%)	Sugar/acid index
SCHT	2020	16.5	4.7	4.7	0.0	0.87	163.14	0.083	38.23	5.40
	2021	15.0	9.9	4.35	5.27	0.47	499.07	0.083	16.61	21.06
SCHB	2020	12.0	12.6	6.5	5.8	0.67	329.7	0.083	13.55	18.81
	2021	12.5	4.35	4.35	0.0	0.80	114.3	0.104	41.94	5.44
Oblachinska	2020	17.6	7.5	7.5	0.0	1.08	161.06	0.163	39.43	6.94
	2021	16.0	8.2	3.85	4.13	0.67	501.6	0.104	84.52	12.24

The data in Figure 1 show an established positive linear relationship with a high coefficient of determination R^2=0.9 between the measured dry matter and fruit weight; fruit size and sugar-acid index; between fruit weight and dry soluble matter; total polyphenols and fruit size of the different genotypes sour cheries.

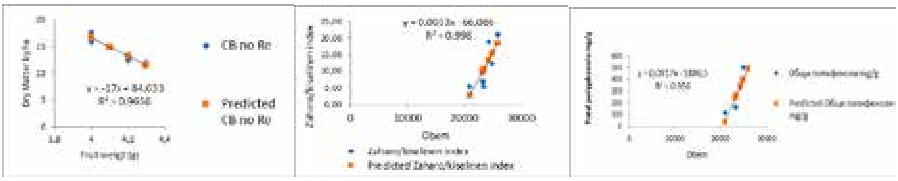

Figure 1. Linear relationship between dry matter and fruit weight; fruit size and sugar-acid index; between fruit weight and dry soluble matter; total polyphenols and fruit size

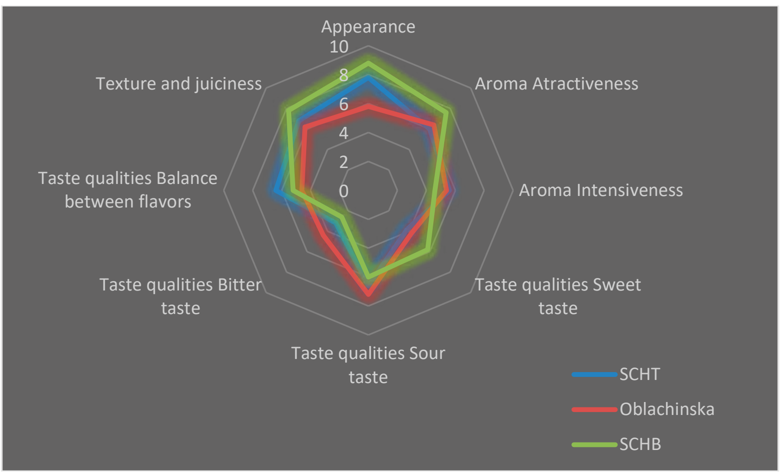

Figure 2. Sensorial analysis of Global Taste Quality

Figure 2 presents the sensory characteristics of the developed genotypes sour cherries. The fruits are evaluated by trained evaluators on indicators of appearance, Aroma attractiveness and intensity, taste qualities - sour, bitter, sweet taste, texture and juiciness evaluation. For all developed variants. In terms of attractive appearance, the SCHBstands out, followed by the SCHT. The highest score (Very good - 6.05) is given to the SCHBgenotype (5.78). It surpasses other genotypes in terms of flavor, taste and texture.

CONCLUSIONS

The described genotypes are adaptive for breeding in the conditions of semi-mountainous and mountainous regions. The forms are appreciated and appreciated by the local population, suitable for both fresh consumption and processing. Due to their attractive appearance, taste and economic qualities, the found and described genotypes would be suitable for inclusion in breeding programs. The SCHT is more common in Troyan, while the SCHT is located mainly among the population in a small village near the town. The fruits of the SCHBhave the greatest weight, height and volume, with dark red fruit flesh and juice, sweeter and more pleasant than others in terms of sensory characteristics.

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BEHAVIOUR OF SOUR CHERRY CULTIVARS TO THE CAUSES OF CYLINDROSPORIOSIS AND SHOT HOLE DISEASE

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Abstract

The study was conducted in the period 2018-2020 on the territory of RIMSA Troyan with three cherry cultivars, such as 'Oblachinska', 'Karneol' and 'M 15', grafted on 'Alkavo' rootstock and planted in 2002 by trench method with local, stockpile organic fertilizing. The planting scheme was 4 x 3 m and they were grown under nonirrigated conditions.
The reaction of the sour cherry cultivars, the intensity of the infestation and the manifestation of cylindrosporiosis and shot hole disease for the optimization of the schemes for sustainable Plant Protection under the climatic conditions of the Troyan region were studied.
'Karneol' was found to have the lowest susceptibility to both diseases studied (shot hole disease <8.7%; white rust 21.3%). The average fruit weight was 5.5 g, it is suitable for fresh consumption, with an extremely balanced taste and with opportunities for organic and sustainable cultivation, and the later ripening period allows to extend the harvesting period.
'M15' had a higher infestation index of Stigmina carpophila (Lev.) Ell (15.3%) compared to the other two cultivars and was more susceptible. Infestation index of Blumeriella jaapii (Rehm.) was slightly higher, but was well below 50%. It has attractive fruits with greater weight, bears abundant fruit and can be included in the cultivar list of modern sustainable technologies.

Key words: *cherries, fungal diseases, climatic conditions, biometric indicators.*

INTRODUCTION

The Carpathian Basin, the Balkans and Asia Minor are considered the main birthplaces of sour cherries as a fruit crop.

A study by Surányi (2021) compared 472 sour cherries cultivars based on 7 relative ecological indicators and 3 biological, where 'Karneol' and 'M 45' rank 5-6, i.e. mountain location (deciduous forest belt), while 'Oblachinska' (on a scale from 1 to 9) is in 7 thermophilic forest belt. The same cultivars are defined as resistant to moderately susceptible to diseases 1-2 (on a scale of 3 degrees) and are moderately drought-resistant (for semi-humid habitats - 5 (on a scale of 12 points).

In Bulgaria, sour cherries are grown as an industrial crop in some regions, such as Plovdiv and Kyustendil and in some districts of the country, where critical temperatures during deep dormancy are not lower than (-25°C) and the risk of return winter and late spring frosts is small (Taseva et al., 2007).

The research and enrichment of sour cherry assortment with new large-sized cultivars for Bulgaria, with increased cold resistance, disease resistance, high fruitfullness and different ripening period in recent years has been supported by the Bulgarian-German project FAMAD. Cultivars with different origin, growth strength, fertility, ripening period, size, fruit color, etc., such as 'Keleris', 'Karneol', 'M15' and others were introduced.

Sour cherry fruits are consumed as fresh, but are used for the production of juices, syrups and alcoholic beverages, confectionery. Due to their high content of vitamin C and other biologically active substances that could act as antioxidants, sour cherries are used to prevent cancer and other cardiovascular diseases, as well as to treat osteoarthritis and gout (Bastos et al., 2015).

It has been established that cherries (*Prunus avium* L.) and sour cherries (*P. cerasus* L.) are seriously endangered by a significant number of phytopathogens affecting the leaves and causing premature defoliation, reduced shoot growth, increased susceptibility to winter injuries, higher mortality of trees. *Blumeriella jaapii* (Rehm.), caused by *Cylindrosporium* *padi*, is one of the most important and economically significant fungal pathogens affecting sweet cherries and sour cherries worldwide and in Serbia (Iličić et al., 2019). The pathogen mainly affects the leaves, thus compromising photosynthetic ability, causing early defoliation, decreasing yields and reducing fruit quality.

Many studies provide information on factors that favour sporulation, spore spreading, spore germination, disease penetration and development. Scientists report that temperatures of 15-20°C are most favourable for the development of *Blumeriella jaapii* (Rehm.), (Valiushkaite et al., 2002). Joshua and Mmbaga (2014) report that the disease caused by *B. jaapii* (Rehm) Arx. is getting more and more economic significance for sour cherry growers in the southeastern United States. In their study, spores were captured in late March, before field symptoms were observed, indicating that the remains of diseased tree leaves are an important source of primary inoculum. Previously infected trees of six cultivars (‘Kwanzan’, ‘Yoshino’, ‘Okami’, ‘Snowgoose’, ‘Autumnalis’ and ‘Akebono’), which overwintered in a controlled environment protected from airborne spores, developed symptoms of the disease in late spring, which indicates that dormant buds can also be a source of primary inoculum.

Disease management should include cultural practices that focus on the propagation of healthy trees and the application of Plant Protection, starting with the fall of flower petals, in order to protect emerging leaves.

The amount and timing of rainfall play an important role in the disease epidemic. The disease usually appears at the beginning of the rainy season due to the presence of airborne pathogens that require high humidity and leaf moisture for its development. Rain can help release or spread pathogens by washing spores from plant and soil surfaces (Khan et al., 2017). In rainy weather, light pink to white masses of conidia (in spots) appear on the underside of the leaf in the center. The spots turn brown and a sufficient number can cause premature defoliation and weakening of the tree. Fruits of trees that are heavily defoliated before harvesting will not be able to ripen normally and are light in color, low in soluble solids, soft and watery. The formation of flower buds and fruits of highly defoliated trees can be reduced for at least two seasons. Trees that defoliate in mid-summer are less resistant to cold and can be damaged by low temperatures in winter.

Tezcan H. (2008) discuss possible causes of disease outbreaks and the effectiveness of some pathogen control methods. After one year with fungicidal control and collection of diseased leaves on the ground with a special machine the incidence decreased from 90% to 10%.

An overview of short-term fluctuations in long-term trends will help to understand the nature and extent of the evolutionary adaptation of plants and pathogens and to determine the fate of plants in future climate change (Burdon & Zhan, 2020). The interest in the effects of climate change on the dynamics of the population of pathogens in agricultural systems is understandable. When climatic conditions are particularly favorable for the development of the disease, the harvest can be completely compromised. Major epidemics are possible, but people can improve their impact on agricultural systems by manipulating certain host factors, environmental parameters or pesticide applications.

The objective of the present study is to trace the infectious process of infection and development and to take into account the infestation index of cylindrosporiosis and shot hole disease in sour cherry cultivars, to optimize the schemes for sustainable plant protection, in the climatic conditions of Troyan.

MATERIALS AND METHODS

The study was conducted in the period 2018-2020 on the territory of RIMSA Troyan with three sour cherry cultivars ‘Oblachinska’, ‘Karneol’ and ‘M 15’, grafted on ‘Alkavo’ rootstock and planted in 2002 by trench method with local, stockpile organic fertilizing. The soil is light gray forest, with a shallow "A" horizon - 10-15 cm, poor in humus (about 1%), with a strong highly gleyed, water impermeable "B" horizon. The annual amount of precipitation for the region is on average about 750 ml. The data on temperatures and precipitation, which are essential for the development of diseases, were used by the Meteorological Station of RIMSA Troyan.

The planting scheme is 4 x 3 m and they are grown under nonirrigated conditions.
Variants of the experiment are the three cultivars and each tree is a replication (5 trees per cultivar were observed). Crown are free-growing and are maintained with annual winter prunings, the soil surface is covered with turf.
Shot hole disease - *Stigmina carpophila* (Lev.) Ell. Symptoms: At first small-sized purple spots appear on the leaves, which grow to small-sized round spots (1-6 mm) with pale brown to ocher color. The tissues around the spots acquire a reddish-brown colour in the form of a ring. In young still growing leaves, the tissues in the middle of the spots necrotize, fall off and perforations form on the leaf blade. On older tissues, the spots are larger and rarely perforated. Purple spots form on the shoots, which grow to rounded or elliptical and slightly concave spots of dark brown colour.
Brown-red spots form on the fruits of cherries and sour cherries. Damaged tissues stop growing and sagging, and the fleshy part of the fruit remains fused with the stone. Epidemiology: The fungus overwinters as mycelium and conidia in infected twigs and buds. At high humidity and temperature above 3 °C on the surface of the infected parts conidia are formed, which in the spring cause primary infections. Due to the low minimum temperature required for the development of the fungus, it is able to multiply in mild winters and dormant periods. The fungus enters the tissues through wounds, pimples and leaf prints formed during the fall, through the stomata or directly through the cuticle.
Dispersal of spores is carried out by rain, wind or insects. Water drops are needed for germination of spores. Any significant wave of infection occurs after prolonged rainy periods (Anon, 2013).
White rust - *Blumeriella jaapii* (Rehm.) v. Arx. (anamorph *Cylindrosporium hiemalis* Higgins) Hosts: Sweet cherry and sour cherry.
Symptoms: Numerous small dots appear on the upper side of the leaves, which are initially purple and later turn brown and burn. On the underside of the leaves, in the places of the spots, a deposit of whitish piles can be seen. Infected leaves begin to turn yellow around the site of damage, then fall off. In highly sensitive cherry and sour cherry cultivars, similar signs are also observed on the stalks and green fruits
Epidemiology: The fungus overwinters in fallen leaves, where ascospores and conidia form in spring. Maturing and discharging of ascospores takes place in rain and moderately warm weather and often coincides with leafing. This period lasted about a month and a half. Conidiospores are also a source of primary inoculum. Mass infections during the vegetation season are carried out only with conidia. They can be dispersed by raindrops and insects (Anon, 2013).
During the mass manifestation of the studied diseases, *Blumeriella jaapii* (Rehm.) and *Stigmina carpophila* (Lev.) Ell, samples of 200 leaves were taken, from each cultivar (variant), 5 trees (replications) from 4 directions and layers of the trees.
The infestation of the studied diseases was reported on the respective score scales (Nedev et al., 1979), according to the spotting of the leaf blade.
The Mc Kenney (1923) formula adopted in phytopathology was used to calculate the infestation index (%).
$I=\Sigma \frac{n.k}{N.K} . 100$, where:
I - infestation disease index in %;
n - number of infected leaves of the respective degree;
k - number of the degree;
N - number of degrees;
K - number of all reported leaves;
Plant protection measures include - winter, post-blossoming and two summer sprays (against aphids, fungal and bacterial diseases). During the last two vegetations, plant protection was carried out in accordance with the requirements for organic fruit production - by using sulfur and copper-containing fungicides.
The following indicators are taken into account
fruit weight and stone weight (g)
fruit size (height, average diameter) (mm)
fruit stalk length (mm)
infestation index (%) for cylindrosporiosis and shot hole disease, determined by the formula of Mc Kynney (1923).
Statistical data processing was performed with A
NOVA (Excel 2019).

RESULTS AND DISCUSSIOINS

The susceptibility to cylindrosporiosis (*Blumeriella jaapii*) (Rehm.) and shot hole disease (*Stigmina carpophila*) of sour cherry cultivars, such as 'Karneol', 'Oblachinska' and 'M 15' was studied.

The shot hole disease infestation was in the range of 6% to 15% for all variants for the three years (Figure 4).

In 2019, when 'Oblachinska' and 'Karneol' had the highest infestation index of shot hole disease, the climatic conditions during the manifestation period (end of June) were T 20°C; 106 mm of precipitation; 80% humidity (Figure 1). In 2020, the index for 'Karneol' and 'Oblachinska' was lower, as T was 19.3°C and the humidity was 80.4% (Figure 2), i.e. they were lower for pathogen development.

In 2021 (Figure 3) the temperatures at the end of June were higher (23.4°C); low air humidity 77.7% and very small amounts of precipitation 18 mm. Despite the appropriate high T, the atmospheric humidity was low and there were no necessary conditions for the development of the fungal shot hole disease. That was the lowest infestation index for the three cultivars (Figure 4). The relatively uniform low values of the index are due to the accumulated infectious background from previous years, because the plantation has been grown according to a biological scheme (excluding RH).

During the study period, 'Karneol' had the lowest rate of shot hole disease <8.7%.

'M15' had a high index of manifestation for the period of the study and showed a stronger and more constant susceptibility to shot hole disease in time.

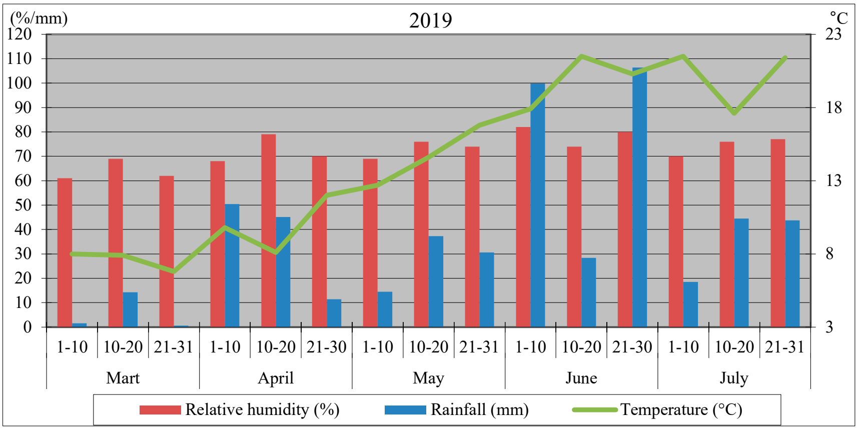

Figure 1. Climatic factors by ten days March-July (2019)

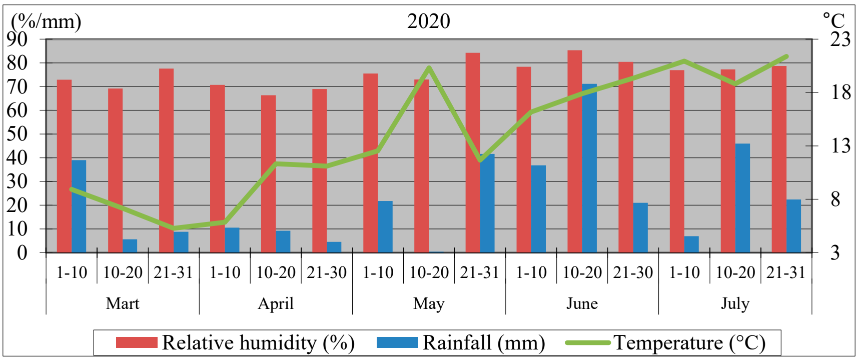

Figure 2. Climatic factors by ten days March-July (2020)

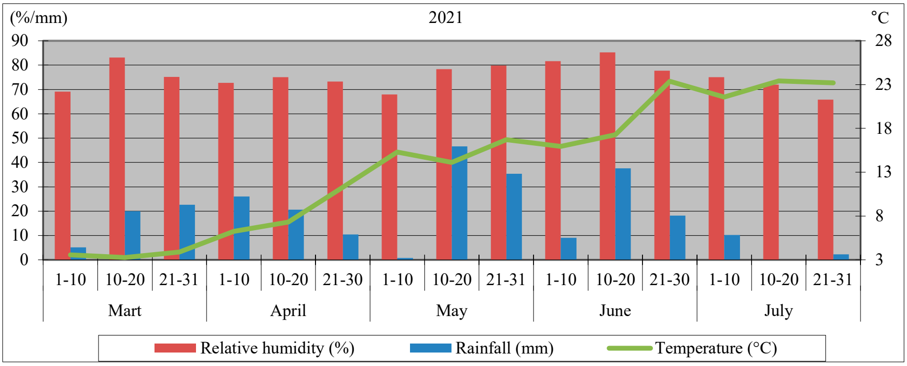

Figure 3. Climatic factors by ten days March-July (2021)

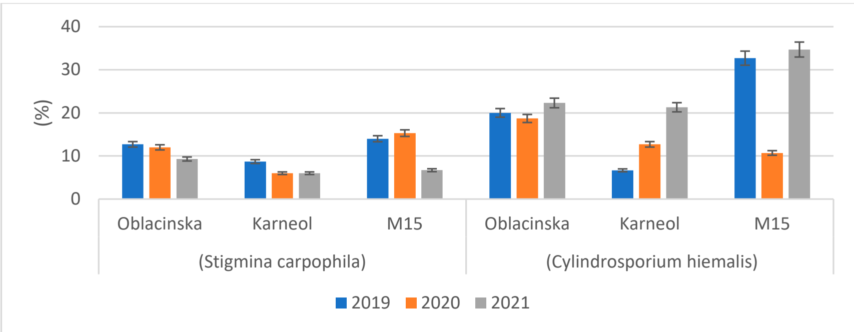

Figure 4. Infestation degree (%) by cultivars (2019-2021)

For white rust the strongest infestation index was reported in 2021 for the three cultivars (Figure 4).

2020 is characterized by low T (11.7°C), during the period of manifestation of cylindrosporiosis, which despite the high humidity (84.2%) did not allow the development of the fungus (Figure 2).

Precipitation for the three years at the end of May was about 35-45 mm, but in 2019 and 2021 there were significantly higher temperatures (>16.5°C) and humidity was 74.0-79.8% (Figures 1 and 3), and this was exactly what favours the development of the disease.

In 2019 and 2021 was the strongest manifestation of cylindrosporiosis, reported in 'M15' (32.7% in 2019; 34.7% in 2021), and in 2021 in all three cultivars was the highest % (Figure 4).

The present study corresponds to the data of Khan et al. (2017), when the average atmospheric temperatures in Kashmir, maximum and minimum were 27.30°C and 8.88°C, respectively, with average relative humidity, maximum and minimum respectively 71.21 and 49.21%, as the maximum value of the infestation index was in the second two weeks of June, which was the favourable temperature, precipitation and relative humidity for the manifestation of the disease. The percentage of leaf disease intensity shows a positive correlation with the average maximum relative humidity and the average precipitation. Thus, both high humidity, optimum temperature and rainfall significantly favor the development of *Blumeriella* leaf spot disease in the Kashmir Valley. The relationship between the development of the disease and meteorological factors reveals that the percentage of disease intensity is strongly and positively correlated with the average maximum relative humidity (r = 0.69), followed by the average precipitation (r = 0.73) (Khan et al., 2017).

Climate change will affect the measures that farmers use to effectively manage disease, as well as the feasibility of certain crop systems in certain regions (Yigal & Ilaria, 2014).

Biometric measurements of the fruits were made. The fruit weight (g) is extremely variable during the 3 years of the study, the smallest was found in ‘Oblachinska’ (2.50-2.99 g), ‘Karneol’ had a fruit weight of 3.74-5.72 g, ‘M 15’ from 4.39 to 7.20 g (Table 1).
It is noteworthy that in 2019 the fruits of the three cultivars had more mass than in the next 2 years, as the lowest weight was registered in 2021. The largest relative share of stone was found in the last year, as for ‘Karneol’ it was 9,98%, and the lowest values were in 2020 (8.08% for ‘Oblachinska’; 2.78% for ‘M 15’) (Table 1).

Table 1. Reproductive characteristics of fruits

	Fruit weight (g)	Stone weight (g)	Relative share of stone (%)	Height (mm)	Diameter (mm)	Fruit stalk length (mm)
2019	X ± St Dev					
‘Oblachinska’	3.46±0.27	0.28±0.07	8.09	15.16±0.86	17.27/16,12	29.66±2.08
‘Karneol’	6.07±0.94	0.44±0.05	7.25	19.90±0.87	22.68/19,83	34.12±2.94
M-15	6.52±0.30	0.42±0.06	6.44	17.91±0.55	22.65/20,34	42.59±3.65
LSD 0,05	*0,57*	*0,06*		*0,75*	*1,22/0,98*	*2.86*
LSD 0,01	*0.77*	*0.08*		*1.01*	*1.65/1.33*	*3.87*
2020						
‘Oblachinska’	2.60±0.49	0.21±0.05	8.08	14.89±0.68	17.34/15.29	24.98±2.59
‘Karneol’	5.53±0.59	0.35±0.05	6.33	16.46±2.86	18.41/16.94	31.04±4.31
M-15	7.20±0.60	0.20±0.04	2.78	17.21±0.71	22.54/20.48	31.88±1.50
LSD 0,05	*0.54*	*0.05*		*1.69*	*1.60/1.42*	*2.93*
LSD 0,01	*0.73*	*0.06*		*2.28*	*2.17/1.92*	*3.96*
2021						
‘Oblachinska’	3.11±0.34	0.27±0.06	8.68	15.09±1.05	20.09/17.81	24.55±2.06
‘Karneol’	4.51±0.45	0.45±0.05	9.98	18.50±0.80	20.09/17.81	32.19±4.82
M-15	4.39±0.34	0.34±0.05	7.74	16.68±0.24	19.81/18.38	37.99±2.98
LSD 0,05	*0.37*	*0.05*		*0.75*	*0.73/0.76*	*3.37*
LSD 0,01	*0.49*	*0.07*		*1.01*	*0.99/1.03*	*4.55*

The length of fruit stalks is different, for Oblachinska 25-29 (mm), for ‘Karneol’ 31-34 (mm), and for ‘M 15’ - 310.04 (mm) in 2020, 43.0 (mm) in 2019.

CONCLUSIONS

The strength of the infestation and the degree of manifestation of fungal diseases, compared to the studied sour cherry cultivars are directly dependent on meteorological factors (temperature, precipitation, humidity).
The strength of the pathological process is determined by the interaction of meteorological factors and the immunity of the cultivars. On this basis, annual models can be prepared for preliminary forecasting of the intensity of the manifestation of fungal diseases and thus to carry out adequate plant protection.
‘Karneol’ had the lowest susceptibility to both diseases studied. The lowest infestation index of shot hole disease was found for the three years of the study (6.7-21.3%). The average fruit weight was 5.5 g, which is suitable for fresh consumption, with an extremely balanced taste and with possibilities for organic cultivation.
For ‘M15’ the infestation index was slightly higher, but was significantly below 50%, had attractive fruits with greater fruit weight, abundant fruit bearing and can be included in the cultivars list of modern sustainable technologies.
For ‘Oblachinska’ the infestation index was relatively high and with constant values, which defines it as susceptible to white rust (cylindrosporiosis).

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THE RESISTANCE OF PEACH TO THE ATTACK OF SOME PATHOGENS IN CLIMATE CHANGE CONDITIONS

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Abstract

The studies were carried out over a period of four years (2018-2021) in a plot at the Research Station for Fruit Growing (RSFG) Constanta. This paper presents the manner in which the ten peach tree cultivars reacted to the climate changes and the attack of pathogen agents: Taphrina deformans Berk et Tull, Cytospora cincta Sacc, Monilinia laxa and Monilinia fructigena Aderh Ruhl Honey. The cultivars with resistance, tolerant (T) to Taphrina deformans (without attack), in the studied period were 'Mimi', 'Catherine Sel.1' and 'Raluca'. The 'Filip' cultivar showed sensitivity (S) towards the Monilinia laxa in 2019 and 2020. As far as for the Cytospora cincta pathogen the cultivars tolerant (T) were 'Collins' in all studied years and 'Filip' in 2018 and 2019. The obtained results demonstrate the importance of choosing the assortment of cultivars, taking into account the favourability of the area, as well as the climate and soil conditions.

Key words: *Cytospora cincta, Monilinia laxa, climate changes, natural infection.*

INTRODUCTION

The southeastern part of Romania is the area that offers the most favorable ecoclimatic conditions for peach cultivation. Unfortunately, this area is also favorable for the development of particularly harmful pathogens such as the fungus *Taphrina deformans* Berk et Tull, *Cytospora cincta* Sacc, *Monilinia laxa* and *Monilinia fructigena* Aderh Ruhl Honey. The territory of the RSFG Constanta is influenced, from a climatic point of view, both by the Black Sea as well as the Danube, which means that spring arrives later, summers are droughty and autumns are long and warm. The introduction within cultures of peach tree cultivars which are more resistant to the attack of the most harmful pathogen agents offers numerous economic advantages and is consistent with the protection of the environment. Therefore, the fruit practice on the one hand and especially the peach breeding program from the Research Station for Fruit Growing Constanţa, set up as a priority objective, the promotion of cultivars and the identification of possible genitors with genetic resistance to *Taphrina deformans* fungus attack.

Cytospora was first introduced by Ehrenberg (1818), which is one of the most important pathogenic fungi of hardwoods and coniferous trees in the world (Adams et al., 2005; Fan et al., 2020).

About 150 species epithets of *Cytospora* are associated with dieback and stem canker on over 130 species of woody hosts (Spielman, 1985; Adams et al., 2005; Kirk et al., 2008; Fan et al., 2020). Trandafirescu (1998, 2007) has studied the resistance to the *Taphrina deformans* within the peach tree and nectarine tree species, detailing the research for each cultivar from the national peach tree collection which was planted in 1981.

According to the estimations of the weather forecasts, there have been presented in the frame of the 4th report of the International Committee for Climatic Changes in the year 2007, the whole Europe and implicit Romania will be confronted in future with a process of global warming, characterized by increasing of temperatures with 0.5 - 1.5°C for the period

2020- 2029 and with 2- 5°C for the period 2029 -2099. In the period 2090-2099 Romania will confront with pronounced drought during the time of summer. Researches from many countries, in the frame of climatic research methodology have the approached aspects regarding climatic changes effects on growth and development of some fruit tree species (Chmielewski and Rotzer et al., 2002; Olensen and Bindi, 2002; Sunley et al., 2006, Chitu et al., 2010).

The introduction within cultures of peach tree cultivars which are more resistant to the attack of the most harmful pathogen agents offers numerous economic advantages (the reduction of production losses, the diminishing of expenses regarding pesticides, the fuel and energy required for the application of treatments, the prolonging of the culture's exploitation period) and is consistent with the protection of the environment(the reduction of the soil's ramming due to the fact that the tractor crosses the orchard a smaller number of times, the reconstruction of the soil's structure, the reduction of the pollutionof the environment and of the fruit, as well as the protection of the consumers' health).

The purpose of this paper is to show the manner in which certain peach tree cultivars reacted to to the attack of pathogens and to recommend said cultivars for extension in production, given the fact that in our country as well as worldwide the main objective is not only the obtainingof fruit of a higher quality, with an elevated yet constant productivity of the trees, but which are also resistant and/or tolerant to the main disease of the species.

MATERIALS AND METHODS

The experimental plot is situated within the RSFG Constanța, with its headquarters in the village of Valu lui Traian, Constanța county, Dobrogea region, Romania. The geographical coordinates are: 44°10' North, 28°29' East, 70-72 m altitude.

During the period of 2018-2021, 10 peach tree genotypes were studied, organised in a demonstrative plot that was created in 2011. The plot has 20 trees per row, with a planting distance of 4 m × 4 m (625 trees/ha), with the canopy shape a vase and the rootstock a wild Tomis 1. Among the studied cultivars there, as well as cultivars promoted in the regional and national assortment, these are: 'Purpuriu', 'Mimi', 'Catherine Sel.1', 'Springold', 'Raluca', 'Cardinal', 'Collins', 'Redhaven', 'Florin' and 'Filip'. The system used for the soil management system was with cultivated strips both between the rows as well as in the row. The soil is a calcareous chernozem (CZka), with a loamy texture and a high, alkaline pH (8.2) in its entire profile. All in all, the climatic conditions were favourable to the growth and fructification of the peach trees.

The study focused on how certain peach tree cultivars reacted to the change in climatic conditions in the winters of the above-mentioned years. The conclusion was that the resistance of the peach tree cultivars differs from year to year because of the climatic changes that have occurred throughout the past few years and that depends on the severity of climatic accidents. During the studied period 2018-2021 the climatic data were recorded with the aid of an automatic meteorological station type WatchDog and were processed as daily averages.

Observations were carried out concerning the behaviour of certain peach tree cultivars towards the attack of the main pathogen agents: *Taphrina deformans* Berk et Tull, *Cytospora cincta* Sacc, *Monilinia laxa* and *Monilinia fructigena* Aderh Ruhl Honey. *Taphrina deformans* causes the blistering of the peach tree's leaves (Figure 1).

Figure 1. The blistering of the leaves -*Taphrina deformans:*
a) Symptoms on the leaves; b) Symptoms on the fruit

The most characteristic symptoms can be noticed on the leaves, although the branches, flowers and fruit can also be attacked. The leaves of the attacked trees become thicker and creased (blistered), the attack being more evident on younger leaves at the tip of the sprouts. The attacked leaves are twice as long and wide (Figure 1a) than the healthy ones and they fall earlier, thus defoliating the tree, which means that the development and the maturing of the branches is disturbed. The fruit also have a weak development and they fall before becoming ripe; sometimes white-yellowish spots can be observed, slightly prominent and with irregular contours (Figure 1b). The young sprouts which are infected stop growing; they remain short, thicker at the base and with leaves only at the tip.

Cytospora cincta, which causesthe perennial cancer of the sprouts is generalised in peach tree crops which have suffered injuries on the bark of the sprouts as a result of other pathogen agents' attacks, such as *Taphrina deformans* or fusicoccum, the attack of insects orhits caused by hail. *Cytospora* installs itself on the already existent wounds and continues its attack on the branches; subsequently, the bark and the wood becomes dry and in some cases ulcerations occur. *Monilinia laxa*, which causes moniliosis or the monilinial drying of the branches manifests itself during spring on all aerial organs of trees of all ages through the withering of the flowers and the drying of the vegetative buds and the sprouts, being accompanied by gumma leakages (Figure 2).

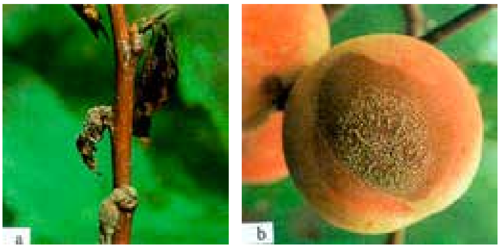

Figure 2. *Monilinia:*
a) Monilinia laxa - the monilinial drying of the branches;
b) Monilinia fructigena - fruit moniliosis

On multi-annual branches we can notice the defoliation and the necrosis of the wood. Upon a weaker attack the trees are able to recover, while upon a stronger one they manifest typical decaysymptoms and, in time, they die. *Monilinia fructigena* causes the moniliosis of the fruit during the period in which the latter begin ripening, the result being that the latter fall androt. In dry weather they remain on the tree, becoming the source of infection for the following year.

From a technological point of view, 8-10 treatments with insecticides and fungicides have been applied each year in the experimental plots, so as to protect against diseases and pests. The attack of these pathogen agents has serious consequences both on the fruit production, as well as on the physiological balance of trees, leading to their debilitation.

These observations were focused on the evolution of the diseases on the leaves, fruit and shoots following. The climatic conditions in this area are favourable to the peach tree culture. Due to its geographic position, Dobrogea is part of the agro-climatic area I, which is warm and droughty and characterised by the most generous thermal resources and the lowest amount of precipitations in comparison to other regions of the country. The behaviour of peach tree cultivars towards the attack of the pathogen agents *Taphrina deformans* Berk et Tull*, Cytospora cincta* Sacc*, Monilinia laxa* and *Monilinia fructigena* Aderh Ruhl Honey - was studied under conditions of natural infections, according to the test created by Crossa Raynaud (1969). The evaluation technique consisted in writing down the frequency of the attacked organs and the intensity with which the symptoms manifested themselves and these two aspects were utilised in assessing the behaviour of the cultivars. The field observations were centred on the calculation of the pathogens' frequency (F %) and intensity (I) on different tree organs such as: leaves, flowers, shoots, branches and fruits. For the intensity of the diseases marks were granted on a scale from 0 to 4.

Depending on the frequency and intensity of the disease, the studied cultivars and hybrids were categorised into 4 classes and 8 groups of resistance according to the following scale (Table 1).

Table 1. Cultivar Categorisation into Classes and Groups of Resistance

Resistance class	**Resistance group**	**Frequency (F%)**	**Intensity (I%)**
1 = tolerant (T)	1	0	0
2 = medium resistance (MR)	2	0.1-11.0	+
	3	11.1-25.0	+
3 = sensitive (S)	4	25.1-34.0	++
	5	34.1-50.0	++
4 = very sensitive (VS)	6	50.1-59.0	+++
	7	59.1-75.0	+++
	8	75.1-100	++++

WA = cultivars without attack (F%= 0 and I= 0); T = tolerant cultivars (F%= 0.1-5% and I= +); WeA = weakly attacked cultivars (F%= 5.1% - 10% and I= +); MA = moderately resistant cultivars (F%= 10.1% - 25% and I= +); S= sensitive cultivars (F%= 25.1 – 50% and I= ++++); VS = highly sensitive cultivars (F%= 50.1% - 100%, I= ++++)

RESULTS AND DISCUSSIONS

The comeback frosts in March-April, which occur after a relatively warm period are more dangerous than those that occur during the obligatory resting period in December-January. Nevertheless, the major climatic changes that have taken place over the past few years have had a significant negative influence on the onset of flowering and fruit setting and implicitly, on the peach tree production.

Analysis was carried out in order to determine the loss of flowering buds caused by temperature variations during winter and the low temperatures during the day.

In 2021 the Springold cultivar recorded the greatest losses caused by frost – 61%; though the trees apparently blossomed abundantly, the pistils were blackened (due to frosts) and there was no fruit setting. Though some of the flowers did indeed show fruit setting, upon careful inspection the core ovary was also blackened. The Cardinal cultivar recorded 54% losses in 2021, while the smallest percentage of affected fruit occurred in the Catherine Sel.1 cultivar - 12% in 2021 (Figure 3).

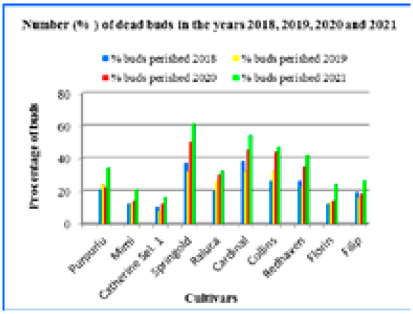

Figure 3. Procentage of peach tree flowering buds perished due to frosts during the winter of 2018, 2019, 2020 and 2021 at Valu lui Traian, Constanța

The peach tree cultivars showed a good resistance to frost during the winters of the four studied years, as follows: Catherine Sel. 1 - 12%, Mimi - 15%, Florin - 16% and Filip - 20%, (Figure 4).

Table 2 presents the relative sensitivity of the ten cultivars from the demonstrative plot created within the laboratory responsible with improving the peach tree concerning the attack of the pathogens: *Taphrina deformans* Berk et Tull, *Cytospora cincta* Sacc, *Monilinia laxa* and *Monilinia fructigena* Aderh Ruhl Honey under natural conditions of infection.

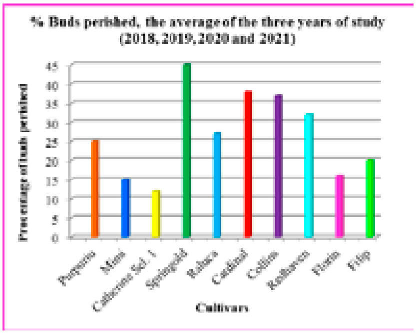

Figure 4. Procentage of peach tree flowering buds perished because of frosts (average over the three years), Valu lui Traian

The analysis of the data in this table highlights a variation in the peach tree cultivars' behaviour towards a pathogen or another. The observations that were carried out under conditions of natural infection with *Taphrina deformans* Berk et Tull for the ten peach tree cultivars displayed the different degrees of resistance.

Table 2. The behaviour of peach tree cultivars towards the attack of the main pathogens in the period 2018, 2019, 2020 and 2021

No.	Cultivar	Year	Intensity of attack (note)			
			Taphrina deformans	*Cytospora cincta*	*Monilinia laxa*	*Monilinia fructigena*
1.	Purpuriu	2018	MR	S	T	T
		2019	MR	S	MR	T
		2020	MR	MR	T	T
		2021	MR	MR	MR	T
2.	Mimi	2018	T	S	T	T
		2019	T	T	T	T
		2020	T	T	T	T
		2021	T	T	T	T
3.	Catherine Sel.1	2018	T	S	T	T
		2019	T	T	T	T
		2020	T	T	T	T
		2021	T	T	T	T
4.	Springold	2018	S	MR	S	S
		2019	S	S	S	S
		2020	MR	MR	MR	MR
		2021	MR	S	MR	MR
5.	Raluca	2018	T	S	T	T
		2019	T	T	T	T
		2020	T	T	MR	MR
		2021	T	T	MR	MR
6.	Cardinal	2018	MR	MR	MR	MR
		2019	T	MR	MR	MR
		2020	MR	T	MR	MR
		2021	T	MR	MR	MR
7.	Collins	2018	MR	T	S	S
		2019	MR	T	S	S
		2020	MR	T	S	S
		2021	MR	T	S	S

8.	Redhaven	2018	S	MR	MR	MR
		2019	S	MR	T	T
		2020	MR	MR	T	T
		2021	MR	T	T	T
9.	Florin	2018	S	MR	MR	MR
		2019	S	MR	MR	MR
		2020	MR	MR	MR	MR
		2021	MR	MR	MR	MR
10.	Filip	2018	S	T	T	T
		2019	S	T	S	T
		2020	S	S	S	T
		2021	S	S	T	T

The Mimi, Catherine Sel.1 and Raluca cultivar was Tolerant (T) in the studied years 2018, 2019, 2020 and 2021 towards *Taphrina deformans*. Purpuriu and Collins displayed a Medium Rezistance (MR) in the studied years 2018, 2019, 2020 and 2021. Redhaven and Florin displayed a Medium Rezistance (MR) in the studied years 2020 and 2021. None of the studied cultivars could be introduced in the classes Very Sensitive (VS).

The Collins cultivar was Tolerant (T) in the studied years 2018, 2019, 2020 and 2021 towards *Cytospora cincta*. The Mimi, Catherine Sel .1 and Raluca cultivars, manifested a good resistance towards *Cytospora cincta* in the studied years 2019, 2020 and 2021. None of the studied cultivars could be introduced in the classes Very Sensitive (VS).

In the studied period 2018-2021 both Mimi and Catherine Sel.1 manifested a good resistance towards *Monilinia laxa*, being basically in the class Tolerant (T). The two studied cultivars displayed an increased resistance towards the attack of *Taphrina deformans* in all studied years.

That is why fruit-growing practices on the one hand and especially the RSFG Constanta programme for improving the peach tree on the other highlighted as main objective for researches the promoting of cultivars and the identification possible genitors with genetic resistance towards the attack of the pathogens *Taphrina deformans* Berk et Tull, *Cytospora cincta* Sacc, *Monilinia laxa and Monilinia fructigena* Aderh Ruhl Honey.

CONCLUSIONS

The results show that the frosts in the winters of 2018, 2019, 2020 and 2021 affected the peach tree species in variable percentages according to the cultivar (approx. 12-45%).

The greatest losses as far as flowering buds are concerned were recorded by he Springold cultivar in 2021 (61%), while the smalles were recorded by 'Catherine Sel.1' in 2019 (8%).

Concerning the attack of the the *Taphrina deformans* the following cultivars were comprised in the class Tolerant (T): 'Mimi', 'Catherine Sel.1' and 'Raluca' cultivar in the studied years 2018, 2019, 2020 and 2021. The three studied cultivars ('Mimi', 'Catherine Sel.1' and 'Raluca') manifested an increased resistance towards the attack of *Taphrina deformans.*

Concerning the attack of the the *Cytospora cincta* the Collins cultivar was Tolerant (T) in the studied years 2018, 2019, 2020 and 2021.

In the studied period 2018-2021 both 'Mimi' and 'Catherine Sel.1' manifested a good resistance towards *Monilinia laxa*, being basically in the class Tolerant (T).

When choosing the assortment of cultivars, one must take into account the favourability of the area for the setting up of fruit-growing plantations.

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THE EFFICACY OF SOME ORGANIC PRODUCTS IN THE CONTROL OF BROWN ROT (*MONILINIA* SPP.) IN EUROPEAN PLUM (*PRUNUS DOMESTICA* L.)

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Abstract

Organic crops in fruit growing are constantly increasing due to the market demand for residue-free products. In this context, successful organic crops require an effective control of major diseases and pests. Brown rot caused by Monilinia spp. is one of the most important and common diseases with great economic impact in plum crop. The objective of this study was to evaluate the efficacy of some organic products in brown rot control into a plum orchard by assessing disease incidence when compared with treatments with conventional fungicides. An experimental scheme of phytosanitary treatments (organic and conventional) was carried out on two plum cultivars ('Stanley' and 'Reine Claude d'Althan'), throughout two consecutive vegetative periods (2020-2021), comprising a total of six treatments with fungicidal effect. The experience was divided in three blocks: organic, conventional and untreated control. According to the results obtained along the two years of study, the average frequency of brown rot damage for both plum cultivars was 9.5% in the conventional variant, 14.3% in organic variant, while untreated variant recorded 23.1%. The results revealed a agood potential for brown rot control by usingthe organic scheme treatments proposed but it's need to be confirmed in a long term study so that to become a suitable and reliable candidate to replace the conventional one.

Key words: *brown rot, control, disease, organic crop, plum.*

INTRODUCTION

Organic cultivation of fruit trees has started to expand due to the increasing consumer demand for organic fruit with no chemical residues (Neelesh & Attika, 2015; Maxim, 2008; Hamm et al., 2002). This cropping system has also been promoted in Romania through the European non-reimbursable funds granted under Measure 4.1.a of the National Rural Development Program 2014-2020. In this context, where fruit tree acreage is constantly expanding in the organic system, some shortcomings are also reported (Sisquella et al., 2014; Lopez-Reyes et al., 2013). The most important issues are related to effective crop protection, since the number and actual effectiveness of new products accepted in organic farming is low (Lacey et al., 2015; Manoj, 2017). Therefore, farmers who practice this type of system are exposed to significant economic losses due to disease and pest infestation.

Plum is one of the most important and widespread fruit crops in Romania, with an annual production of over 750,000 tons, ranking the second in the world after China, according to the latest technical data (FAO, 2020). The plum crop is affected by various harmful organisms that can endanger the fruit production if an efficient protection against diseases and pests is not carried out. Among the most common diseases of plum that causes significant losses is brown rot (Byrde & Willetts, 1977; Van Leeuwen, 2000; Hrusti'c et al., 2012). In Europe, the most encountered species from the genus *Monilinia* are: *Monilinia fructigena*, *Monilinia laxa* and *Monilinia fructicola,* the last one being a quarantine pathogen in Europe (EPPO, 2007; Usall et al., 2015). This disease affects flowers, leaves, shoots, and fruits and has a negative

impact on the quality and quantity of production (De Cal et al., 2009; Byrde & Willetts, 1977). Without an adequate phytosanitary protection, this mycosis can lead to complete yield loss in years with extremely favorable climatic conditions for the fungus. Local microclimate is an element that plays a crucial role in the initiation and maintenance of the disease (Miessner & Stammler, 2010; Hong et al., 2000; Hong & Michailides, 1999).
The aim of this study was to verify the efficiency of some organic products in the control of brown rot in European plum.

MATERIALS AND METHODS

During vegetative periods of 2020-2021, at Fruit Research & Development Station Bistrita was tested an experimental model in order to evaluate the impact of an ecological treatment scheme to control brown rot. The experimental plot was established into a plum orchard eight years old with two cultivars: 'Stanley' and 'Reine-Claude d'Althan'. The design of experiment consisted in three blocks: organic, conventional and untreated variant, each block included a number of nine trees of each cultivar: 'Stanley'and 'Reine-Claude d'Althan', divided into three repetitions. The treatment scheme included six sprays with fungicides, in both organic and conventional variants. The products were applied by spraying with an atomizer. The organic products with fungicide effect tested were the following: 20% copper sulfate, 60% *Mimosa tenuifolia* and 20% citrus extract, 20% citrus seed extract and mimosa tree bark extract 80%. The chemicals products with fungicid effects tested in this study were: Copper hydroxide + 50% Cu metallic copper, 26.7% boscalid + 6.7% pyraclostrobin, tebuconazole 250 g/L, mancozeb 80%, diphenoconazole 250 g/liter , chlorothalonil 500 g/l. The concentrations used for all products applied in the experiment were in accordance with the manufacturer's recommendations.
To determine the damage degree of brown rot symptoms on fruits, the methodology used in plant protection was applied. The frequency (F%) was noted for each repetition of each variant on 300 fruits, than the average for each variant was calculated. The statistical differences between cultivars and treatment variants were checked using One-way Anova test (Microsoft excel).

RESULTS AND DISCUSSIONS

The results obtained along the vegetative periods 2020-2021 depend on treatment variants in terms of frequency of brown rot infection, being influenced by climatic conditions. Abundant rainfall correlated with humidity and positive temperatures created a favorable environment for mycosis development. Thus, according to the climatic conditions registered in FRDS Bistrita, 2021 proved to be a more favorable year for the occurrence and development of brown rot than 2020.
The dynamics of climatic factors in the two years of study did not affect the way in which the plant protection treatments were carried out, since they were adapted to the weather conditions in order to achieve the maximum effectiveness. The results of this study regarding the efficacy of tested products on the occurrence of brown rot in plum orchards are shown in figure 1.

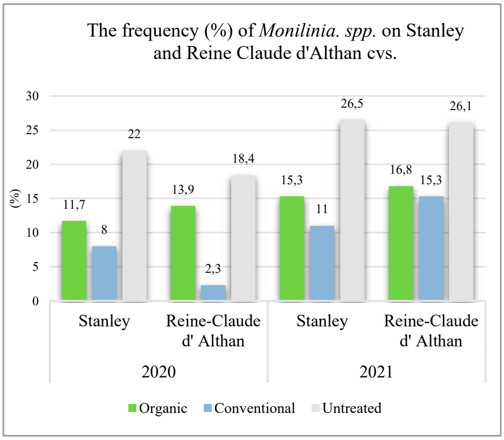

Figure 1. Incidence of brown rotin plum cultivars (2020-2021)

The incidence of *Monilinia.* spp. in the organic variant of 'Stanley' in 2020 was 11.7%, close to the conventional variant, while the untreated control variant had an incidence twice as high. Regarding 'Reine-Claude d'Althan', the difference between the organic and conventional variants was higher.

According to the 2020 results, which were quite similar to those of 2021, the incidence of brown rot in the organic variant was close to that in the conventional, whereas in the untreated control was almost twice as high.
The average frequency over the two years of the study once again highlighted differences depending on the treatment variant and cultivar (Figure 2).

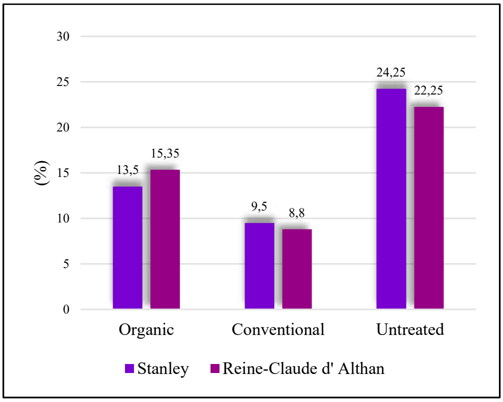

Figura 2. The average frequency of *M. spp.* along two consecutive vegetative periods (2020-2021).

The One-way Anova test performed on the average values of the frequency of brown rot revealed the existence of significant differences between both treated variants (organic and conventional) and the untreated control in the year 2021 (F value is bigger than critical F value or 2 df and α=0,05 in both cases). Regarding the year 2020, only treated trees by applying conventional scheme displayed significantly less damage than the untreated control. In this case calculated F value (19.93) is bigger than critical F value (18.51) for 2 df and $\alpha = 0.05$ while for the organic variant the calculated value is smaller and therefore the differences are not significant. For both years there were no significant differences between the two treated variants for 2 degrees of freedom and $\alpha = 0.05$.
Our data show increased damage in 2021 in all three variants and both cultivars and this could be due to the bigger number of rainy days in August 2021 (14 days) than in August 2020 (6 days) and a total of 35 rainy days in the second part of the summer 2021 compared to 28 rainy days in the same period of 2020 (July-September). Rainy days and a warm weather in preharvest period are providing optimal conditions for the fungus to spread and develop (EPPO, 2020). As Figure 3 shows, the year of 2021 offered better conditions for the fungus.

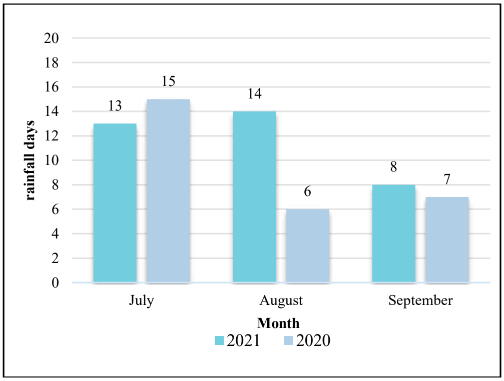

Figure 3. Distribution of the number of days with precipitations in the second part of the summer, in the two years 2020-2021

CONLUSIONS

The organic scheme treatments applied in our experimental plot against *Monilinia* spp. revealed its potential for disease control, encouraging the ecological way of brown rot control. However, this kind of scheme treatments need to be confirmed in a long term study so that to become a suitable and reliable candidate to replace the conventional one.

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PRELIMINARY RESULTS REGARDING THE BEHAVIOUR OF DIFFERENT PEACH VARIETIES UNDER DIFFERENT PLANTING DENSITY

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Abstract

The paper shows preliminary results of the study regarding the behaviour of peach varieties cultivated in intensive system, aiming to find the best solution in terms of economic and sustainability results. The study was carried out on plantation established in 2019 at FRDS Băneasa, farm Moara Domnească, county Ilfov in the Vlăsiei Plain. In 2019, three peach varieties ('Catherine Sel. 1', 'Filip', 'Sprincrest') with different ripening periods were planted. Rootstock used: Tomis 1. The tree canopy is: Bi-Baum®. The peach trees were planted at 4,0×1.5 m, 4.0×2.0 m and 4.0×2.5 m, upon a randomized block design, with drip irrigation. Between the rows, soil was kept tilled and without grass. In 2020 and 2021 measurements were made on early stage growth of tree for: the trunk diameter increase, the shoot length and the trunk cross-sectional area of tree. During our study we found that the planting distance influenced especially average shoot length increase and average trunk diameter and average shoot length are linked together. The study will continue especially to find out the density influence on productivity.

Key words: *peach, varieties, densities, growth dynamic.*

INTRODUCTION

Peach cultivation has been attested in our country since the 14th and 15th centuries according to a series of documents mentioned in Pomologia vol. I (1963), in the whole hilly area of Moldovia, Wallachia and Transylvania, along with other species (Ghena et al., 2010). Peach [*Prunus persica* (L.) Batsch] is a thermophilic fruit tree species that is highly appreciated in Romania, but its production area is relatively restricted from de climate point of view (Septar et al., 2021).

Fruit Research and Development Station Băneasa has a history of studying this specie because it is located in the southeast of the country, an area suitable for peach cultivation.

According to the FAO ranking, regarding the dynamics of peach production worldwide, we can realize that Romania is not in the first positions, both in terms of production and cultivated areas.

The countries that occupy the leading positions in this top have the advantage of the climatic factor with which Romania cannot compete. In order to revitalize the current situation of peach cultivation in our country and to keep up with international trends regarding the strategic objectives of the research-development-innovation system for the field of horticulture, this paper aims to present the preliminary results of the study regarding the behaviour of peach varieties cultivated in intensive system, with the aim of finding the best solution in terms of economic and sustainability results.

Traditional orchards involve long planting distances, which allows the trees a wide and vigorous development.

The conventional orchard system for growing peaches is the Open Vase (DeJong et al., 1999; Çetinbaș et al., 2021). Traditionally peaches are planted in low density (6 × 6 m, 7 × 7 m) resulting low yield per unit area (Lal et al., 2018). These practices are no longer advantageous today, as the resource crisis is increasingly being discussed, with land and labour becoming more expensive and less available.

The different planting system have been successfully demonstrated in different fruit crops: peach and nectarine (Lal et al., 2018), apricot (Guerriero & Scalabrelli, 1989; Kumar et al., 2013; Moloșag et al., 2021), apple

(Comănescu et al., 2012), pear (Machado et al., 2014), cherry (Stănică and Eremia, 2014). Tree cultivation systems are constantly evolving, today there is a great diversity among them, each with its advantages and disadvantages. Super high-density (SHD) systems also known as hedgerows (Diez et al., 2016).
Various studies have been made to find out the effect of different training systems, rootstocks and planting systems on early fruit yield and quality for peach growing (DeJong et al., 1999; Farina et al., 2005; Caruso et al., 2015; Sobierajski et al, 2019; Souza et al., 2019; Çetinbaş et al., 2021).
The advantages of these systems consist in an improved orchard management, by satisfying the need of early production (Robinson, 2007), minimum pruning strategies, light interpretation and distribution (Robinson, 2007; Hoza et al., 2015) and improved tree canopies.

MATERIALS AND METHODS

We conducted the study at the Experimental Base Moara Domnească, within the Research and Development Station for Fruit Growing (RDSFG) Băneasa, located N-E of Bucharest in Afumați, Ilfov County, part of Vlasiei Plain, a subunit of the Roman Plain (44°50' Northern latitude and 26°24' Eastern longitude and 70 m above the sea level). The annual mean temperature is 12°C and the total annual amount of precipitation is ranging between 550 and 600 mm, the maximum occurring between May and July, torrential rains being common. The dominant air circulation direction is from the East and North-East in winter, and from the West in the rest of the year, with a maximum wind speed of 12.6-14.4 km/h and the zonal soil type reddish luvisol. In the depressed areas and in the crevices there are reddish luvisols and stagnosols.
Three peach varieties ('Catherine Sel. 1', 'Filip', Sprincrest') with different ripening periods: were planted in 2019. All peach varieties were grafted on rootstock: Tomis 1. The canopy that was considered adequate for testing is: Bi-Baum®. The apricot trees were planted at 4.0 × 1.5 m (1.666 trees ha^{-1}), 4.0 × 2.0 (1.250 trees ha^{-1}) m and 4.0 × 2.5 m (1.000 trees ha^{-1}), upon a randomized block design. The irrigation system is provided by drip pipes, with a flow rate of 1.6 l/hour-1.75 l/hour. Between the rows, the soil was kept tilled and without grass.
Our experience is a bifactorial one and is carried out in order to observe the agrobiological potential of the varieties studied, aiming to intensify the cultivation technologies, through high density.
The chosen rootstock is Tomis 1, a generative Romanian rootstock for peaches, obtained by selection in 1979 by Indreiaş Alexandra, approved in 1997. It has good affinity for grafting with all varieties in the assortment, induces medium vigour, fruiting precocity and good fruit productivity and quality of grafted varieties.
In order to identify the most suitable variety and planting distance with the final goal of crop intensification, corroborated with the degree of maximization of the tree density, determinations were made on early stage growth of tree. The determination of observed characteristics was made in 2020 and 2021, at the end of the growth cycle, with electronic calliper and roulette for shoot length. Several tree growth indicators were analysed: the average trunk diameter increase (ATDI, mm), the average shoot length increase (ASLI, mm) and the trunk cross-sectional area of tree (TCSA, cm^2) was calculated by using formula TCSA = Girth2/4π (Westwood et al., 1963).
The collected data were processed with the facilities of MSExcel 2010 and are presented as tables and charts. The tables include statistical indicators as average, standard deviation and variation coefficients.

RESULTS AND DISCUSSIONS

A closer look in Tables 1 and 2 reveals the influence of planting distance on the three synthetic growth indicators: the average trunk diameter increase (ATDI, mm), the average shoot length increase (ASLI, cm) and the trunk cross-sectional area of the tree (TCSA, cm^2).
When the peach was planted at 1.5 m apart in the trees line, the (ATDI, mm) was 1.20 mm per season (STDEV=1.1348; VAR=94.5641), with the lowest increase 0.13 mm on 'Filip' variety and the higher increase 2.39 mm on 'Springcrest'.

Table 1. Annual growth dynamic. Average shoots length increase related to the planting distance

Variant	Intra-row planting distance [m]	Variety / Rootstock	Average shoots length [cm]	Average shoots length [cm]	*Average shoots length increase [cm]*
V1	1,5	CATHERINE SEL. I / TOMIS 1	43,38	63,63	20,25
	1,5	FILIP / TOMIS 1	24,00	52,25	28,25
	1,5	SPRINGCREST / TOMIS 1	15,88	30,50	14,63
Indicators		**AVG**	**27,75**	**48,79**	**21,04**
		STDEV	*14,1283*	*16,8311*	*6,8469*
		VAR	*50,9129*	*34,4959*	*32,5398*
V2	2,0	CATHERINE SEL. I / TOMIS 1	30,25	47,25	17,00
	2,0	FILIP / TOMIS 1	30,88	45,75	14,88
	2,0	SPRINGCREST / TOMIS 1	35,00	49,88	14,88
Indicators		**AVG**	**32,04**	**47,63**	**15,58**
		STDEV	*2,5810*	*2,0879*	*1,2269*
		VAR	*8,0551*	*4,3841*	*7,8730*
V3	2,5	CATHERINE SEL. I / TOMIS 1	29,38	43,50	14,13
	2,5	FILIP / TOMIS 1	24,25	49,63	25,38
	2,5	SPRINGCREST / TOMIS 1	28,38	41,38	13,00
Indicators		**AVG**	**27,33**	**44,83**	**17,50**
		STDEV	*2,7167*	*4,2836*	*6,8431*
		VAR	*9,9390*	*9,5544*	*39,1035*

Table 2. Annual growth dynamic. Average trunk diameter and trunk cross-sectional area of tree increase related to the planting distance

Variant	Intra-row planting distance [m]	Variety / Rootstock	Average trunk diameter [mm] 01.10.2020	Average trunk diameter [mm] 06.04.2021	*Average trunk diameter increase [mm]*	TCSA [cm²] 01.10.2020	TCSA [cm²] 01.10.2021	*Average TCSA increase [cm²]*
V1	1,5	CATHERINE SEL. I / TOMIS 1	14,92	16,00	1,08	17,47	20,10	2,62
	1,5	FILIP / TOMIS 1	15,12	15,25	0,13	17,95	18,26	0,31
	1,5	SPRINGCREST / TOMIS 1	10,33	12,72	2,39	8,38	12,70	4,32
Indicators		**AVG**	**13,46**	**14,66**	**1,20**	**14,60**	**17,02**	**2,42**
		STDEV	*2,7096*	*1,7186*	*1,1348*	*5,3940*	*3,8498*	*2,0150*
		VAR	*20,1359*	*11,7258*	*94,5641*	*36,9474*	*22,6223*	*83,3107*
V2	2,0	CATHERINE SEL. I / TOMIS 1	12,77	15,75	2,98	12,80	19,47	6,67
	2,0	FILIP / TOMIS 1	15,68	15,75	0,07	19,30	19,47	0,17
	2,0	SPRINGCREST / TOMIS 1	13,40	16,00	2,60	14,10	20,10	6,00
Indicators		**AVG**	**13,95**	**15,83**	**1,88**	**15,40**	**19,68**	**4,28**
		STDEV	*1,5310*	*0,1443*	*1,5818*	*3,4400*	*0,3597*	*3,5742*
		VAR	*10,9748*	*0,9116*	*83,9918*	*22,3391*	*1,8279*	*83,4780*
V3	2,5	CATHERINE SEL. I / TOMIS 1	12,68	13,25	0,57	12,62	13,78	1,17
	2,5	FILIP / TOMIS 1	12,63	14,40	1,77	12,52	16,28	3,76
	2,5	SPRINGCREST / TOMIS 1	12,40	14,63	2,23	12,07	16,80	4,74
Indicators		**AVG**	**12,57**	**14,09**	**1,53**	**12,40**	**15,62**	**3,22**
		STDEV	*0,1498*	*0,7393*	*0,8567*	*0,2948*	*1,6139*	*1,8452*
		VAR	*1,1922*	*5,2461*	*56,1764*	*2,3769*	*10,3317*	*57,3177*

Under similar conditions, (ASLI, cm) was 21.04 cm per season (STDEV=6.8469; VAR=32.5398), with the lowest increase 14.63 mm on ‘Springcrest’ variety and the higher increase 28.25 cm on ‘Filip’ and (TCSA, cm^2) was 2.42 cm^2 per season (STDEV=2.0150; VAR=83.3107), with the lowest increase 0.31 cm^2 on ‘Filip’ variety and the higher increase 4.32 cm^2 on ‘Springcrest’.

When the peach was planted at 2.0 m apart in the trees line, the (ATDI, mm) was 1.88 mm per season (STDEV=1.5818; VAR=83.9918), with the lowest increase 0.07 mm on ‘Filip’ and the higher increase 2.98 mm on ‘Catherine Sel. 1’ variety. Under similar conditions, (ASLI, cm) was 15.58 cm per season (STDEV=1.2269; VAR=7.8730), with the lowest increase 14.88 cm both on ‘Filip’ and ‘Springcrest’ varieties and the higher increase 17.00 cm on ‘Catherine Sel. 1’ variety and (TCSA, cm^2) was 4.28 cm^2 per season (STDEV=3.5742; VAR=83.4780), with the lowest increase 0.17 cm^2 on ‘Filip’ variety and the higher increase 6.67 cm^2 on ‘Catherine Sel. 1’.

Finally, when the peach was planted at 2.5 m apart in the trees line the (ATDI, mm) was 1.53 mm per season (STDEV=0.8567; VAR=56.1764), with the lowest increase 0.57 mm on ‘Catherine Sel. 1’ variety, and the higher increase 2.23 mm on ‘Springcrest’. Under similar conditions, (ASLI, cm) was 17.50 cm per season (STDEV=6.8431; VAR=39.1035), with the lowest increase 13.00 cm on ‘Springcrest’ variety and the higher increase 25.38 cm on ‘Filip’ variety and (TCSA, cm^2) was 3.22 cm^2 per season (STDEV=1.8452; VAR=57.3177), with the lowest increase 1.17 cm^2 on ‘Catherine Sel. 1’ variety and the higher increase 4.74 cm^2 on ‘Springcrest’.

The data collected and assessed by calculation of determination and regression coefficients leader to highlight of a relationship between the average trunk diameter increase (ATDI, mm) and the average shoots length increase (ASLI, cm). These were R^2=0.5055 and r=0.7110**, which is statistically insured even at 12 pairs of values and n=12-2 liberty degrees of liberty. The relationship between the two assessed parameter is described by the equation of ascendent tendency line y=2.1716x + 9.2107 (Figure 1).

Another important relationship was found between annual trunk diameter increase (ATDI, mm) and trunk cross section area (TCSA, cm^2). The coefficients were R^2=0.9771** and r=0.9885**, which are very significant statistically insured even at 12 pairs of values and n=12-2 liberty degrees (Figure 2).

A very closed relationship was found between trunk cross section area (TCSA, cm^2) and the average shoots length increase (ASLI, cm). The coefficients found were R^2=0.1223 and r=0.3497, at n=12 pairs of values and 12-2 liberty degrees which is described by the equation: y=-0.8055+20.7050 (Figure 3). The descendent trend of the line suggests that the both parameters are influenced not only by the variety-rootstocks combination and planting distances but also by applied maintenance technology (irrigation, fertilisation, pruning, etc.). These aspects and additional data will be collected, processed and interpreted in the coming years.

CONCLUSIONS

Based on the presented data we found that the planting distance influenced all tree growth indicators that were analysed in all three studied peach varieties.

For the varieties planted at 1.5 m apart in the trees line, the ‘Sprincrest’ variety recorded the highest values of the indicators analysed, with 4.32 cm^2 (TCSA, cm^2) value.

For the varieties planted at 2.0 m apart in the trees line, the ‘Catherine Sel. I’ variety recorded the highest values of the all indicators analysed, with 6.67 cm^2 (TCSA, cm^2) value.

For the varieties planted at 2.5 m apart in the trees line, the ‘Springcrest’ variety recorded the highest values of the indicators analysed, with 4.74 cm^2 (TCSA, cm^2) value.

By using the synthetic growth indicator TCSA, will be possible to correlate the productivity with the planting distances in the future studies.

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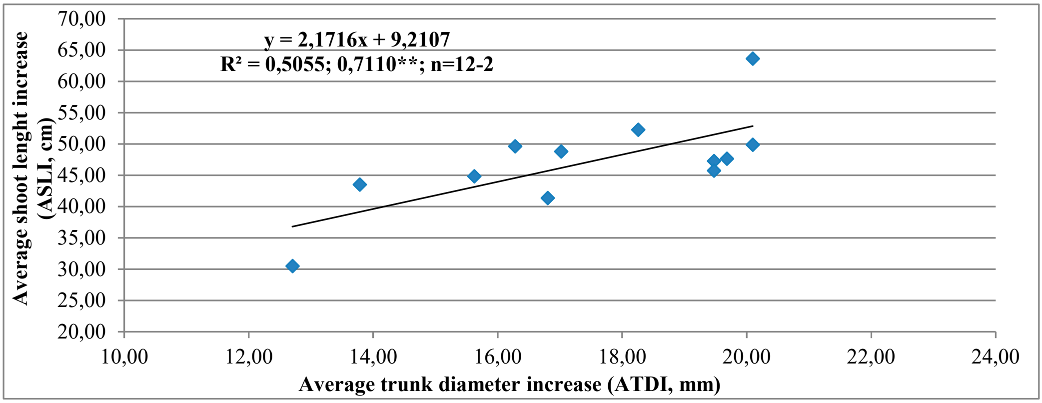

Figure 1. Correlation between average trunk diameter increase (ATDI, mm) and average shoot length increase (ASLI, cm)

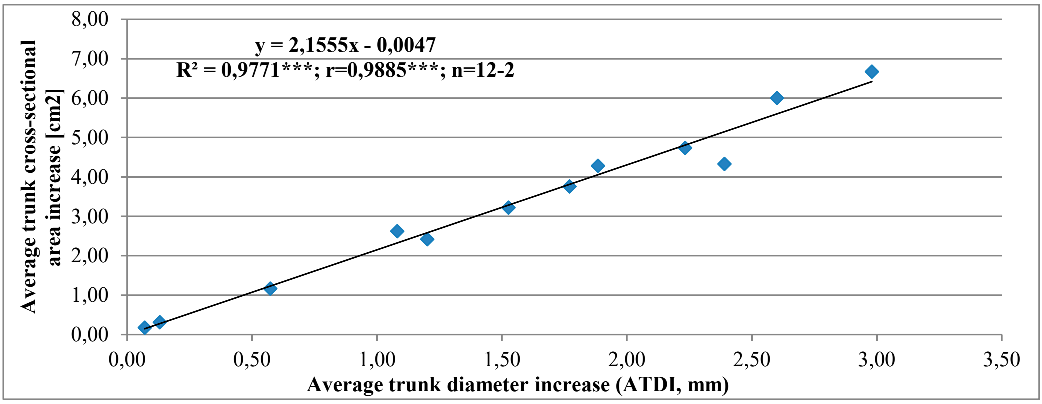

Figure 2. Correlation between average trunk cross- sectional area increase (TCSA, cm^2) and average trunk diameter increase (ATDI, mm)

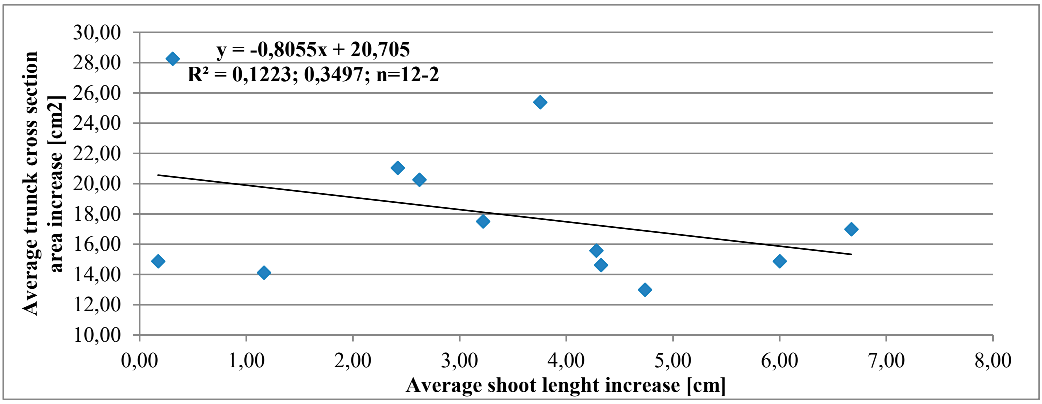

Figure 3. Correlation between average trunk diameter increase (ATDI, mm) and average shoot length

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PROPERTIES OF GRANULAR ORGANO-MINERAL FERTILIZER AND THE INFLUENCE OF ITS APPLICATION ON SOME CHEMICAL PROPERTIES OF THE SOIL IN AN APPLE PLANTATION, JONATHAN VARIETY

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Abstract

The physical-mechanical properties, as pH and compressive strenght, determined for the fertilizer granules obtained by enriching the compost from urban sludge with mineral fertilizers with N, P, K, fall within the norms imposed on organo-mineral fertilizers. Applying organo-mineral fertilizer to acidic soil in an apple orchard improved the physico-chemical properties of the soil. The experimental factor was the fertilizer dose, placed randomly, in five variants and four repetitions. One year after fertilization, the soil reaction was significantly improved (from pH = 5.4 to pH = 5.80). The mobile phosphorus content in the soil increased significantly in the fertilized variant with 40 t fertilizer / ha. The humified organic matter was at a high level in variant with 60 t/ha (humus = 4,25%) compared to the control variant (humus = 1.58%).

***Key words**: biosolid. organo-mineral, fertilizer, properties, soil.*

INTRODUCTION

Due to the nutrient content and the important intake of organic matter, large amounts of sludge can be used in agriculture as fertilizer. The use of sludge in agriculture could solve or alleviate a pressing problem: the removal of residual sludge from wastewater treatment facilities to avoid incineration or other costly and polluting processes (Adugna, 2016; Bowszys et al., 2015; Wei & Liu, 2005).
The application of residual sludge in fruit growing is an inexpensive method and is in line with the ecological principles of waste recycling, and at the same time can be a method of improving the physical, chemical and biological properties of the soil (Aggelides & Londra, 2000). However, its effect apparently depends on pedological conditions and cultivated plant species. In order to minimize the negative effects of residual sludge on a soil, the characteristics of a given sewage sludge and the critical concentrations of heavy metals must be taken into account.
By digesting and stabilizing of raw sewage sludge, in order to reduce the concentrations of pathogens and toxic chemicals below the established levels so that they can be used as fertilizers without harming the health of plants, soil and groundwater, so-called biosolids are obtained (Kominko et al., 2018; Kumar et al., 2017).
Organo-mineral fertilizers obtained by combining organic (biosolids) and mineral fertilizers offer various advantages (Parent et al., 2003; Lee & Bartlett, 1976; Tishkovitch et al., 1983)
The most common presentation form of organo-mineral fertilizers is the granular form which ensures, among other things, the reduction of storage space, the reduction of pollution due to dust and allows precise application (Deeks et al., 2013).
In this paper, the properties of biosolid-based fertilizer granules were determined and the influence on some soil properties of the application of this type of fertilizer was studied.

MATERIALS AND METHODS

The biosolid-based granular fertilizer used for experiments was made by reactive extrusion according to a manufacturing recipe in which the organic part is provided by biosolids, protein hydrolyzate and molasses (Cioica et al, 2020).

The granules are characterized by a water content of 1.33-1.70% and a bulk density of about 850 kg/m^3 (Nagy et al., 2021).

In order to determine the pH of the biosolid based fertilizer, samples of 2 g granules were dissolved in 20 ml of distilled water, after 20 min the pH was measured with pH indicator paper from Merck. Five repetitions were made and the average value was calculated.

The compression tests (Figure 1) were performed for biosolid based granules in initial state and for dried granules with a manual press equipped with a 5 kN force transducer, a Spider 8 data acquisition plate and a mechanical dial indicator, the compression test being videotaped. One granule was used for the measurements, the measurements being repeated 5 times to establish the average value of the compressive strength. The measured values of the granule deformation were taken with a resolution of 0.01 mm in the video recording compression tests. The drying process of the granules was performed with an AXIS 100 thermobalance, at a temperature of 80 °C until at least 3 consecutive equal values of the mass were obtained.

Figure 1. Compression test

The study regarding the effect of the biosolid based fertilizer on some soil properties was carried out at the Research Institute for Fruit Growing Pitesti, Mărăcineni (44° 51′ 30” N, 24° 52” E), in an apple plantation. The experiment was organized on a wet phreatic aluviosol, formed on fluvial deposits, with a loamy-sandy texture. The field was located in a meadow terrace of the Argeș River. The planting distance of the apple trees is 3.5 m x 1.25 m and the density is 2,285 apple trees/ha.

A single-factor experiment was designed (five experimental variants with four replicates), the experimental factor being the dose, with the following graduations:

V_1 = 0 t/ha (unfertilized control);
V_2 = 20 t/ha;
V_3 = 40 t/ha;
V_4 = 60 t/ha;
V_5 = 80 t/ha.

The five experimental variants were placed randomly, in four replicates.

The biosolid applied in the experimental variants comes from the sludge obtained at the Mioveni wastewater treatment plant. The quality indicators of the biosolid are presented in Table 1.

Table 1. Biosolid quality indicators (Test report, 2018)

No.	Quality Indicators	U. M.	Determined values
1	Dry matter	%	67.86
2	Volatile substance	%	35.34
3	pH measured at 20.6^{0}C	pH units	7.09
4	Nitrogen	% DM	1.52
5	Organic carbon	% DM	21.5
6	P_2O_5	% DM	1.38
7	K_2O	% DM	0.675
8	CaO	% DM	0.35
9	Cadmium	mg/kg DM	1.04
10	Chromium	mg/kg DM	44.8
11	Copper	mg/kg DM	74.3
12	Nickel	mg/kg DM	26.5

Before applying the compost, the soil chemical properties were analyzed using the following methods described by Florea et al. (1987): total nitrogen by Kjeldahl method (Kjeldahl, 1883); extractable phosphorus (P-AL) by Egner - Riem Domingo method (Egnér et al., 1960), by which the phosphates are extracted from the soil sample with a solution of acetate-ammonium lactate at pH=5.75, and determined colorimetric phosphate anion extracted as molybdenum blue (Egner et al., 1960); exchangeable potassium (K-AL) by Egner - Riem Domingo method by which the hydrogen and ammonium ions of the extraction solution replace by exchange the exchangeable potassium ions in the soil sample which are thus passed into the solution (Egnér et al., 1960). Potassium dosing in the solution thus obtained is done by flame emission photometry. Organic carbon is done by- wet oxidation method followed by titrimetric dosing by Walkley – Black with the Gogoasa modification (Edu et al.,2013) and humus (deduced by calculation from organic carbon); soil pH, soil: water ratio = 1: 2.5 by the potentiometric method; mobile aluminum by the Sokolov method (Sokolov, 1939); sum of exchangeable bases and hydrolytic acidity by Kappen method (Soil Survey Laboratory Methods Manual, 1996), base saturation degree (determined by calculation, depending on the sum of bases). Statistical analyses was performed with an IBM SPSS (SPSS 14) software. The results of field estimates and chemical analyzes performed on soil were processed using the variance analysis method and the multiple comparison method. In order to systematize and process the large data volume of analyzes on the physical and chemical characteristics of soil generalizations were made, with the data being presented in tables representing their variations depending on the experiment and agrochemical-agrotechnical measures applied.

RESULTS AND DISCUSSIONS

In general, most cultivated plants prefer soils with a neutral, slightly acidic or slightly alkaline pH (6.3-7.5). At the same time, the consumption of nutrients by plants depends directly on the pH. Calcium and magnesium are easily assimilated by plants at pH 7-8.5, nitrogen at pH 6.0-6.8, phosphorus at 6.5 - 7.5, potassium at higher pH of 6, and the trace elements are assimilated more easily in the acidic environment and more difficult in the alkaline environment. Therefore, any organo-mineral fertilizer must ensure that the pH is kept within optimal limits for plants.

The values of pH, measured for a solution of 20 ml of distilled water and 2 g of biosolids fertilizer, are between 6.5-7.0, with a mean value of 6.75.

The granular form of biosolids based fertilizers contributes to the improvement of their transport, storage and application properties. To ensure these properties it is important to know the compressive strength of these granules.

According to the results obtained in case of granules in the initial state (Figure 2), at a humidity of 1.33-1.70%, a very good concordance of the measured values is observed for three of the five tested samples. The measured values of the granule deformation were taken with a resolution of 0.01 mm in the video recorded compression tests. Thus, the polynomial regression function of third-degree, determined for the 5 measurements, explains in a proportion of 99.63% the behavior of the granules at the compression test. The polynomial equation obtained shows an almost linear behavior of the deformation of the granules in relation to the applied compression force. Regarding the values obtained, the predominantly linear character is observed up to a stress of 150 N in which case the average deformation is about 0.36 mm. Above this value, the polynomial character is observed up to a value of maximum 250 N at which the deformation reached a value of 0.68 mm. Above this value of the compressive force, during the mechanical stress, the granules did not deform anymore.

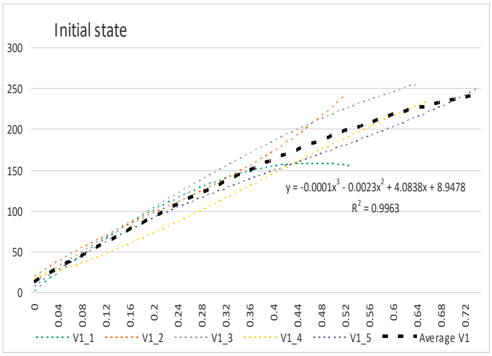

Figure 2. Compressive strength of biosolid based granules- initial state

In case of dry granular material (Figure 3) there is a turning point in the representation of the polynomial regression function of third-degree which explains the behavior of the material in a proportion of 98.5%. The value of this point is found at a force of about 110 N. And in this case there is a very good concordance of the values measured in three of the five tests performed. From the measured values a lower deformation of the granules is observed up to a value of about 80N which corresponds to a deformation of about 0.12 mm, followed by an area of high deformation up to about 125 N corresponding to a deformation of 0.5mm followed again by an area of lower deformation. The maximum value of the force to which the granules deformed was about 200 N at a deformation of 0.68 mm.

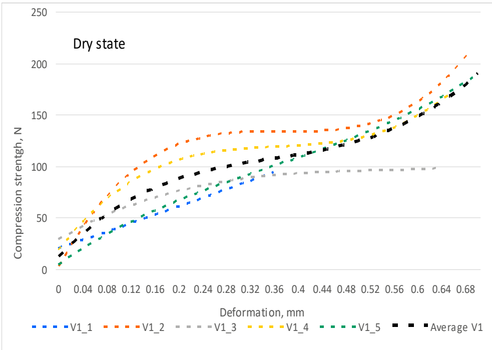

Figure 3. Compressive strength of biosolid based granules - dry state

During the measurements, the deformation of the granules was plastic (Figure 4).

Figure 4. Granules after compression test

The tests show similar deformation for the two humidities of the tested granules, a higher value of the maximum force in the case of dry granules and the influence of humidity in the deformation-stress behavior for the two humidities used.

All these elements lead to the need to extend the tests in order to identify the possibility of using the biosolid based granules for the intended purpose.

The soil properties of the apple plantation, from an agrochemical point of view, falls into the class of acidic soils, with an acidic to strongly acidic reaction, a low-very low humus content in the arable horizon, a very low nitrogen supply, low phosphorus and potassium contents, as can be seen in Table 2.

Table 2. Agrochemical soil properties

Soil depth, (cm)		0-20	20-40
Acidity indicators	pH	5.4	5.2
	Sum of bases SB (me/100 g sol)	14.21	6.0
	Extractable Acidity Ah (me/100 g sol)	2.67	9.7
	Base saturation V (%)	84.19	38.21
	Mobile aluminum Al^{3+} (mg/kg)	11.00	11.81
Fertility indicators	Total nitrogen content N_t (%)	0.08	0.07
	Total phosphorus content P_2O_5-P (mg/kg)	20	18
	Potassium content K_2O-K (mg/kg)	148	130
	Humus content (%)	1.58	1.20

One of the most important factors controlling the immobilization (sorption) and mobility of nutrients in the soil is the pH. Acid soils are characterized by a deficiency of nutrients and toxicity of metals such as Mn, Fe and Al, aluminum being the main limiting factor for plant growth and development in acid soils (Kochian et al., 2004, Gupta et al., 2013).

At a low pH (around 4.3) trivalent aluminum Al^{3+} predominates, which has the greatest impact on plant growth. In contrast, precipitated or chelated aluminum with organic compounds is not toxic to plants (Nogueirol et al., 2015). At a pH higher than 5-6, the dominant species are $Al(OH)_2^{+}$, $Al(OH)^{+2}$ which are not as toxic as Al^{3+} (Kinraide, 1991, Delhaize and Ryan, 1995, Hagvall et al., 2015, Kisnieriene and Lapeikaite, 2015). Polycationic Al (charge >2) is rhizotoxic as are other polyvalent cations (Kinraide, 1991).

In the case of the soil fertilized with increased doses of biosolid (60-80 t/ha), the high level of aluminum in the biosolid is not found as a high content in the soil, most probably because these are forms of aluminum adsorbed in the structure of the biosolid or in the clay-humic complex (Nicola C., 2021).

From Figure 5 we observe that increasing the dose of biosolid to 40-60 t/ha leads to an increase of the soil pH from 5.4 to 5.8.

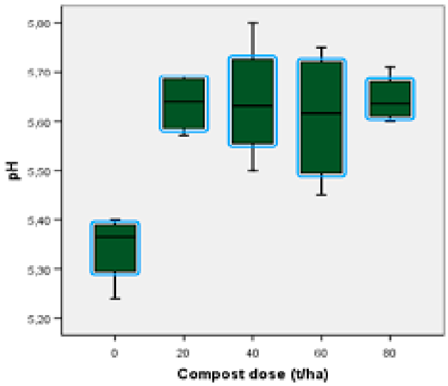

Figure 5. The pH level of the soil

Phosphorus availability is influenced by soil organic matter, pH and Al, Fe, Ca (Smithson, 1999).

Soil reaction pH, Al, Fe, P are properties of the soil related to each other. Their role in plant growth and development is very important. In acid soils the soluble inorganic phosphorus is fixed by aluminum and iron.

Limestone and humus are commonly used amendments to increase soil pH and phosphorus solubility and to suppress the solubility of aluminum and iron in the soil.

Biosolids brings large amounts of calcium into the soil, but also aluminum and iron.

In the 40-60 t/ha biosolid variants, the mobile phosphorus content increased about eight times compared to the unfertilized control, from 25 ppm to 225 ppm (Figure 6).

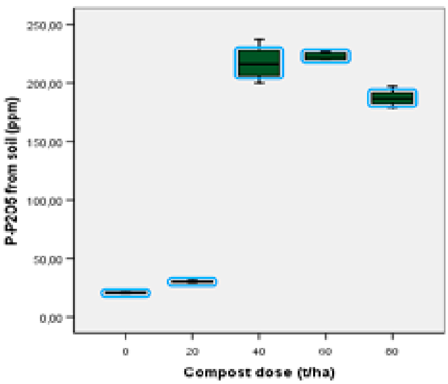

Figure 6. The mobile phosphorus content

Regarding the humus content, it is observed (Figure 7) that a dose of 60 t / ha biosolid ensures an increase of about 2.5 times compared to the unfertilized control.

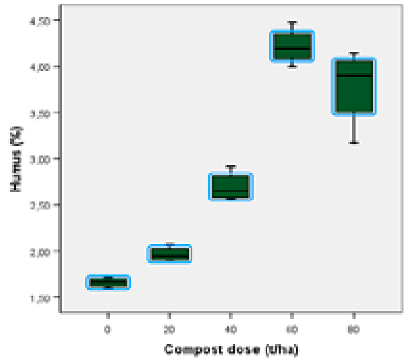

Figure 7. The humus content

CONCLUSIONS

The knowledge of some characteristics of biosolids based granular fertilizers allow the identification of some additives in order to optimize their performances.

The study of the biosolids based granular fertilizers obtained by reactive extrusion showed that the pH obtained of about 6.75 corresponds to the agrotechnical needs.

The values of the compressive strength obtained after the tests, up to 200 N ensure the improvement of their transport, storage and application properties.

The tests show similar deformation for the two humidities of the tested granules, a higher value of the maximum force in the case of dry granules and the influence of humidity in the deformation-stress behavior for the two humidities used.

Experiments have shown that as a consequence of the application of 40-60 t / ha of biosolids the soil pH increased from 5.4 to 5.8., the mobile phosphorus content increased about eight times compared to the unfertilized control and for a dose of 60 t/ha the humus content increase of about 2.5 times compared to the unfertilized control.

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FRUIT QUALITY OF TWO PLUM CULTIVARS GRAFTED ON 'DOCERA 6' (*PRUNUS DOMESTICA* L. X *PRUNUS CERASIFERA* EHRH.) ROOTSTOCK

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Abstract

The most commonly used rootstock for plum cultivars in Bulgaria is the seedling P.cerasifera Ehrh. In studies concerning the resistance of some rootstocks to the Plum Pox Virus, good results were obtained for the clonal rootstock 'Docera 6 '. Considering that plum fruit quality depends mainly on the scion genotype, but could be influenced by the rootstock as well, the objective of the current study was to evaluate the fruit quality of the plum cultivars 'Jojo' and 'Topgigant Plus', when grafted on 'Docera 6'. The same cultivars grafted on the seedling rootstock P. cerasifera were used for comparison. The trees were planted in 2016 at the Fruit Growing Institute of Plovdiv, Bulgaria. In 2020-2021 fruit biometrical data, fruit flesh firmness, TSS (°Brix), skin colour and fruit flesh colour were measured. For evaluating the consumer acceptance and nutritional value of the fruits, sensory and chemical analyses were performed. All of the studied parameters were significantly influenced by the cultivar. The largest fruits were measured for the combination Topgigant Plus/Docera 6 (71.53 g). The content of sugars and organic acids were the highest in 'Jojo' fruits.

***Key words**: European plum, evaluation.*

INTRODUCTION

European plum (*Prunus domestica* L.) is grown in temperate climate zones, mainly in Central, Southern and South-Eastern Europe. The Balkans is forming centre of the species, and therefore plum is a fruit of great importance. The plum (*Prunus domestica* L.) is one of Bulgaria's main stone fruit species. It is widely grown in all country regions and is the third after walnut and sweet cherry in areas occupied (Agrostatistical handbook, 2020).

Along with the species' wide distribution, a major disease related to it is endemic in our country. The Sharka disease, caused by the Plum pox virus (PPV), is among the most harmful and a major limiting factor for plum production (Milusheva et al., 2015). As there is no effective chemical control against viral diseases, tolerance or resistance to PPV is one of the most wanted characteristics in the newly bred plum cultivars and rootstocks.

'Docera 6' is a rootstock obtained due to the breeding program of the University of Munich. Its primary purpose was to create rootstocks resistant to the PPV suitable for stone fruit species. The 'Docera' series plants are interspecific hybrids between *Prunus domestica* and *Prunus cerasifera* (Neumüller et al., 2012). The laboratory and field tests of 'Docera 6', conducted at the Fruit Growing Institute - Plovdiv, define it as a promising rootstock in its reaction to the virus (Milusheva et al., 2015). Therefore, the new 'Docera 6' rootstock is of interest to all plum producing countries where PPV is spread.

The resistance of the rootstock to specific diseases is significant, but it is not the main criterion for its introduction into orchards. The most important features are the agronomic and biological characteristics that the rootstock induces to the grafted cultivar and on which the producer's incomes depend. In addition, the rootstock has a significant impact on the development of the trees, when they start fruiting, the quality and quantity of their fruit production.

As roots absorb water containing dissolved minerals, it can be assumed that the rootstock will influence the ability to provide nutrients for the whole plant. Thus, nutrient sufficiency is also an essential factor affecting fruit production and quality. The relationships between different rootstocks and differences in leaf nutrient levels have been documented in other fruit species (Tsipouridis and Thomidis, 2005, North and Cook, 2008). Described is the effect of eleven apple rootstocks on the fruit weight, quality, soluble solids (Tomala et al., 2008).

In Germany for 5-year-old trees, Hilsendegen (2018) reported that 'Docera 6' reduced the growth strength of 'Jojo' and 'Čačanska Lepotica' by 12-14% compared to the same cultivars grafted on 'Wavit' rootstock. In Poland, Sitarek and Machlańska (2019) also described the early development of 'Jojo' and 'Čačanska Lepotica' trees and compared them to trees grafted on 'Wavit' and 'Weiva'. However, the authors report a stronger vegetative growth of the trees grafted on 'Docera 6'. After obtaining the first fruits, the varieties grafted on the test rootstock had a lower average yield and smaller fruits.

Fruit production is the most important for growers, processors and consumers. Therefore, this research aimed to study the influence of 'Docera 6' on the fruit quality of the cultivars' Topgigant Plus' and 'Jojo' and to compare it with the fruit quality of the same cultivars grafted on the standard for Bulgaria rootstock.

MATERIALS AND METHODS

The current research was conducted in a trial orchard at the Fruit Growing Institute – Plovdiv, Bulgaria. The cultivars 'Jojo' and 'Topgigant Plus' grafted on 'Docera 6' were compared to the same cultivars grafted on the standard for our country rootstock, the seedling *Prunus cerasifera* Ehrh. The trees were planted in 2016. In 2020-2021 fruit biometric, colourimetric and sensory analyses were performed. An average sample of 25 fruits was taken and fruit biometry was measured using Mitutoyo 500-196-30 Digimatic Absolute Caliper 150 mm. Average fruit weight was measured with a digital scale. Plum fruits were subjected to colour evaluation in the CIEL*a*b* system. Using handheld colourimeter PCE-CSM 2, colour parameters of the equatorial part of 10 fruits with and without natural wax bloom were measured. The 0 L* values represent the black colour, 100- represents a perfect reflecting diffuser (bright). A positive a* value indicates red and a negative a* value - green colour, a positive b* value indicates yellow and a negative b* value blue colour. The Hue angle expresses the colour nuance and values are defined as follows: red-purple: 0°, yellow: 90°, bluish-green: 180°, and blue: 270° (McGuire, 1992; Voss, 1992). The Chroma defines the saturation of the colour. Fruit firmness was measured using a PCE-PTR-200N digital penetrometer. The total soluble solids content was measured using digital refractometer Kern ORF 85BM. In 2021 fruit chemical analyses were performed. Chemical analyses of the fruit flesh included determining the sugar content-total, inverted and sucrose, by the method of Schoorl-Regenbogen, the acid content - titrimetrically and the active acidity (pH) - potentiometrically, by pH meter.

For sensory evaluation of the fresh fruits a methodology developed at the Fruit Growing Institute Plovdiv was used. A total number of 8 characteristics were scored by a group of trained consumers using the following scale: 1-3 - bad; 3-5-satisfactory; 5-7 good; 7-8 - very good, above 8 - excellent. Each characteristic was multiplied by a coefficient, established by the scientists of section "Breeding, genetic resources and biotechnology" at the Fruit Growing Institute Plovdiv, depending on its importance: 0.175 for appearance, 0.050 for aroma attractiveness and 0.225 for aroma intensity, 0.200 for sweetness, 0.125 for sour taste, -0.125 for bitterness, 0.200 for taste balance, and 0.150 for fruit flesh texture and juiciness (Neshev et al., 2021).

Data were statistically processed by Duncan's multiple range test of the IBM SPSS statistics 26 software. The independent samples t-test was applied in this study for determining the significance of the difference in the chemical analyses.

RESULTS AND DISCUSSIONS

The quality of food products is the most important feature for anyone involved in their production and processing. Fruit size is one of the leading fruit characteristics affecting market value and consumer preferences (Martins et al., 2021). Although the fruit quality depends mainly on the cultivar, it can also be influenced by the rootstock (Scalzo et al., 2005). The dimensional fruit sizes of the scion/rootstock combinations are presented in Table 1.

Table 1. Fruit biometric analyses

Scion/rootstock combination	Fruit height (mm)	Fruit width (mm)	Fruit thickness (mm)	Fruit weight (g)	Peduncle length (mm)	Stone weight (g)	Sone relative share (%)
Topgigant plus/ Docera 6	58,09 a	44,22 a	46,41 a	71,36 a	9,75 a	2,73 a	3,83 b
Topgigant plus/ *P. cerasifera*	56,04 a	43,93 a	45,05 a	66,40 a	8,33 b	2,48 b	3,73 b
Jojo/Docera 6	48,28 b	36,60 b	35,07 b	38,74 b	8,71 ab	1,99 c	5,17 a
Jojo/*P. cerasifera*	49,71 b	34,83 c	35,98 b	40,89 b	8,42 b	1,98 c	4,84 a

Different letters in the same column indicate statistically significant differences ($p < 0.05$) according to Duncan's test.

Significant differences are observed only between the different plum cultivars. The largest fruit weight was measured for the scion/rootstock combination Topgigant Plus/Docera 6 (71.53 g).
The difference with the same cultivar grafted on *P. cerasifera* is 4.96 g, and it is statistically non-significant. The peduncle of the fruits obtained by the cultivars grafted on 'Docera 6' is longer compared to the ones obtained from the grafted on the standard rootstock.
The well-informed consumer demands delicious fruits with high nutritional value. Chemical analyzes are a part of the efficient production of quality fruits. The total soluble solids content, sugars and acids are essential for the taste quality and the value of the fruits for the human nutritional diet. All of them are influenced by the overall development of plants, which in turn depends on the scion/rootstock combination. Fruit flesh firmness is important quality relevant to fruit shelf life and consumer acceptance.
Fruit flesh firmness is a combination of skin and flesh strength, and in general, genotypes with the firmest fruits are preferred (Hend et al., 2009).

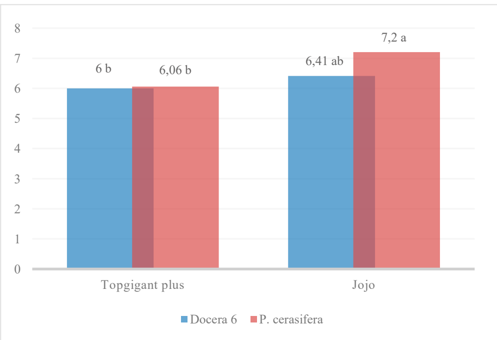

Figure 1. Fruit flesh firmness (kg/cm^2)

The TSS content is important for dried fruits production. Its high content is associated with a higher amount of sugars and increases the yield and quality of the dried product. Non-significant differences were observed for fruit flesh firmness and TSS content between the cultivars grafted on the different rootstocks.
'Jojo' had a higher fruit flesh firmness and TSS content compared to the other cultivar 'Topgigant Plus'.

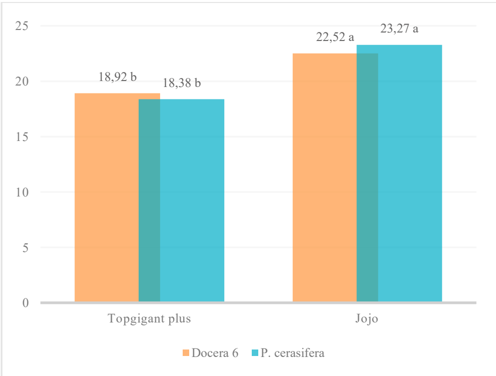

Figure 2. Total soluble solids content (°Brix)

The highest fruit firmness and TSS content were measured for 'Jojo' when grafted on the seedling *P. cerasifera* (Figures 1 and 2).
In some cases, the rootstocks could significantly affect the minerals content, sugars (sucrose and fructose) and organic acids in the fruit.
If there are such differences, it could affect the commercial value of the fruits and guide the choice of rootstock when planning and establishing an orchard (Caruso et al., 1996).
In a previous study, no substantial differences were observed in the main nutrients content (N, P, K) in the leaves of the trees grafted on *P. cerasifera* and 'Docera 6' (Akova et al., 2019).
After analyzing the fruit chemical content of the fruits, non-significant differences between the cultivars grafted on different rootstocks were observed, too (Table 2). Although the non-significant differences, 'Topgigant Plus'/ *P.*

cerasifera had higher sugars and acids content compared to the same cultivar grafted on 'Docera 6'. 'Jojo'/*P. cerasifera* had higher sucrose and acids content.

Table 2. Fruit chemical analyses

Scion	Rootstock	Sugars % Total	Sugars % Inverted	Sugars % Sucrose	Acids %	pH
Jojo	*P.cerasifera*	14,82	10,44	4,16	1,21	3,53
	Docera 6	14,82	11,76	2,91	0,94	3,28
Difference significance		ns	ns	ns	ns	*
Topgigant Plus	*P. cerasifera*	12,96	9,04	3,72	1,40	2,81
	Docera 6	11,46	7,92	3,36	1,21	2,85
Difference significance		ns	ns	ns	ns	ns

*Asterisks indicate significant differences between the tested cultivars by independent samples t-test at P≤0.05, ns – non-significant difference.

The data of the chemical analyses and TSS content in the fruits are in correspondence with their sensory evaluation. When grafted on *P. cerasifera* the fruits of 'Jojo' had the highest TSS and sucrose and their sweet taste was highly appreciated by the consumers (Table 3). The fruits obtained of 'Jojo' grafted on the standard seedling rootstock had the highest grade for their attractive and intensive aroma, sweetness and balanced taste.

Skin colour is an essential indicator of fruit maturity and quality, and consequently, it is a decisive consumer preference and acceptance parameter (Crisosto et al., 2003; Romano et al., 2006). Chromatic parameters of both cultivars grafted on 'Docera 6' and *P. cerasifera* rootstocks are presented in Table 4. Both cultivars have very similar skin colours before and after removing the wax bloom. Significant differences between rootstocks were observed for the chromatic parameters b and Chroma index of the fruit flesh colour of 'Jojo'. The grafted on *P. cerasifera* 'Jojo' had brighter and richer yellow coloured fruit flesh.

Table 3. Fruit sensory analyses

Scion/rootstock	Appearance	Aroma: Attractive	Aroma: Intensive	Taste: Sweet	Taste: Sour	Taste: Bitter	Taste: Balanced taste	Fruit flesh texture and juiciness	Total grade	
Top gigant plus /Docera 6	1,49	0,38	0,36	1,10	0,63	-0,03	1,18	1,45	6,55	Good
Top gigant plus/ *P. cerasifera*	1,53	0,38	0,36	1,20	0,59	-0,03	1,14	1,35	6,52	Good
Jojo/Docera 6	1,40	0,24	0,30	1,20	0,56	-0,03	1,09	1,55	6,33	Good
Jojo/ *P. cerasifera*	1,49	0,45	0,45	1,35	0,44	-0,03	1,23	1,65	7,03	Very good

Table 4. Fruit colour

	Chromatic parameter	Topgigant Plus/Docera 6	Topgigant Plus/ *P. cerasifera*	Jojo/Docera 6	Jojo/*P. cerasifera*
With wax bloom	L	33,34 a	33,02 a	34,82 a	34,58 a
	a	0,11 a	-0,41 a	0,12 a	-0,12 a
	b	-7,26 a	-7,15 a	-4,57 a	-6,67 a
	Chroma	7,41 a	7,37 a	6,11 a	6,77 a
	Hue	273,21 a	269,84 a	275,19 a	271,52 a
Wax bloom removed	L	17,81 b	18,02 b	21,67 a	19,50 ab
	a	4,93 a	3,60 a	3,83 a	4,50 a
	b	0,80 a	0,45 a	0,53 a	0,49 a
	Chroma	5,14 a	3,78 a	4,01 a	4,63 a
	Hue	147,89 a	109,24 a	114,87 a	79,69 a
Fruit flesh	L	42,52 a	43,12 a	44,61 a	46,69 a
	a	1,17 b	1,51 b	3,88 a	5,19 a
	b	23,88 c	21,90 c	29,99 b	35,57 a
	Chroma	23,96 c	22,17 c	30,37 b	35,99 a
	Hue	86,86 a	85,10 a	83,09 a	81,85 a

Different letters in the same row indicate statistically significant differences ($p < 0.05$) according to Duncan's test.

CONCLUSIONS

All studied characteristics of the fruits obtained from the two cultivars grafted on 'Docera 6' are similar to the features of the grafted on *Prunus cerasifera*. A tendency for better taste qualities of the 'Jojo' cv. when grafted on *P. cerasifera* was observed. The clonal rootstock 'Docera 6' did not significantly affect the fruits' appearance, size, and chemical characteristics. Their quality is similar to the grafted on the standard rootstock cultivars.

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RESEARCH ON GENETIC RESISTANCE OF THE 'ELMAR' APRICOT VARIETY AT LOW TEMPERATURES

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Abstract

The south-eastern part of Romania, especially the one of influence of the Constanta Fruit Resort, has faced in the last years with serious problems generated by the late and the return frosts. Late frost that occurred in spring affect apricot plantations, causing irregularities in the constancy of fruit production and their quality, gummy leaks and sensitivity of trees to the attack of apricot-specific pathogens. The research was conducted in an orchard of apricot aged 9 years, located at 18 km away from Constanta. Trees planted at a distance of 4x4m were studied during the vegetation season paying particular attention to resistance to low temperature. In the winter of 2017, temperatures decreased to -17 degree C and produced losses of flower buds. In the spring of 2018, the 'Elmar' variety bloomed well, 100% of flower buds being resistant to negative temperatures from winter. In 2019 was a warm winter but the temperature decreased in January to -17 degree C and the trees suffered a thermal shock. Following, resistance to frost decreased and the percentage of flower buds affected was 76.6%. In 2020 'Elmar' variety was not affected. In February of 2021 were, recorded temperatures of -16 degrees C associated with very cold wind, the flower buds were affected in 60%.

Key words*: thermal shock, temperatures, flower buds.*

INTRODUCTION

The apricot is a species appreciated for fruit quality and is grown successfully in areas with high temperatures (Cociu V. et al., 2000; Sestraș R. et al., 2004). In Romania, grows in the S - E part of the Dobrogea area. In this part, the apricot, do not make the fruits every year because of the low temperatures that affect the flowers (Braniște N. et al., 2007). The 'Elmar' is a new variety introduced in crop and after these studies this variety proves to us that it has a genetic resistance to low winter temperatures and temperatures during the flowering period. We made a trial with 'Elmar' variety to study resistance to winter cold, fructification and fruit quality. As a result of the researches, we were able to establish the resistance limit at low temperatures and we observed that the 'Elmar' variety behaves differently from one year to another depending on the evolution of the temperatures. In cold winters associated with wind, the productions are kept in relation to the warm winters. Researchers are concerned about obtaining apricot varieties that start late in vegetative and resist winter frost.

MATERIALS AND METHODS

The study was carried out in apricot demonstrative plots at Research Station for Fruit Growing Constanta, located in South-Eastern Romania, near the Black Sea. The site is located at 44°10' Northern latitude and 28°29' Eastern longitude, and 70 m above sea level. Climate is continental with warm and droughty summers, frequent dry winds all the year round and temperate winter generally without snow. The mean annual temperature is 12.0°C and the total active temperature is 3988°C, out of which 3170°C during the growing season; the annual precipitation amount is 400 mm, out of which during the growing season (April 1 to September 30), 240.7 mm. The lowest winter temperatures below -20°C are not very often: 1 out of 10-15 years and so are the spring frosts susceptible to cause apricot yield damage. The climatic water deficit reaches as much as 400 mm/year, so irrigation application is needed for apricot.

The zonal soil type is a calcaro-calcic chernozem formed on loess, with loam texture and a proper capacity of water preserving, holding and circulation. The humus content ranges between 2.5 and 4%; pH of the soil is poor alkaline (7.0-8.1).

There are twenty-four cultivars planted in demonstrative lots (4 m × 4 m scheme) in 2011 with North-South row orientation and the crown shape is improved vase. The apricot trees are grafted on ‘Constanta 14’ rootstock described by Indreias et al. (2010).

The agronomic and fruit-quality characteristics were evaluated four years (2018 to 2021).

The trees and fruit characteristics were evaluated according to the Methodology for trying new varieties of fruit trees, berries and rootstock in order to approve the homologation and International Union for the Protection of New Varieties of Plants (UPOV) guidelines.

To record the temperatures during winter and during the vegetation period we used our own meteorological data from the weather station.

During 2018-2021 the fruit yield was recorded starting with the 6th year after planting, when fruit production was considered stable. The average yield was evaluated by weighing the fruit of five apricot trees of each cultivar (kg/tree) and then as kg/ha. The fruits were weighed in the laboratory and analyzed for determinate the average fruit weight, color, pulp weight and kernel weight. The fructification pruning was made during the vegetative rest period. Soil has been kept clean by weeds by manual and mechanical work. To determine the percentage of buds affected by low winter temperatures, we made observations on the field. Observations were made in November (before frost), in February, at the beginning of the vegetative period (March) and during the vegetative period. After the flowering we determined the percentage of flowers formed to determine the influence of winter cold on fruit buds. During the vegetation period, we made observations on the percentage of fruit formed, the production of the fruit obtained and the qualities of the fruits.

RESULTS AND DISCUSSIONS

Research into the resistance of fruit buds to winter cold began in the winter of 2018 when the trees were 5 years old. In the spring of 2018 (Table 1) the apricot variety ‘Elmar’ was very little affected by the late frosts, thus a percentage of 94% of viable flowering buds was registered. The year 2018 was a normal year from the thermal point of view and the trees resisted very well during the winter.


Figure 1. ‘Elmar’ cv. Apricot

Figure 2. ‘Elmar’ cv. apricot

Table 1. Behavior of the ‘Elmar’ variety in the 2018-2021 period

Year	No. buds analyzed	% of viable fruit buds	% of affected buds
2018	311	94	6
2019	307	23.4	76.6
2020	290	97	3
2021	295	40	60
Average	300.75	63.6	36.40

In 2019 the winter temperatures varied a lot in very short periods of time, so if in January we recorded temperatures of -17^{0}C, in February we recorded days with temperatures above +12^{0}C, followed by temperatures of -7^{0}C in March. For this reason, the 'Elmar' apricot variety suffered, the flower buds were affected in a proportion of 76.6% according to Table 1.

Table 2. Fruits production and fruit characteristics at 'Elmar' cv. in 2018-2021 period

Year	Average fruit weight (g)	Weight of pulp (g)	Weight of stone (g)	Fruit production (kg/tree)
2018	49	46	3	22
2019	60	59	5	13
2020	53	50	3	21
2021	59	55	4	12
Average	55.25	52.5	3.75	17

In 2020, the 'Elmar' apricot variety was not affected by winter frosts, the variety having a good tolerance to thermal shocks (table 1). In 2021, from the analysis of the flower buds analyzed between January and February, we notice that the apricot variety 'Elmar' was 60% affected by the winter frosts and very little by the return frosts or late frosts.

Observations regarding the genetic resistance of flowers at low temperatures during bloom showed that the 'Elmar' variety very well tolerates low temperatures. In years with high production, when winter frost did not destroy fruit buds, and the temperature drop gradual the flowers were not affected by low temperatures during the flowering period.

In 2018 and 2020 during the bloom when the flowers were opened, during the night when temperatures were -1^0 ... -4^{0}C, the apricot flowers were not affected. The results regarding the influence of low temperatures on the percentage of fruits formed are presented in Table 2. The fruits were analyzed morphologically and was determined the production on the tree (Table 2). From the analyzed data we observe an average production during the four years of study of 17 kg/tree, which places this variety above the average production.

CONCLUSIONS

'Elmar' is constant in terms of fruiting, having good results even in unfavourable years for apricot cultivation. 'Elmar' variety is a relatively resistant variety to low winter temperatures and resistance depends on how low the temperature is. Under thermal shock, fruit buds can be affected to 60% to 80%, and warm winter's causes fruit bud losses. If the fruit buds are not affected during the winter, the 'Elmar' variety fructify normally, being resistant to low temperatures during the flowering period. It shows good plasticity to the accentuated climatic changes of the last years.

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RESEARCH CONCERNING MORPHOLOGY AND PRODUCTIVITY OF SOME STRAWBERRY VARIETIES MULTIPLIED BY BIOTECHNOLOGY

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Abstract

Strawberry (Fragraria grandiflora) production has been developed in the past 10 years due to superior economic advantages and relatively simple culture technology. Easily commercialization on the market and industrial products obtained by processing, make from strawberry (Fragaria grandiflora) culture a passion and a profitable business. Were studied very old strawberry (Fragaria grandiflora) varieties and new Romanian varieties that could be grown all over the world. Variety Red Gauntlet is a productive variety with quality fruits and production can reach 20 t/ha (cultivated in V2 variant) and 36 t/ha (cultivated in V4 variant). The new varieties are very productive and can produce in optimal conditions of culture 40 t/ha (Delicious and Viva). The same performance has and the variety Mara. Fruit production increased with the application of organic fertilizer and soil mulching material. New varieties have a very high yield potential and technological measures stimulated the production and fruit quality.

Key words: *strawberry, variety, productivity.*

INTRODUCTION

The strawberries and cherry fruits are the first fruit who arrive to ripening (May-June). Strawberries are early species, producing fruit in the first year after planting, if the plants are planted in July. Its possible to obtain a earlier production or the later than that obtained in the field. By using varieties with continue fructification (with several harvests per year) we can get strawberries and autumn until October.

With a low hight (15-40 cm) the strawberries can grow in very small spaces in the garden, and on large surfaces or in greenhouses. Strawberry production in greenhouses and solariums has some advantages and disadvantages (Lieten, 2001).

Was made observations on morphological characteristics of the five varieties of strawberry fruit featured for quality and yields per hectare.

The morphological characteristics studied were plant vigor, inflorescence structure and average fruit weight. Productivity was determined for each variety studied to highlight the influence of genetic material on productivity.

Plant height is used as a criterion of vigour (Bologa, 1997).

MATERIALS AND METHODS

The strawberry varieties studied were obtained in the laboratory using artificial culture medium (Murashige & Schoog) and acclimatized in the field.

They were studied five varieties of strawberry (Gorella, Red Gauntled, Delicious, Viva and Mara), grown in four variants:

V1 - classic culture system directly into the ground;

V2 - land cover with agro-textile material mulch). Agro-textile material was installed in the planting moment;

V3 - classical culture fertilized with organic fertilizer (leaf compost);

V4 - culture with agro-textile mulch and fertilized with organic fertilizer.

The Gorella variety is a very old variety, created in the Netherlands in 1960, particularly appreciated for its qualities.

The Gauntlet Red variety was obtained and introduced in culture in England in 1957, it is well known and appreciated in our country.

The Delicious variety is a romanian variety (Dana x Red Gauntlet) obtained at SDP Satu Mare (Romania), homologated in 2003, and has big fruits dark-red colored.

Viva is a romanian variety (Addie x Red Gauntlet) obtained at SDP Satu Mare (Romania), homologated in 2003.
Mara is a romanian variety (Pajaro x Red Gauntlet) obtained at SDP Satu Mare, Romania, homologated in 2003. The fruit matures in the first 20 days of June.
The fertilization was made in the spring before flowering.
The trials were conducted in the years 2014 - 2016 in the field of Faculty of Horticulture in Bucharest.
Planting was carried out in early September 2014, and data on the morphology and productivity varieties were registered in 2015-2016.
Planting was done in simple rows distances 40 x 25 (100000 plants/hectare).
Observations concerning the vigor were made when the plants reached full development by measuring plant height from ground level.
The number of inflorescences per plant and the number of the flowers in the inflorescence was determined by counting them.
The average weight of the fruit was obtained by weighing the output of each plant in relation to the number of fruit formed on the plant.
Using the production obtained on the plant and number of plants on hectare we calculated the yields.

RESULTS AND DISCUSSIONS

In 2019, after planting (September) was applied to the specific technology (irrigated by drip and protection against diseases and pests) and observations began in 2020. Planting was carried out at the optimum time, and after resumption of vegetation were made measurements concerning the foliage developments.
The vigor of the plant is influenced by the culture system. The V3 and V4 variants promotes plant growth due to organic fertilizer.

Table 1. The vigor of studied varieties (average 2020-2021)

Variety	Plant height (cm)				No. leaves per plant			
	V1	V2	V3	V4	V1	V2	V3	V4
Gorella	38	42	40	45	8	6	7	6
Red Gauntlet	28	37	40	40	5	5	5	5
Delicios	30	34	35	35	7	7	6	5
Viva	26	26	30	32	8	7	6	6
Mara	25	28	30	35	7	6	7	6

Table 2. Echeloning the flowering to strawberry (average 2020-2021)

Variety	Opening of the first flowers			
	V1	V2	V3	V4
Gorella	14.V	9.V	12.V	9.V
Red Gauntlet	12.V	8.V	10.V	9.V
Delicios	12.V	8.V	12.V	8.V
Viva	14.V	10.V	12.V	10.V
Mara	14.V	10.V	11.V	10.V
	Staggering blooming (Nr. of days)			
Gorella	11	9	10	12
Red Gauntlet	10	13	12	12
Delicios	8	9	10	10
Viva	8	6	9	9
Mara	7	7	8	10

Table 3. The study of strawberry inflorescences (average 2020-2021)

Varierty	Inflorescence length (cm)				The average number of flowers on inflorescence			
	V1	V2	V3	V4	V1	V2	V3	V4
Gorella	17	16	20	25	11	10	11	13
Red Gauntlet	18	15	18	22	9	10	12	12
Delicios	15	17	19	24	9	9	11	13
Viva	20	23	20	26	6	8	9	10
Mara	16	17	20	21	8	10	10	13

Table 4. The flowering capacity

Varierty	Nr. inflorescences per plant			
	V1	V2	V3	V4
Gorella	1	1	2	2
Red Gauntlet	2	2	2	2
Delicios	2	2	2	2
Viva	3	2	3	3
Mara	2	2	2	2

Number of flowers open was different from one variety to another and was influenced on culture solution. The fertilizing with organic fertilizer, influenced the number of flowers formed (V3 and V4). The fertilization has not affected the number of inflorescences formed by each variety. Viva Variety formed the lower number of flowers / inflorescence, but formed the big fruits in V4 variant.

Table 5. Elements of productivity (average 2020-2021)

Variety	Nr. fruits per plant				The average weight of the fruit (g)			
	V1	V2	V3	V4	V1	V2	V3	V4
Gorella	11	10	15	17	12	15	17	17
Red Gauntlet	11	10	14	18	18	20	20	20
Delicios	18	18	18	20	16	18	20	20
Viva	18	16	18	20	14	17	19	20
Mara	16	19	20	19	12	14	18	21

The productivity is determined by the size of the inflorescence, the number of flowers on inflorescence and the weight of the fruit. The studied varieties formed 1-3 inflorescences per plant. The strawberry is a species with high productive potential. The production is influen-

ced by variety, culture system and climatic conditions during the growing season.
The quantity of fruits obtained on plant depends on the size and number of fruit formed per plant.

Table 6. Production of strawberries depending on the variant of culture (2020-2021 average)

Variety	The yield /plant (g)			
	V1	V2	V3	V4
Gorella	132	150	225	289
Red Gauntlet	198	200	280	360
Delicios	288	324	360	400
Viva	252	272	342	400
Mara	192	280	360	399
Media	212.4	245.2	313.4	369.6

Table 7. Production of strawberries (average 2020-2021)

Variety	The yield (kg/m^2)			
	V1	V2	V3	V4
Gorella	1,32	1,50	2,25	2,89
Red Gauntlet	1,98	2,00	2,80	3,60
Delicios	2,88	3,24	3,60	4,00
Viva	2,52	2,72	3,42	4,00
Mara	1,92	2,80	3,60	3,99
Media	1,82	2,45	3,14	3,69
	The yield (t/ha)			
Gorella	13,2	15,0	22,5	28,9
Red Gauntlet	19,8	20,0	28,0	36,0
Delicios	28,8	32,4	36,0	40,0
Viva	25,2	27,2	34,2	40,0
Mara	19,2	28,0	36,0	39,9
Media	21,24	24,52	31,3	36,9

Applying organic fertilizers (V3) ensures high yields. If organic fertilization is associated with mulching with agro-textile we can obtain the highest yields. The Red Gauntlet variety in these conditions very well and Romanian varieties have achieved production of 40 t / ha. In the field conditions, without a adequate technology, who no ensuring all factors of vegetation at the optimum level, is not use full capacity of production of the variety.
To achieving high yields of fruit per unit area are important a number of factors, including variety, cultivation technology and climatic conditions.
Harvests in the 4 experimental variants have demonstrated that a biennial crop of strawberries is very cost effective. The use of organic fertilizers stimulate capacity of fruiting and material agro-textile control the weeds, fruit damaged and preserves soil moisture.

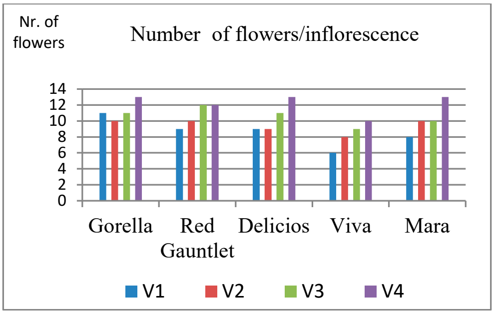

Figure 1. The fructification capacity

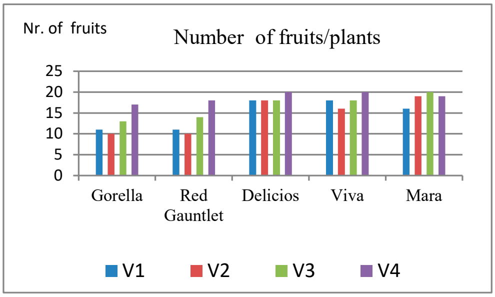

Figure 2. The fructification capacity

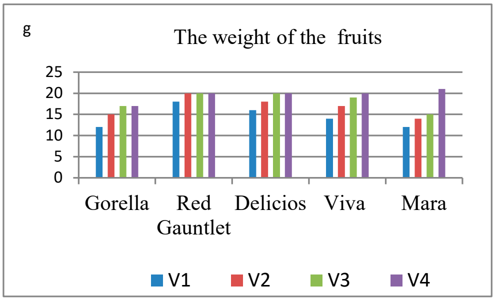

Figure 3. The average weight of the fruits

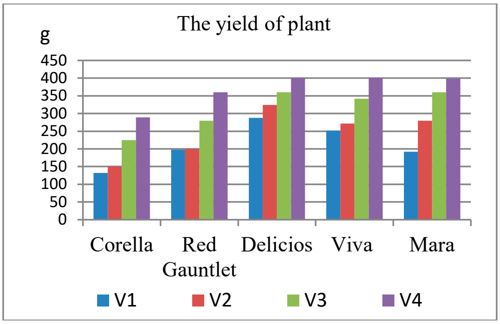

Figure 4. The plant fructification capacity

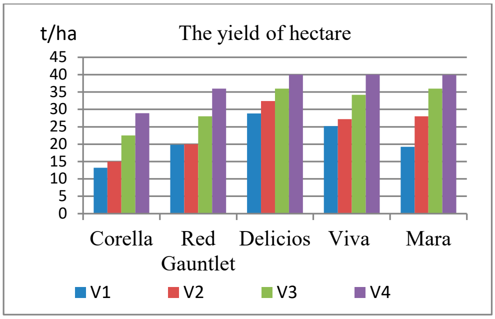

Figure 5. The potential yield per hectare

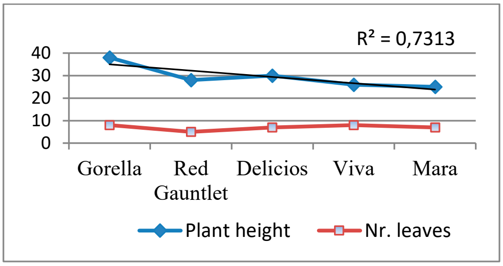

Figure 6. The correlation between plant height and number of leaves per plant (V1)

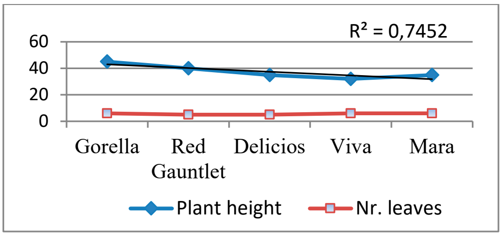

Figure 7. The correlation between plant height and number of leaves per plant (V4)

The analysis of the correlation between the different characteristics showed that there is a direct correlation between plant height and number of leaves per plant in each variant and there is no correlation between other characteristics analyzed.

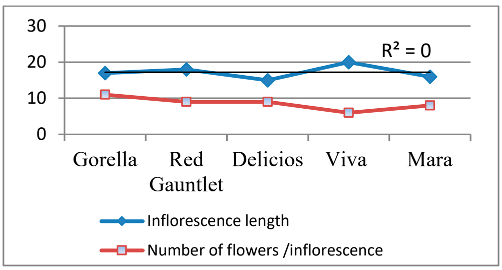

Figure 8. The correlation between inflorescence length and number of flower per inflorescence (V1)

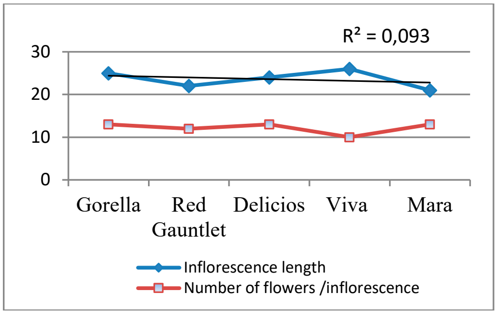

Figure 9. The correlation between inflorescence length and number of flower per inflorescence (V4)

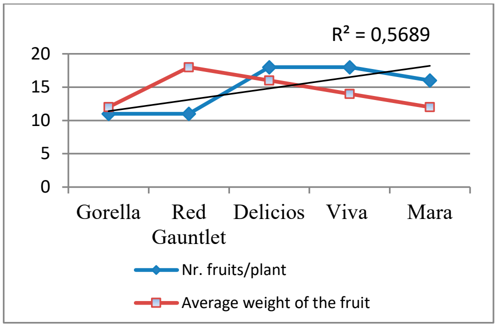

Figure 10. The correlation between number of fruits per plant and average weight of the fruit (V1)

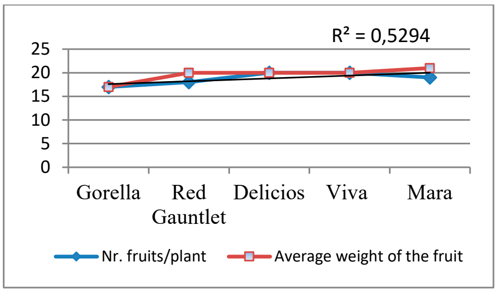

Figure 11. The correlation between number of fruits per plant and average weight of the fruit (V4)

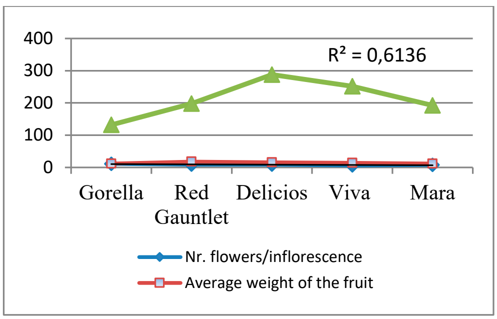

Figure 12. The correlation between number of flowers per plant, the average weight of the fruit and the yield per plant (V1)

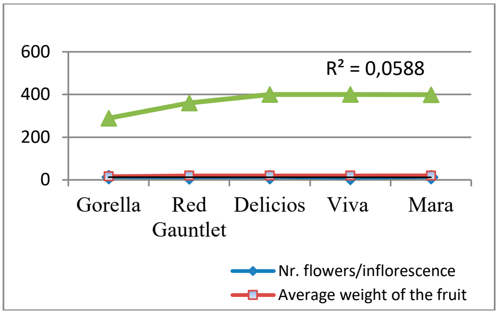

Figure 13. The correlation between number of flowers per plant, the average weight of the fruit and the yield per plant (V4)

CONCLUSIONS

The quality of the biological material used influences greatly the percentage of gripping. The biological material used had a planting gripping between 96-98%.

In the classical culture of strawberries are produced high yields of fruits, but culture variant with agro-textile and organic fertilization provides very high yields.

Fruits obtained in V2 variant were higher compared to fruit obtained in conventional culture (V1 variant), but higher values provides variants V3 and V4.

Among the varieties studied, the most productive varieties were Delicios and Viva (40 t/ha in variant V4) and Mara (39.9 t/ha).

The variety with the lowest production was the variety Gorella (13.2 t/ha in the classical system of culture).

Strawberry production remains a profitable crop, regardless of the manner of obtaining the fruit, because investments are recovered from the first crop of fruit.

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IMPACT OF SOIL MAINTENANCE SYSTEMS ON THE VEGETATIVE AND REPRODUCTIVE MANIFESTATIONS OF 'KATINKA' CULTIVAR

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Abstract

The study was conducted in RIMSA-Troyan in the period 2014-2018, in a plum plantation of 'Katinka' cultivar, under non-irrigated conditions, on pseudo-podzolic gray forest soils, poorly stocked with nutrients. The impact of different soil surface management systems (fallow, natural grassland, artificial grassland) on vegetative and reproductive indicators of plum trees was observed. Data showed that the largest growth of the trunks was registered in soil managed as an artificial grassland (trunk section 2014-30.01cm²; 2015-39.01cm²; 2017-60.56cm²; 2018-70.92cm²) and the highest annual growth was observed for each year of the study period, formed by higher number of annual twigs. In the formation of the volume and projection of the crowns, no patterns have been established regarding the soil management way. The highest yields were reported in 2017 with 14 kg/tree and in 2018 with 9.1 kg/tree from an artificial turf as a soil management system, which had a positive effect on other reproductive indicators, such as weight and size of fruit.

Key words: *plum, soil management systems, growth manifestations, reproduction.*

INTRODUCTION

The plum is widespread throughout Bulgaria. The main plum plantations are located in the semi-mountain and mountain regions of Bulgaria. In addition to agrotechnical events, the system of soil surface menagement system is of major significance for the good development and optimal fruit bearing of trees (Dinkova et al., 2005; Taseva, 2005; Petrov et al., 2008).
The most widely used systems for soil surface management systems in the orchards of Bulgaria are fallow and natural grassing. Prolonged soil management as a fallow land, especially without the introduction of organic matter, dramatically impairs its agrophysical characteristics (Merwin, 2004). Natural grass-cover is a preferred system that has a positive effect on erosion processes, especially in mountain and semi-mountain areas of Bulgaria with wet soils and sloping terrain (Gergov et al., 2001; Petrov et al., 2008).
In recent years, modern fruit growing has introduced environmentally friendly systems related to improving soil fertility, maintaining nutrient balance and limiting erosion through artificial grass-cover of rows and inter-row spacings and rows with legume species (Fiener & Auerswald, 2007; Watson & Evans, 2007; Prasuhn, 2012; Poláková et al., 2018).
For the mountain conditions of Bulgaria, the mixture of common bird's-foot-trefoil (*Lotus corniculatus* L.) with red fescue (*Festuca rubra* L.) and Kenthucky bluegrass (*Poa pratensis* L.) is determined as the most suitable for grass-covering of raspberries, blackcurrants and plums plantations (Petrov & Minkov, 2006; Vitanova & Petrov, 2010; Bozhanska et al., 2019).
The turf-mulched system and living mulch have a slight inhibitory effect on the growth of plum trees, and the fruit bearing is not significantly reduced (Dinkova et al., 2004).
Applying a properly selected soil menagement system can ensure good growth, quality and high yields of fruit plants, reduce erosion processes, improve soil fertility and ensure optimal nutrition (Bozhanska et al., 2019, 2017; Hristova et al., 2017).
A number of scientists have studied biologically and economically viable systems for soil surface management and their impact on the growth, development and fruit bearing of fruit crops (Glenn & Welker, 1989; Merwin et al., 1994; Parker & Meyer, 1996; Neilsen et al., 1999; Miletic et al., 2010)

The research workers at RIMSA Troyan have been working for decades on the study of appropriate environmentally friendly systems for soil surface management in plum plantations (Gergov et al., 1998; Dinkova et al., 2004), monitoring their impact on vegetative and reproductive capabilities of trees and fruit quality (Petrov & Dinkova, 2004; Dinkova & Petrov, 2004).

The aim of the present study is to determine how the different soil surface management systems affect the vegetative and reproductive manifestations of 'Katika' plum cultivar.

MATERIALS AND METHODS

To study the impact of different systems of soil surface management on the growth and reproductive performance of plum trees, an experiment with the following variants was set in Troyan RIMSA:

- Fallow - the interrows are maintained as a fallow by disking;
- Natural grassland - the interrows are covered by turfgrass of natural perennial grasses;
- Artificial grassland - interrows are covered by turfgrass of grass mixture from legume and grasses in ratio (1:1) with bird's-foot-trefoil (*Lotus corniculatus* L.) and red fescue (*Festuca rubra* L.) at a seeding rate of 5 kg/da;

The experiment was conducted in 2014-2018 in a plum orchard with 'Katinka', grafted on a Fereley rootstock, established in 2010, on pseudo-podzolic soils with poor nutrient soils. Inter-row spacings were covered with turf (Natural grassland, Artificial grassland) in 2013.

The vegetative and reproductive indicators of the fruit trees are taken into account, in the different variants of soil management:

- Trunk cross section (cm²); Crown volume (m³); Crown projection (m^2); Annual shoot length growth (cm); Total annual shoot length growth (cm); Number Annual shoot length;
- Fruit weight (g); Fruit stone (g); Yield per tree (kg); Fruit stone (% of the fruit); Fruit and stone dimensions (mm);

RESULTS AND DISCUSSIONS

Inter-row spacings was covered by turf and sown with grass species in 2013. Their impact on the vegetative and reproductive indicators of the trees was evident after 2014, when they reached the optimal for their species root system and aboveground mass.

In the first two years of the experiment (2014-2015), there was a large increase in the trunk cross section of the trees from the artificial grassland variant, followed by the fallow variant.

In 2016 the trees of the three variants are approximately equal in trunk cross-section, but in natural grassland there was a maximum trumk increase by 18 cm^2, compared to 2015. In the next two years (2017-2018), a larger increase was observed in the trunks of the grass-covered variants, as the largest was in trees grown on artificial grassland in 2017 (60.56 cm^2), 2018 (70.92 cm^2) (Table 1).

Table 1. Vegetative characteristics of trees (2014-2018)

		Trunk cross section (cm²)	Crown volume (m³)	Crown projection (m^2)	Annual shoot length growth (cm)	Total annual shoot length growth (cm)	Number Annual shoot length
2014	Clean cultivation	26.88	1.16	1.43	38.51	401.17	10.42
	Natural grassland	25.98	1.48	1.58	31.07	513.69	16.53
	Artificial grassland	30.1	2.35	2.25	37.73	1007.91	26.71
2015	Clean cultivation	36.31	4.42	4.69	29.14	305.67	10.49
	Natural grassland	31.56	4.8	4.85	33.05	361.00	10.92
	Artificial grassland	39.01	2.82	3.04	43.10	538.67	12.5
2016	Clean cultivation	48.74	3.81	3.56	20.73	370.80	17.89
	Natural grassland	49.6	5.04	4.97	23.50	229.40	9.76
	Artificial grassland	46.22	5.29	4.71	11.00	258.60	23.51
2017	Clean cultivation	50.45	6.30	5.49	26.34	394.20	12.51
	Natural grassland	56.99	7.64	6.39	23.75	319.80	15.00
	Artificial grassland	60.56	5.74	5.73	28.45	359.40	16.80
2018	Clean cultivation	53.17	14.26	11.10	22.35	310.20	14.80
	Natural grassland	68.65	10.13	7.88	21.28	250.60	13.60
	Artificial grassland	70.92	9.49	8.84	24.94	280.50	15.75

The highest increase in trunk cross-section was measured in the artificial grassland management system, in all years of the study.
When the experiment was set in 2014, the trees of the artificial grassland variant had larger crowns (2.35 m^3) and a projection (2.25 m^2).
The following year, these differences were erased, with the volume and projection of the crown lagging behind. In 2016, the volume and the projection had higher values again in the grass-covered variants.
The largest crowns were observed in the fallow management system in 2018 (volume-14.26 m^3; projection-11.10 m^2) (Table 1), as no impact on the shape and volume of the crowns were established in relation to soil surface management system.
According to Costes et al. (2004) the growth that is formed each year is a variable that determines the size of the trees. In the first years of the study (2014-2015), the highest one-year growth was measured in the artificial grassland variant, formed by a larger number of annual twigs, except for 2016, the trend was maintained until the end of the study. Our studies correspond to various studies, according to which grass-covering of inter-row spacing does not negatively affect the growth manifestations of plum trees (Dinkova et al., 2008; Petrov et al., 2008).
The main morphological characteristics of plums are shape, size, weight.
With the gradual entry of the plantation into full fruit bearing, the fruit weight and yields increased annually. The data are presented in Table. 2.

Table 2. Reproductive performance of 'Katinka' cultivar (2015-2018)

Variant	Fruit weight (g)	Fruit stone (g)	Yield per tree (kg)	Fruit stone (% of the fruit)
2015				
Clean cultivation	19.71	0.93	7.22	4.74
Natural grassland	17.83	0.98	7.07	5.49
Artificial grassland	14.51	0.76	6.41	5.22
$LSD_{0.05}$	*1.11*	*0.08*		
St Dev	*2.89*	*0.13*		
2016				
Clean cultivation	18.95	0.88	6.40	4.66
Natural grassland	18.78	0.84	3.90	4.49
Artificial grassland	21.88	0.92	5.80	4.18
$LSD_{0.05}$	*1.51*	*0.08*		
St Dev	*2.35*	*0.90*		
2017				
Clean cultivation	22.36	0.88	6.50	3.92
Natural grassland	23.00	0.91	3.50	3.96
Artificial grassland	24.28	1.01	9.50	4.15
$LSD_{0.05}$	*4.69*	*0.42*		
St Dev	*3.33*	*0.12*		
2018				
Clean cultivation	16.76	0.89	7.90	5.11
Natural grassland	19.32	0.96	7.40	4.98
Artificial grassland	21.18	0.92	9.10	4.34
$LSD_{0.05}$	*1.80*	*0.08*		
St Dev	*2.49*	*0.12*		

The fruit weight varied according to variants and during the years from 14.5 to 24.2 g. These data correspond to Blazek and Pistekova, (2009) where for the conditions of Holovousy, ‘Katinka’ weighed 21.8 g and variation was 17-24 g. Milatovic et al. (2018) confirmed the weak growth force of ‘Katinka’, and gave 22.9 g per fruit weight. For the years of the present study, such a weight was reported in 2017, when the fruits were the largest (22.36-24.28), regardless of the soil surface management system. In the same study, ‘Katinka’ was defined as a high-yielding cultivar. For the Bulgarian conditions the highest yields on average for the three variants were reported in 2018 (7.40-9.10 kg per tree).

The relatively small variation in the stone weight among the different variants by less than 0.2 g is impressive.
In 2015 the highest weight and the highest yield were reported in the fallow variant (Table 2). In the following years the highest fruit weight was measured in the grass-covered variants, as in the artificial grassland larger fruits with higher yield were formed. In 2017, the largest fruit weight of 24.28 g and the highest yield of 9.5 kg/tree were reported for the variant, compared to the other variants for the study period.
Low yields were reported in the natural grassland variant, regardless of the larger size of the fruit, and in 2016 the yield was half less than in the other two variants. This is probably because of some suppression and competition of grass vegetation.
The relative share of stone by variants over the years is 4-5.5%, which is close to most plum cultivars and is not affected by the soil surface management system.
The fruit sizes varied very little, comparing them by variants and years. The stone size varied much less, with a thickness of less than 0.5 mm between variants.
Slight variation in the fruit size and stones shows that they are not affected by the soil management system (Table 3).

Table 3. Sizes of fruit and stone (2015-2018)

	Fruit size (mm)			***Stone fruit size (mm)***		
	height	width	thickness	height	width	thickness
2015						
Clean cultivation	35.20	27.90	28.10	20.40	11.90	6.10
Natural grassland	34.90	28.20	28.00	20.20	12.10	6.20
Artificial grassland	34.50	27.40	27.90	20.80	12.00	5.90
$LSD_{0.05}$	*0.98*	*0.85*	*0.92*	*0.84*	*0.30*	*0.42*
St Dev	*2.68*	*2.29*	*3.14*	*1.26*	*0.39*	*0.60*
2016						
Clean cultivation	36.30	28.60	28.30	20.60	12.50	6.50
Natural grassland	35.80	28.20	28.30	20.80	12.30	6.30
Artificial grassland	38.10	31.00	30.20	21.00	12.50	6.90
$LSD_{0.05}$	*1.25*	*1.43*	*1.46*	*1.39*	*0.74*	*0.52*
St Dev	*2.41*	*2.34*	*2.16*	*1.60*	*0.83*	*0.50*
2017						
Clean cultivation	39.07	32.33	29.87	21.80	13.87	7.00
Natural grassland	39.40	32.67	29.53	22.36	14.13	6.86
Artificial grassland	35.00	35.00	33.33	23.93	14.87	7.00
$LSD_{0.05}$	*3.91*	*2.28*	*1.95*	*2.50*	*1.35*	*0.49*
St Dev	*2.67*	*1.91*	*2.41*	*2.67*	*1.91*	*0.37*
2018						
Clean cultivation	37.27	29.20	27.67	22.46	13.62	6.54
Natural grassland	37.93	30.53	29.53	23.31	13.69	6.77
Artificial grassland	39.40	31.73	30.33	23.08	13.92	6.92
$LSD_{0.05}$	*1.45*	*1.25*	*1.07*	*0.83*	*0.52*	*0.39*
St Dev	*2.49*	*1.93*	*1.70*	*2.21*	*0.72*	*0.51*

For the period of the study, the fruitfullness rate for 'Katinka' was calculated for different soil management systems. In the first year (2015) in all three variants it was higher, as the highest value was registered for the natural grassland variant (0.22 kg/cm^2). In the following years, under the influence of the grass vegetation of the natural grassland, the fruitfullness decreased drastically (Figure 1).

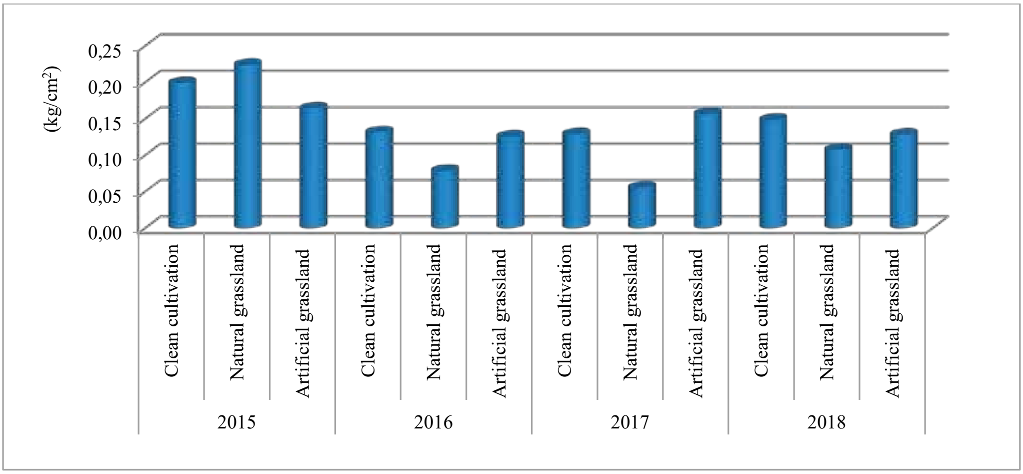

Fig. 1 Fruitfullness coeficient (kg/cm^2) (2015-2018)

CONCLUSIONS

Maintaining the soil surface in artificial grassland had a positive effect on the growth of trees with higher trunk growth and high annual growth, formed by a large number of annual twigs. The volume and projection of the crowns were not affected by the soil management system.

Higher yield formed by fruits with higher weight with high fruitfullness rate was gathered when 'Katinka' was grown on artificial grassland.

Maintaining the inter-row spacing in natural grassland had a negative effect on the reproductive performance of this cultivar due to the competition of grass vegetation.

The highest efficiency on the vegetative and reproductive manifestations of 'Katinka' cultivar was registered in the artificial grassland. This makes this system promising.

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DEGREE OF CONTROL OF WEEDS UNDER THE INFLUENCE OF AGRO-TECHNICAL MEASURES AND HERBICIDES IN A PEACH PLANTATION (*PRUNUS PERSICA* L.)

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Abstract

The research was carried out on a family farm in Periam, Timis County, and studied the Redhaven peach variety. The soil where the orchard was placed is a chernozem, moist phreatic, moderate hyposodic between 50-100 cm, weak carbonate, deep on medium loessoid materials, medium loam / medium loam. The value of the apparent density (AD) is extremely low in the Atk surface horizon with a value of 1.10 g / cm^3, very low in the range of 18-75 cm, and low in the Amk2 horizon with a value of 1.35 g / cm^3. For this experiment, the randomized block method was used, and 8 experimental variants were chosen, which were placed on 4 rows of trees, resulting in 4 repetitions with 32 experimental plots. The surface of an experimental plot was 12.20 m^2. From the obtained data, can be observed that the effectiveness of the weed control measures materialized through the productions obtained in the two experimental years. Following the application of weed control measures, the production obtained in 2019 had values between 12.78 t / ha and 20.65 t / ha, and in 2020 the production was between 11.50 t / ha and 18, 65 t / ha.

***Key words**: peach, herbicide, weed infestation, pest control, production.*

INTRODUCTION

Weed control in peach plantations (*Prunus persica* L.) continues to be a major problem, especially for young plantations (Botu et al., 2003; Hoza, 2000; Drăgănescu 2002; Gradinariu, 2002).

EU farming systems have become more vulnerable and less sustainable due to excessive herbicide dependence and the huge growth of herbicide-resistant weeds (Tataridas et al., 2022).

Human health, biodiversity and plantation sustainability can be affected by the toxic substances of many chemical pesticides, which have been blamed for soil and water degradation. The recent European Union (EU) legislative frameworks set out the needs and requirements of citizens as a major task for the organization of the agricultural sector in the Member States (Peeters et. al., 2020). It is said that climate change will cause temperatures to rise by at least 2 ° C in the 21st century and will negatively affect crop production by increasing biotic and abiotic stress (Raza et al., 2019). The increase in heavy rainfall is another factor that predicts production losses. As stated by Maes et al., 2019, the EU will have more drought events, and temperatures will put pressure on agroecosystems. However, uncertainty remains high as climate change is a continuing phenomenon (Tubiello, 2007). Sustainable crop production is a complex link of decisions and resources that must be properly managed to mitigate the long-term impact of climate change. This task is even more difficult in respect of organic farming systems, where chemical pesticides (Alda, 2007; Cârciu, 2006; Manea, 2006) are not used. The situation is even worse in organic farming, where low tillage is applied and there are perennial weeds, which are normally managed with effective synthetic herbicides, such as glyphosate (Scedei et al.,2021), in conventional systems, but are absent in conventional systems organic culture (Melander et al., 2013). A future scenario implies that crops that are susceptible to more pests and diseases are likely to face higher yield differences in organic farming compared to conventional production (Rasche, 2021).

Herbicides have been and are being applied for a long time in agricultural systems, representing an important tool for crop protection, especially in the years to come, when weeds are expected to cause more problems in agricultural systems (Kudsk, 2003; Neve et al., 2018).

The total use of herbicides in the EU27 since 1990 indicates that the goal of reducing chemical pesticides by 50% by 2030 remains a major challenge (Figure 1). However, the consecutive use of herbicides is not only observed in the EU, it is also the major component for crop protection in developed and developing countries (Gianessi, 2013; Shattuck, 2021).

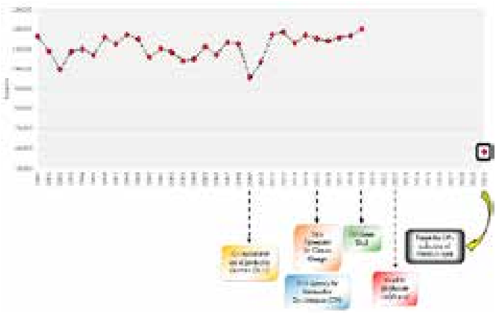

Figure 1. Total herbicide use in the EU_{27} between 1990–2019 and the important milestones and targets for chemical pesticide reduction by 50% by 2030 (https://www.fao.org/faostat)

The question is what is the fate of glyphosate and are there alternatives? Glyphosate is not in line with several sustainable development goals (Krimsky, 2021) and its appearance in the markets beyond 2022 is still unknown, while several effective herbicide generic products have overwhelmed the markets after the expiration of patents (Shattuck, 2021). However, we wonder if there are effective alternatives to glyphosate that need to be integrated into a complex decision-making system for crop protection to keep low costs and maintain ecosystem services (Fogliatto et al., 2020; Kanatas et al., 2021). Concern about the future of herbicides extends beyond the fate of glyphosate and is appropriately stated by Beckie et al., 2020, wondering what the future holds for other successful herbicides.

MATERIALS AND METHODS

The field studies were carried out in a family plantation of peach (Redhaven variety), (*Prunus persica*), from Periam (46 ° 01′41 ″ N 20 ° 53′35 ″ E), Timis county. The value of the apparent density (AD) is extremely low in the Atk surface horizon with a value of 1.10 g / cm^3, very low in the range of 18-75 cm, and low in the Amk2 horizon with a value of 1.35 g / cm^3 (Ianoș, 1995).

The 8 experimental variants were (V1 - untreated, not hoed; V2 - Agro Glyfo Green (4 l / ha) + 1 manual hoeing; V3 - Glyphogan 480 SL (4.5 l / ha) - 1 manual hoeing; V4 - Typhoon 360 SL (5 l / ha) + 1 manual hoeing; V5 - Glyphotim (4 l / ha) + 1 manual hoeing; V6 - Clean Up Xpert (5 l / ha) + 1 manual hoeing; V7 - Roundup Classic Pro (4 l / ha) + 1 hoeing; V8 - Fusilade Forte EC (1.3 l / ha) + 1 manual hoeing) and these were placed on 4 rows of trees (using the randomized block method), in 4 repetitions with a number of 32 plots. The surface of an experimental plot was 12.20 m^2.

The determination of the floristic composition was determined by weed mapping, which is a very complex work and includes several main links, weed species determination, weeding level, weeding mapping and so on. based on them, control measures were established. Each method has its advantages and disadvantages.

Herbicides were administered on each experimental variant. Their application was done manually, with the help of the vermorel type sprayer, using 400 l of solution on an area of 1 ha. They were administered in May.

An herbicide efficacy test has been established in accordance with the standard method of the European and Mediterranean Plant Protection Organization (EPPO) (EPPO / OEPP, 2020).

The optimum time for cultivation is 7 to 21 days after the application of post-emergent herbicides, to allow the translocation of the herbicide into the root of perennial weeds.

The processing and interpretation of the experimental data was done by analyzing the variance (Săulescu, 1967).

RESULTS AND DISCUSSIONS

In the two experimental years (2019-2020) the climatic conditions showed very large oscillations. Temperatures had close values, compared to the amount of precipitation that showed different values from one month to another, which influenced both the level of weeding and production.

The weeding level in 2019 was 114,75 weeds / m^2. The most common weeds were: *Elymus repens* 20,02 weeds / m^2 (17,45%), *Setaria glauca* 18,04 weeds / m^2 (15,72%), *Amaranthus retroflexus* 16,00 weeds / m^2 (13,99%) and *Veronica hederifolia* 14,60 weeds / m^2 (12,72%).

The species *Stellaria media* (0,89%), *Cardaria draba* (0,52%), *Cirsium arvense* (0,21%) and *Digitaria sanguinalis* (0,05%) had a low frequency (Figure 2.).

Monocotyledons accounted for 38,83%, while dicotyledons accounted for 61,17%.

In 2019, 14 plant species were identified, included in 8 botanical families. The *Poaceae* family includes 4 species: (*Elymus repens, Setaria glauca, Cynodon dactylon*, and *Digitaria sanguinalis*). The *Brasicaceae* family includes 3 species (*Capsella bursa pastoris, Sinapis arvensis* and *Cardaria draba*). The *Asteraceae* family includes 2 species (*Sonchus arvensis* and *Cirsium arvense*). The families *Amaranthaceae*,*Caryophyllaceae,Chenopodiaceae, Convolvulaceae* and *Scrophulaceae* include a single species of weed.

The highest participation percentage of 28,58% belongs to the *Poaceae* family (Figure 3.).

The average field after the application of weed control measures was 31,55 weeds / m^2 (27,49%).

Monocotyledonatae holds 10,79% (12,37 weeds / m^2) and dicotyledonatae 16,70% (19,16 weeds / m^2) (figure 4).

The percentage of uncombined monocotyledonous ranges between 3,47% (V7 - Roundup Classic Pro (4 l / ha) + 1hoeing) and 19,57% (V8 - Fusilade Forte EC (1.3 l / ha) + 1 hoeing). The percentage of uncombined dicotyledonous lies between 5,43% and 29.34%

The number of weeds / m^2 in the control variant was 114,75. The average experimental field was 17,38 t / ha.

The absolute production oscillates between 12,78 t / ha, in the V8 variant - Fusilade Forte EC (1,3 l / ha + 1 manual plow and 20,65 t / ha in the V7 variant - Roundup Classic Pro (4 l / ha) + 1 manual hoe (V6 - Clean Up Xpert (5 l / ha) + 1 manual hoeing), the yield obtained was 20,16 t / ha (Table 1).

The relative production is between 73,53% and 118,81%, respectively.

The production increase oscillates between, 1,56 t / ha (V4 - Taifun 360 SL (5 l / ha) + 1 manual hoe) and 3,27 t / ha (V7 - Roundup Classic Pro (4 l / ha) + 1 manual hoeing).

The degree of weed control in variants where the active substance was glyphosate, shows close values. The percentage of weed control is between 57,38% (V8 - Fusilade Forte EC (1.3 l / ha) + 1 manual hoeing) and 92,25% (V7 - Roundup Classic Pro (4 l / ha) + 1 manual hoeing). In variant V2 - Agro Glyfo Green (4 l / ha) + 1 manual hoeing, the degree of weed control is 90,12% (Figure 5.)

The number of uncontrolled weeds ranged between 8,90 weeds / m^2 and 48,91 weeds / m^2, respectively.

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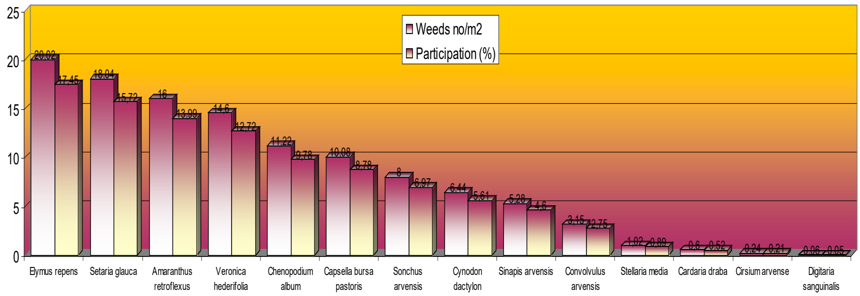
The number of uncontrolled weeds ranged between 8,90 weeds / m^2 and 48,91 weeds / m^2, respectively.



Monocotyledonatae: 44,56 weeds /m^2 (38,83%) Dicotyledonatae: 70,19 weeds/m^2 (61,17%)

Figure 2. Number of weeds and percentage of participation, in variant V1 - untreated, not hoed, in 201

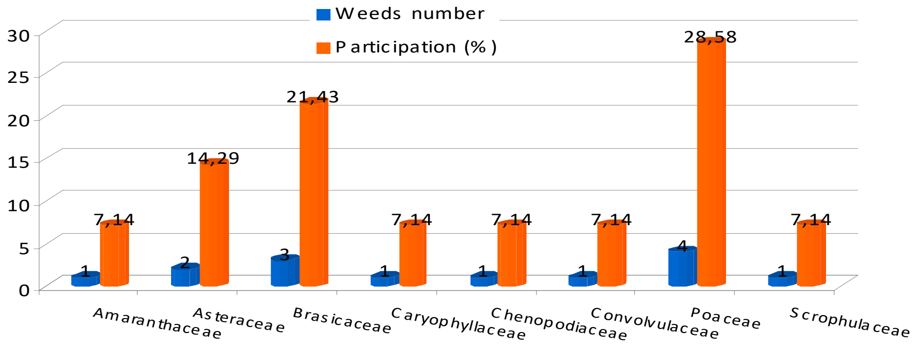


Figure 3. The level of weeding according to the botanical family, in 2019

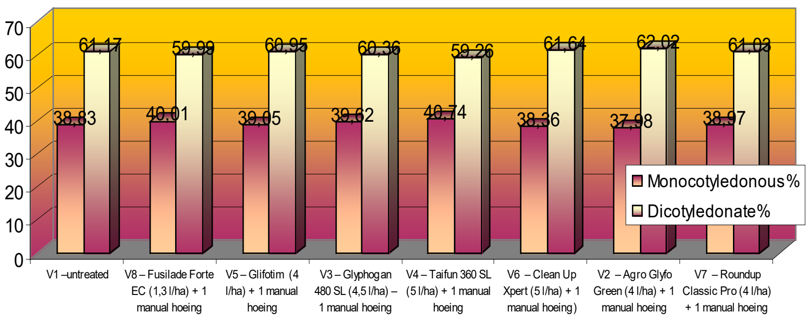
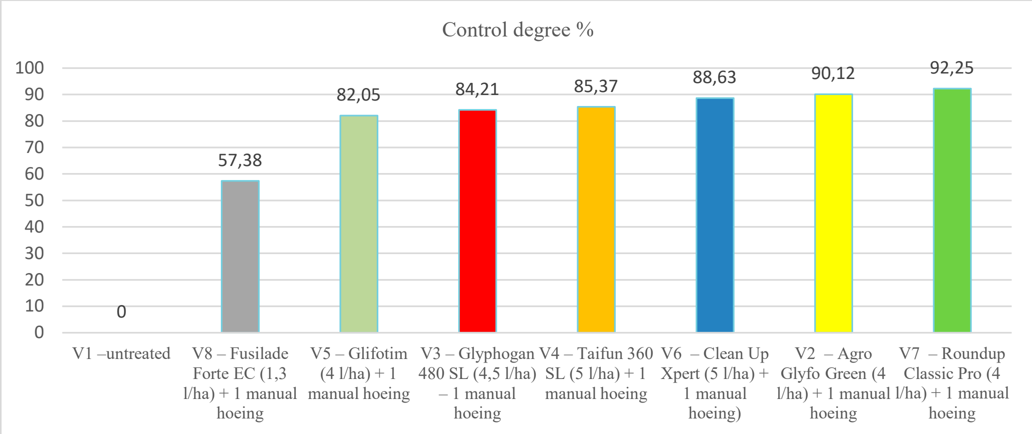


Figure 4. The distribution of weeds in groups, following the application of control measures, in 2019



DL $_{5\%}$ = 5,93 weeds/m^2; DL $_{1\%}$ = 8,00 weeds/m^2; DL $_{0,1\%}$ = 10,62 weeds/m^2

Figure 5. Intensity of agrotechnical measures, on the level of weeding, in 2019

Table 1. The influence of weed control measures on peach production (t / ha), in 2019

Treatment	Absolute yield (t/ha)	Relative yield (%)	Yield difference (t/ha)	Significance
V_7 – Roundup Classic Pro (4 l/ha) + 1 manual hoeing	20,65	118,81	+3,27	Xx
V_6 – Clean Up Xpert (5 l/ha) + 1 manual hoeing)	20,16	115,99	+2,78	Xx
V_2 – Agro Glyfo Green (4 l/ha) + 1 manual hoeing	19,60	112,77	+2,22	X
V_3 – Glyphogan 480 SL (4,5 l/ha) – 1 manual hoeing	19,35	111,33	+1,97	X
V_4 – Taifun 360 SL (5 l/ha) + 1 manual hoeing	18,94	108,97	+1,56	-
X – Mean	**17,38**	**100,00**	**Mt.**	**-**
V_5 – Glifotim (4 l/ha) + 1 manual hoeing	17,23	99,13	-0,15	-
V_8 – Fusilade Forte EC (1,3 l/ha) + 1 manual hoeing	12,78	73,53	-4,60	00
V_1 – untreated, unhoed	10,40	59,83	-6,98	000

DL $_{5\%}$ = 1,96 t/ha; DL $_{1\%}$ = 64 t/ha; DL $_{0.1\%}$ = 4,61 t/ha.

In 2020 the weeding level was 128,04 weeds / m^2. *Elymus repens* (17,57%), *Cynodon dactylon* (15,81), *Convolvulus arvensis* (14,07%) and *Veronica hederifolia* (12,86%) were very frequent (figure 6.). With low presence we have the species *Chenopodium album* (0,83%), *Cardaria draba* (0,09%), *Polygonum aviculare* (0,06%) and *Stellaria media* (0,03%). Monocotyledonous represent 42,55% and dicotyledonous 57,45%.

The weeds mapped in 2020 belongs to 9 botanical families. The *Poaceae* family includes 4 species (*Elymus repens, Cynodon dactylon, Echinochloa crus-galli* and *Digitaria sanguinalis*), with a participation rate of 28,58%.

The *Asteraceae* family includes 2 species (*Cirsium arvense* and *Sonchus arvensis*) (Figure 7), the participation percentage being 14,29%. Also 2 species of weeds belonging to the family *Brasicaceae* (*Capsella bursa pastoris* and *Cardaria draba*).

The other 6 families include weeds: *Convolvulus arvensis, Veronica hederifolia, Amaranthus retroflexus, Chenopodium album, Polygonum aviculare* and *Stellaria media*, with a participation rate of 42.86%. Following application of control measures, the average value of field in respect to uncontrolled weeds was 30.74%.

The average of uncombined monocotyledonous species represents 13,06% (16,73 weeds / m^2), and dicotyledonous represent 17,68% (22,64 weeds / m^2).

The number of uncontrolled monocotyledonous weeds ranges between 6,03 weeds / m^2 (41,30%) in the V7 variant - Roundup Classic Pro (4 l / ha) + 1 manual hoeing and 25,80 weeds / m^2 (43,25%) in the variant V8 - Forte EC rifles (1.3 l / ha) + 1 manual hoeing. The number of uncontrolled dicotyledonous weeds ranged between 8,57 weeds / m^2 (58,70%) and 33,87 weeds / m2 (56,75%) (Figure 8.).

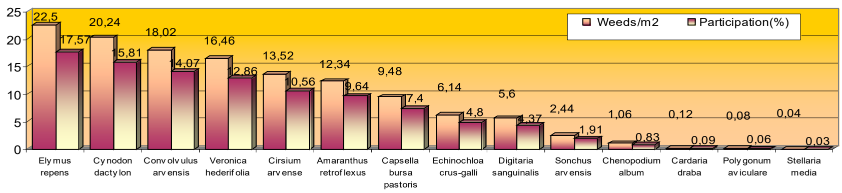
Compared to 2019, in 2020 the effectiveness of weed control measures was lower

Compared to 2019, in 2020 the effectiveness of weed control measures was lower, the degree of weed control ranges between 53,40% (V8 - Fusilade Forte EC (1.3 l / ha) + 1 manual hoeing) and 88,60% (V7 - Roundup Classic Pro (4 l / ha) + 1 manual hoeing). With a high percentage of weed control level, of 86,75%, the variant V2 - Agro Glyfo Green (4 l / ha) + 1 manual hoeing (Figure 9.) is also highlighted.

Following the application of weed control measures, the level of weeds is significantly reduced from 128,04 weeds / m2 (V1 - untreated, not hoed) to 14,60 weeds / m^2 (V7 - Roundup Classic Pro (4 l / ha) + 1 manual hoeing).

The yield obtained in 2020 was lower than that obtained in 2019. The average field, assumed as a control variant, was 15,80 t / ha.

The absolute production obtained for peaches lies between 11,50 t / ha in the V8 variant - Fusilade Forte EC (1,3 l / ha) + 1 manual hoeing and 18,65 t / ha in the V2 variant - Agro Glyfo Green (4 l / ha ha) + 1 manual hoeing. A production of 18,07 t / ha was obtained in the V7 variant - Roundup Classic Pro (4 l / ha) + 1 manual hoeing, with an increase of production compared to the control variant, up to 2,27 t / ha (Table 2). The relative production is between 72,78% and 118,03%, respectively. The highest increase in production was 2,85 t / ha, very significant.



Monocotyledonous: 54,48 weeds/m^2 (42,55 %); Dicotyledonous: 73,56 weeds/m^2 (57,45 %)

Figure 6. Distribution and percentage of weed participation, in variant V1 - untreated, unhoed, in 2020

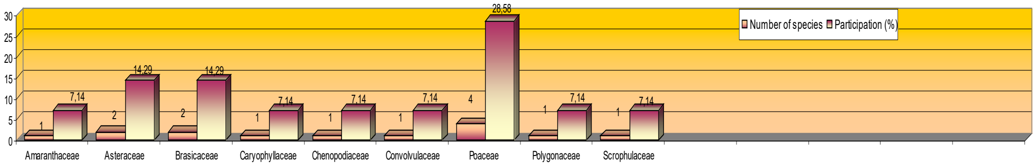


Figure 7. Distribution of weed species according to the botanical family, in 2020

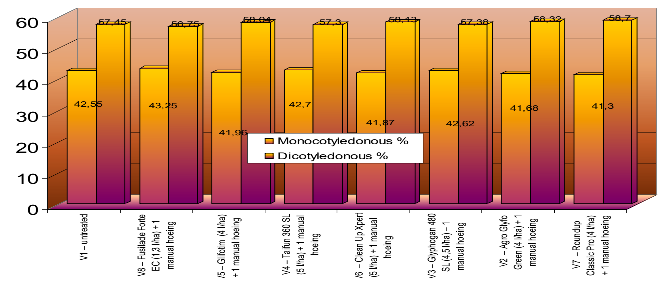
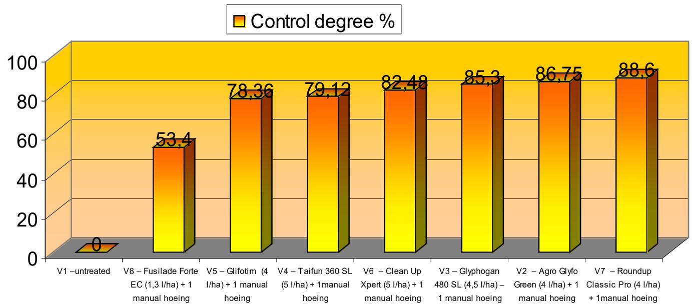


Figure 8. Arrangement of weed species in groups, in the year 2020



DL $_{5\%}$ = 6,12 weeds/m^2 DL $_{1\%}$ = 8,25 weeds/m^2 DL $_{0,1\%}$ = 10,95 weeds /m^2

Figure 9. The influence of agrotechnical measures on the degree of weed control (%), in 2020

Table 2. The effect of weed control measures on peach production (t / ha) in 2020

Treatment	Absolute yield (t/ha)	Relative yield (%)	Yield difference (t/ha)	Significance
V_2 – Agro Glyfo Green (4 l/ha) + 1 manual hoeing	18,65	118,03	+2,85	xxx
V_7 – Roundup Classic Pro (4 l/ha) + 1 manual hoeing	18,07	114,36	+2,27	Xx
V_3 – Glyphogan 480 SL (4,5 l/ha) – 1 manual hoeing	17,80	112,65	+2,00	X
V_4 – Taifun 360 SL (5 l/ha) + 1 manual hoeing	17,22	108,98	+1,42	-
V_6 – Clean Up Xpert (5 l/ha) + 1 manual hoeing)	16,98	107,46	+1,18	-
V_5 – Glifotim (4 l/ha) + 1 manual hoeing	16,47	204,24	+0,67	-
X – Mean	**15,80**	**100,00**	**Mt.**	**-**
V_8 – Fusilade Forte EC (1,3 l/ha) + 1 manual hoeing	11,50	72,78	-4,30	000
manual hoeing	9,75	61,70	-6,05	000

DL $_{5\%}$ = 1,53 t/ha; DL $_{1\%}$ = 2,06 t/ha; DL $_{0.1\%}$ = 2,73 t/ha.

CONCLUSIONS

Following the research conducted in the two experimental years (2019 - 2020), the following conclusions were drawn.

Different climatic conditions in the two experimental years, especially in terms of precipitation, impacting the effectiveness of weed control methods, the degree of control and the production obtained.

In 2019 the weeding level was 114,75 weeds / m^2, and in 2020 128,04 weeds / m^2. The higher level of weeding in 2020 was recorded in May 2020 due to the higher amount of rainfall (128,00 mm).

In 2019 the most widespread weeds were: *Elymus repens* (17,45%), (15,72%), *Amaranthus retroflexus* (13,99%) and *Veronica hederifolia* (12,72%). In 2020, the species *Elymus repens* (17,57%), *Cynodon dactylon* (15,81), *Convolvulus arvensis* (14,07%) and *Veronica hederifolia* (12,86%) were dominant.

In 2019, monocotyledonous represented 38,83%, and dicotyledonous 61,17%. In 2020, the percentage of monocotyledonous increased, which was 42,55%, while that of dicotyledonous was 57,45%. In both years 2019 and 2020, 14 weed species were identified, included in 8 and 9 botanical families, respectively.

The degree of weed control in 2019 ranged between 57,38% and 92,25%. The V7 variant - Roundup Classic Pro (4 1 / ha) + 1 manual hoeing), proved to be the most efficient with a control percentage of 92,25%.

In 2020, the degree of weed control had values between 53,40% and 88,60%, much lower compared to 2019.

The effectiveness of the weed control measures materialized through the productions obtained in the two experimental years. Following the application of weed control measures, the production obtained in 2019 had values between 12,78 t / ha and 20,65 t / ha, and in 2020 the production was between 11,50 t / ha and 18, 65 t / ha. The production increase compared to the field average, in 2019 was 3,27 t / ha, and in 2020 it was 2,85 t / ha.

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EFFECT OF THE RADICULAR AND FOLIAR FERTILIZER ON FRUIT QUALITY IN THE PEACH ORCHARD

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Abstract

Nutrient management is a determining element of the technology in fruit quality. Significance of foliar fertilization has been increased continuously over the last years, as it can directly improve the vegetative and generative performance of the trees. In this study we aimed to evaluate the effect of the radicular (NPK+S) and foliar (Cropmax) fertilizer on fruit quality parameters in a peach orchard during 2019 -2021 period. According to our results, the weight of the fruits increased by 40% in the b1 treatment and 64% in the b2 treatment at Filip cultivar and by 27% in the b1 treatment and 49% in the b2 treatment at Catherine sel. 1 cultivar in the three years of study compared to the control treatment. In conclusion, the fruit quality can be improved in peach orchard using radicular and foliar fertilizers.

Key words: *climate conditions, fertilization, peach, weight.*

INTRODUCTION

Fertilization of fruit trees is an essential element of the technology due to its significant influence on the quality and yield. Nowadays foliar fertilization has become a basic management tool in intensive orchards. Foliar fertilizers have an interesting potential to improve fruit quality, with relatively low costs and low environmental impact (Csihon et al., 2021). Application of sprays supplies nutrients to plants more rapidly than the soil fertilization (Tagliavini et al., 2002; Nagy et al., 2012, 2019). The use of biostimulators is increasing all over the world. They can be considered as natural growth regulators. Beneficial effect of the biostimulators on the fruit quality is confirmed by several studies (Hudina et al., 2003; Basak & Mikos-Bielak, 2008; Nagy et al., 2019; Csihon et al., 2013, 2021).

The aim of this work was to provide data on the effect of 'Cropmax' foliar biostimulator on the fruit quality in a peach orchard.

MATERIALS AND METHODS

Experimental site. The studied orchard is located in Valu lui Traian commune, district Constanta, Dobrogea region, Romania. This is a semi-arid region with a climatic water deficit (WD), calculated as a difference between annual precipitation (P) and Penman-Monteith reference evapotranspiration (PM-ETo), ranging from about -400 mm on the Black Sea coastal area to -320 mm (Paltineanu et al., 2007). The soil is a calcareous chernozem with a loamy texture and alkaline pH in topsoil which has a good soil structure (0-60 cm depth, with 27-32% g/g clay content, 1.6-2.8% g/g humus content, 1.5-6.8% g/g carbonate content), while in the non-structured subsoil, the humus content is lower than 1% g/g and the carbonates from 9 to 14% g/g; land slope is between 2.0 and 2.5% (Paltineanu et al., 2011).

The peach orchard was planted in spring of 2011. Trees were grafted on Tomis 1 rootstock and designed with spacing of 4 x 2.5 meter with north-south row orientation. The canopy shape was a classic vase with the height of 2.5 m. The experiment design was based on the split-plot method with three treatments: b1) radicular fertilizer with NPK+S, 15:15:15+ 12.8; b2) radicular and foliar fertilizer (Cropmax); b3) control, each one containing four replicates. The soil management system is represented by clean cultivation both between tree rows and in the row. Plant protection refers to the principles of integrated pest management.

The climatic data: solar radiation, air temperature, relative humidity, wind speed at the height of 2 m, precipitation (P) and Penman-Monteith reference evapotranspiration have been recorded by an automatic weather station (iMetos, IMT 300, Pessl Instruments, Austria) by a 1-h step. These data have been periodically transferred to a laptop and processed as diurnal means and used in calculations.

Applied treatments. The main objective of this study was to evaluate the effect of radicular and foliar fertilizer on the fruit quality in a peach orchard. The trial was consisted of three treatments (control, radicular fertilizer and radicular + foliar fertilizer). The first treatment with foliar fertilizer was applied in full bloom in 2019 and 2020, then the treatments were repeated 3 times. In 2021, the first treatment with foliar fertilizer was applied later, 11.05, followed by two replicas (Table 1). Biostimulant treatment was applied to peach orchard in the vegetation phases with 0.5 l/ha dosage (Figure 1).
Every spring, in the peach orchard was applied to the soil the complex NPK fertilizer enriched with easily absorbable S (15: 15: 15+ 12.8), 500 kg/ha respectively. Each treatment was consisted of 16 trees.

Table 1. Foliar fertilizer application times (2019-2021)

2019	2020	2021
12.04.2019	24.04.2020	11.05.2021
13.05.2019	15.05.2020	09.06.2021
31.05.2019	29.05.2020	02.07.2021
18.06.2019	17.06.2020	

Figure 1. Foliar fertilizer applied at peach orchard

Applied materials. The peach tree (*Prunus persica* L. Batsch) has been selected for this study as it is one of the most cultivated fruit tree species worldwide, especially in the warm temperate climate regions. Filip is the first peach cultivar with flat fruit "sandwich" and white pulp, registered in Romania, in 2002 at Research Station for Fruit Growing (RSFG) Constanța (Figure 2). Catherine sel. 1 is the first clingstone peach cultivar registered in Romania, in 2001 at RSFG Constanta (Figure 3).

Figure 2. Filip cultivar

Figure 3. Catherine sel. 1 cultivar

NPK complex fertilizers, with a balanced formula in the content of Nitrogen (N), Phosphorus (P), Potassium (K), guarantee a fertilization rich in nutrients with direct results on increases in the quality and quantity of fruit products.
Cropmax is a complex super concentrate nutrient for foliar fertilization, which is 100% natural, produced by Farming Holland BV. Cropmax fertilizer presents the following chemical composition: growth plant stimulator (auxines, citochinine, gibberellines), organic amino acids, vegetal vitamins, vegetal enzymes, macro-elements: N0 - 2%, P - 0.4% , K - 0.02%, Fe - 220 mg, Mg - 180 mg, Zn - 40 mg, Mn - 45 mg, Cu - 5 mg, other elements: B, Ca, Mo, Co, Ni - 10 mg. Being a natural product, Cropmax is well absorbed by leaves and stems. Influence on photosynthesis leads to the increase of carbohydrates amount. Also are reduced metabolic deficiencies.

Assessed parameters. Each year average samples of 15 fruits/treatment were tested. Fruit growth was monitored by measuring longitudinal and transversal fruit diameter and fruit height after harvest. The measurements were performed using a metric digital caliper (Insize Co., Ltd. China). The average weight of a fruit was determined by weighing 10 fruits/treatment and dividing by the number of weighed fruits. The weighing of the fruit was performed with a precision balance (Kern & Sohn GmbH, Germany). Fruits of Filip cultivar were harvested between the 11th to the 12th of July in 2019, from 15th to the 16th of July in 2020 and 26th to the 27th of July in 2021. Fruits of Catherine sel. 1 cultivar were harvested between the 23rd to the 24th of July in 2019, from 27th to the 28th of July in 2020 and 2nd to the 3rd of August in 2021.

Data analyses. SPSS 14.0 software and Microsoft Office Excel were used for the analysis of variance and various calculations for fruit quality properties. Different letters in the graphs indicate significant differences for the probability (P) $\leq$ 0.05 according to Duncan's multiple range test.

RESULTS AND DISCUSSIONS

Climate conditions. During the growing season in the experimental period the mean yearly maximum and minimum air temperatures were 26.4 and 12.7°C, respectively, versus the long-term yearly means of 25.8 and 13.3°C.

The mean annual air temperature was 19.5°C, versus 19.7°C for long-term. In the growing season, the mean annual precipitation amount was 213.8 mm, versus 270.7 mm for long-term, almost similar, and mean annual reference evapotranspiration was 714.0 mm, versus 722.7 mm for long-term, indicating a normal period, both in terms of rainfall and evapotranspiration. ETo values were on average 134.0, 150.3 and 134.7 mm month-1 during June, July and August, respectively.

The average value of climatic water deficit (WD) in the growing season of the study period was -500.2 mm, versus -452.0 for long-term. The mean value of yearly and monthly climate data during the growing season in the experimental period is shown in the Tables 2 and 3.

Table 2. The mean value of annual climate data during the growing season in the 2019÷2021 experimental period versus the long-term, 1980 ÷ 2015, Valu lui Traian, Romania

Climatic data	Growing season	
	2019÷2021	1980÷2015
Mean air temperature, T_{med} (°C)	19.5	19.7
Mean maximum air temperature, T_{max} (°C)	26.4	25.8
Mean minimum air temperature, T_{min} (°C)	12.7	13.3
Precipitation, P (mm)	213.8	270.7
Reference evapotranspiration, PM-ET_0 (mm)	714.0	722.7
Water Deficit/Water Excess, WD/WE (mm)	-500.2	-452.0

Table 3. The mean value of monthly climate data during the growing season in the experimental period 2019÷2021, Valu lui Traian, Romania

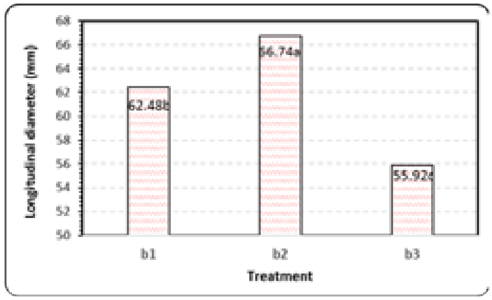
Climatic data	Growing season					
	April	May	June	July	August	September
Mean air temperature, T_{med} (°C)	10.0	16.6	22.3	24.0	23.9	19.4
Mean maximum air temperature, T_{max} (°C)	16.7	23.0	29.3	31.4	31.7	26.5
Mean minimum air temperature, T_{min} (°C)	3.6	10.6	16.0	16.5	16.7	12.6
Precipitation, P (mm)	34.5	41.2	55.0	26.0	9.7	47.4
Reference evapotranspiration, PM-ET_0 (mm)	86.0	123.0	134.0	150.3	134.7	86.0
Water Deficit/Water Excess, WD/WE (mm)	-51.5	-81.8	-79.0	-124.3	-125.0	-38.6

The period of experiment (2019÷2021) was considered as a relatively normal period, with monthly temperature means of 22.3°C in June, 24.0°C in July and 23.9°C in August, respectively.

Biometrical measurements and weight to peach fruits. After harvesting, the fruits of experience have been subjected to biometrical measurements and fruits weighing. The values presented represent average values of the three years of study.

Thus, peach fruits on Filip cultivar had a longitudinal diameter of 55.92 mm to 66.74 mm. The smallest longitudinal diameter was found in b3 treatment. Figure 4a shows that there were significant differences between the treatments studied on fruit's longitudinal diameter, as indicated by different letters according to the probability (P) ≤ 0.05 according to Duncan's multiple range test. As with fruit longitudinal diameter, the transversal diameter determined on the fruits of the studied treatments had the same trend. The highest value was obtained in b2 treatment, 63.24 mm and the lowest value in b3 treatment, 53.27 mm, respectively. Figure 4b shows significant differences, written with different letters, between the treatments studied regarding the fruits transversal diameter. The height of the fruits determined by the studied treatments had the same trend. The peach fruits had a height of 30.54 mm to 36.96 mm. Figure 4c shows significant differences between the treatments studied regarding the fruits height. As with fruit biometrical characteristics, the fruit weight determined on the fruits of the studied treatments had the same trend. The highest value was obtained in b2 treatment, 85.15 g and the lowest value in b3 treatment, 51.95 g, respectively. Figure 4d shows significant differences between the treatments studied regarding the fruits weight.

The peach fruits of the Catherine sel. I cultivar had a longitudinal diameter of 53.8 mm to 69.7 mm. The smallest longitudinal diameter was found in b3 treatment. Figure 5a shows that there were significant differences between the treatments studied on fruit's longitudinal diameter, as indicated by different letters according to the probability (P) ≤ 0.05 according to Duncan's multiple range test.



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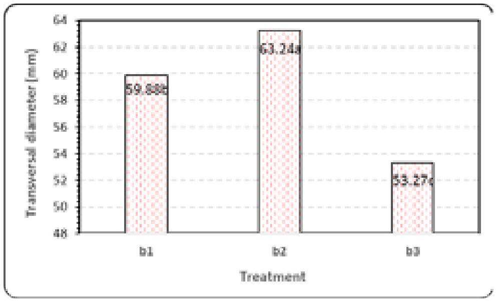

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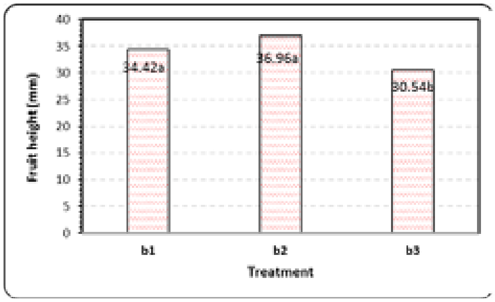

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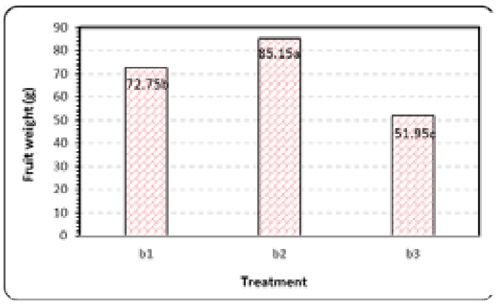

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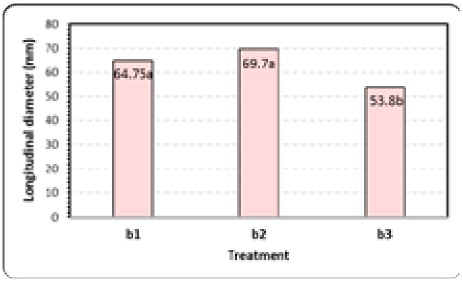
Figure 4. Longitudinal diameter (a), transversal diameter (b), fruit height (c) and fruit weight (d) of the fruits to Filip cultivar, 2019-2021

As with fruit longitudinal diameter, the transversal diameter determined on the fruits of the studied treatments had the same trend. The highest value was obtained in b2 treatment, 64.0 mm and the lowest value in b3 treatment, 50.3 mm, respectively. Figure 5b shows significant differences, written with different letters, between the treatments studied regarding the fruits transversal diameter. The height of the fruits determined by the studied treatments had the same trend. The peach fruits of the Catherine sel I cultivar had a height of 45.1 mm to 56.6 mm. Figure 5c shows significant differences between the treatments studied regarding the fruits height. As with fruit biometrical characteristics, the fruit weight determined on the fruits of the studied treatments had the same trend. The highest value was obtained in b2 treatment, 172.4 g and the lowest value in b3 treatment, 115.4 g, respectively. Figure 5d shows significant differences between the studied treatments regarding the fruits weight.



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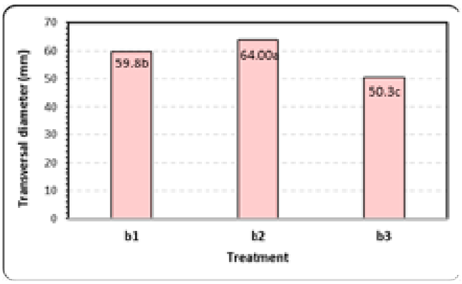

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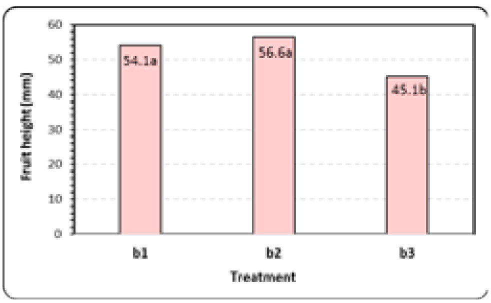

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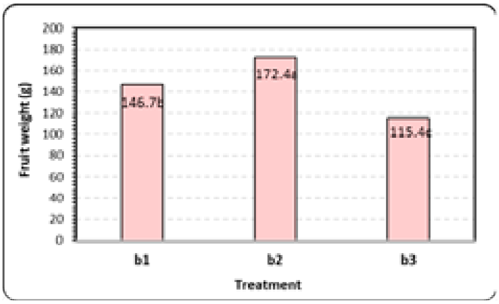

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Figure 5. Longitudinal diameter (a), transversal diameter (b), fruit height (c) and fruit weight (d) of the fruits to Catherine sel. 1 cultivar, 2019-2021

CONCLUSIONS

The study found that both radicular and foliar fertilization help to improve the quality of the fruits of the two studied cultivars.

Radicular and foliar fertilization with Cropmax has led to a higher increase in fruit weight in Filip and Catherine sel. 1 cultivars compared to radicular fertilization.

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OLD LOCAL APPLE GENOTYPES THREATENED WITH EXTINCTION

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Abstract

During research expeditions in the Central Balkan Mountain region, trees of late-ripening (mid-October), rare local cultivars, and forms of apples with valuable pomological and biological qualities were marked. In 2021, all marked forms bore abundant fruit. The highest yield (280 kg per tree) was registered in 'Meka Shekerka' cultivar (MS F1). The fruit weight was on average 59.50 g, obloid form, low percentage of dry matter (12%), a high percentage of total sugars (18%), and the highest glucoacidimetric coefficient. BF1 was distinguished by yields of 140-150 kg per tree, very good taste of the fruit (lowest acid content 0.27%), attractive appearance (green-yellow with a blush on the sunlit side), white fruit flesh, pleasant taste with a pronounced aroma of green apple, abundant waxy coating, with a mass of 86.17g and globose fruit shape. Our scientific data are compared with the information obtained from the local population on age, origin, fruitfulness, use, and distribution in the region.

Key words: *apple, local cultivars and forms, fruit weight, fruit skin colour, chemical composition.*

INTRODUCTION

The traditional cultivars of fruit species are the wealth and natural heritage of each country. They are important for economic and biological reasons, especially recently due to the growing interest of consumers in the consumption of fruits produced without the use of chemicals. Many rare and locally distributed cultivars have become extinct and are difficult to find today.

Old traditional cultivars (autochthonous and cultural) are almost neglected and represent an important part of Bulgarian natural and cultural heritage. Regardless of that most orchards of old cultivars are neglected. Efforts have recently been made to restore and protect them, as their fruit market is slowly emerging. Traditional cultivars are more resistant to plant diseases, pests, and other forms of abiotic stress.

Long-term research in the region of the Central Balkans has been carried out to establish the type of this region's fruit species and local cultivars and forms and to propose a program for their preservation and renewal. Our previous expeditions in the area of the village of Stanchov Khan and the surrounding villages were described by Dragoyski et al. (2012).

Because of the closed nature of municipalities and neighborhoods in the past, the same forms and primitive local cultivars of apples have been exchanged and propagated among the locals. The gardens are managed only around the few inhabited houses, with trees over 60-70 years old predominating.

These farms have a rich gene pool of unique, endangered cultivars that need to be preserved. Due to their increased resistance to diseases, they can be included in organic fruit orchards, as their production can be successfully used for the preparation of traditional healthy foods such as dried fruits, pestil, marmalades, and fruit brandies typical of the Balkan region. Single trees from the old local apple cultivars, such as 'Kantarka', 'Yovovka', 'Vlashka', and 'Skrinyanka' were found. Old local cultivars of pears 'Summer Pear', 'Vodnik', and others were found in the observed villages.

Many of the collected cultivars can be donors in the selection for disease resistance, high acidity, pectin, and biologically active substances. Their fruits are suitable for fresh consumption and the preparation of traditional healthy foods. Such production could be very attractive for tourists and would help the development of this business in the area.

Nesheva et al. (2019) also surveyed this area of the Central Balkan National Park. The total area was approximately 530 ha, at more than 750 m above sea level. During the investigation, wild apple species *Malus sylvestris* Mill., *M. dasyphylla* Borkh., *M. praecox* (Pall.) Borkh. and wild pear species *Pyrus pyraster* Burgsd., *P. amygdaliformis* Vill., *Pyrus nivalis* Jacq. and *Pyrus elaeagrifolia* have been identified.

Due to the preservation of the assortment, the morphological and pomological characteristics of the fruits of the most common traditional apple cultivars from the region of Bjelovar-Bilogora County were studied by Vujević (2016). Based on the study of pomological characteristics were determined cultivars, such as 'Jonatan', 'Kanada', 'Šampanjka', 'Zlatna zimska parmenka', and 'Božićnica'. These cultivars can be recommended for propagation and renewal of the production range in the study area, due to the variety of pomological characteristics, fruit quality, and resistance to adverse abiotic and biotic factors.

A significant part of the diversity in the area of the forest park of Starčevica, Banja Luka, was studied by Antić et al. (2021). Analysis of the site and related tree species in the community was performed based on representative samples belonging to the wild relatives of the cultivated species, wild cherry (*Prunus avium*), wild apple (*Malus sylvestris*), wild pear (*Pyrus communis*). For each fruit species, abundance and degree of presence were determined, and a map was created based on GPS coordinates collected from the field. A total of 1059 trees at 37 landfills were inventoried, of which 39 were wild apple trees, 27 wild pears, and 118 wild cherries. Other trees of other forest species are mostly hornbeam (*Carpinus betulus*), beech (*Fagus sylvatica*), and oak (*Quercus petraea*). The presence of wild fruit trees in the Starčevica forest park was noted at an average value of 2 by the Braun-Blanquet method and the abundance of 10-25% of fruit trees in the study area compared to the presence of other forest species.

Extensive research were done by Ghosh et al. (2012) given the high genetic diversity in the genus *Malus*. The age of the plants varied between 13 and 70 years, and the yield potential varied from 650 to 1,085 kg/tree. The fruit weight varied between 130 and 225 g, the dry matter content (Brix) varied between 15.0 and 18.4°, the acidity 1.7 to 4.6%, and the total sugar content varied between 5.1 and 14.3%.

For the region of Apriltsi, Marishnitsa, near the town of Troyan, Vitkov (2015) marked, described, and studied 38 apple cultivars and forms and their reproductive characteristics. The fruits of most of them have an attractive appearance, rich skin color, juicy and tasty fruit flesh, a rich chemical composition, valuable nutritional and dietary qualities, and good storage in ordinary home conditions.

With the extinction of old cultivars of trees, Bulgarian natural and cultural heritage, genetic diversity is lost, and those cultivars that have adapted to local agroecological conditions over the years might be at the heart of organic fruit growing (Janjić, 2016).

The fruits of the traditional old apple cultivars from the Topusko region were studied by Babojelić et al. (2014). They differ significantly in quality and physico-chemical characteristics. Given the many benefits of growing traditional cultivars, it is important to preserve them, because the extinction of old trees of certain cultivars and the loss of a significant source of genetic resources, impoverishes the assortment of apples on the market.

The present work aims to study the pomological characteristics of old local cultivars and forms of endangered apples.

MATERIALS AND METHODS

During the research period, research expeditions were conducted in the Central Balkan Mountains region (Stoynovska neighborhood) and trees of late-ripening (mid-October), rare local cultivars and forms of apples with valuable pomological, and biological qualities were marked.

The marked trees were in abandoned orchards, in depopulated villages and neighborhoods and were not maintained and applied agricultural techniques, so they were introduced into alternative fruiting. They were over 100 years old, with damaged trunks, and falling bark, but still show high yields (good reproductive performance in a full fruit-bearing year), which does not significantly interfere with the size of the fruit.

To be protected from wild animals and unfavorable climatic conditions, the trees were grafted by cuttings over 1.5 m high from the soil surface, the diameter of the trunk is over 2 meters, the crowns are free-growing, erect, tall (12-15 m and more), globular in some and pyramidal (Figure 1).

Individual single trees of these shapes were found in different neighborhoods in the region of Troyan. The most important thing was to preserve and reproduce them because they are a valuable genetic resource.

Figure 1. Old apple tree

A visual assessment of the attitude of the observed cultivars and forms to the economically significant diseases, such as scab and powdery mildew on leaves and fruits was performed. Slight susceptibility based on single spots or no spots of scab on the leaves and fruits was reported, and no symptoms of powdery mildew were found.

Monitored indicators

Vegetative - diameter of the trunk (m); size and shape of the crown (m);

Reproductive - yield (kg/tree), fruit weight (g), fruit size, and stalk (mm);

Chemical composition of the fruit at the ripening stage:

- Dry matter in refractometrically (%)
- Sugars (%) (total, invert and sucrose) - according to Schoorl and Regenbogen method, (Donchev et al., 2000)
- Organic acids (malic, citric) (%) -by titration with 0.1N NaOH (Donchev et al., 2000)
- vitamin C (mg/%) to Tilman method (Donchev et al., 2000)
- Pectin (%) extraction of pectin substances to calcium pectate (Melitz)

Chemical composition of the fruit after a month after fruit harvesting:

- Total Soluble Solids (%)
- physical - density (firmness) of fruit flesh (kgf/cm^2) - determined with a digital penetrometer FHT-15 (3.5 mm), by measuring on both sides of 25 randomly selected fruits. Fruit skin of measured fruit was removed.
- pomological characteristics - taste, aroma, color (SC-30, Colorimeter for color difference)

RESULTS AND DISCUSSIONS

Vegetative indicators

The crowns of all the described shapes were free-growing. Forms SF 1 was with the slightest growth, whose trunk circumference had 64 cm, a spherical crown with an average diameter of 5 m and a height of 5.5 m, and SF 2, which had a larger trunk circumference and wider and a high spherical crown. MS F1 had a trunk circumference of over 2 m, over 10 m of tree height, the crown was upright, free-growing with an average diameter of 6-6.5 m. MSF2, KS, BF1, and BF2 also had the largest sizes.

The skeletal branches of BF forms (1, 2, 3) were firmly connected to the guide, and the angle of deviation from the stem is 45°. In other forms of ‘Shekerka’, the overgrown wood was more upright (with a sharper angle to the stem).

Reproductive indicators

Form BF 1 was characterized by a fruit weight of 86.17 (g) and a globose fruit shape (UPOV 2005), followed by SF 1 and SF 2 with a fruit weight of 83.17 and 81.67 (g, respectively). The exception was ‘Newtown Pippin’ with 101 (g) (Table 1). BF 1 also had the largest fruit height (53.37 mm) and diameter sizes (60.34-63.89 mm), followed by BF 2 (height - 51.13 mm; diameters 58-60 mm).

Figure 2. Apple fruit Momina Cheska

The average diameter of the fruit was the largest in the 'Newtown Pippin' (61.55-64.68 mm). The smallest were the fruits of 'Momina cheska' (average diameter 50.85 mm), with a yield of 130 kg of wood.

The fruits ripened a little earlier than other cultivars, fall off, but remained for a long time without damage and rot.

They were suitable for processing (Figure 2).

Table 1. Biometric indicators of fruit

	Yield (kg/tree)	Fruit weight (g)	Height (mm)	Average Diameter (mm)	Fruit stalk length (mm)
BF 1	140	86.17	53.37	62,12	13.11
BF 2	165	78.13	51.13	59,50	11.26
BF 3	150	70.67	49.51	57,00	9.89
MS F1	280	59.50	46.54	53,60	11.28
MS F2	260	56.17	44.71	52,21	11.62
KS	200	62.00	46.79	53,06	14.12
'Zimna koravka'	210	76.33	44.84	58,62	9.57
'Newtown Pippin'	120	101.00	50.53	61,10	14.93
SF 1	85	83.17	49.06	58,74	16.71
SF 2	65	81.67	47.79	59,22	12.38
'Karastoyanka'	110	65.67	42.48	55,78	6.47
'Momina cheska'	130	52.67	42.89	50,82	10.75
LSD 0,05		*11.12*	*3.27*	*2.31*	*2.87*
St Dev		*19.16*	*4.14*	*3.99*	*3.44*

Chemical composition at the time of fruit ripening included the content of dry matter (%), total sugars and acids (%), and pectin (%) (Table 2). The highest percentage of dry matter was in the variety "Zimna koravka' at 18%, while the forms of 'Korava Shekerka' and SF 1 had 17%.

Forms BF 1, 2, 3 had a very low dry matter content of 11.5-12.5%, but their pectin content was 1.0-1.17%, the highest for the group.

Regarding the total sugars 'Karastoyanka' and 'MS F1' contained 18.05% each, followed by 'Korava Shekerka' with 15% and 'SF1' with 14.5%. 'MS F1' was characterized by the highest content of sucrose (5.32%), and there were cultivars and forms without sucrose, which was an extremely important indicator determining the consumption of these fruits by people with high blood sugar.

These were 'BF 3', 'Newtown Pippin' and 'Momina cheska'. The lowest pectin content was registered in 'Momina cheska' and 'SF 2' (0.22-0.26%).

Table 2. Chemical composition of fresh fruit (2021)

	Dry matter (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Acids (%)	vit. C (mg/%)	Pectin (%)
BF1	12.00	10.05	6.65	3.23	0.27	17.60	1.17
BF 2	12.50	13.45	10.90	2.42	0.40	17.60	0.63
BF 3	11.50	11.75	11.75		0.34	14.08	1.00
MS F1	12.00	18.05	12.45	5.32	0.27	17.60	0.61
MS F2	11.50	14.30	11.75	2.42	0.47	14.08	0.57
'Korava Shekerka'	17.00	15.15	12.60	2.42	0.34	17.60	0.72
'Zimna Koravka'	18.00	13.45	12.45	0.50	0.27	12.32	0.47
'Newtown Pippin'	14.00	13.45	13.45		0.40	17.60	0.73
SF 1	17.00	14.50	13.45	1.00	0.40	14.08	0.41
SF 2	16.00	13.45	10.90	2.42	0.20	15.84	0.26
'Karastoyanka'	15.50	18.05	13.45	4.37	0.34	12.32	0.39
'Momina cheska'	15.50	13.45	13.45		0.34	15.84	0.22
St Dev	*2.40*	*2.27*	*1.91*	*1.50*	*0.07*	*2.10*	*0.28*

After one month of fruit storage, the value of soluble solids increased by 1 to 4%, as form BF1 from 11.50% (Table 2), reached 15.20% (Table 3). In 'Zimna koravka', the value of soluble solids during the harvest period was 18%, and a month later 21.8%. Most likely, this was the reason for the long period of storage of this form (May-June next year). The forms with green-coloured skin, covered with abundant wax, thin and strong, such as BF 1, 2, 3, had white fruit flesh, juicy, and slightly sour, pronounced apple flavor and were suitable for consumption in the ripening stage. The density of the fruit flesh was on average 8.5 kgf/cm^2. MS F1, 2 were similar in description, but without a pronounced aroma, they were significantly sweeter and preferred by certain consumers. These forms could also be preserved (Table 3). The presence of abundant wax deposits increased storage time in ditches covered with ferns.

Cultivars and forms with red skin color and green fruit flesh were suitable for processing and consumption after storage and resistant to long-term storage. Form SF1 was on an intermediate position in the present study, whose fruits were suitable for consumption at the ripening stage and then subject to long-term storage. The density of the fruit flesh was 8 kgf/cm^2. The density of the fruit flesh of all the described forms was from 7.02 to 13.06 (kgf/cm^2) (Table 3). 'Zimna Koravka' had the densest fruit flesh (13.06 kgf/cm^2), followed by SF2 with 12.45 kgf/cm^2, and 'Karastoyanka' with 11.27 kgf/cm^2 (Table 3).

Table 3. Pomological and physical characteristics of the fruit

	Fruit skin	Fruit flesh	Fruit flesh density (kgf/cm^2)	Soluble Solids (%)
BF 1	Green, red on the sunlit side, with white dots. Oily, covered with abundant wax, thin, strong	White, soft, juicy, slightly sour, with a pronounced apple aroma	8.24	15.00
BF 2	White-green, with blush on the sunlit side, covered with abundant wax, thin	White, juicy, sour with aroma	8.76	13.20
BF 3	Light green, on red clouds on the sunlit side, with abundant waxy coating, tender, thin	White, soft, juicy, not coarse, sour, with a pronounced aroma	10.90	15.20
MS F1	Main colour - yellow-green, covered almost entirely with discontinuous and continuous red stripes, thin, tough	White, soft but not floury, slightly dry, sweet, odourless	7.62	13.80
MS F2	Green with blush on the sunlit side, with darker red stripes, thin, strong	White, soft, juicy, sweet, tender, odourless	7.40	12.80
'Korava Shekerka'	Green, covered with red discontinuous and continuous stripes, thick but not coarse	Green, soft, dry, sweet	7.90	17.00
'Zimna koravka'	Green, with blush, thick, firm	Green, slightly coarse, without stony cells, juicy, sweet, odourless	13.06	21.80
SF 1	Dark red, slim but tough	White to creamy, grainy, tender, juicy, slightly sour, slight aroma	8.00	19.80
SF 2	Green, with broken red stripes on the sunlit side, firm but not thick	Green, slightly coarse, juicy, sour, with aroma	12.45	18.60
'Karastoyanka'	Reddish in stripes, firm, tough	Greenish, juicy, firm, slightly sour with aroma	11.27	18.80
'Newtown Pippin'	Dark green with rust, thick, firm	Green, firm, to slightly coarse, fibrous, highly sour with aroma	9.27	18.00
'Momina cheska'	Dark red, firm, tough	White, soft, dry, without a pronounced aroma	7.02	15.00
St Dev			*2.07*	*2.85*

CONCLUSIONS

In 2021, all marked forms bore abundant fruit. BF1 was distinguished by yields of 140-150 kg per tree, very good taste of the fruit, attractive appearance (green-yellow with a blush on the sunlit side), white fruit flesh, pleasant taste with a pronounced aroma of green apple, abundant waxy deposit, with a weight of 86.17 (g) and a globose fruit shape.

'Meka Shekerka' (MS F1) had the highest yield (280 kg per tree). The average fruit weight was

59.50 g (Table 1), obloid shape, a low percentage of dry matter (12%), high percentage of total sugars (18%).

'Korava Shekerka' (KS) with a fruit weight of 62.00 g, SF 1 with a weight of 83.17 g were characterized by dense fruit flesh and thick skin, which prolongs the storage period. They can be consumed till May next year.

'Newtown Pippin' and 'Momina cheska' and BF 3 were sucrose-free forms, which makes them suitable for diabetics.

Due to the variety of pomological characteristics, fruit quality and resistance to adverse abiotic and biotic factors, it is important to preserve traditional apple cultivars as a source of genetic diversity in the area where they grow. The described cultivars and forms should be preserved and propagated because of their suitability for fresh consumption and processsing and their inclusion in selection programs.

Our scientific data were compared with the information obtained from the local population on age, origin, fertility, use and distribution in the region. It is advisable to create small, sustainable, orchards from the local forms of apples.

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BEHAVIOR OF PLUM VARIETIES GROWN IN DRYANOVO EXPERIMENTAL STATION TO ECONOMICALLY IMPORTANT DISEASES

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Abstract

The behavior of plum varieties grown in Dryanovo Experimental Station for the period 2018-2020 to the economically important diseases of the plum (Polystigma; Monilinia fructigena; Stigmina carpophila, Tranzschelia-pruni spinosae) was studied.
Sensitive to red leaf spots were the plum varieties Yoyo, Chachanska lepotitsa, Chachanska najbolie and Nevena, according to the Stanley standard (slightly sensitive). With regard to late brown rot and powdery mildew, a high degree of sensitivity manifested variety Tegera. Nevena and Balvanska Slava proved to be practically resistant to these diseases. Plum rust tolerant plums (Tranzschelia-pruni spinosae) include the varieties Yoyo, Balvanska Slava, Nevena, Gabrovska, Tegera and Hanita. From the study on the conditions of Dryanovo, Bulgarian varieties show low to medium susceptibility to economically important diseases.

Key words: *Plum, economical important diseases, Polystigm, Monilinia fructigena, Stigmina carpophila, Tranzschelia-pruni spinosae.*

INTRODUCTION

The plum is an economically valuable fruit species with traditions in our country. The biological and economic qualities of plum allow it to take a leading place in our country. It is a traditional fruit crop, widespread at 300-700 m in the foothills and mountainous regions of the Central Stara Planina, in the region of Troyan, Dryanovo, Gabrovo. This is due to the favorable soil and climatic conditions that the plum orchard finds in foothill and mountain conditions (Anzin, 1956; Enikeev, 1960) (Djouvinov and Vitanova, 2002). Plum (*Prunus domestica* L.) is characterized by high productivity opportunities varieties with different ripening periods and relatively low production costs. Valuable nutritional, medicinal and dietary qualities make it a desirable fruit, both in the domestic and foreign markets. (Mladenova et al., 2017).
In the recent past, the most widespread varieties were Kyustendil Blue Plum and Stanley. Plum varieties selected in a number of European countries have become widespread in the recent years. The most common diseases in plum culture are early and late brown rot, red leaf spots, fungal powdery mildew, plum rust (Iliev and Stoev, 2008; Iliev et al., 2011). The selection of varieties for the creation of a new orchard is an important moment in the preparation for a profitable fruit growing agribusiness. Choosing the right variety reduces the cost of growing fruit trees, reduces the risk of disease and allows for rhythmic annual harvesting. In addition, the breeder can more flexibly meet the requirements of the variety set market (Stoev et al., 2017).
The present study aims to trace the attitude of some varieties grown in the Dryanovo region to the studied economically important diseases.

MATERIALS AND METHODS

In the period 2018-2020, a study was conducted covering 13 plum varieties with different agrobiological characteristics and their relationship to economically important fungal diseases. The data on temperatures and precipitation, which are essential for the development of the studied diseases, were used by the Meteorological Station in OSS-Dryanovo (Table 1), (experimental station for plum culture), which is a branch of RIMSA-Troyan, Bulgaria.

Humidity during the growing season is one of the main factors in the development of fungal diseases. Regarding the amount of precipitation, the region of Dryanovo is within the average amount of rain for the country, which varies around 410 l/m² for the period from March to August. The only exception is 2020, when the amount of precipitation for Dryanovo for the months from March to August was 343 l/m^2. The obtained results are averaged by the statistical methods of Lidanski (1988).

Table 1. Degree of infestation of introduced and our plum varieties to the diseases: powdery mildew, *Plum rust* red leaf spots, late brown rot (%)

Сорт	***Polystigma Rubrum (%)***		***Tranzschelia-pruni spinosae***		***Stigmina carpophila***		**Monilia fructigena**	
	x	± S x	x	± S x	x	± S x	x	± S x
Altanova renkloda	7.41	0.52	11.00	10.92	13.87	3.52	19.60	11.76
Balvan's glory	8.60	3.89	3.20	1.60	9.33	0.58	1.80	0.85
Gabrovska	11.07	4.69	5.07	0.99	16.80	7.79	8.27	5.67
Yo Yo	9.73	3.87	3.93	0.70	11.73	3.04	13.40	11.43
Nevena	13.47	2.12	6.67	3.43	9.60	0.20	14.10	0.14
Pacific	13.87	2.14	7.33	5.46	11.53	0.42	14.27	10.43
Stanley	3.07	0.90	11.27	4.11	10.60	1.64	16.80	13.22
Strinava	8.40	2.42	3.87	0.12	8.07	0.42	15.33	12.47
Tegera	16.20	7.45	4.00	2.23	8.47	2.14	13.50	11.74
Hanita	13.13	3.64	4.33	0.70	16.00	2.65	12.40	7.16
Chachanska lepotica	23.23	10.14	10.73	8.73	19.20	6.42	7.47	4.06
Chachanska najbolia	17.73	3.21	8.33	5.61	10.40	0.87	5.00	4.16
Chachanska rodna	11.67	1.89	10.27	8.81	10.93	3.13	5.73	4.09

The field experiments were carried out in the Experimental Station in the town of Dryanovo. The region is 300 m above sea level and belongs to the Pre-Balkan climatic region of Bulgaria. The plantations are established on pseudo-podzolic gray forest soil at a planting distance of 5x5 m. being grown according to the adopted technology for plum culture under non-irrigated conditions. The climat conditions have a significant impact on the growth and fruiting of the plum crop. The plantation is maintained in accordance with the methodology for the study of plant resources by Yoncheva et al., (1979). The degree of sensitivity to red leaf spots, fungal powdery mildew, plum rust and late brown rot was reported on 200 annual and short twigs on a scale (Yoncheva et al., 1979).

The observed trees were not treated with insecticides and fungicides. Excluding pesticide use facilitates the accumulation of infection in the orchard and the attack of trees by *Polystigma rubrum, Tranzschelia-pruni spinosae Stigmina carpophila Monilia fructigena*. The following indicators were taken into account in the study: Indicators of resistance to these diseases are: the condition of the fruit in the presence of measles and brown rot, as well as signs on the leaves of the observed diseases (Yoncheva et al., 1979).

The obtained results were subjected to mathematical and The obtained results are averaged by the statistical methods of Lidanski (1988) analysis using the software products used during the study was “MS Excel Analysis ToolPak Add-Ins”.

RESULTS AND DISCUSSIONS

During 2018 the meteorological conditions in the region of Dryanovo were favorable for the development of red leaf spots. This is due to the higher temperatures in April (16.7 ºC) and May, compared to the base period. Most of the varieties have over 10% attack of red leaf spots. The highest degree was reported for the variety Chachanska lepotitsa (21.3%), followed by Chachanska najbole and Hanita, and the lowest grade was Stanley - 2.2%.

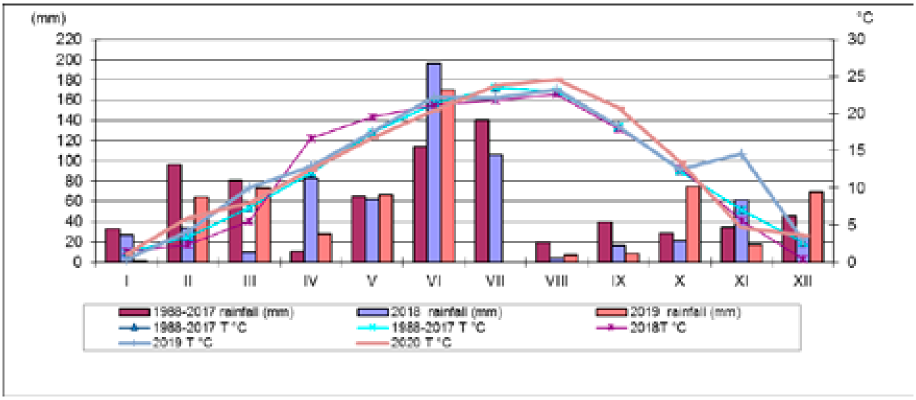

Figure 1. Climatic factors: temperature (°C), precipitation (mm), humidity (g/m^3).

In 2019, the amount of precipitation in April (83.0 l/m^2) is higher compared to 2018 (10.6 l/m^2) and 2020 (27.3 l/m^2) and are a prerequisite for a stronger attack by *Polystigma rubrum* (Figure 1). This pathogen inflicted the greatest damage on the Chachanska Lepotitsa variety - 34.8%. In most varieties the percentage is from 10 to 14.4%. Values below 10% were reported for the cultivars Altanova Renkloda, Balvanska Slava, Gabrovska, Yoyo, Strinava, and the lowest was in the cultivar Stanley - 3%. In 2020, lower temperatures in late March and early April affect the development of pathogens, especially those that appear at the beginning of the growing season. One of these pathogens is *Polystigma rubrum*, whose development coincides with the phases of flowering and the appearance of leaves in plums. During this period, the conditions for infection are not suitable as there are days with temperatures below 10ºC, which stops the flight of ascospores. The unusually high degree of infestation by *Polystigma rubrum* has been reported in low-susceptibility varieties, probably due to the later appearance of the leaves. The biggest damages were inflicted on the varieties Tegera - 24.8% and Chachanskaya najbloya - 20.8%. In most varieties the percentage varies from 10.2 to 16.2%. The lowest values were reported for the varieties Altanova Renkloda - 7.2% and the variety Stanley - 4% (Figure 2).

The highest values were reported in 2018 and 2019 for the Chachanska Lepotitsa variety - 21.3% and 34.2%. In 2020, the same variety reported only 14.2% attack rate. This is probably due to the biological peculiarities of the variety, as the beginning of the vegetation begins 2-3 days earlier than that of the varieties Tegera and Chachanska naibolya. In all other varieties the infestation rate is below 20%.

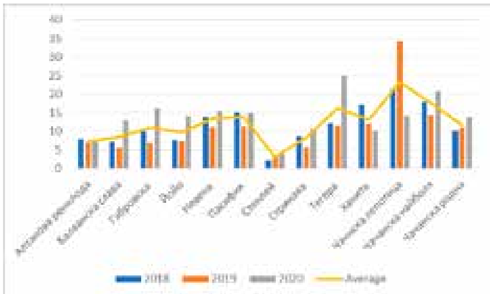

Figure 2. Infection rate (%) of *Polystigma rubrum*

The varieties Stanley, Strinava and Altanova Renkloda are defined as weakly sensitive, as their damage index does not exceed 11% throughout the study period. In the varieties Balvanska Slava, Gabrovska and Yoyo the degree varies widely and in 2018 and 2019 values below 11% were reported and we can define them as weakly sensitive. But in 2020, the same values were reported - Gabrovska variety 16.2%, Yoyo - 14.2% and Balvanska slava - 13%. This shows that these varieties in some years may show a medium sensitivity to red leaf spots. The strength of the attack by the pathogen depends very much on the meteorological conditions that affect its development and spread.

During the summer months of June and July 2018, large amounts of precipitation were measured - 114.51 l/m^2 and 140.51 l/m^2. High humidity and soil are beneficial for the development of the fungus (Figures 1 and 3). Plum rust was manifested with a high degree of infestation in varieties - Altanova Renkloda (23.6%), Chachanska native (20.4%) and Chachanska lepotitsa is 20%. The varieties Balvanska Slava, Yoyo and Hanita have the lowest level of infestation below 5%. For other varieties, the percentage varies from 6 to 18%. This year is indicative of varieties that show moderate sensitivity.

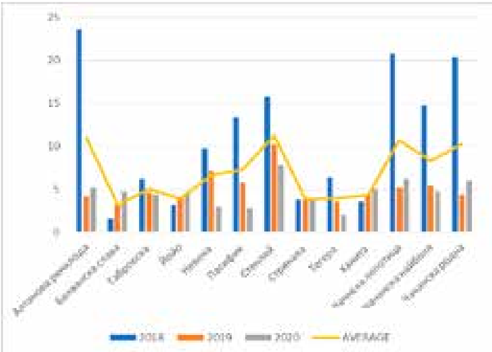

Figure 3. Infection rate (%) of *Tranzschelia-pruni spinosae*

In 2019, the months of August and September there was a drought and retention of higher temperatures, especially in September, which affected the spread of rust. The highest value is in the sensitive Stanley variety - 10.2%, and in other varieties the percentage is between 7 and 3%. Degree of attack below 5% was reported in the varieties Strinava, Tegera, Hanita, Yoyo, Gabrovo and Altanova Renkloda, and the lowest degree in Balvanska Slava - 3.2%.

In 2020, the months of July (0 l/m^2), August and early September, there was atmospheric drought and retention of higher temperatures, which affected the spread of rust. The highest value is for the sensitive Stanley variety - 7.8%, and for other varieties the percentage is between 2% and 6%. A degree of infestation below 5% was reported for the varieties Strinava, Balvanska Slava, Hanita, Yoyo, Gabrovska, Nevena, Pacific, Chachanska naibolya and Altanova renkloda, and the lowest degree in Tegera - 2%.

In 2018, the most serious damage from shotgun is in the variety Chachanska lepotitsa - 26.6%. In five varieties over 10% were reported, and in the other varieties a lower percentage was reported and respectively the Tegera variety had the lowest - 6.6% (Figure 4). This is a year with heavy rainfall, especially in June and July, when it is a prerequisite for a longer period of infection with powdery mildew. The lesions of fungal powdery mildew in 2019 are weak compared to the previous one. In all varieties the degree of infestation is similar and the values are between 8% for Tegera variety (lowest) and 17% for Hanita (highest). The damage from fungal powdery mildew in 2020 was less pronounced, probably due to the atmospheric drought in July. In most varieties the degree of infestation is similar and the values are from 7.6% for the variety Chachanska rodna, as the lowest, to 12.4% for Stanley. For another year, the highest damage was inflicted on the Hanita variety by 18%, and Chachanska lepotitsa with a close value of 15.8%. In 2018, the sensitive varieties Gabrovska and Chachanska lepotitsa showed the disease with values over 25%. Over the next two years, the damage index ranged from 12.4% for Gabrovo to 15.8% for Chachanska Lepotitsa. The varieties Yoyo, Pacific, Hanita and Altanova Renkloda are of medium sensitivity. The other varieties show low sensitivity and in the indicative year (2018) the lowest values were measured at Tegera - 6.6% and Strinava - 7.6%.

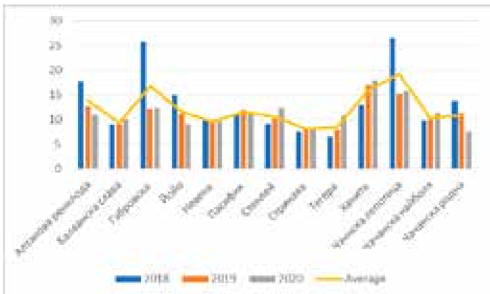

Figure 4. Infection rate of *Stigmina carpophila* (%)

Significant rainfall in June and July (2019) provoked the development of the fungus *Monilinia frutigena*. The largest lesions are in the varieties Altanova Renkloda, Pacific, Strinava and Tegera, as they reported values between 20% and 28.2% fruit rot. Only in the two varieties Yoyo and Chachanska the value below 10% were reported. The Altanova Renkloda and Pacific varieties have very low yields and have also been severely affected by late brown rot. Significant rainfall in June provoked the development of the fungus *Monilinia frutigena.*

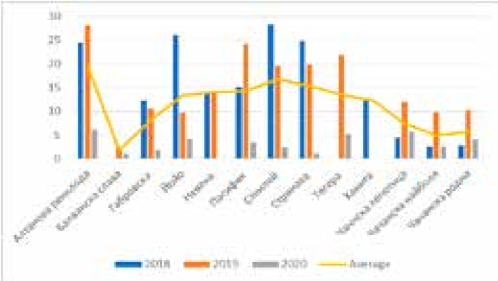

Figure 5. Infection rate (%) of *Monilia fructigena*

In July, a drought was observed, which affected the spread of brown rot spores. The largest lesions are in the varieties Altanova Renkloda, Chachanska Lepotitsa, and Tegera, with values between 6.2% and 4.8% of fruit rot reported. Values below 15% were reported for all other varieties. In the varieties Nevena and Hanita there is a very low yield (single number of fruits), due to which no damage index is reported.

CONCLUSIONS

Global climate change requires a new concept in the selection and selection of varieties and the study of their susceptibility to disease.

Opal is sensitive to red leaf spot, while Nevena, Pacific, Gabrovo, Hanita, Chachanska Lepotitsa and Chachanska Rodna are moderately sensitive. The varieties Altanova Renkloda, Balvanska Slava, Yoyo, Tegera, Strinava, Chachanska Naibolya and Stanley are defined as weakly sensitive.

The group of tolerant and insensitive varieties to rust on plums includes Yoyo, Altanova Rencolada, Balvanska Slava, Nevena, Opal, Gabrovska, Tegera and Hanita. The Stanley variety is very sensitive.

Compared to the powdery mildew, the varieties Chachanska lepotitsa and Gabrovska belong to the sensitive ones. The varieties Yoyo, Hanita and Altanova Renkloda sensitivity have medium sensitivity, and Stanley, Chachanska native, Chachanska naibolya, Balvanska slava, Nevena and Strinava are tolerant.

The Stanley, Yoyo and Altanova Rencloda varieties have the disadvantage that their fruits can be affected by late brown rot. The varieties Stanley, Altanova Renkloda, Yoyo and Strinava were very sensitive to late brown rot, and in some years the damage was over 25%. These varieties fall into the group for fresh consumption, which requires the application of a modern sustainable plant protection system to obtain quality attractive fruits and high sustainable regular yields.

With the best results in terms of tolerance or low sensitivity to the studied pathogens, were found in Yoyo, Pacific, Tegera, Hanita and Chachanska best. The Stanley, Chachnska Naibolya, Pacific and Altanova Renkloda varieties bear fruit regularly and have good fruit qualities. The varieties Nevena, Gabrovska and Strinava selected in Experimental station of plum-Dryanovo are characterized by complex resistance to economically

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THE INFLUENCE OF CHANGES IN CLIMATIC CONDITIONS ON THE BIOLOGY OF THE APPLE WORMS LEPIDOPTERA - TORTRICIDAE

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Abstract

The pest Cydia pomonella L. is present in all countries where the apple tree is grown (CABI 2021), the damage done by this species being considerable.The lower threshold of development of the species under study is 9°C that influences the appearance in apple plantations of butterflies, it was recorded in the analyzed area on May 9 in 2019 and May 1 2020, a period during which the sum of the temperature degrees did not meet the thermal constant (K) of the species worth 624°C (Rosca I. et al., 2011). The research was carried out between 2019-2020 at the Research Station for Fruit Growing Iasi, Romania using AtraPom traps (ICCRR Cluj-Napoca) to determine the biological cycle of the Cydia pomonella L. species in correlation with the influence of changes in climatic conditions.

Key words: *apple, traps, biological cycle, thermal constant.*

INTRODUCTION

Climatic and environmental conditions have contributed to the emergence and development of the studied pest agent confirming its presence in 36 countries (CABI, 2021) causing damage especially to the apple crop but also to the crops of pear, plum, quince, peach, apricot and walnut.
Although the insecticides used can combat this pest, they must be applied taking into account the biology of the pest in order to have the desired effectiveness, the apple worm having a spread flight throughout the growing season of the trees, representing a danger to the harvest.
The pest is very dangerous when insecticides are not used, with damage of up to 80%, causing damage from the first to the last generation (Mitrea I. et al., 2010) this causes increased attention in apple plantations.
The aim of this work is to follow the biological cycle of the species according to climatic conditions, so that we can determine the time when the pest occurs and its period of activity, with a significant change in recent years, due to mild winters and higher temperatures. The moment of the pest occurrence and the period of its activity give us the possibility to apply the phytosanitary treatments precisely.

MATERIALS AND METHODS

The studies were carried out within the Research and Development Station for Fruit Growing Iasi on its plantations for a period of two years (2019-2020) in which researches were carried out on the *Cydia pomonella* L. species.
The experience took place on a batch of 5 ha of apple trees that includes several varieties (Idared, Jonagold, Generos) where AtraPom traps marketed by the "Raluca Ripan" Institute of Chemistry Cluj-Napoca (Figure 3) were placed in order to determine the flight curve of the affected agent pursued.
The recording of climate data was carried out using the Adcon Telemetry weather station addVANTAGE A840, the processing of the data in the field being highlighted with the help of the Microsoft office package.
The actual temperature favorable to the appearance and development of *Cydia pomonella* L. in 2019 and 2020 was analyzed according to the method used in plant protection (Săvulescu A., 1978).

RESULTS AND DISCUSSIONS

The biological cycle of the apple worm is conditioned by the fulfillment of the lower development threshold of 9 ^{0}C of the species, it varies depending on the climatic conditions of each year, so in 2019 it was reached on March 8 and in 2020 on March 3.

The activity of adult insects is influenced by the thermal constant (K) which in the apple worm is worth 624^{0}C identified in the two years of study at different dates, so in 2019 it was recorded in June and in the year 2020 in July. The actual temperature amount of 2019 totaled 1707.3^{0}C allowing the development of two full.

generations of *Cydia pomonella* L. and an incomplete one. In the year 2020 it reached the sum of 1931.4^{0}C, which signifies the development of two complete generations and an incomplete one resulting in a reserve of pests for the following year.

The sum of the actual temperatures is different from one year to the next so during the same period of the year from April to October, the value was 1707.3^{0}C in 2019 and in 2020 the value being 1931.4^{0}C which indicates a different climatic situation from year to year influencing the biological cycle of the species. This can also be seen in the evolution of the average monthly temperatures that in 2019 were between 10.5^{0}C and 21.8^{0}C and in 2020 between 11.5^{0}C and 23.6^{0}C.

The overall temperature sum for Miroslava is $\Sigma(t_n\text{-}t_0)$ = 4143.62^{0}C in 2019 and $\Sigma(t_n\text{-}t_0)$= 4428.7^{0}C in 2020 (Figure 1).

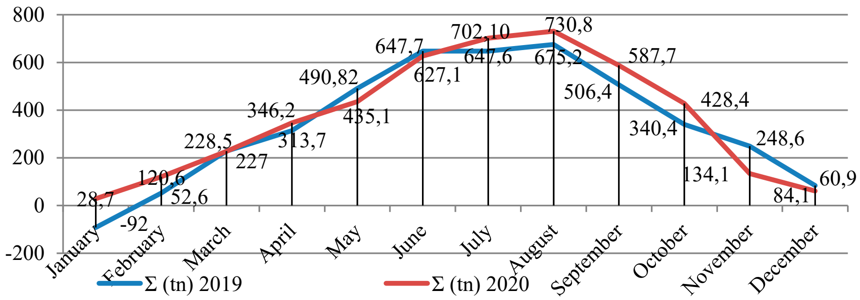

Figure 1. The sum of the actual and non-effective average monthly temperatures of 2019 and 2020

According to the biological threshold 9^{0}C of the apple worm the overall amount of temperature is represented by the sum of the actual and non-effective temperature. In this case, in 2019, the global sum of temperatures is made up of the effective sum of temperatures of 1707.3^{0}C and the sum of the non-effective temperatures of 2436.32^{0}C compared to 2020 when the effective sum of temperatures is 1931.4^{0}C and the sum of the non effective temperatures of 2497.3^{0}C.

Analyzing Figure 2, in 2019 the first catches were recorded on May 9, the traps being placed on May 3. On May 9, when the first butterflies were captured, the sum of the temperature degrees was 105^{0}C, the fulfillment of the thermal development constant K= 624^{0}C being achieved on June 30.

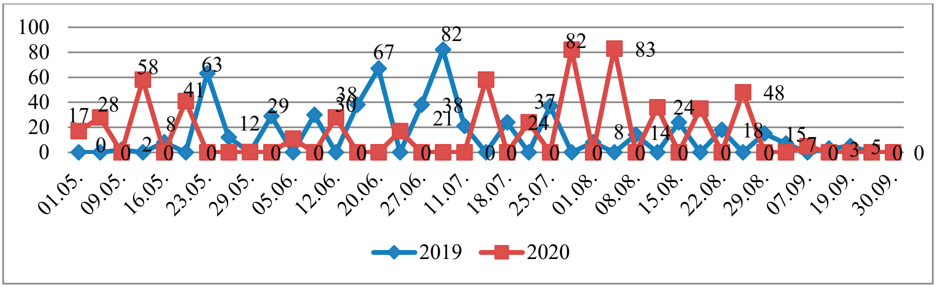

Figure 2. Flight curve

Figure 3. Trap AtraPom (original photo)

During this period (May 9-June 30) the maximum flight curve was reached on May 23, the flight of the first generation being for a period of 52 days, after June 30, the flight of the second generation began with the maximum of the flight curve on July 4, the second generation meeting the thermal constant (K) on August 12, the flight is for a period of 43 days. From 12 August to the last recorded catches on 19 September, 300.1^{0}C have accumulated. The cessation of the appearance of the pest is carried out at the sum of 1548.1^{0}C. The thermal constant does not favor the development of a complete generation, instead achieving a third generation incomplete.

In 2020, pheromone traps to monitor the appearance of the pest were placed on 22 April and the first catches were recorded on 01 May. The fulfillment of the K=624^{0}C thermal constant of the pest's development took place on July 3 when the first complete generation of the species was realized, with a maximum of the flight curve on May 15 (Table 1). In the case of the first generation of the apple worm, the butterflies had a sleazy flight over 64 days. The evolution of the stages of the biological cycle of the second generation lasted until August 16, when 1248^{0}C accumulated. The flight of the second generation was over a period of 44 days.

Table 1. Effective temperature in 2019 and 2020 for Research Station for Fruit Growing

Month	No. of days	Temperature (t_n) 2019	t_n-t_0	Σ(t_n-t_0) partial*	Σ(t_n-t_0) cumulated	Temperature (t_n) 2020	t_n-t_0	Σ(t_n-t_0) partial*	Σ(t_n-t_0) cumulated
January	**31**	-3,0	-	-	-	0,9	-	-	-
February	**28**	1,9	-	-	-	4,2	-	-	-
March	**31**	7,4	-	-	-	7,3	-	-	-
April	**30**	10,5	1,5	43,8	43,8	11,5	2,5	76,2	76,2
May	**31**	15,8	6,8	211,7	255,5	14,0	5,0	156,2	232,4
June	**30**	21,6	12,6	377,7	633,2	20,9	11,9	357,0	589,4
July	**31**	20,9	11,9	368,6	1001,8	22,7	13,7	423,2	1012,6
August	**31**	21,8	12,8	396,2	1398,0	23,6	14,6	451,7	1464,3
September	**30**	16,9	7,9	236,4	1634,4	19,6	10,6	317,7	1782,0
October	**31**	11,4	2,4	72,9	1707,3	13,8	4,8	149,4	1931,4
November	**30**	8,3	-	-	-	4,5	-	-	-
December	**31**	2,7	-	-	-	2,0	-	-	-

*)=values of this column rezult from **(t_n-t0)**×no. of days of each month, where: -t_n=montly average temperature; -t_0=biological threshold (9^{0}C)

The activity of the apple worm did not cease until September 7, when 1538.2^{0}C was achieved, signifying an incomplete third generation because the thermal constant of 624^{0}C is not met with a value of 290^{0}C, the pest being active for a period of 22 days, during this period presenting a danger of attack, especially since it synchronizes with the ripening period of the fruit.

CONCLUSIONS

In 2019 the pest *Cydia pomonella* L. had an activity carried out over 131 days while it started its activity on May 9 and the sum of the annual temperature degrees reached the value of 4143,62^{0}C. During the period in which the worm activated the sum of the temperature degrees totaled 1548.1^{0}C, which according to the thermal constant developed two complete generations and an incomplete one not meeting only 300.1^{0}C, which signifies a prolonged activity of the pest due to climatic conditions.

In the second year of study the sum of the annual temperature degrees was 4428.7^{0}C and the number of days of activity was 130, starting its activity on May 1st and ceasing on September 7th when the sum of the effective temperature degrees was 1538.2^{0}C which favored the development of two full and third incomplete generations.

In the two years of study, climatic conditions favored the development of an incomplete third generation, which took place over 36 days in 2019 compared to 22 days in 2020. In terms of the sum of the actual temperature degrees, there is no view, a difference being in the amount of 290^{0}C in 2019 and 300.1^{0}C in the year 2020 although the number of days in which they have added up is inversely proportional to the value of $\Sigma(t_n-t_0)$.

We can conclude that in both years of study the pest represented a danger in the plantation throughout the vegetation period, a good knowledge of the pest's biology is important in order to apply the phytosanitary treatments rationally and at the right time.

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THE PHYTOVIRAL STATUS OF SOME NEW ESTABLISHED SWEET CHERRY ORCHARDS IN MOLDOVA REGION, ROMANIA

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Abstract

Ten new established sweet cherry orchards in Moldova region were surveyed in the summer of 2021 to assess the phytoviral status. Nine of these were established with abroad propagated material from Italy, Belgium, Czech Republic and Netherlands, and the other one with material produced in Romania. Two blocks of 200 trees from each orchard were visually monitored for virus-like symptoms and then ten samples per orchard were randomly collected for laboratory testing. Sampling trees were tested for the presence of the following viral pathogens: Prune dwarf virus (PDV), Prunus necrotic ring spot virus (PNRSV), Apple mosaic virus (ApMV), Apple chlorotic leaf spot virus (ACLSV), Plum pox virus (PPV), Arabis mosaic virus (ArMV), Cherry leaf roll virus (CLRV), Raspberry ringspot virus (RpRSV), Strawberry latent ringspot virus (SLRSV) and Tomato black ring virus (TBRV) by DAS-ELISA using BIOREBA antiserum kits. The presence of Little cherry virus-1(LChV-1) on three samples which expressed symptoms of potentially infections was checked by RT-PCR. One out of ten surveyed sweet cherry young orchards found to be infected by one virus. PNRSV was detected in one orchard with an occurrence of 10%. The average of infection with PNRSV in the surveyed orchards from Moldova was 1%. No other infection was found in the surveyed orchards. The newly established orchards in Moldova region have generally a very good phytoviral status which is an important prerequisite for their success. However, there are some cases with virus infected old orchards in the proximity of the newly established orchards which might represent potential source of infection.

Key words: *propagation material, serological and molecular assays, survey, sweet cherry, virus.*

INTRODUCTION

Sweet cherry is the third fruit species cultivated in Romania with a harvesting area of 3,200 ha, after plum and apple crops, and ranks the fourth place in both fruit production and yield, with 37,640 tonnes, and 11.76 t/ha, respectively (FAOSTAT, 2020).

The sweet cherry (*Prunus avium* L.), member of *Rosaceae* family, genus *Prunus*, is often affected besides fungal and bacterial diseases by many viruses, some of them with economic importance regarding quality of fruits and also productivity of crops (Nemeth, 1986; Hadidi & Barba, 2011). The viruses are dangerous intracellular pathogens that can affect yield and fruit growth (Pavliuk et al., 2019). A suitable management of viral diseases targets limiting the losses of fruits by using prevention measures, because once infected by viruses trees cannot be treated in orchard. The using of the healthy planting material, setting up the new orchards far away from sources of infection, chemical treatments applied against virus vectors, using of resistant cultivars (if there are), represent useful preventive measures that contribute to limit the viral diseases in pome and stone fruits orchards. Because sometimes viral infections do not express symptoms, rigorous monitoring and removal of infected trees are required, followed by their replacement with virus free trees. Surveys and regular testing of the orchards are recommended especially for viruses that are pollen and seed transmitted (Caglayan et al., 2011).

In Romania, in the last years were established many sweet cherry orchards with planting material produced both in our country and in different European countries.

The aim of this study was to get information about the initial phytoviral status of planting material by assessing the occurrence of the viruses in some new sweet cherry orchards in Moldova region, and to assess their potential success depending on the viral status.

MATERIALS AND METHODS

Field surveys

Ten newly established sweet cherry orchards (1-6 years old) from five counties (Bacău, Galați, Iași, Vaslui and Vrancea) in Moldova region were surveyed in the summer of 2021 to assess the phytoviral status. Nine of these were established with abroad propagated material, produced in Italy, Belgium, Czech Republic and Netherlands, and the other one with material produced in Romania. The orchards surveyed were mainly established with valuable cultivars, such as Regina, Kordia, Tamara, Bigarreau Burlat and Carmen. Two blocks with a total of 200 trees (100 trees per block) from each orchard were individually monitored by visual observation for potential viruses-like symptoms. Then, randomly were collected ten samples per orchard for laboratory analyses. Each sample consisted in 5-10 leaves. Additionally, when symptomatic leaves were observed, samples from these trees were analyzed in laboratory by serological or molecular assays. Also, when was the case of presence of old sweet cherry trees or orchards in the proximity of the newly established orchards, those were surveyed in order to check the potential viral external inoculum sources. When virus-like symptoms were observed, a few symptomatic trees were sampled and laboratory tested.

Serological and molecular assays

Sampled trees were serological tested for the presence of the following viral pathogens: *Prune dwarf virus* (PDV), *Prunus necrotic ring spot virus* (PNRSV), *Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV), *Plum pox virus* (PPV), *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Raspberry ringspot virus* (RpRSV), *Strawberry latent ringspot virus* (SLRSV) and *Tomato black ring virus* (TBRV). Serological assays were performed by Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Clark & Adams, 1977) using commercial polyclonal antiserum against to all viruses mention above, according to the manufacturer's instructions (Bioreba, Switzerland). Absorbance values were measured at 405nm after 1h substrate hydrolysis. Samples were considered positive if their absorbance values were more than twice those of the negative control. Then, the occurrence was established for each virus.

Molecular assay was performed by RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) in order to check the presence of *Little Cherry virus*-1 (LChV-1) in some suspect trees. For total RNA extraction was used Spectrum Total Plant RNA kit. The iScript cDNA Synthesis Kit was used for reverstranscription and FastStart Taq DNA Polymerase kit for amplification, according to the manufacturer's instructions. Specific primers for detection of LChV-1 were used according to Bajet et al. (2008). The amplification products were separated by electrophoresis in 1.5% agarose gel stained with RedSafe and visualized under UV light using Gel Doc XR.

RESULTS AND DISCUSSIONS

Field surveys

More than 2,000 trees were visually inspected for the potential viral symptoms in ten new sweet cherry orchards in Moldova region. Generally, no typical virus-like symptoms were observed on inspected trees. However, chlorotic rings and necrotic spots on leaves were sporadically observed in only one orchard from Bacău County. In another orchard, from Galați County, reddish of the leaves and rolling of the leaf edges upwards were observed on some trees. In both cases, the sampled leaves were analyzed by serological or molecular assays.

DAS-ELISA test

A total of one hundred samples (ten from each orchard) were tested by DAS-ELISA. Based on the obtained results, the viruses occurring in surveyed sweet cherry orchards in Moldova region was determined (Table 1).

Virus infection was detected in one out of ten new sweet cherry orchards surveyed. More precisely, PNRSV was detected inside an orchard from Bacău County. The occurrence of PNRSV in this orchard was calculated at 10%.

No other infections were detected in the new sweet cherry orchards surveyed in Moldova region according to DAS-ELISA test.

Table 1. The occurrence of viruses based on DAS-ELISA test in some new sweet cherry orchards in Moldova region

Orchard location/ county code	Provenance of plant material	The occurrence of viruses (%) based on DAS-ELISA test									
		PPV	PDV	PNRSV	ACLSV	ApMV	ArMV	CLRV	RpRSV	SLRSV	TBRV
1. Pădureni (IS)	Netherlands	0	0	0	0	0	0	0	0	0	0
2. Podu Iloaiei (IS)	Czech Republic/ Belgium	0	0	0	0	0	0	0	0	0	0
3. Plopana (BC)	Romania	0	0	**10**	0	0	0	0	0	0	0
4. Valea Mare Ivănești (VS)	Italy	0	0	0	0	0	0	0	0	0	0
5. Grumezoaia (VS)	Italy	0	0	0	0	0	0	0	0	0	0
6. Crasna (VS)	Italy	0	0	0	0	0	0	0	0	0	0
7. Vârlezi (GL)	Netherlands	0	0	0	0	0	0	0	0	0	0
8. Urechești (VN)	Netherlands	0	0	0	0	0	0	0	0	0	0
9. Cotești (VN)	Netherlands	0	0	0	0	0	0	0	0	0	0
10. Odobești (VN)	Belgium	0	0	0	0	0	0	0	0	0	0

The overall occurrence of PNRSV in Moldova regions was calculated at 1%. No infections with PDV, ApMV, ACLSV, PPV, ArMV, CLRV, RpRSV, SLRSV and TBRV were found in the surveyed sweet cherry orchards in Moldova region.

RT-PCR assay

Samples from three trees that suggested potential infections with LChV-1, tested by RT-PCR assay, revealed the absence of this virus (data not shown). Most probably, the symptoms were caused by other factors.

Inoculum source nearby

In the proximity of two newly established orchards were found old trees or orchards which expressed symptoms on leaves of possible viral diseases (chlorotic diffuse mottling, chlorotic rings or spots, chlorosis of veins). DAS-ELISA test revealed the presence of PDV in eight samples collected from the proximity of two newly sweet cherry orchards (two samples nearby of orchard no. 3, and six samples nearby of orchard no. 6). These old orchards might represent potential source of viral infection for the newly established sweet cherry orchards.

Studies about the occurrence of viruses in commercial orchards, variety collections or nurseries of pome and/or stone fruits were performed in many countries during time, such as Albania (Digiaro et al., 1994), Serbia (Mandic et al., 2007), Turkey (Ulubas, 2008; Ulubas & Ertunc, 2008), Bosnia and Herzegovina (Matic et al., 2008), India (Brakta et al., 2012), China (Ni et al., 2012), Latvia (Gospodaryk et al., 2012), Lebanon (Nassar et al., 2012), Bulgaria (Kamenova et al., 2019; Borisova et al., 2021) and different percentage of infections with viruses were found. However, our study focused on some newly sweet cherry commercial orchards (1-6 years old). Similar studies were performed in some newly plum orchards in the same region (Zagrai et al., 2021), as well as in some newly sweet cherry and plum orchards from Transylvania (Zagrai et al., 2022 - in preparation).
Corroborating these results we will be able to create an image about the phytoviral status of some new established orchards of plum and sweet cherry in these regions of Romania.

CONCLUSIONS

Newly established sweet cherry orchards in Moldova region generally revealed a very good phytoviral status. This situation represents an important prerequisite for a successful start of cherry industry in this important fruit area of Romania.

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DURABLE VITICULTURE DEVELOPMENT IN DRĂGĂȘANI VINEYARD BY USE THE AHP METHOD TO ASSESSS AND RANK THE MOST SUITABLE GRAPEVINE VARIETIES

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Abstract

The work was intended to identify the most valuable grapevine varieties for Drăgășani vineyard by means of an analytical hierarchical process (AHP). Eight grapevine varieties zoned for this grapevine growing area were included in the AHP exercise: Negru de Drăgășani, Pinot Noir (for red wines), Crâmpoșie selecționată, Sauvignon, Pinot Gris, Fetească regală, Riesling Italian (for white wines) and Azur (for table grapes). Of these, the results recommended Crâmpoșie selecționată, Negru de Drăgășani and Azur as the most valuable varieties in Drășășani vineyard. These varieties can develop the viticulture in Oltenia and, also, in Romania, offering local alternatives for wine and table grapes. The analyses were carried out using the Expert Choice Desktop software package.

Key words: analytical hierarchical process, pairwise comparison, *Vitis*, durable viticulture, wine region IIII.

INTRODUCTION

Drăgăşani vineyard is part of Hills of Vallachia and Oltenia viticultural region (Region III). There are four wine centers within the vineyard: Drăgăşani, Gușoeni, Măciuca (in Vâlcea county), Iancu Jianu (in Olt county) (Order no. 1205/ June 22, 2018 for the approval of the Nomination of the viticultural areas and the classification of the localities by viticultural regions, vineyards and wine centers).

Drăgăşani vineyard, the oldest and most famous in the Carpatho-Danubian area of Oltenia, includes the lands located between the Getic Subcarpathians to the north and the Romanian Plain to the south and south-east, being found between the parallels 44°30' and 44°55' northern latitude and meridians 23º55' and 24º15' eastern longitude. The territory of the vineyard, which covers an area of 16,000 ha, is located on the terraces and slopes of the subunit known as the Olteţ Plateau (Figure 1). The age of the vineyards can not be specified, being the first plantations made by the Geto-Dacians.

Depending on the grapevine growing areas established at the European Union level, Drăgăşani vineyard is located in the C I wine zone (Bucur, 2011).

Figure 1. Map of vineyards in Romania
Source: https://guideandtravel.pl/en/w-wine/

The climate is of moderate continental temperate type, generated by the geographical position, where some mediterranean influences from the south and south-west are felt, which generate a closeness in climate terms with the Dealu Mare vineyard, especially in terms of heliothermic resources. The relatively large distances between the centres determine appreciable differences from this point of view, the smallest heliothermic resources being registered in the north of the vineyard, and the largest in the southern part. The sum of the hours of sunshine during the active vegetation period is 1,660 hours, the global thermal balance is 3,400°C, and the annual rainfall is 480 mm, with an uneven distribution, most falling in the spring and autumn. Excessive rainfall in autumn favours the attack of rot, which in some years leads to a decrease production of about 50%. The hail is also very common (Stroe, 2012). Spring hoar-frosts and frosts are relatively common around April 20th (Popa et al., 2015).

An important beneficial influence on the vineyard has the Olt river, Drăgășani vineyard being located on its right side (60 km long and 20 km wide), and on the terraces and hillocks from the river basin of its affluents (Pesceana, Dâlga, Mamu, Beica, Cerna, Olteț) (Gheorghiță et al., 2016).

The relief consists of hills and hillocks with altitudes ranging from 200-300 m to 400-500 m (Figure 2).

Figure 2. Image from Drăgășani vineyard (original)

In this vineyard there is a large variety of soils, as: Eutricambosol, Stagnic Luvosols, Aluvisols, Geoerodic Regosols, Aluvisols (Entic, Batigleic), Vertosols, Gleisols (Toti et al., 2017).

Regarding the production direction, the old assortment that brought the fame of this vineyard consisted of the varieties: Crâmpoşie, Braghină, Gordan, Tămâioasă românească for white wines, and for red wines: Negru moale, Negru vârtos, as well as some local varieties: Balaban, Roşioară, Slaviţă. Currently, in the Drăgășani vineyard, due to the diversity and variability of the ecological conditions, the production directions are also different, the assortment being mainly made up of wine varieties and to a small extent, of table grape varieties (Stroe, 2012; https://www.agro.basf.ro/ro/stiri/fermier-in-romania/ghidul-principalelor-regiuni-viticole-si-podgorii-din-romania.html). Mention the zoned varieties for the Drăgășani vineyard as follows: for white wines - Chardonnay, Crâmpoșie selecționată, Fetească regală, Pinot gris, Riesling italian, Sauvignon, Vilarom; for flavored wines - Negru aromat, Șarbă, Tămâioasă românească, Muscat Ottonel and for red wines - Alutus, Cabernet Sauvignon, Merlot, Negru de Drăgașani, Novac, Pinot Noir. From the group the table grapes, the zoned varieties are: Victoria, Augusta, Azur, Călina, Muscat D'Adda, Muscat de Hamburg (Order no. 225/2006 for the approval of the Zoning of noble fruitful grapevine varieties admitted for cultivation in the viticultural areas of Romania).

The aim of this work is the application of a hierarchical methodology (AHP) to give a scientific contribution to a durable viticulture, by assessing and ranking some grapevine varieties of white and red wine, and table grapes that exploit well the potential of the Drăgășani vineyard.

MATERIALS AND METHODS

In this work AHP was used to study eight grapevine cultivars (*Vitis vinifera* L.), belonging to the following production directions: red wine (Negru de Drăgășani, Pinot Noir cvs.), white wine (Crâmpoșie selecționată, Sauvignon, Pinot Gris, Fetească regală, Riesling Italian cvs.) and table grapes (Azur cv.).

AHP is a multi-criteria decision-making method consisting of the decomposition of the decision problem into simpler components or levels and the definition of a hierarchy setting by pairwise comparison between the proposed levels (Saaty, 1977).

In order to determine the most important grapevine varieties for Drăgășani vineyard, 16 criteria with a scale of 8 levels each were used in the AHP exercise, as follows: criterion 1 - harvesting period (from 1: the shortest harvesting period to 8: the longest harvesting period); criterion 2 - portfolio of derived products (from 1: the smallest number of derived products to 8: the highest number of derived products); criterion 3 - harvested quantity by one worker in 8 hours (from 1: the lowest quantity to 8: the highest quantity); criterion 4 - harvesting cost (from 1: the lowest cost to 8: the highest cost); criterion 5 - knowledge for recognition (from 1: most recognizable product to 8: hardest recognizable product); criterion 6 - knowledge for harvesting (from 1: the less knowledge necessary to 8: most knowledge necessary); criterion 7 - tools needed for harvesting (from 1: the least to 8: the more); criterion 8 - complexity of harvesting process (from 1: lowest to 8: highest); criterion 9 - distribution range (from 1: lowest to 8: highest); criterion 10 - market potential (from 1: low to 8: high); criterion 11 - transport from the harvesting point to the storage center (from 1: the most easy to 8: the most complicated); criterion 12 - perishability (from 1: lowest to 8: highest); criterion 13 - “celebrity” of the product on the market (from 1: the least known to 8: the most popular); criterion 14 - biotic threats (from 1: the fewest threats to 8: the most threats); criterion 15 - abiotic threats (from 1: the fewest threats to 8: the most threats); criterion 16 - development of the process of harvesting (from 1: undeveloped to 8: extremely developed). The analyses were obtained using the Expert Choice Desktop software (v. 11.5.1683).

These criteria were used else were to identify the most important grapevine varieties from Hills of Banat, Huși vineyard, Danube Terraces, viticultural center Stefanesti, Calafat, Odobești (Buciumeanu et al., 2020a,b; 2021; Vizitiu et al., 2020; 2021).

Also, having a high a degree of generality, these criteria have been used in the forestry research field (Blaga et al., 2019; Cântar & Dincă, 2019; Ciontu et al., 2018; 2019; Pleșca et al., 2019; Tudor & Dincă, 2019; Vechiu & Dincă, 2019; Enescu & Dincă, 2020).

RESULTS AND DISCUSSIONS

All grapevine varieties studied are zoned for Drăgășani vineyard, according to Order no. 225/2006.

The AHP alternative ranking (the average of the marks given on each criterion), ensued from experts’judgment, is presented in Table 1.

According to the AHP results, the grapevine varieties with the highest potential for Drăgășani vineyard were: Crâmposie selecționată, Negru de Drăgășani and Azur (Figure 3). These varieties were obtained at Research and Development Station for Viticulture Drăgășani (Figure 4), being certificated in 1972, 1993 and 1984 respectively. Also, Azur variety was patented - Patent no. 96475 – 06/30/1988.

Table 1. AHP alternative ranking

Criterion	Grapevine varieties							
	Negru de Drăgășani	**Azur**	**Crîmpoșie selecționată**	**Sauvignon**	**Pinot Gris**	**Fetească regală**	**Pinot Noir**	**Riesling italian**
1	6	1	3	2	8	5	7	4
2	3	1	2	7	6	4	8	5
3	8	5	7	1	4	6	2	3
4	6	8	7	3	4	2	5	1
5	5	8	2	1	7	3	6	4
6	7	6	4	3	5	2	8	1
7	6	8	3	2	5	1	7	4
8	1	7	2	5	6	3	4	8
9	8	1	7	2	4	3	6	5
10	7	1	8	5	6	2	4	3
11	1	2	6	4	7	3	8	5
12	5	8	7	1	2	4	6	3
13	5	1	8	6	7	3	2	3
14	6	8	4	1	7	2	3	5
15	2	8	3	7	6	5	4	1
16	7	4	8	1	3	2	5	6

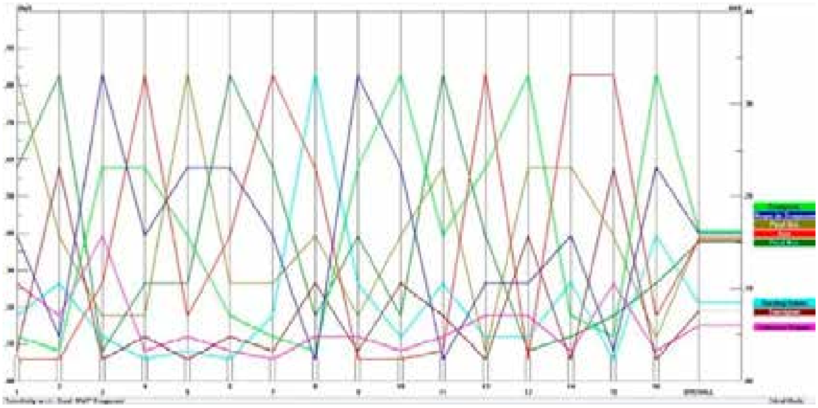

Figure 3. The ranking of the eight grapevine varieties (Negru de Drăgășani, Azur, Pinot Noir, Crâmpoșie, Sauvignon, Pinot Gris, Fetească regală, Riesling italian) cultivated in Drăgășani vineyard

Azur variety is zoned for Drăgășani vineyard only, but it is cultivated in many viticultural centers in the south of the country, and the other two varieties are more widespread. Crâmpoșie selecționată variety can be found in Drăgășani, Dealul Bujorului, Panciu, Sâmburești, Dealurile Craiovei, Murfatlar vineyards. Outside from Drăgășani vineyard, Negru de Drăgășani variety is zoned for the vineyards: Dealu Mare, Ștefănești, Sâmburești, Dealurile Craiovei, Plaiurile Drâncei (Order no. 225/2006). Azur variety exploited very good the climatic and pedological resources of the Iaşi Vineyard (Filimon et al., 2020).

These genotypes have a high growth vigor, generally show good resistance to stress factors and have high grapes production (Table 2). Therefore, they are very suitable for the ecological conditions of Drăgășani vineyard, being studied over time from a pedoclimatic point of view, the number of phytosanitary treatments being much diminished in the normal years.

These varieties selected in the Drăgăşani vineyard can develop the viticulture in Oltenia and in Romania, implicitly, offering local alternatives for wine and table grapes (Gorjan, 2013).

The selected varieties are important sources of valuable genes both for the Drăgășani vineyard and for the other vineyards in Romania and, therefore, recommending their use in the programs of grapevine genetic improvement in Romania and abroad.

Crâmpoșie selecționată

Negru de Drăgășani

Azur

Figure 4. Crâmpoșie selecționată, Negru de Drăgășani and Azur grapevine cultivars (after Măcău and Gorjan, 2016)

Table 2. Behaviour to stress factors and production characteristics of Crâmpoșie selecționată, Negru de Drăgășani and Azur grapevine varieties (adapted after Stroe, 2012; Ampelografia României IX, 2018; https://www.madr.ro/docs/cercetare/Rezultate_activitate_de_cercetare/SCDVV_Dragasani.pdf.)

Grapevine cultivar	Origin	Behaviour to stress factors	Characteristics of the yield
Crâmposie selecționată	Free fecundations of Crâmpoșie cv.	Good resistance to downy mildew, powdery mildew, gray rot and medium tolerance to frost (-22°C…-20°C).	- ripening stage: the 5th; - vegetation period: 190-200 days; - grapes yield: 16.5 t/ha, in average; - bunch: medium to large size (190-250 g); berries: medium size (2.5-2.7 g); - Sugar accumulation in grape berries at full maturity: 186-209 g/l; 186-209 g/l; must acidity: 4.2-4.6 g/l H_2SO4.
Negru de Drăgășani	Negru vârtos x Saperavi	Good resistance to frost (until -22°C), drought, downy mildew, powdery mildew; very good resistance to gray rot.	- ripening stage: the 5th; - vegetation period: 180-206 days; - grapes yield: 11-15t/ha, in average; - bunch: medium size (180-200 g); berries: medium size (2.0-2.5 g); - high potential of sugar accumulation in grape berries (195 -199 g/l); acidity: 4.7‰.
Azur	Coarnă neagră x Cardinal	Good resistance to powdery mildew and downy mildew and poor resistance to gray rot; good resistance to cold and drought.	- ripening stage: the 3rd - 4th; - vegetation period: 175-185 days; - grapes yield: 20.5t/ha, in average; bunch: medium size (250-400 g); berries: medium size (1.80-3.28 g); - sugar accumulation in grape berries at full maturity:154 g/l; acidity: 3.4 g/l H_2SO4.

The rootstocks recommended for Crâmpoșie selecționată, Negru de Drăgășani and Azur varieties are: SO4-4, Berlandieri x Riparia Kobber 5 BB - Crăciunel 2 Selection, Dragasani M-70.

Berlandieri x Riparia Kobber 5 BB - Crăciunel 2 Selection rootstock has a very good resistance to moisture and drought, with a very good vigour. By grafting using this rootstock, a very good percentage of standard grapevines were obtained.

CONCLUSIONS

According to AHP results, based on pairwise comparisons of 16 criteria having a high degree of generality, Crâmpoșie selecționată, Negru de Drăgășani and Azur varieties were selected as the most important grapevine varieties for Drăgășani vineyard, under the conditions in which all eight grapevine varieties taken into the study are zoned for this area. These genotypes generally show good resistance to stress factors and have a high grapes yield.

Although the vineyard assortment consists mainly of wine varieties and to a lesser extent of table grape, the selected varieties are both for wine (Crâmpoșie selecționată, Negru de Drăgășani) and for table grapes (Azur).

Due to the diversity and variability of the ecological conditions, Drăgășani vineyard benefits of favorable eco-climatic conditions for grapevine cultivation and the use of AHP results can contribute to the development of a sustainable viticulture.

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THE MEASUREMENTS OF STARCH AND TOTAL SUGARS CONTENT IN SOME GRAPEVINE VARIETIES (*VITIS VINIFERA* L.) DURING DORMANCY THROUGH DIFFERENT METHODS

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Abstract

Two extraction and measurement methods usually employed to analyze the total sugar and starch contents in plant tissues were investigated with the view to streamlining the process of total sugar and starch determination. Seven-year-old grapevines (table grapes varieties - 'Muscat de Hamburg', 'Napoca', 'Cardinal', 'Perla of Csaba', and wine varieties - 'Fetească regală', 'Muscat Ottonel', 'Pinot noir', and 'Fetească neagră') were marked before winter. During dormancy (October-February), starch level in canes was appreciated through iodine in the potassium iodide method and the anthrone method, both methods using the same biological material (grape canes). Through the anthrone method, the soluble sugars, starch, and total carbohydrates were quantified as g % dry substance. Among the used methods, there were similarities regarding the results. The presented selected methods for starch content determination in plant materials are commonly used and sufficiently accurate in measurements made of engineering works.

Key words: *anthrone method, dormancy, grapevine, iodine, starch, soluble sugar.*

INTRODUCTION

Starch is the most important carbohydrate stored in woody plants (Chapin et al., 1990), the presence of starch in plants is different depending on species, organs, or seasons. The monitorization of carbohydrate reserves (mostly starch, but we have soluble sugar as well) in the grapevine during dormancy is important because the number of reserves will influence the shoot growth and reproductive development in the following season (Bennett et al., 2005).

In the literature, carbohydrates reserve status from different parts of plants, was described by various authors (Schaefer, 1986; Warmund et al., 1986; Koblet and Perret, 1990; Koblet et al., 1993; Candolfi-Vasconcelos et al., 1994, 1995; Warmund et al., 1986; Schumann and Schaefer, 1988; Vršič, 1996).

The use and storage of carbohydrates (starch and soluble sugars) during the growing season are variable depending on environmental conditions such as temperature -cool or hot climate (Londo and Kovaleski, 2019; Santos et al., 2020), water reserve (Pellegrino et al., 2014), light level (Schreiner et al., 2012 and plant factors such as photosynthetic characteristics of grapevine leaves (optical properties of leaves and leaf morphology (Bates et al., 2002). Winter resistance is related to the accumulation of carbohydrates in grapevine stem tissues depending on the vigor of the shoots or canes (Samra, 2008). The presence of starch indirectly influences winter tolerance, as plants use starch to produce other metabolites or provide energy for cold hardiness. (Noronha et al., 2018). In grapevines, carbohydrates accumulated in perennial organs in the previous year will support the early development of annual organs. Considering the above facts, the aim of this study was to understand the dynamics of starch reserves during dormancy in some grape varieties and to compare two different methods of starch content determination.

Strategies for examination of samples for starch for the most part require a quantitative breakdown of the polymer to the monomer, glucose, which is at that point quantitatively decided, frequently colorimetric. Different strategies for the starch examination have involved polarographic (Hopkins, 1934) or chromatographic (Englyst et al., 1983) procedures. The starch-iodine response has moreover been consolidated into strategies (Blakeney and Matheson, 1984), but is rarely used individually because is not a quantitative determination.

Considering the above-mentioned facts, the present study aims to understand the dynamics of the starch reserve during the dormancy season of some table and wine grapevine varieties. Two extraction and measurement methods usually employed to analyze the total sugar and starch contents in plant tissues were investigated with the view to streamlining the process of total sugar and starch determination. The results obtained by the two methods were compared.

MATERIALS AND METHODS

The experiment was conducted during 2019-2020 dormancy period, on *Vitis vinifera* L., on four table grapes cultivars: 'Muscat de Hamburg', 'Napoca', 'Cardinal', 'Perla de Csaba' and four wine grapes 'Fetească regală', 'Muscat Ottonel', 'Pinot noir' and 'Fetească neagră'. The vineyard was planted in 2013 at the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca, Romania (46°46′N; 23°35′E). Grapevines were Guyot pruned (2 canes of 8-9 buds/vine and 2-3 spur) with the same trunk height (0.7-0.8 m). Planting densities were 4545 vines/ha (2.0 m x 1.1 m). The soil of the vineyard is haplic luvisols, deep and fertile, with high water holding capacity. Monthly temperature and precipitation are reported in Table 1.

Samples of one-year-old canes were collected from five vine plants from each variety during dormancy (October, November, December, January, and February).

Table 1**.** The climatic data at UASVM Cluj-Napoca during the analyzed period (2019-2020)

Month/ climate	X	XI	XII	I	II
		2019		**2020**	
Mean T°C	12.1	6.2	-0.1	-1.7	2.0
Min T°C	5.2	-12.0	-13.0	-16.0	-7.0
Max T°C	22,7	13.0	2.5	1.5	7,2
Precipitation (mm)	57.85	17.02	56.14	55.88	17.53

The first method used to determine the insoluble carbohydrate reserve concentrations (starch) was the treating of wood samples with iodine in potassium iodide after cutting into small pieces of 70-90 µm (Cordea, 2014; Călugăr et al., 2019b). The starch content was appreciated by grading the sections on a scale from 0 to 5 depending on the presence of the starch in the canes' tissue as follows:

0 - when there is no starch in any cane tissues;
1 - when the starch is present only in medullary ray up to the xylem area;
2 - when the starch is in medullary ray, a few in xylems and not found in phloem;
3 - when the starch is present in medullary ray, xylem, and a few in phloem;
4 - when the starch can be identified in all tissues section, but without filling up all cells;
5 - when all cells of sections are filled with starch.

All sections were analyzed with a 10x microscope objective and evaluated in a range from 3 to 5 in all eight *Vitis* cultivars that were analyzed (Figure1).

The current experiment did not have any section with 0, 1 or 2. This first method is simple, incipient, low cost, easy, and is used to evaluate the starch content for different grape varieties before spring pruning or inbreeding fields.

The second method was the determination of carbohydrates by the chemical method with the anthrone reagent, described by Cimpoi et al., 2020. This method was also been used by Călugăr et al., 2019a, and Călugăr et al., 2021. The content of soluble sugars, respectively of the starch of the analyzed material expressed as glucose and related in percentage to the dry material.

a b c

Figure 1. The starch content on sections evaluated from 3 (a), 4 (b) to 5 (c) under 10x objectiv

The total carbohydrate was calculated as the sum of the soluble sugars and starch. According to Iliescu et al., 2012, after the calculated total carbohydrate (THC g%) for analyzed material, it can be stated that if:

THC g% < 11 g% = very low content;
11 - 13 g% = low content;
13 - 14 g% = sufficient content;
14 - 16 g% = good content;
>16g% = very good content.

The color intensity obtained (with transparent blue-green color shades) is measured colorimetrically, using UV-VIS spectrophotometer at a wavelength of 620nm (Comsa et al., 2013; Călugăr et al., 2010).

Statistical analysis

The data were analyzed using variance analysis (ANOVA) and the Duncan test ($p \leq 0.05$) was performed to emphasize the significant differences between moments of analysis.

RESULTS AND DISCUSSIONS

The experimental field was settled in Cluj-Napoca area, in central Transylvania in the Somes Mic Corridor, located within three major geographical units: the Transylvanian Plain, the Somes Plateau, and the Apuseni Mountains (Boancă et al., 2018). Voevod et al., 2020 showed that temperature and precipitation in Cluj-Napoca show a clear warming trend over the last three decades. In the summer of 2019, the temperature rose by +2.7% to the mean temperature of the Cluj area. Regarding the precipitations, during the fall of 2019 were registered the lowest amounts of precipitations, with 52.7% smaller than the mean values (Voevod et al., 2020).

During the dormancy period 2019-2020, in Cluj Napoca area, the lowest temperatures were registered at the end of November (-10…-12°C), in the middle of December (-12...-13°C), in January (-11...-16°C). During the dormancy of the analyzed period, there were very short periods with temperatures below -10°C, far from grapevine temperature frost resistance (-18±3°C). Cluj-Napoca is not included in the wine-growing areas of Romania, according to Order no. 225/2006 regarding the approval of the zoning of the *Vitis vinifera* grape varieties admitted for planting. In time, there were some table grape varieties ('Napoca', 'Cetățuia', 'Timpuriu de Cluj') created for this area to satisfy consumers' need for local products given the appropriate climate conditions of the area.

The determination of carbohydrates is a reliable laboratory indicator for assessing the degree of maturation of rootstock and scion cuttings for grafting and for obtaining a good planting material (Iliescu et al., 2011; Călugăr et al., 2019a; Călugăr et al., 2021).

In Figure 2 and Figure 3, the two extraction and measurement methods usually employed to analyze the total sugar and starch contents in plant tissues were investigated with the view to streamlining the process of total sugar and starch determination obtained results were similar. Using the first method (treating of wood samples with iodine in potassium iodide) by analyzing each grapevine variety, it has been evaluated that the starch content level varied between varieties. During October, the highest starch level was recorded in 'Napoca' and 'Fetească regală' varieties studied were statistically equal (4.90) (Figure 2).

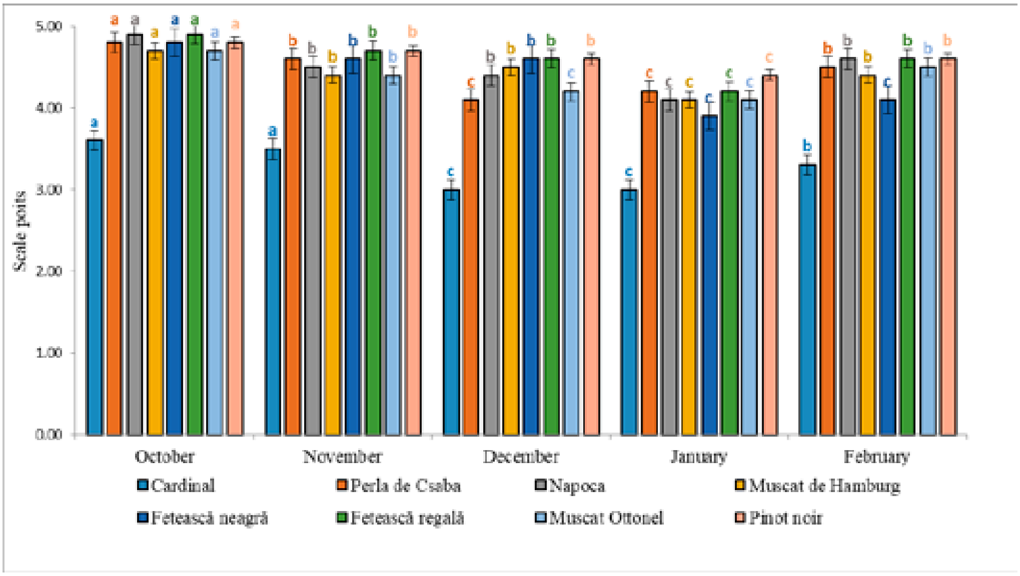

Figure 2. Starch level in canes over the 2019/2020 winter conditions according to the method 1 (the treating of cane with iodine in potassium iodide) The difference between any two columns with the same color, followed by at least one common letter, is insignificant ($p \leq 0.05$). Error bars indicate the standard error of the mean at the 5 % level of probability (n=5)

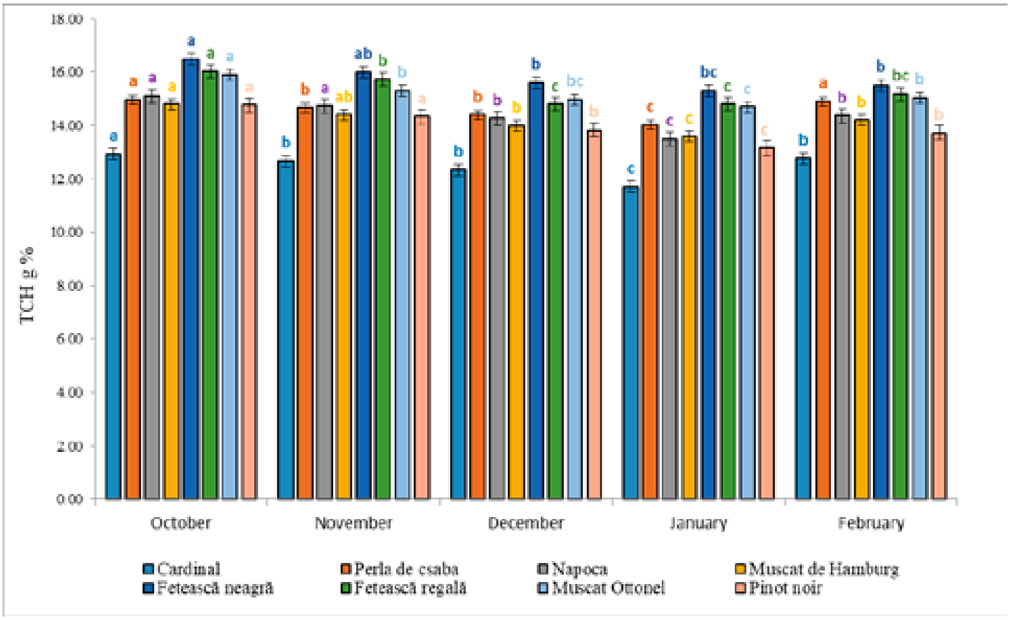

Figure 3. THC % level in canes over the 2019/2020 winter conditions according to the method 2 (the determination of carbohydrates by the chemical method with the anthrone reagent) The difference between any two columns with the same color, followed by at least one common letter, is insignificant ($p \leq 0.05$). Error bars indicate the standard error of the mean at the 5 % level of probability

Using the second method (chemical method with the anthrone reagent) showed a similar result as the previous method, with the highest content of TCH g% for 'Fetească regală' (16.03 g%) and for 'Napoca' (15.03 g%) as in Figure 2. A lower starch content level was recorded in the case Cardinal variety with 3.60 using the first method and a TCH g% of 12.93 g% which means a low content of total carbohydrates.

In the following months, the level of starch was dropping, no matter the used method, with the lowest values in January for all grapevine varieties. Between the level of starch determined in October and the level of starch determined in November, there is a significant difference for all analyzed varieties, according to the Duncan test (Figure 2 and Figure 3). The same pattern is maintained till January when the lowest level of starch was determined. In the middle of dormancy, the content of starch was appreciated with 3.0 for 'Cardinal' variety (when the starch is present in medullary ray, xylem, and a few in phloem) and was determined the content of 11.70 g% TCH. Between grape varieties, in the middle of January, the highest content of starch was for 'Pinot noir' (4.40), followed by 'Fetească regală' and 'Perla of Csaba' (4.2) using the first method of determination. Interesting, there were some differences in results between the two methods, during testings in January. If through the first method, the level of starch for 'Pinot noir' was evalueted with 4.40 (a good appreciation of starch), the results for the second method, were 13.70 g% TCH which means sufficient content of carbohydrate.

In a study on the same varieties and on the same location, but in a previous dormancy period, Călugăr et al., 2019b state that, due to the temperature during winter in Cluj-Napoca, between 0 and -5…-7°C, and the abundant snow precipitations, surprisingly, starch content in canes starts to resynthesize, thus, in the middle of January, it was significantly higher than in November. In the middle of January (2019), the highest content of starch was appreciated for 'Perla de Csaba', 'Muscat Ottonel', and 'Fetească regală' (4.90), significantly higher than for 'Napoca' (4.68), 'Cardinal' (4.63), and 'Pinot noir' (4.55). After another month, in the middle of February (2019), the level of starch appreciated was the highest for 'Napoca' and 'Fetească neagră' (5.00), very close to 'Perla of Csaba' (4.97) and 'Muscat de Hamburg' (4.95). The lowest level of starch appreciated in canes in February was for 'Muscat Ottonel', 'Pinot noir' (4.86), and 'Fetească regală' (4.84).

The results obtained in the following dormancy period (2019/2020) on the same varieties as presented by Călugăr et al. 2019b are lower regarding the appreciation of starch content, mainly due to the climatic condition during the vegetation period, the phytosanitary state, and also, during winter (with lower temperatures).

Air temperature, relative humidity, daily thermal amplitude, and genotype are considered to be the main triggering factors of this pattern according to the studies undertaken by Londo and Kovaleski, 2017; Antivilo et al., 2017. 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).

Grapevine varieties that genetically tend to have more carbohydrates also tend to be more frost resistant during the winter.

During February, the content of starch starts to increase, the highest value was recorded for 'Napoca', 'Fetească regală' and 'Pinot noir' (4.6) and the highest content of TCH g% (14.37 g% for 'Napoca' and 15.17 g% for 'Fetească regală'). In this month, 'Cardinal' (table grape variety) was evaluated with 3.30 as the first method determination and 12.77 g% TCH (low content carbohydrate) according to the second method.

This could be considered as a new polymerization process that takes place due to increasing temperatures, thus in spring, the amount of starch and soluble sugars would be in balance. Kovaleski et al., 2018, has demonstrated that wild grapevine species with northern spreading tend to exhibit higher cold resistance and responsiveness to temperature fluctuations than southern species. The same authors suggest that future grapevine improvements can be sustained through the investigation and mapping of temperature responsiveness. Wood ripening of grape canes has been related to resistance to winter frost and bud viability, but it was also associated with the biological material (Dobrei et al., 2015).

Our results can be compared with those by Cimpoi et al., 2020, for the three varieties, the best results were obtained by the 'Golia' grape variety, which recorded a TCH of 15.64 g% followed by 'Aromat de Iași' (TCH of 14.97% and 'Chasselas doré' (TCH of 13.83 g%).

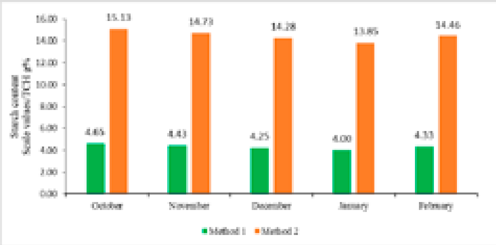

Figure 4. The appreciation of wood ripening through two different methods (Method1 - treating of wood samples with iodine in potassium iodide (with scale from 0-5); Method 2 - the chemical method with the anthrone reagent - results expressed as TCH g%)

As seen in Figure 4 between the two methods of testing starch content are similarities in results. The differences between methods are from an economically and time-consuming point of view. The first method of treating wood samples with iodine in potassium iodide (scale from 0-5) is more rapid in obtaining the results, but it needs a special microscope and a trained person. The second method (the chemical method with the anthrone reagent) is time-consuming and may pollute the environment by using several chemical substances (as presented in chapter Materials and Methods) but is more precise in the result as the researcher can find the starch and soluble sugar content as g%.

The results of the present study indicate that, although the studied varieties are table and wine grape, there is no distinct difference depending on the direction of production as for the starch content. Therefore, the results show the different potential of the genetic determinants for wood ripening which may be correlated with frost resistance as demonstrated by Dobrei et al. (2015).

CONCLUSIONS

The presented selected methods for starch content determination in plant materials are commonly used and sufficiently accurate in measurements made of engineering works. The starch analysis involves mainly determinations of its content as well as the shape and size of its granules.

The findings of the present research show the different potential of the genetic determinants for wood ripening which may be correlated with frost resistance. Also, the study presents the results regarding the two methods used for testing wood ripening. The results obtained through both methods are similar, but the difference arises from working methods (time, materials and environmental).

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USE OF VEGETABLE PROTEINS AS ALTERNATIVES TO PVVP AND CASEINATE FOR REMOVING POLYPHENOLS FROM WHITE GRAPE MUSTS

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Abstract

Polyvinilpolypirrolidone (PVPP) and potassium caseinate (KCas) are the standard treatments used for removing before fermentation a part of the polyphenolic compounds from white grape musts, in order to prevent their oxidation in the resulted wines. As PVPP is a synthetic polymer and KCas is an animal protein with allergenic potential, in line with the movements toward more natural and vegan products, alternative fining agents are being proposed in the form of vegetal proteins. In this study the fining potential of pea and potato proteins was evaluated compared to PVPP and KCas, by determining the change in colour (CIELab parameters) and the amount of total polyphenols removed from the wines of Welsch Riesling. Each fining agent was applied to the must before fermentation in doses of 10, 20 and 30 g/hl and their effect in wine was analysed. The treatments applied tend to reduce wine colour yellowness (parameter b), shift more toward greener (parameter a), and decrease colour saturation (C). For each treatment the parameters determined spectrophotometrically were in direct correlation with the dose used, even though the total colour differences (ΔE values) of the musts were not perceivable by the naked eye, in the young wines. However, clarification of must with any fining agent significantly removed a part of the total polyphenols in a dose-dependent manner, the efficiency of the fining agents being in the following order: PVPP>Pea>Potato=KCas>Control. To also evaluate the economic impact of using these new alternatives, sensory analysis was also carried out and the costs of treatments were determined.

Key words: *white wine, pre-fermentative, fining agents, CIELab, total polyphenols.*

INTRODUCTION

Modern white winemaking relies more and more on treatments applied during pre-fermentative stages to ensure a better clarification of the grape juice and to remove certain undesirable solids or compounds before the alcoholic fermentation begins. Commercial pectolytic enzymes are used to lower the viscosity of juice by breaking down the pectin macromolecules, increasing in the same time the yield of juice (Claus and Mojsov, 2018). Lowering the viscosity of grape juice improves the rate of sedimentation of many undesirable solid particles such as dust, fungicides and microorganisms. Many winemakers support the natural sedimentation or the flotation procedure by adding fining agents, which accelerate these processes and can also bring some more benefits such as lowering the concentration of polyphenols, improving the colour of the final product and extending the shelf life of the product (OIV, 2021a).

Nowadays, alternatives are sought, because these materials tend to be rejected by the general public due to their synthetic or animal or origin.

The PVPP is very effective for removing compounds which confer undesirable wine pigmentation or bitterness, but it is a synthetic homopolymer (Ribéreau-Gayon *et al.*, 1999; Laborde *et al.*, 2006; Cosme *et al.*, 2012). Potassium caseinate is a very effective treatment for oxidized juices and wines, reducing browning and maderisation due to its capability of casein-quinone conjugates formation, but it is a phosphoprotein derived from milk (Hurrel *et al.*, 1982; Kroll *et al.*, 2003; Ribéreau-Gayon *et al.*, 1999; Cosme *et al.*, 2012). Due to the risk of allergenic reactions and the requirement of the mention for the use of animal proteins on the label, many winemakers tend to avoid casein and use PVPP as an alternative treatment.

Indeed, some residues of allergenic fining agents can be present in some wines, but most of the wines prepared for bottling were free of allergenic proteins (Peñas *et al.*, 2015). Casein is not usually an issue, but fining agents containing egg white proteins can leave more frequently residues than those based on milk proteins (Peñas *et al.*, 2015). Following the OIV guidelines for the use of fining agents with allergenic potential (OIV, 2014), helps very much in limiting the presence of allergenic residues in the final wine.

On the other hand, synthetic polymers such as PVPP or PVP raised some safety concerns, as they can allegedly contain certain impurities, residual monomers or degradation products with potential effects on human health (Schubert and Glomb, 2010; EFSA ANS Panel, 2020). However, the latest conclusion of the European Food Safety Agency regarding the use of typical doses of PVP and PVPP as food additives, revealed they are not of safety concern (EFSA ANS Panel, 2020). Nevertheless, natural alternatives to synthetic polymers or to potentially allergenic proteins are still desired, especially for the application to organic wine production. Renouncing to these fining procedures is not an option, as the management of phenolic compounds during white wine production is essential to achieve the sensorial characteristics of quality modern wines. Vegetable proteins are receiving lately a lot of attention, as they have similar functions as PVPP and caseinate (Marangon *et al.*, 2019). Pea and potato proteins, for example, are allowed as fining agents for conventional or organic wine production (EU Regulation 2021/1165). Even though certain vegetal proteins, including from pea and potato, may cause allergic reactions as well, these are less frequent than in the case milk casein (Taylor *et al.*, 2021; Castells *et al.*, 1986; Seppälä *et al.*, 1999; Martorell *et al.*, 2006). In accordance to European legislation, the use of fining agents derived from pea and potato may not be mentioned on the wine labels, as they are generally non-allergenic protein isolates (Peñas *et al.*, 2015; EGTOP, 2015). As such, these vegetal proteins may be good alternatives to PVPP and caseinate in white wine production, but more evaluation is needed to determine their other possible effects on sensory wine quality.

MATERIALS AND METHODS

The grapes of Welsch Riesling variety were harvested in 22 September 2020 from Săhăteni Pietroasa wine region. The processing of grapes followed the classical white winemaking protocol: destemming, crushing and then the must was separated using a vertical hydraulic press. A dose of 50 mg/l SO_2, using a solution of 10% w/v potassium metabisulphite, and a dose of 3 g/hl pectolytic enzyme (Zimafruit, Enologica Vason) were added in to the resulted free-run juice (100 l) to speed up clarification. To evaluate the effect of several fining agents and doses, the homogenized must was transferred in 39 containers of 2-liter capacity, thus producing 13 experimental variants with 3 replicates for each. The experimental samples are described in the Table 1.

Table 1. Experimental variants produced to test the effect of fining agents and doses added

Experimental variants	Fining agent	Dose, g/hl
Control	-	-
KCas_10 KCas_20 KCas_30	Potassium caseinate (Clarito Spray Dry, Enologica Vason)	10 20 30
PVPP_10 PVPP_20 PVPP_30	Polyvinylpolypyrrolidone (PVPP, Enologica Vason)	10 20 30
Pea_10 Pea_20 Pea_30	Pea protein (Proveget 100, Agrovin)	10 20 30
Potato_10 Potato_20 Potato_30	Potato protein (Proveget Fine, Agrovin)	10 20 30

Colloidal dispersions of 5% w/v concentration were prepared in distilled water for each fining agent. The following amounts of dispersions were added to must: 4 ml of dispersion for the dose of 10 g/hl, 8 ml for the dose of 20 g/hl and 12 ml for the dose of 30 g/hl.

The samples thus treated, were well homogenized, kept at 10°C for 48 hours and then racked off the lees. Subsequently, 1.4 liters of each limpid must variant and repetition was transferred into 1.5 liters containers. The physio-chemical parameters of the limpid free run juice are presented in the Table 2.

Table 2. Physio chemical parameters of the limpid free run juice

Brix, %	20.2
pH	3.38
Total titratable acidity, g/l expressed as tartaric acid	5.81
Sugars, g/l	194
Potential alcohol, % vol.	11.8

The musts were inoculated with 25 g/hl active dry yeast Fermol Arome Plus, AEB and 24 hours later, a dose of organic nutrient of 40 g/hl (Nutristart ORG, Laffort) was added to each sample. The alcoholic fermentation continued for 7 days at a constant ambiental temperature of ≈20ºC. The resulted wines were racked off, treated with 70 mg/l SO_2, using 10% w/v $K_2S_2O_5$ solution and 0.6 g/l granulated sodic bentonite, using a 5% w/v gel to remove thermolabile proteins. Then, they were left for cold stabilization at -4ºC for 14 days. After that, the wines were racked again and transferred in 0.75 l bottles, then stored for 6 months in the cellar, to assess if their sensory qualities preserve well.
After the storage period, all the samples were sensorially evaluated and spectrophotometrically analyzed using CIELab method (OIV, 2021b) and the measurement of OD_{280} of a 10% diluted sample. The sensorial analysis was performed by a panel of 5 experienced winetasters, using a methodology developed in our laboratory and patented at the Romanian Office of Inventions and Trademarks (Antoce and Nămoloşanu, 2007). The CIELab method and OD_{280} were carried out running the software WinAspect version 2.2.7. coupled with a double beam UV-VIS spectrophotometer Specord 250 from Analytik Jena AG. All the spectrophotometric determinations were performed in quartz or glass cuvettes and conventionally referred to the optical path of 10 mm.

RESULTS AND DISCUSSIONS

After 6 months of storage in bottles, all the fining agents applied to the must before alcoholic fermentation led to an increase in the lightness parameter (L) and decrease of colour saturation (C). These changes can be observed in Figure 1. The effect of lightness increase and colour saturation decrease is enhanced by the quantity of the fining agent added, in a dose-dependent manner, confirmed by the linear regression equations included in Figure 1, with coefficients of determination varying from 0.86-0.99. In accordance to the equation slopes, the maximum effect is produced by potato protein or potassium caseinate. The samples treated with PVPP and pea protein showed milder effect on these changes.

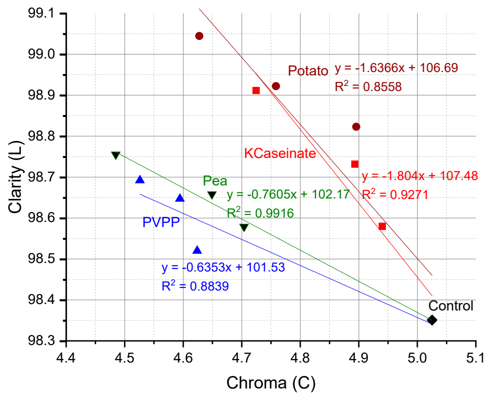

Figure 1. Variations of clarity (L) and chroma (C) parameters in experimental samples depending on fining agent type and dose

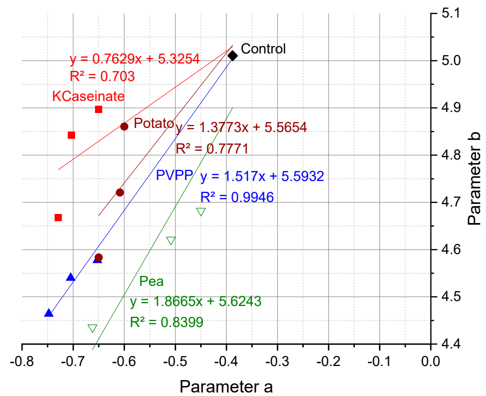

Figure 2. Variations of *a* and *b* parameters in experimental samples depending on fining agent type and dose

On the other hand, we can observe in Figure 2 the effect of fining agents on parameters *a* and *b*. The applied fining agents showed a reduction of yellowness (parameter *b*) and a shift more toward greener (parameter *a*). These effects were more evident in the samples treated with higher doses. The pea protein and PVPP were able to reduce more the parameter *b* (yellowness) than potato protein or potassium caseinate at similar doses of treatment. Thus, the most effective treatments to obtain lower values for parameter *b* remain those with pea protein and PVPP.

However, the PVPP seems to change the most the colour towards greener, by decreasing more the value of the parameter *a*.

In Table 4 are presented the average values of the Total Polyphenolic Index (TPI) along with standard deviations.

The TPI showed a decreasing trend with an increase of the fining agent dose. The most powerful fining agent in reducing the TPI was PVPP and followed by pea protein.

Table 3. The effect of dose and of clarifying agent on TPI, CIELab parameters and colour difference

	TPI	**Clarity (L)**	**Parameter a**	**Parameter b**	**Chroma (C)**	**hab°**	****ΔE Total colour difference**
Dose effect	$F_{(2, 262.335)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.93$	$F_{(2, 34.823)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.64$	$F_{(2, 21.792)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.52$	$F_{(2, 43.336)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.69$	$F_{(2, 37.279)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.66$	$F_{(2, 33.347)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.63$	$F_{(2, 45.741)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.72$
Control (without)	6.32±0.02[d]	98.35±0.09[d]	-0.388±0.03[c]	5.010±0.12[c]	5.025±0.12[c]	94.43±0.46[c]	-
Low dose (10 g/hl)	6.13±0.07[c]	98.63±0.13[c]	-0.588±0.09[b]	4.754±0.14[b]	4.791±0.14[b]	97.05±1.04[b]	0.476±0.10[c]
Medium dose (20 g/hl)	6.00±0.08[b]	98.74±0.13[b]	-0.631±0.09[b]	4.681±0.12[b]	4.724±0.12[b]	97.68±1.06[b]	0.603±0.09[b]
High dose (30 g/hl)	5.91±0.08[a]	98.85±0.15[a]	-0.697±0.05[a]	4.537±0.11[a]	4.591±0.11[a]	98.74±0.69[a]	**0.780±0.09[a]**
Fining agent effect	$F_{(3, 96.596)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.88$	$F_{(3, 38.880)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.75$	$F_{(3, 30.854)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.70$	$F_{(3, 42.764)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.77$	$F_{(3, 43.691)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.77$	$F_{(3, 27.629)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.68$	$F_{(3, 6.408)}$ $p<0.05$ $\hat{\omega}_p^2 = 0.32$
Control (without)	6.32±0.02[d]	98.35±0.08[d]	-0.388±0.03[d]	5.010±0.12[c]	5.025±0.12[c]	94.43±0.46[d]	-
KCas	6.05±0.10[c]	98.74±0.16[b]	-0.694±0.04[a]	4.802±0.13[b]	4.852±0.12[b]	98.24±0.59[ab]	0.562±0.17[b]
PVPP	**5.90±0.11[a]**	98.62±0.08[c]	-0.702±0.05[a]	4.527±0.05[a]	4.581±0.04[a]	98.82±0.65[a]	0.644±0.11[ab]
Pea	6.01±0.09[b]	98.67±0.09[bc]	-0.540±0.10[c]	4.579±0.11[a]	4.613±0.10[a]	96.75±1.44[c]	0.571±0.17[b]
Potato	6.09±0.10[c]	98.93±0.11[a]	-0.619±0.04[b]	4.720±0.12[b]	4.760±0.12[b]	97.48±0.60[bc]	**0.702±0.14[a]**

*Average values ±SD and **Two-way ANOVA – Tukey HSD (p<0.05).

Table 4. Total polyphenolic index and total colour differences towards control samples

Variants	**TPI**	**ΔL**	**Δa**	**Δb**	****ΔE Total colour difference** $F_{(2, 9.927)}$, $p<0.05$ $\hat{\omega}_p^2 = 0.73$
Control	6.32 ± 0.02	-	-	-	-
KCas_10	6.17 ± 0.02	0.23 ± 0.09	-0.262 ± 0.03	-0.113 ± 0.13	0.389 ± 0.03[e]
KCas_20	6.04 ± 0.03	0.38 ± 0.18	-0.316 ± 0.03	-0.168 ± 0.17	0.554 ± 0.12[bcde]
KCas_30	5.95 ± 0.06	0.56 ± 0.12	-0.341 ± 0.06	-0.342 ± 0.09	**0.746 ± 0.11[abc]**
PVPP_10	6.05 ± 0.03	0.17 ± 0.05	-0.265 ± 0.03	-0.433 ± 0.12	0.541 ± 0.09[bcde]
PVPP_20	5.89 ± 0.01	0.3 ± 0.08	-0.317 ± 0.03	-0.470 ± 0.11	0.648 ± 0.07[abcd]
PVPP_30	**5.79 ± 0.04**	0.34 ± 0.06	-0.360 ± 0.04	-0.546 ± 0.13	**0.744 ± 0.09[abc]**
Pea_10	6.11 ± 0.03	0.23 ± 0.09	-0.062 ± 0.02	-0.328 ± 0.13	0.418 ± 0.10[de]
Pea_20	6.00 ± 0.02	0.31 ± 0.08	-0.121 ± 0.05	-0.389 ± 0.14	0.526 ± 0.08[cde]
Pea_30	**5.92 ± 0.01**	0.41 ± 0.11	-0.274 ± 0.10	-0.575 ± 0.14	**0.770 ± 0.10[ab]**
Potato_10	6.22 ± 0.02	0.47 ± 0.15	-0.212 ± 0.04	-0.151 ± 0.10	0.558 ± 0.08[bcde]
Potato_20	6.09 ± 0.02	0.57 ± 0.03	-0.220 ± 0.03	-0.291 ± 0.12	0.687 ± 0.03[abc]
Potato_30	5.98 ± 0.02	0.69 ± 0.02	-0.262 ± 0.01	-0.428 ± 0.14	**0.863 ± 0.07[a]**

*Average values ±STDEV.S and **One-way ANOVA – Tukey HSD (p<0.05).

The changes induced by fining agents on the CIELab parameters, TPI and total colour difference were statistically analysed using additive model Two-way ANOVA coupled with post-hoc test Tukey HSD ($p<0.05$), in order to evaluate, on one hand, the main effects of fining agents irrespective of the dose and, on the other hand, the main effect of a low, medium or high dose applied, irrespective of the type of fining agent (Table 3). Both of factors were statistically significant, which means that they affect the parameters of wine at $p<0.05$, inducing reductions of total polyphenol index (TPI) and differences in total colour (ΔE). The effect sizes were large for both factors with bigger values for the dose effect (TPI $\hat{\omega}_p^2 = 0.93$; ΔE $\hat{\omega}_p^2 = 0.72$) and slightly lower values for the fining agent effect (TPI $\hat{\omega}_p^2 = 0.88$; ΔE $\hat{\omega}_p^2 = 0.32$), meaning that the correction of TPI or ΔE can be easier achieved by adjusting the dose of any of the tested fining agents (Table 3). In the case of the CIELab parameters (L, a, b, C, hab°), the dose and fining agents were also statistically significant ($p<0.05$), but the effect magnitude was opposite comparing with the case of TPI and ΔE. The effect magnitude was large for both factors, with bigger values for the fining agent type (L $\hat{\omega}_p^2 = 0.75$; parameter a $\hat{\omega}_p^2 = 0.70$; parameter b $\hat{\omega}_p^2 = 0.77$; C $\hat{\omega}_p^2 = 0.77$; hab° $\hat{\omega}_p^2 = 0.68$), followed by the effect of the applied dose (L $\hat{\omega}_p^2 = 0.64$; parameter a $\hat{\omega}_p^2 = 0.52$; parameter b $\hat{\omega}_p^2 = 0.69$; C $\hat{\omega}_p^2 = 0.66$; hab° $\hat{\omega}_p^2 = 0.63$) meaning that to achieve a certain correction for any specific CIELab parameter, selecting the appropriate fining agent is more efficient than changing the dose (Table 3). However, it is clear from the Table 3 that the use of any fining agent, even at low dosage, may induce significantly favourable changes of any of the parameters, when compared with the parameters of control sample. Obviously, the higher the dose, the bigger the effect on colour and polyphenols, but the stripping effect of these fining agents on aroma and mouthfeel may also increase, lowering complexity of the wine. Nevertheless, each grape variety, vintage and terroir will lead to a different level of phenolic compounds in the must, therefore the dose of the fining agent should be adjusted in accordance with the desires wine style. For a lighter body and a fruity white wine of Welsch Riesling, the lowest dose of any of the tested fining agent was determined to be enough. For selecting the appropriate fining agent to be used during the pre-fermentation stage, the following features can be exploited, taking into account their effectiveness (Table 3):

- **to lower TPI:** PVPP > Pea > KCas= Potato > Control.
- **to increase clarity/lightness (L):** Potato > KCas ≥ Pea ≥ PVPP > Control.
- **to shift the colour towards green (parameter a), thus reducing the red component associated with oxidation:** PVPP = KCas > Potato > Pea > Control.
- **to reduce yellowness (parameter b), also associated with some phenolic oxidation:** PVPP = Pea > Potato = KCas > Control.
- **to reduce colour saturation (C):** PVPP = Pea > Potato = KCas > Control.
- **to induce a beneficial total colour difference (ΔE):** Potato ≥ PVPP ≥ Pea = KCas.

The total colour difference (ΔE) indicated in Table 3 and 4, has values less than 1, so, for all samples a human observer will not see with the naked eye the colour differences between the wines.

These total colour differences (ΔE) are statistically significant (Table 3), but can be observed only by means of the spectrophotometer. Even not perceivable now, these colour differences have technological importance, as they show a reduction of oxidisable polyphenols from the must, thus reducing their presence in the wines, where in time, after a period of storage, could lead to visible effects. The evolution of the experimental samples over time could make a difference through slow oxidation reactions and in the presence of different oxidation substrates.

These small changes of the CIELab parameters and lowering TPI could be even more important for certain white wines which are produced from varieties more susceptible to oxidative phenomenon such as browning and pinking, as it is demonstrated that the fining agents could reduce to a certain degree the concentration of small-molecule phenolic compounds and even anthocyanins (Salacha *et al.*, 2008; Cojocaru and Antoce, 2019; Cosme *et al.*, 2019;). Browning rate is significantly correlated with flavanol concentration ($r^2=0.84$) and with total phenolic ($r^2=0.79$) (Salacha *et al.*, 2008).

The results presented in Table 4 show that the total colour changes (ΔE) were influenced the most by the highest dose (30 g/hl) of any fining agent, practically no significant differences being obtained. For potato protein and PVPP, however, the medium dose of 20 g/hl produced also a similar effect as the doses of 30 g/hl. This is an important finding, as reducing the dose has also a beneficial economic impact.

According to the data indicated in Figure 3 we can observe that Potato protein increase the most the price of resulted wine, being followed by the PVPP. However, the Pea protein alternative is more or less close to K Caseinate, with only a lower price increase as compared to the other fining agents.

From a sensory point of view, with the exception of bitterness, the perception of taste parameters (acidity, sweetness, astringency, extract) and visual (colour intensity) were statistically insignificant (Figure 4). The tasters pointed out that the minimum doses (10 g/hl) better preserved the complexity of the Welsch Riesling wines, while the higher doses, especially in the case of Potato protein, led to a reduction in complexity of the resulted wines. Following the sensory analysis, the overall quality of the wines was established to be in the following descending order of the applied treatments: PVPP > Pea > KCas > Potato > Control.

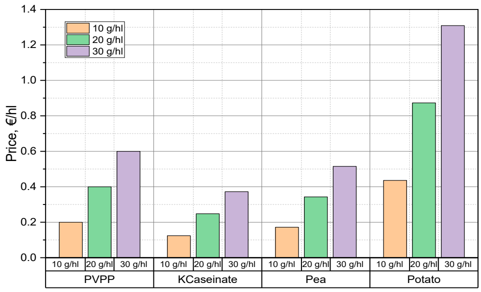

Figure 3. Price increase per hl of wine due to the application in musts of the fining agents in certain doses

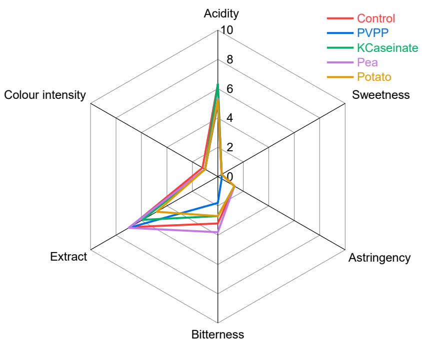

Figure 4. The overall effect of the fining agents on taste and the perceived colour intensity (average values)

Considering the price increase induced by these treatments (Figure 3) and the sensory panel agreement that the Potato protein reduces the wine complexity, the other vegetal protein, the Pea protein, appears to be a good alternative for PVPP and KCas.

By applying the principal components analysis (PCA) to the results of the sensory analysis, some correlations of fining agents to the sensory attributes can be highlighted (Figure 5). As there were no significant changes in the main taste characteristics except bitterness, only this one was included in the PCA chart, along with the perceived aroma attributes.

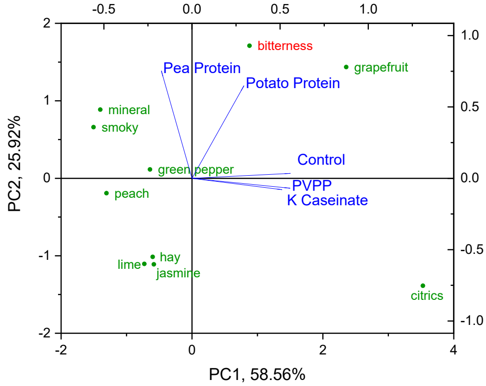

Figure 5. The overall effect of fining treatments on wines sensory characteristics

This technique reduces the data complexity and brings about some patterns and associations between the measured variables. It can be pointed out that the experimental variants treated with PVPP and those treated with KCas have certain sensory characteristics in common with the control samples, so they keep the complexity of the wines, similar to the control samples. These variants were associated with citrus, grapefruit and less with the mineral or smoky character. Experimental samples treated with Potato protein were more strongly associated with grapefruit and bitterness, while those treated with pea protein were more strongly associated with mineral, smoked and even green pepper. In all experimental samples, regardless of the treatment, there were highlighted dominant aromas of peach, hay and lime.

CONCLUSIONS

The pre-fermentative operations and treatments during white winemaking are important decisions for the quality of the final product. The reduction of TPI and inducing beneficial total colour differences can be achieved by using several fining agents in dose dependent manner, the higher the dose, the higher the produced effect. However, to fine tune the CIELab colour parameters not only the dose, but also the type of fining agent is important. Also, the sensory quality of the final wine, as well as the price, are significantly influenced by the fining agent used. The treatments with PVPP or KCas proved to be efficient and cost-effective, justifying their widespread use in spite of their drawbacks as being synthetic or of animal origin, respectively. The present sensory analyses results, along with the spectrophotometric ones, highlighted that the Pea protein is a very good alternative to the treatments with PVPP or K Caseinate, while the Potato protein, even in low doses, was found to reduce too much the complexity of white wines and to be also the most expensive of all. The commercial products based on pea protein are allergen-free isolates, which give comparable results with PVPP or K Caseinate fining agents. Moreover, the pea protein isolates are allowed to be used in organic winemaking and the treatment cost is similar with the regular treatments with PVPP or K Caseinate.

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THE RESVERATROL CONTENT IN BLACK GRAPES SKINS AT DIFFERENT DEVELOPMENT STAGES

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Abstract

The resveratrol (3,5,4'-trihydroxystilbene) is a phytoalexin belonging to the class of polyphenolic compounds called stilbene, produced in response to stress factors by a wide variety of plants, including Vitis vinifera. This study presents the high performance liquid chromatography (HPLC) system detection of the forms of resveratrol (cis- and trans-) isomers in the skin of the Cabernet Sauvignon and Merlot black grapes from the Şimnicu de Sus wine grape-growing area during the 2019 and 2020 period. The resveratrol isomers were determined throughout the period of maturation of the studied varieties (15 July to 5 September) of the grape berries skin samples. The resveratrol content of grapes skin decreased constantly from the green phase until the full maturity for both varieties, reaching zero for cis-resveratrol (0.043mg/L for Cabernet Sauvignon variety in 2020) in the matured fruits.

Key words: *cis- and trans- resveratrol, black grapes, grape skin.*

INTRODUCTION

The resveratrol was isolated from the grape berries, skin and seeds in 1976 (Wu et al., 2013). In 1992, Siemann and Creasy reported that the resveratrol could appear in grape products and especially in wine being considered responsible, at least partially for the effects of wine on coronary artery disease. Other studies confirmed this aspect as well (St Leger et al, 1979; Rimm et al., 1991; Hertog et al., 1995; Rimm et al., 1996; Arthur et al., 1997; Hao et al., 2004; Nakata et al., 2012; Timmers et al., 2012; Mazza et al., 2021). Some varieties showed a *trans*-resveratrol content in skins of two to three times higher than the average content in all varieties (Torchio et al., 2013; Nguyen et al., 2020). Besides, resveratrol play a role in the prevention of human pathological processes such as inflammation, atherosclerosis and carcinogenesis (Stivala et al., 2001; Donna et al., 2009; López-Nicolás et al., 2009; Holthoff et al., 2010; Shingai et al., 2011; Stark et al., 2011; Mobasheri et al., 2012; Carter et al., 2014). The *trans*-resveratrol was considered to have antimutagenic and chemoprotective effects against cancer proliferation (Melzoch et al., 2001; Borra et al., 2005; Chandra et al., 2015; Munir et al., 2015; Jiang et al., 2017), antiseptic, antioxidant and anti-inflammatory characteristics (Park et al., 2000; Pinto et al., 2005; Szewczuk et al., 2005; Bist et al., 2010; Zhu et al., 2012; Goutzourelas et al., 2015; Benayahoum et al., 2015; Nunes et al., 2017). As a result of these studies, the resveratrol is considered one of the constituents of black grapes and red wine that can be used to make pharmaceutical preparations in order to improve human health (Delmas et al., 2005; Ahn et al., 2007).

The in-depth study of resveratrol isomer stability was conducted by (Jeandet et al., 1991; Căpruciu et al., 2007; Cayuela et al., 2009; Kutil et al. 2015; Novelle et al., 2015; Oliveira et al., 2017; etc). Another important aspect for the synthesis of resveratrol is the influence of the viticultural area on the total polyphenols dynamics.

The influence of the viticultural area has been studied by many authors who have established direct relationships and correlations between climatic conditions (temperature, precipitations), soil conditions and resveratrol. It has been established that the duration of sunshine, precipitation, slope exposure and winds are essential factors for the synthesis and dynamics of total polyphenols (Sautter et al.,

2008; Rastija et al., 2009; Ubalde et al., 2010; Duque et al., 2011; Geana et al., 2015; Cichi et al., 2016; Leeuwen et al., 2016).
The quality-oriented practices in viticulture produce grapes with high levels of stilbene (Bavaresco, 2003; Yaman et al., 2016; Storchi et al., 2019; Rocchetti et al., 2021).
It has been found that grape vines synthesize the resveratrol as a response to stress factors: hitting impact, high level of UV radiation (Rodriguez et al., 2006; Choi, 2011), fungal infections (Hoos and Blaich, 1990, Dai et al., 1995; Schoonbeek et al., 2001; Pezet et al., 2004), the presence of heavy metals such as copper, etc.
The exposure to UV radiation produces irreversible isomerization of *trans*-resveratrol in *cis*- resveratrol which exhibits less stability than the isomer which is not exposed to light (Delmas et al., 2011; Wu et al., 2013).
When *Botritys cinerea* attacks, the resveratrol is produced in grape as a means of self-defense. Some studies have shown that a 160 mg/L trans-resveratrol concentration is sufficient to inhibit the growth of the *Botritys cinerea* mycelium (Adrian & Jandet, 2012).
The objectives of this study were to determine through high performance liquid chromatography (HPLC) system, the *cis* and *trans* resveratrol from the grapes of Cabernet Sauvignon and Merlot cultivars at different stages of their development, as well to do statistical analysis of the obtained results at full grapes maturation.

MATERIALS AND METHODS

Sampling Sites

The experiments were organized in parcels placed in identical orographic conditions (plateau conditions), on a slightly levigated reddish brown soil. Geographically, the area is located in South West of Romania, at 44°24'23 "N, 23°48'09" E and it is characterized by mild winters and long summers, with high sunshine duration and low rainfall (Table 1), climatic conditions required for the synthesis of anthocyanins and total polyphenols, including the resveratrol.

Table 1. Description of the sampling sites for blake grapes

Sampling sites	Șimnicu de Sus
Altitude (m)	175
Average July temperature (°C)	+22°C … +23°C
Radiation	Hight
Precipitation (mm)	565
Soil	terraced forest brick soils or heavily eroded slopes with moderate slopes rich in carbonates

The organization of the experiments was done by the randomized block method, with 2 variants, representing the studied varieties, respectively 15 vine per variety in 4 repetitions. Soil preparation and grapevine work was done uniformly for both varieties.
The varieties included in the study are grafted on Berlandieri x Riparia Kober 5BB rootstock, with 2 x 1.2 m planting distance, semi-high growth with multiple Guyot cutting system, with a crop load of 40 buds/vine.

Fruit Sampling

The biological material used for analysis was the grape berries skins of `Cabernet Sauvignon` and `Merlot` cultivars, which are basic varieties in the vineyards of south-western Romania, used in the production of high quality red wines.
The samples were harvested at 15 days between the veraison phase and full maturity (15 July - 5 September), the analyzed varieties being in the same stage of development.
For each variety, 100 grape berries were harvested at the same specified date, in 3 repetitions and were analyzed in viticulture lab of the Faculty of Horticulture, University of Craiova (3 samples).

Reagents

The pure resveratrol (*trans* - 3,5,4' - trihydroxystilbene) was purchased from Sigma Chemical Co. and the stock solution (1000 mg/L was prepared by dissolving 25.00 mg of commercial product, without previous purification in 25 mL of methanol. The acetonitrile, methanol and acetic acid were used for liquid chromatography (Merck). The used water was ultra-pure, Basic TWF.

The used protocol to resveratrol determination by HPLC

A. *Extraction-purification method based on solvent extraction*

The extract obtained from the skin of 100 grapes berries (representing a sample) was used in order to identify the cis and trans-resveratrol forms by HPLC Agilent 1000. The grape berries obtained from the three samples were manually separated, their skin was weighed and placed in 50 mL of 96% ethanol. After homogenization, the samples were kept in the dark for 48 hours. To obtain the extracts, the centrifugation method was used at 4000 rpm for 20 minutes followed by filtration through 0.45 µm filters. Centrifugation was performed with Centurion Scientific Centrifuge LTD K2 R Series. The samples obtained were kept in the dark until the extract was subject to chromatographic analysis.

B. *HPLC method for determination*

This study involves a simple, accurate, sensitive and reproducible high performance liquid chromatographic method for the determination of resveratrol (*cis*- and *trans*-3,5,4' -trihydroxystilbene) in the skin of Cabernet Sauvignon and Merlot grapes. The calibration curves of *trans*-resveratrol and *cis*-resveratrol were obtained by plotting the peak area of each standard against concentration in the range of 5-100 and 2-50 mg/L, respectively. The number of calibration points was eight for *trans*-resveratrol and five for *cis*-resveratrol. Each calibration point was the average of three independent measurements.

The chromatographic separation was performed on an Econosil C18 10 U column (250 mm x 4.6 mm i.d., 5 µm particle size) at room temperature (20°C) and with thermostatic control. The total gradient time was 35 minutes, with a flow rate of 1.0 mL/min, with an injection volume of 5 µL. The gradient elution system consists of water (A) and acetonitrile (B) (H_2O/CH_3CN 2: 8 v/v). The column effluent was monitored by the UV detector.

The *cis*-resveratrol is detected at absorbance of 280 nm and the *trans*-resveratrol at 307 nm absorbance. The identification was based on retention time and spectrum data. In order to confirm the accuracy of the method, repetitive analyzes were performed, calculating the mean relative standard deviation (RSD) for three replicated determinations. The calculation of the limit of detection (LOD), signal-to-noise ratio (S/N) = 3 of the individual compounds was reached at their maximum absorption.

Statistical Analysis

In order to determine the content of *trans*- and *cis*-resveratrol in the grapes of the Cabernet Sauvignon and Merlot, the analysis of variance (ANOVA) was used. The differences between the means values of *trans*- and *cis*- resveratrol were tested by the Duncan test (using the SPSS 16 program). The results were expressed in mg/L, as mean values ± standard deviation (SD). Also, the coefficient of variation (CV %) was calculated.

RESULTS AND DISCUSSIONS

The results of the study regarding the dynamics of *trans*- and *cis*- resveratrol in the grape berries skins are presented in Table 2 and Table 3.

Table 2. The variability of *trans*- resveretrol (mg/L) in grape berries skin analyzed in Șimnicu de Sus viticultural areas during the 2019-2020

Calendar dates	Variety of grapes											
	Cabernet Sauvignon						Merlot					
	2019			2020			2019			2020		
	Mean	S.D.	CV %	Mean	S.D.	CV%	Mean	S.D.	CV %	Mean	S.D.	CV %
15 July	9.152	0.76	8.3	8.463	0.27	3.2	11.083	0.17	1.5	10.443	0.37	3.5
29 July	5.828	0.65	11.2	4.539	0.17	3.7	7.581	0.25	3.3	6.778	0.27	4.0
8 August	3.316	0,40	12.1	2.822	0.19	6.7	4.363	0.26	6.0	3.169	0.21	6.6
22 August	1.997	0.10	5.0	1.240	0.18	14.6	2.491	0.42	16.8	1.897	0.20	10.5
5 Sept.	0.633	0.08	12.7	0.430	0.04	9.3	0.996	0.04	3.9	0.675	0.06	8.4

Table 3. The variability of *cis*- resveretrol (mg/L) in grape berries skin analyzed in Șimnicu de Sus viticultural areas during the 2019-2020

Calendar dates	Variety of grapes											
	Cabernet Sauvignon						Merlot					
	2019			2020			2019			2020		
	Mean	S.D.	CV %	Mean	S.D.	CV %	Mean	S.D.	CV %	Mean	S.D.	CV %
15 July	7.119	0.13	1.8	6.459	0.13	2.0	8.071	0.14	1.7	6.994	0.3	4.3
29 July	4.910	0.14	2.8	3.531	0.11	3.1	5.573	0.09	1.7	5.178	0.02	0.4
8 August	2.213	0.04	1.9	1.788	0.1	5.7	3.828	0.02	0.6	2.123	0.01	0.7
22 August	1.083	0.06	4.6	1.045	0.03	2.6	1.095	0.03	2.6	1.105	0.03	2.6
5 Sept.	0.065	0.01	19.6	0.043	0.01	23.3	0.086	0.01	15.4	0.058	0.01	16.6

The presented data show differences in the resveratrol accumulation between both isomers and varieties, which change from one determination to another. The results of the study show that a maximum of *cis*- and *trans*-resveratrol content was recorded at the beginning of the grape maturation period for both Cabernet Sauvignon and Merlot varieties in both years of study (2019 and 2020). There is a continuous decrease in the content of *trans*- and *cis*-resveratrol from the initial stage of grape berries ripening to full maturity for both studied varieties. Jeandet et al. (1991), find that there is a decrease in the capacity of grape fruits to synthesize the resveratrol during the fruit maturation, and this appears to be produced at the skin level. Thus, a maximum content of 9.152 mg/L for *trans*-resveratrol was recorded on 15 July 2019 and 8.463 mg/L in 2020 for Cabernet Sauvignon in Șimnicu de Sus, the values continuing to decrease continuously until the full maturity in both years of study. A higher amount of *trans*-resveratrol quantity was extracted from the Merlot variety skins in comparison with the Cabernet Sauvignon variety under the same orographic conditions and climate of the Șimnicu de Sus vineyard plantation, i.e. 11.083 mg/L in the 2019 crop, and 10.443 mg/L in 2020. A continuous decrease in the *trans*-resveratrol content was observed during maturation also by Giuffrè (2013) on the four varieties of red grapes grown in the south-west Calabria (South Italy). The coefficient of variation of *trans*-resveratrol in the Cabernet Sauvignon variety skins is low or medium in all the analyzed data, between 5% and 12.7% in 2019 and between 3.2% and 14.6% in 2020 (Table 2). The variation coefficient of *trans*-resveratrol in the Merlot variety is low for most determinations periods, ranging from 1.5% to 16.5% in 2019 and from 3.5% to 10.5% in 2020 (Table 2). Higher levels of *trans*-resveratrol content in the Merlot variety have been observed throughout the study compared to the Cabernet Sauvignon variety, with a higher dynamic in 2020. It can also be observed that at the end of the maturity process, the lowest value of *trans*-resveratrol is detected by the HPLC system at the Cabernet Sauvignon variety in 2020 with a value of 0.430 mg/L In another study, the highest concentration of *trans*-resveratrol detected in the Cabernet Sauvignon grapes at full maturity in the Tekirdağ region of Turkey was 0.443 mg/L (Yaman et al., 2016). The resveratrol concentration in skins ranged from 0.5 to 14.1 μg/g fresh skin, with an average of 4.12 μg/g for the 36 skin samples from grapes grown in Japan (Okuda & Yokotsuka, 1996). The content of *cis*-resveratrol in Șimnicu de Sus recorded a maximum value at the beginning of the maturation period on July 15 for both Cabernet Sauvignon and Merlot. The content of *cis*-resveratrol decreases continuously for both varieties both in 2019 and in 2020 until September 5 (last date of the analysis). Lower values were observed at the technological maturity compared with *trans*-resveratrol content (Table 3). The coefficient of variation of *cis*-resveratrol for the Cabernet Sauvignon variety is low in most analyzed data, ranging from 1.8% to 4.6% in 2019 and between 2% and 5.7% in 2020 and it has medium values (19.6 %) at the end of the maturation period, in 2019 and very high values (23.3%) in 2020 (Table 2). The Merlot variety is characterized in both years of study by a higher capacity of *cis*-resveratrol synthesis compared to the Cabernet Sauvignon variety. The coefficient of variation of *cis*-resveratrol for Merlot variety is also low in most of the analyzed data, ranging between 0.6% and 2.6% in 2019 and between 0.4% and 4.3% in 2020 and medium at the end of the maturation period, both in 2019 (15.4%) and 2020 (16.6%) (Table 3). Cui et al. (2015), after a study on grapes and wine from three major wine regions in China, show a resveratrol content in Merlot wines higher than that recorded in Cabernet Sauvignon wines. A comparative analysis of the results obtained in the two analyzed varieties in the two years of study (Table 4 and Table 5) was performed on September 5 (the last date of the analysis). Thus, on September 5 2019, the Cabernet Sauvignon variety recorded an average content of 0.633 mg/L of *trans*-resveratrol, with 36.4% lower than the Merlot variety, which had an average content of 0.996 mg/L the negative difference being statistically assured to a significant degree. Also, in 2020, the Cabernet Sauvignon variety recorded an average content of 0.43 mg/L in *trans*-resveretrol, lower with 36.3% than the Merlot variety, which had an average content of 0.675 mg/Lbut the negative difference was not statistically assured (Table 4). Similar data for the *trans*-resveratrol content of the Cabernet Sauvignon and Merlot varieties in the Șimnicu de Sus wine grape-growing region were obtained at the end of the maturation period also by Geana et al. (2015), for the same varieties grown in Murfatlar vineyards in Dobrogea, the viticultural area located in the south-east of Romania.

Also, in 2020, the Cabernet Sauvignon variety recorded an average content in *cis*-resveratrol of 0.043 mg/L, lower with 25.9% than the Merlot variety, which had an average content of 0.058 mg/L the difference was not statistically assured.

The two varieties studied in the two years recorded similar *cis*-resveratrol content on September 5, the differences were not assured in any of the experimentation year (Table 5).

Table 4. The synthesis of the results on the *trans*- resveretrol content for the in grape berries skin analyzed in the Șimnicu de Sus viticultural area on full maturity

Variety	2019		2020	
	Content of *trans*-resveratrol mg/L	Relative content %	Content of *trans*-resveratrol mg/L	Relative content %
Cabernet Sauvignon	0.633[b]	63.6	0.430[a]	63.7
Merlot	0.996[a]	100.0	0.675[a]	100.0

Note: Means separation by Duncan test at p≤0.05. Means with the same letter are not statistically significant.

Table 5. The synthesis of the results on the *cis*- resveratrol content for the in grape berries skin analyzed in the Șimnicu de Sus viticultural area on full maturity

Variety	2019		2020	
	Content of *cis*-resveratrol mg/L	Relative content %	Content of *cis* -resveratrol mg/L	Relative content %
Cabernet Sauvignon	0.065[a]	75.6	0.043[a]	74.1
Merlot	0.086[a]	100.0	0.058[a]	100.0

Note: Means separation by Duncan test at p≤0.05. Means with the same letter are not statistically significant.

In 2019, the Cabernet Sauvignon variety recorded on 5 September a *cis*-resveratrol average content of 0.065 mg/L lower with 24.4% than the Merlot variety with an average content of 0.086 mg/L the difference not being statistically assured.

CONCLUSIONS

Studying the dynamics of resveratrol from the beginning of the fruit maturation period until the technological maturity, it was observed that the *trans*-resveratrol form is quantified more than the *cis*-resveratrol form for the studied varieties, with the highest values for the Merlot variety in the Șimnicu de Sus viticultural area in the 2019 crop.

The *cis* and *trans*-resveratrol forms of the black grape varieties in the Șimnicu de Sus wine grape-growing area were on a downward trend during the maturation process, the highest values being recorded in the first maturation period. The resveratrol dynamics analysis of the studied varieties highlights the high potential of synthesis for this substance at the skin level, requiring further studies of these aspects. Thus, the Șimnicu de Sus viticultural area becomes a point of reference within the wine-growing area of South-West Oltenia region, Romania, for obtaining quality red wines. The resveratrol content in the grape berries skin at the time of technological maturity being a proof in this respect.

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THE EFFECT OF THE PRE-FERMENTATIVE SKIN CONTACT ON THE COLOUR CHARACTERISTICS AND TOTAL PHENOLS OF WHITE WINES

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Abstract

Skin contact for aromatic grapes at low temperatures is essential for the quality of the resulted wines, Sometimes, even non-aromatic grapes can benefit from skin contact so that the wines obtained achieve the desired mouthfeel and aroma. In this study we have evaluated the effect of the pre-fermentative skin contact on a blend of white grapes consisting of 80% of a non-aromatic Romanian variety, Feteasca alba, and 20% of the aromatic variety Muscat Ottonel. The macerations were conducted at controlled temperature for 6 hours (T6) and 12 hours (T12), while for control, no skin contact was allowed (T0). The effects of maceration on the CIELab parameters and total polyphenols (TPI) of resulted wines were evaluated. The wines with 12 hours of maceration (T12) were significantly different from the samples with no maceration (T0) and samples with short time skin contact (T6). The colour differences can be easily perceived by an inexperienced observer, as long as the total colour differences ΔE values (T12-T0) = 3.90 ± 0.98 and, respectively, (T12-T6) = 2.44 ± 0.97. The TPI results suggest that the skin contact period, favours more polyphenol extraction, but also promotes oxidation of polyphenols and then their precipitation.

Key words: *CIELab, pre-fermentative maceration, total polyphenols, white wine.*

INTRODUCTION

The pre-fermentative skin contact on white grapes is occasionally performed in order to extract certain primary aroma compounds, along with some phenolic compounds important for wine texture. However, when extracting more polyphenols, later on, they should be protected from oxidation (Jones *et al.*, 2008; Bueno *et al.*, 2010; González-Barreiro *et al.*, 2015; Esti & Tamborra, 2006). Most importantly, for white winemaking the well-balanced phenolic compound extraction and harmonious preservation is decisive for the wine quality modern consumers expect. With or without maceration, white wines are preferred with supple tannins, with one notable exception represented by orange wines (Schneider & Chichua, 2021), for which the long maceration on skins leads to a higher extraction and oxidation. Moreover, lack of temperature control during skin contact will change dramatically the rate of the extraction of volatile and phenolic compounds. For an adequate management of skin contact, most authors recommend keeping the temperature around 10-15ºC or even lower, to limit the extraction of phenolic compounds, while enhancing the extraction of aroma precursors or beneficial volatile compounds (Ramey *et al.*, 1986). The pre-fermentative maceration at temperatures lower than 15ºC limited the extraction of both excessive tannins and proteins, lowering the browning capacity and the required dose of bentonite necessary to achieve the commercial heat stability of young wines (Ramey *et al.*, 1986). In the case of the aromatic grape varieties, the scope of maceration is mainly the extraction of aroma compounds. The varieties from the Muscat family, as it is the case of Muscat Ottonel which was used in our blend with the non-aromatic Feteasca alba (20% and 80% respectively), the characteristic varietal aroma is determined by over 50 monoterpene alcohols and derivatives identified in the berries (Mateo & Jiménez 2000). These terpene alcohols exist either in free forms or as glycosides (Versini *et* *al.*, 1994; Carrau *et al.*, 2008), the latter being released by enzymic hydrolysis in the presence of β-glycosidase or by chemical hydrolysis at low pH (Williams *et al.*, 1992; Skouroumounis & Sefton, 2000; Boido *et al.*, 2002; Swiegers *et al.*, 2005). Nevertheless, yeast can also have influence on the aroma, including on the terpenic aroma, a recent study showing that certain yeast strains of *Saccharomyces cerevisiae* can produce monoterpene alcohols in a simple chemically defined medium, even in the absence of precursors such as the terpenic glycoconjugates (Carrau *et al.*, 2005). Irrespective of the aroma compounds found in grapes, their free or precursor concentrations are increased by pellicular maceration, as compared to the case of directly pressing musts, as Peyrot des Gachons (2002) showed in their study on Sauvignon blanc. The extraction is potentiated by the maceration temperature, higher concentrations of the skin located compounds being obtained at 18ºC as compared with 10ºC (Peyrot des Gachons, 2002).

In modern winemaking, however, keeping the temperature at higher values is not always desirable, as this is also accompanied, during pre-fermentative phase, by higher polyphenol extraction and by the growth of unwanted microorganisms and certain enzymatic reactions (Gómez-Míguez *et al.*, 2007; Marais, 1998; Salinas *et al.*, 2005). Conducting the skin maceration at lower temperatures is thus preferred in modern winemaking and it comes also with the advantage of reducing the concentrations of sulphur dioxide during this phase. Whenever possible, during pre-fermentative phase, the effect of oxygen, which is more soluble at lower temperatures, may be reduced by adding carbonic ice (Carillo *et al.*, 2011; Roussis *et al.*, 2007). Other effects observed as a result of the skin contact is an increased extraction of potassium ions, leading later on to more potassium bitartrate precipitation, thus, to lower final titratable acidity and higher pH (Ough, 1969; Boulton, 1980; Sokolowsky *et al.*, 2015), requiring sometimes other interventions for correction.

As few studies regarding the effect of length of pre-fermentative skin contact have been conducted on Romanian grape varieties, this research was dedicated to the evaluation of the colour characteristics and total phenols of musts for white wines with maceration based on Feteasca alba as the main variety (Moroşanu *et al.*, 2016; Moroşanu *et al.*, 2018).

MATERIALS AND METHODS

This study was carried out on a blend consisting of 80% Fetească alba and 20% Muscat Ottonel grapes, harvested on September 10th, 2018 from a vineyard located in Dealu Mare - Boldesti Scaeni DOC (Denomination of Controlled Origin). The experimental variants were done in triplicate and the pre-fermentative technological steps (I-VII) conducted accordingly to Table 1.

Table 1. Pre-fermentative technological steps for the production of white wine with maceration

Pre-fermentative technological steps and parameters	**Must variants** depending on the duration of skin maceration		
	T0	**T6**	**T12**
I. Grape mash (crushed grapes)	5 kg	5 kg	5 kg
II. Treatment with SO_2 on grape mas	50 mg/kg	50 mg/kg	50 mg/kg
III. Skin contact	0 hours	6 hours	12 hours
IV. Temperature during skin contact	8-10°C	8-10°C	8-10°C
V. Reserved grape must after press	3 litres	3 litres	3 litres
VI. Clarification time by settling at 10°C	3 hours	3 hours	3 hours
VII. Reserved clarified grape must for fermentation	2 litres	2 litres	2 litres

After maceration, the separated and clarified musts were allowed to ferment with their natural yeasts, at temperatures between 15 and 20°C. The resulted wines were racked off the lees and stored for two months at cellar temperatures before they were analysed. The classical analyses conducted on musts and wines were in accordance to the OIV recommended methods (OIV, 2018). The CIELab parameters and Total Polyphenolic Index were determined with a UV-VIS Specord 250 spectrophotometer from Analytik Jena AG (Germany) equipped with Chroma software Ver. 2.0. The CIELab parameters were measured in glass cuvettes of 10 mm path length (OIV, 2018), while the Total Polyphenol Index (TPI) was measured in a quartz cuvette of 10 mm optical thickness at a wavelength of 280 nm on 10% diluted samples. The TPI results were multiplied with 10.

RESULTS AND DISCUSSIONS

The physico-chemical parameters determined on two different stages of winemaking (Table 2) showed a tendency for pH increase and a total acidity decrease in direct relation to the length of skin contact time. These changes in titratable acidity and pH are well explained by the higher extraction of potassium cations from the skins with the longer maceration times.

The results presented in Table 2 are in agreement with other previous studies (Ough, 1969; Boulton, 1980; Sokolowsky *et al.*, 2015). The pH and total titratable acidity values are also affected by some potassium bitartrate precipitation and to a certain degree by microorganism metabolism.

Table 2. Physico-chemical analyses of musts and wines obtained with maceration

Winemaking phase	Variants	Physico-chemical parameters*		
		Sugars, g/l	Total acidity, g/l tartaric acid	pH
Grape must after settling	T0	205 ± 2.35[a]	5.39 ± 0.15[a]	3.10 ± 0.02[b]
	T6	215 ± 4.10[a]	4.67 ± 0.06[b]	3.34 ± 0.05[a]
	T12	208 ± 5.56[a]	4.76 ± 0.42[ab]	3.41 ± 0.11[a]
Wine after cold treatment	T0	0.84 ± 0.69[a]	5.73 ± 0.21[a]	3.23 ± 0.03[b]
	T6	0.57 ± 0.19[a]	5.17 ± 0.70[a]	3.33 ± 0.02[a]
	T12	0.76 ± 0.15[a]	5.16 ± 0.61[a]	3.32 ± 0.05[ab]

*Average values ± Standard Deviations. Different letters indicate significant differences at $p<0.05$ determined by One-Way ANOVA and Tukey HSD test.

That is why the wine titratable acidity is not anymore correlated with the maceration time, the samples stabilizing all around a similar value. The values of acidity in our wines are not significantly different than in the case of the correspondent musts, due to several conditions during fermentation.

Generally, the titratable acidity decreases after alcoholic fermentation as a result of ethanol accumulation and storage at low temperature, which both affect the solubility of potassium bitartrate leading to crystallization and precipitation.

However, it is not uncommon to observe a rise in total titratable acidity of wines due to various yeast fermentations under certain conditions during alcoholic fermentation (temperature, nutrient, oxygen, etc.) or presence of strains which promote succinic acid and/or lactic acid production, as normal fermentation by-products (Thoukis *et al.*, 1965; Vilela, 2019; Mendes-Ferreira & Mendes-Faia, 2020; Sainz *et al.*, 2022). Another cause of titratable acidity increase may be the volatile acidity production by unwanted microorganisms and uncontrolled winemaking process (Zoecklein *et al.*, 2012; Chidi *et al.*, 2018), but this is not our case. Sugar concentration of the resulted musts showed small variations between the variants, with average of around 5 g/l (Table 2).

After wine cold stabilization and racking the colour and TPI index were measured, to determine the relationship between maceration and the wine quality. The results (Table 3) showed a statistically significant decrease of TPI in macerated wines as compared to control wine. This was surprising at first, as many studies show that polyphenols tend to increase in white musts and wines produced with pre-fermentative skin contact, the effect being more evident as the time and temperature of maceration increase (Cheynier *et al.,* 1989; Marais, 1998; Darias-Martın *et ́ al.,* 2004; Gomez-Miguez *et al.,* 2007).

Table 3. Total polyphenol index and CIELab parameters of wines after cold stabilization

Variants	TPI*, UA	Colour parameters*				
		Clarity (L)	Parameter a	Parameter b	Chroma (C)	hab°
T0	7.28 ± 0.14[a]	98.69 ± 0.04[a]	-0.156 ± 0.06[b]	5.23 ± 0.09[c]	5.23 ± 0.09[c]	91.72 ± 0.65[b]
T6	6.13 ± 0.59[b]	98.22 ± 0.05[a]	-0.384 ± 0.03[a]	6.88 ± 0.08[b]	6.89 ± 0.08[b]	93.19 ± 0.18[a]
T12	6.52 ± 0.39[b]	96.41 ± 0.50[b]	0.133 ± 0.05[c]	8.38 ± 0.90[a]	8.39 ± 0.90[a]	89.11 ± 0.28[c]

*Average values ± Standard Deviations. Different letters indicate significant differences at $p<0.05$ determined by One-Way ANOVA and Tukey HSD test.

The decrease we have observed could be due to a natural process of stabilization, where the phenolic-protein interaction under cellar temperature conditions caused the precipitation of these aggregates, especially because the wines were not treated with bentonite to remove unstable proteins.
It is well known that the pH, alcohol content and the concentration of polyphenols and proteins in the wine may affect colloidal stability and therefore cause spontaneous precipitation (Siebert & Lynn, 2003; Charlton *et al.* 2002; Adamczyk *et al.*, 2012). Moreover, phenolic compounds are demonstrated to be among the factors involved in protein haze formation, as they were found, for example, in the natural proteinaceous precipitate in a Sauvignon blanc wine (Esteruelas *et al.*, 2011).
The colour CIELab parameters are also included in Table 3. Clarity or lightness parameter (L) is very good for all wines, with a tendency to slightly decrease with skin contact, but a significant difference is observed only in the case of 12 hours skin contact. In the same time, with the decrease in lightness, the chromaticity of the samples increases.

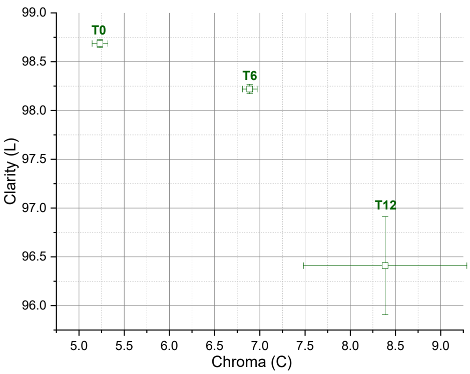

Figure 1. Placement of wines in the CIELab space described by parameters Clarity and Chroma

In Figure 1, an inverse direct relationship can be observed between the lightness parameter (L) and chroma (C), with the increase in the skin contact time. The experimental white wines resulted from long time skin contact had deeper colour, with enhanced colour saturation. The colour saturation itself (Chroma in Table 2) significantly increased with the maceration time (T) for all the experimental variants, the chromaticity being extremely well correlated to the time of skin contact (C=5.256+0.263*T; R^2=0.999). Even though the lightness was affected to a lesser extent than chroma (L=98.91-0.19*T; R^2=0.896), both parameters contribute to total colour difference (ΔE), a practical parameter for interpretation of the overall colour. The values of parameter a (showing the position of the sample colour between red and green) showed statistically significant differences among the samples, indicating that the colour loses some of its green component with the time of maceration, at 12 h maceration even a slight red component being present (Table 3 and Figure 2).

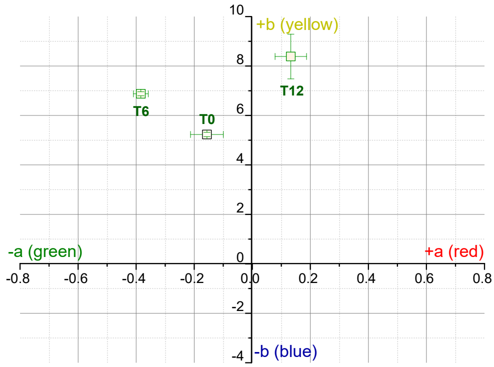

Figure 2. Placement of wines in the CIELab space described by parameters a and b

On the other hand, the parameter b (showing the position of the sample colour between yellow and blue) was also significantly different for all the experimental samples, the values increasing linearly with the skin contact time, indicating an increase of yellowness in macerated wines (Table 3 and Figure 2). Nevertheless, in white wines, the parameter b is generally more important than parameter a, having the most influence on the colour saturation (C), from which it does not differ much (Table 3).
The positioning of the wine samples in the colour diagram described by the parameters a and b (Figure 2) reveals that control wines, resulted from direct pressing musts, have the least intense yellow component and a green component almost imperceptible for the naked eye, while the 6 hours macerated wine had a slight increase in yellow, but a noticeable increase in the green component. On the other hand, the 12 hours macerated wines showed a

higher increase in the yellow colour component, with a noticeable shift towards the red space of the diagram, thus suggesting an increased oxidation compared with the other experimental variants, due to the extraction of more oxidizable polyphenols.
To have an overall idea of the changes in colour induced by the maceration, the total colour differences (ΔE) were calculated and included in Table 4, along with the differences of the main CIELab parameters. The total colour difference between control wines (T0) and wines with 6h maceration (T6) samples is ΔE = 1.73 ± 0.14, suggesting that, being higher than 1, but lower than 2, can only the noticeable for observers with trained eyes.

Table 4. Colour differences among the experimental wine samples

	Colour differences			
Variant comparison	ΔL	Δa	Δb	Total colour difference ΔE*
T6-T0	-0.466 ± 0.05	0.228 ± 0.05	1.648 ± 0.15	**1.73 ± 0.14**^{b}
T12-T0	-2.276 ± 0.54	0.289 ± 0.01	3.154 ± 0.81	**3.90 ± 0.98**^{a}
T12-T6	-1.810 ± 0.53	0.517 ± 0.05	1.507 ± 0.95	**2.44 ± 0.97**^{b}

*Average values ± Standard Deviations. Different letters indicate significant differences at $p<0.05$ determined by One-Way ANOVA and Tukey HSD test.

The wine samples resulted through a longer maceration time (T12) compared to control wines (T0) had a total colour difference of ΔE = 3.90 ± 0.98, clearly noticeable by any observer, even inexperienced. Comparing the wines with different maceration times, T6 and T12, a colour difference of ΔE = 2.44 ± 0.97 is obtained, generally meaning that most inexperienced observers would be able to perceive it. Clearly, the difference of total colour is increasing with the time of skin contact.

CONCLUSIONS

The influence of skin contact time on the physico-chemical parameters of must and wine emphasizes that the longer the time of contact, the more noticeable increase is seen in pH and decrease in total acidity, even though the final values cannot be entirely predicted because of other influences such as yeast metabolism and potassium bitartrate precipitation during cold stabilization.
The influence of skin contact time on the polyphenol content evaluated by Total Polyphenol Index. The measurement of TPI showed a statistically significant decrease of polyphenol concentration in the macerated wines, as a result of wines stabilization through the precipitation of some phenolic compounds and proteins extracted through skin contact. As the musts and wines were not treated with bentonite for protein removal, a possible polyphenol-protein interaction and finally spontaneous precipitation could occur and explain the decrease in the total polyphenolic index.
The influence of skin contact time on colour measured by CIELab parameters showed a higher Clarity (L, lightness) in non-macerated wines, while the skin contact time increased this parameter, meaning the samples with maceration were less transparent than the control. The decrease in lightness of 12 hours skin contact wines was statistically significant compared with the other experimental variants. The CIELab parameters a and b showed that the colour of controls with no skin contact (T0) is pale-yellow, while after a maceration of 6 hours (T6) a slightly more intense yellow-green colour resulted, which is desired by most winemakers because it suggests and is usually correlated with a higher mouthfeel. After a 12 hours maceration (T12), a higher value for the yellow component is obtained, with a perceivable pale reddish component. This deeper colour, with less green component than the other variants, suggests the possibly that the higher content of extracted polyphenols was also more prone to oxidation. These long-macerated wines, darker in colour and with enhanced colour saturation (C), are generally not desired by the winemakers, as this incipient oxidation can affect the general perception of wine quality.
The influence of skin contact time on total colour difference. The calculated ΔE clearly shows an influence of the maceration ΔE values on the colour of the resulted wines. There are differences of colour for both variants obtained with various times of maceration (T6, T12) as

compared with the control wines (T0). Especially in the case of wines with longer maceration (T12), the total colour difference ΔE = 3.90 ± 0.98 is easily recognized by any untrained wine consumer, which is not a good thing, as lighter coloured wines are preferred by most consumers. However, the differences in colour between the short-macerated wines (T6) compared to control wines are not so obvious for an inexperienced wine consumer, even though trained professionals would perceive the difference.

Considering the observed and discussed consequences of the skin contact time on the production of white wines, it can be concluded that a short maceration time of 6 hours is better for the wine quality as compared either with no maceration or with a longer maceration of 12 hours.

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AMPELOGRAPHIC AND AGRONOMIC VARIABILITY WITHIN THE 'TĂMÂIOASĂ ROMÂNEASCĂ' CULTIVAR

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Abstract

The 'Tămâioasă românească' variety (syn. Muscat à petits grains blancs) is one of the oldest and most famous varieties for aromatic wines in Romania. This prospective study aimed to evaluate the variability of some morphological, agrobiological and qualitative features of 'Tămâioasă românească' cv. and of the two clones 'Tămâioasă românească' 104 Dg. and 'Tămâioasă românească' 36 Pt., in the specific conditions of the Drăgășani vineyard, Romania. Also, it aimed to identify some ampelographic descriptors useful for the discrimination between the two clones on the one hand and between the clones and the 'Tămâioasă românească' variety on the other hand. Based on the 48 ampelographic and ampelometric descriptors used and analyzed, our partial results show that there is an important phenotypic variability within the population of the 'Tămâioasă românească' variety.

***Key words**: ampelometric descriptors, clones, grapevine, phenotypic variability.*

INTRODUCTION

The genetic diversity of the *Vitis* genus has always been a very valuable source for obtaining new genotypes, useful from a viticultural and oenological point of view, but also an alternative for adapting the varieties to the effects generated by disturbing abiotics and biotics factors from the viticultural ecosystem (Eibach and Töpfer, 2015; Riaz et al., 2018).

The conservation, assessment and use of genetic diversity in grapevines are a topical concern both worldwide (Lacombe et al., 2004; Žulj Mihalević et al.,2013 ; Marković et al., 2017; This et al., 2006) and nationally (Bodea et al., 2009 ; Bucur and Dejeu, 2018; Cichi et al., 2015; Popescu et al., 2017; Stroe, 2016).

Intra-varietal variability in grapevines is a valuable germplasm source, on which clonal selection and breeding of grapevines are based (Hajdu et al., 2011; Mannini, 2000; Oprea & Moldovan, 2007; OIV, 2017).

Various international studies and programs are being conducted to test and validate grapevine phenotyping methodologies, as well as to identify simple, fast, and low-cost methods to identify a large volume of grapevine accessions (Boursiquot et al., 1995; Rustioni et al., 2014; This et al., 2004; Volk, 2010).

Several phenotyping methodologies have been used and tested, including ampelometric characteristics of leaves (Bodor et al., 2013, 2018; Chitwood et al., 2014), bunch and berry morphology (Diago et al., 2013; Ferreira et al., 2018; Grimplet et al., 2019) as well as grape biochemical composition (Bigard et al., 2018; Escudier et al., 2016; Russo et al., 2009).

The grown of grapevines and the production of wine have a very long history and tradition in Romania (Teodorescu, 1964). However, the number of native varieties and the cultivated areas with these varieties are currently low (Antoce and Călugăru, 2017; Cichi et al., 2020). There is a real risk of diminishing the national viticultural germplasm fund, with all the consequences deriving from it in the current context of the challenges posed by climate change (Bucur et al., 2016; Cichi, 2006; Duchene et al., 2010), the limited number of varieties currently used for planting in Romania, the interest of many grape growers oriented towards international varieties, the lack of autochthonous planting material and of a deficient financing regarding the conservation and efficient use of the local genetic resources (Cichi et al., 2019).

The Drăgășani vineyard has a very old viticulture history and tradition in Romania.

According to Teodorescu I.C. (1943) the existence of vine on the territory of the current vineyard is related to the existence of the first plantations made by the Geto-Dacians (1st century BC). It is located in the Muntenia and Oltenia Hills wine-growing region, between the Getic Subcarpathians to the north and the Romanian Plain to the south and south-east, being located between 44°30' and 44°55' parallels north latitude and between 23°55' and 24°15' meridians eastern longitude (Olteanu et. al., 2002).
'Tămâioasă românească' cv. (syn. Muscat à petits grains blancs, Tămâioasa alba românească, Tămâioasa alba de Drăgasani) is one of the oldest and most famous varieties for aromatic wines in Romania, being considered a local variety and a reference variety for the Drăgășani vineyard (as it appears from the synonymy with the variety). The Cotnari vineyard and the Pietroasa viticultural center are also the Romanian traditional cultivation areas of the 'Tămâioasă românească' variety (Constantinescu et al., 1960).
At present there are still controversies regarding the geographical and the genetic origin of this variety (Popescu et al., 2017). About the 'Tămâioasă românească' cv. and the wines produced from this variety in Drăgășani, there are written documents that attest to its cultivation a long time before to Phylloxera (Istocescu et al., 2004). These documents refer to the wine producers, the quality of the wine and the medals obtained by the wines at international and national wine exhibitions and competitions. In this regard, Iorga N. (1925), quoted by Teodorescu (1943), in the paper *History of Romanian Trade* (published in Bucharest, 1925, p.131), mentioned that in 1545 Greek merchants in Wallachia were stopped to sell in large quantities a sweet wine like *Malvazia*, specific to the Drăgășani region, in Sibiu (across the border at that time). Given the specifics of the qualitative potential of the 'Tămâioasă românească' cv. to produce natural semi-sweet and sweet wines, we appreciate that, most likely, the sweet Drăgășani wine mentioned above came from the 'Tămâioasă românească' variety.
Although it is a very old variety in culture in Romania, only four clones were obtained for the 'Tămâioasă românească' variety: 'Tămâioasă românească' 104 Dg. in 1982 at the Research and Development Station for Viticulture and Oenology Dragasani, 'Tămâioasă românească' 36 Pt. in 1982 and 'Tămâioasă românească' 5 Pt. in 1989 (at S.C.D.V.V. Pietroasa) and 'Tămâioasă românească' 24 Cot. in 2009 at S.C. Cotnari S.A.
Various researches carried out at national level regarding the 'Tămâioasă românească' variety focused mainly on the agronomic, technological characteristics and on the quality of the wines obtained from this variety in different cultivation areas (Popescu et al., 2009; Stoica et al., 2008, 2009; Vișan et al., 2014), but also on the SSR markers (Ghețea et al., 2010; Popescu et al., 2017) or several ampelographic characteristics (Giugea et al., 2019; Gorjan, 2012; Rotaru, 2009). Research on the evaluation of the phenotypic traits of the 'Tămâioasa românească' clones is limited (Stroe et al., 2009; Stoica et al., 2017).
In this context, this prospective study aimed to evaluate the variability of some morphological, agrobiological and technological features of 'Tămâioasă românească' cv. and of the two clones 'Tămâioasă românească' 104 Dg. and 'Tămâioasă românească' 36 Pt., in the specific conditions of the Dragasani vineyard. Also, it aimed to identify some ampelographic descriptors useful for the discrimination between the two clones on the one hand and between the clones and the 'Tămâioasă românească' variety on the other hand.

MATERIALS AND METHODS

Plant material. The plants used in the present study were the cultivar of *Vitis vinifera* L. 'Tămâioasă românească', and 'Tămâioasă românească' 104 Dg. and 'Tămâioasă românească' 36 Pt. clones. The vines were 18 years old. Ten vines per cultivar and clones were selected for the study, in three replications. The vines were cultivated under the same growing conditions using rootstock Kober 5 BB, with the 2.0 x 1.2 m spaces, semi-tall shape of the stem (with a trunk of 0.6 m), Double Guyot pruned, 12 bud/m^2, without irrigation.
Location and climatic characteristics. The study was conducted for three consecutive years (2018-2020) in the Drăgășani vineyard,

Drăgășani -Dealul Olt area. The main climatic characteristics during the experimentation period are shown in Table 1. The weather data were obtained from the Drăgășani meteorological Station, located at approximately 3 Km from the experimental site. In terms of heliothermal resources, the studied years were particularly favourable for the grapevine. Although the volume of precipitation was within normal limits in relation to the multiannual values, there is still a semi-arid aspect during the growing season based on De Martonne Aridity Index (Table 1).

Table 1. Main climatic indexes of the experimental site

Climatic Index	Average 2018-2020	Class
Winkler Index	1810	Moderate temperate
Huglin`s heliothermal index (IH)	2525	IH5-Warm
Annual Rainfall (mm)	750	Normal for region
Rainfall in the growing season (mm, April 1st to September 30th)	425	Normal for region
De Martonne Aridity Index (IDM)	33	Slightly- arid
De Martonne Aridity Index growing season (IDM, April 1st to September 30th)	14.53	Semi-arid
Cool nights Index (IF)	13.04	CI_{+1}- Cool night

Ampelographic descriptors. The observations were carried out for three consecutive years. The ampelographic descriptors were recorded in accordance with OIV standardized descriptors and methods (OIV, 2009), at different stages of the growth cycle : 25 for mature leaf (OIV 067- 068, OIV 077-080, OIV 093-094, OIV 601- 617), 2 for inflorescence (OIV 152-153); 7 for bunch (OIV 202-204, OIV 206-209); 8 descriptors for berry (OIV 220-223; OIV225-226; OIV 236, OIV 238), 2 for vegetative growth (OIV 353-354) and 4 for grape yield (OIV 502-503, OIV 505-506).

Sampling measurement and analyses. Ten bunches for each clone/cultivar, 10 berries from the middle part of bunches, in 3 replicates, were used for measurements and analyses of bunch and berry traits, at full maturity. Sugar content (°Brix values) was measured using Kruss Optronic Hand Refractometer Hrot 32. Total acidity of must (g/L H_2SO_4) was determined by the titration method, NaOH 0,1N until pH 7.0. Sugar content and total acidity of must measurements were done in five replicates.

Fertility. The observations were made after flowering, targeting the number of inflorescences/vine (Ni), number of total shoots/vine (Nts), number of fertile shoots/vine (Nfs) and it was expressed by the relative fertility index (Rfi) and absolute fertility index (Afi). The two fertility indices were calculated according to the following formula:

Rfi = Ni/Nts;

Afi = Ni/Nfs.

Statistical analysis. Each variable was examined by analysis of variance (ANOVA). All variables that were significant in the F test were analysed by HSD Tukey`s test to means separation and to establish if there were significant differences among the clones and among the clones and 'Tămâioasă românească' cv.

RESULTS AND DISCUSSIONS

Along with SSR markers, the adequacy of the use of ampelographic descriptors of adult leaves, grapes and berries has been demonstrated as valuable tools in the identification of grapevine varieties and the evaluation of clonal polymorphism (Atak et al., 2014). The leaf is one of the most important vegetative organs used in phenotypic descriptions and morphological identification of grapevine cultivars (Bodor et al., 2013).

Regarding the phenotypic homogeneity of the ampelometric characteristics of the adult leaf in the 'Tămâioasă românească' cv. population, an obvious variability can be recorded. The coefficient of variation had values between 8.65% (OIV 601- lenght of vein N1, 104.91 ±9.08 mm) and 33.15% (OIV 611- lenght of vein N5, 24.05±7.98 mm). An important variability in 'Tămâioasă românească' cv. was also found in terms of the depth of the upper lateral sinuses, CV% = 23.66 for length petiole sinus to upper lateral leaf sinus (OIV 605, 43.79±10.36 mm). Chitwood et al. (2016) mention a variability in distal sinus depth associated with colder, drier climates during the growing season.Lenght of tooth of N2 (OIV 612) and width of tooth of N2 (OIV 613) had a high degree of variation both in 'Tămâioasa românească' cv.

and in the two clones, the coefficient of variation recording values between 20.07% (OIV 613 at 'Tămâioasa românească' 36 Pt.) and 31.84% (OIV 613 at 'Tămâioasa românească' cv.).

The lenght of vein N1 (OIV 601), CV% with values between 7.78% ('Tămâioasa românească' 36 Pt.) and 8.72% ('Tămâioasa românească' 104 Dg.) had the lowest degree of variation in both the 'Tămâioasa românească' variety and the two clones. In Table 2 there are presented only the coded OIV descriptors with numerical evaluation showing the distinct features among 'Tămâioasă românească' cv., 'Tămâioasă românească' 36 Pt. and 'Tămâioasă românească' 104 Dg. clones.

Table 2. Main distinct phenotypical characteristics evaluated by OIV descriptors

OIV Code	'Tămâioasă românească' cv.	'Tămâioasă românească' 36 Pt.	'Tămâioasă românească' 104Dg.
067	4	5	4
078	7/5	7/5	3
093	1	3/5	3
094	7	7	5
152	3	3	3
153	2	2	2
204	7	7	7
208	2	1/2	1/2
209	2	2	2
223	2	2	2
236	2	2	2
601	3	3/5	3
602	3	5	5
603	5	5	5
605	3	3	5
606	3	3	5
607	7	7	5/7
608	3/5	5	5
609	5	7	5
610	3	5	3
611	3	1	3
612	5	5	3
614	3/5	3/5	3
615	3	5	3/5
617	3	5	5

Regarding the fertility, as a valuable trait of grapevine varieties to reproductive performance and capitalize on specific biotope conditions, one can notice a high variability of the relative fertility of the shoots (the ratio between the number of inflorescences/vines and the total number of shoots/vine) in the 'Tămâioasă românească' variety, in which CV% = 28 % . A medium variability of the absolute fertility (Afi) is observed both in 'Tămâioasă românească' cv. as well as in the two clones. This is partly explained by the response of genotypes to the variability of environmental conditions specific to the three years of study. Under the same experimental conditions, the 'Tămâioasă românească' 104 Dg. clone was noted in terms of relative (Rfi) and absolute fertility (Afi), the differences being statistically significant compared to 'Tămâioasă românească' 36 Pt. ($p \leq 0.01$).There are also differences in the length of internodes (OIV 353). 'Tămâioasă românească' 104 Dg. has the longest internodes, the differences being statistically significant compared to 'Tămâioasă românească' and Tămâioasa românească' 36 Pt. ($p \leq .01$).

The production of useful biomass and the efficiency of the use of pedoclimatic resources are important objectives in grapevine breeding programs, but also an important criterion in the choice of plant material by the vine growers. Results of various studies showed a genetic variability and different clonal responses to soil water availability (Tortosa et al., 2020), to use rootstock (Boso et al., 2010), to bud load or pruning system (Feitosa et al., 2018).Both the 'Tămâioasă românească' 104 Dg. clone and the 'Tămâioasă românească' 36 Pt. clone have a shorter length of bunch compared to the 'Tămâioasă românească' cv., the differences being statistically significant ($p\leq .01$). The weight of bunch is also lower in the 'Tămâioasă românească' 104 Dg. and 'Tămâioasă românească' 36 Pt. clones (Table 3), the differences being significant compared to 'Tămâioasă românească' cv. ($p\leq .01$).The biometric characteristics of the grape had an medium variation, except for the weight of berry (OIV 503) at 'Tămâioasă românească' 104 Dg., in which a high degree of variation was noticed (CV% = 28.87). 'Tămâioasă românească' cv. and 'Tămâioasă românească' 36 Pt. have berries larger in length (OIV220) and width (OIV 221) compared to the 'Tămâioasă românească' 104 Dg. clone, the differences between the means being statistically significant ($p \leq .01$). Of the two clones, the highest berries weight was recorded at 'Tămâioasă românească' 36 Pt., the differences being statistically significant

Table 3. Main morphological, agro-biological and quantitative characteristics

Characteristics	U.M.	'Tămâioasă românească' cv.			'Tămâioasă românească' 36Pt			'Tămâioasă românească' 104 Dg.		
		Mean	SD	Min→Max	Mean	SD	Min→Max	Mean	SD	Min→Max
Relative fertility index (Rfi)	ratio	0.75^{ab}	0.21	0.43→1.31	0.67^{b}	0.14	0.40→1.31	0.81^{a}	0.16	0.46→1.23
Absolute fertility index (Afi)	ratio	1.25^{b}	0.25	0.81→1.77	1.13^{b}	0.23	0.73→1.65	1.47^{a}	0.22	0.97→1.81
Length of internodes	cm	8.48^{b}	1.59	6.53→12.6	8.86^{b}	1.49	6.82→12.54	12.14^{a}	1.98	9.21→14.3
OIVnotation	353	3		*1→5*	3		*1→5*	5		*3→7*
Diameter of internodes	mm	8.69^{a}	1.30	6.46→11.47	8.97^{a}	1.23	6.34→12.03	8.94^{a}	1.19	6.98→11.52
OIVnotation	354	3		*3→5*	3		*3→5*	3		*3→5*
Length of bunch (peduncle excluded)	mm	197.52^{a}	21.16	157.46→223.00	170.24^{b}	20.73	129.31→200.27	164.13^{b}	21.71	118.39→207.53
OIVnotation	202	7		*5→7*	5		*3→7*	5		*3→7*
Weight of bunch	g	266.85^{a}	45.68	183.5→359.51	196.10^{b}	49.67	138.89→368.20	184.07^{b}	24.15	138.31→286.20
OIVnotation	502	3		*3*	1/3		*1→3*	1/3		*1→5*
Length of berry	mm	14.14^{a}	1.73	11.45→18.6	14.71^{a}	1.33	12.66-16.76	12.34^{b}	1.5	8.94-15.23
OIVnotation	220	3		*3→5*	3		*3→5*	3		*1→3*
Width of berry	mm	13.82^{a}	1.39	11.06→16.10	14.15^{a}	1.36	11.7→16.24	12.29^{b}	1.43	9.47→14.60
OIVnotation	221	3		*3→5*	3		*3→5*	3		*1→3*
Weight of berry	g	2.15^{a}	0.32	1.24→3.28	2.29^{a}	0.27	1.61→3.25	1.42^{b}	0.41	0.69→2.2
OIVnotation	503	3		3	3		3	3		*1→3*
Length of pedicel	mm	4.32	0.69^{b}	3.16→5.94	4.78^{ab}	0.88	3.21→6.59	4.99^{a}	1.06	3.10→6.51
OIVnotation	238	1/3		1-3	1/3		*1-3*	1/3		*1-3*

Note: Means separation by HSD Tukey`s test at $p \leq 0.05$. Means with the same superscript are not statistically significant

Table 4. Main qualitative characteristics at full maturity (Average 2018-2020)

Cultivars/Clone	Sugar content (° Brix)			Total acidity of must (g/L H_2SO_4)		
	Mean	SD	Min→Max	Mean	SD	Min→Max
'Tămâioasa românească' cv.	21.97^{a}	1.78	19.33→24.56	4.71^{a}	0.52	4.00→5.62
'Tamaioasa romaneasca' 36 Pt.	22.17^{a}	1.75	20.8→24.60	4.74^{a}	0.57	3.72→5.48
'Tamaioasa romaneasca' 104 Dg.	20.24^{b}	1.16	18.98→22.78	4.17^{b}	0.30	3.60→4.77

Note: Means separation by HSD Tukey`s test at $p \leq 0.05$. Means with the same superscript are not statistically significant

compared to 'Tămâioasă românească' 104 Dg. ($p \leq .01$). Similar results regarding the morphological traits of cluster and berries at 'Tămâioasă românească' cv. were reported by Popescu et al. (2015).

The phenotypic expression of different grapevine genotypes in terms of the complexity of the quality of grapes and wines obtained in various climatic contexts of the different viticultural regions, represent important research concerns in various fields (Moutinho-Pereira et al., 2009; Neethling et al. 2012; Neumann et al., 2014).

With a multiannual average over the three years of study of 22.17 ° Brix and an average total acidity content of 4.74 g/L H_2SO_4, the 'Tămâioasă românească' 36 Pt. clone had the highest sugar content in must and the highest acidity, the differences being statistically significant compared to 'Tămâioasă românească' 104 Dg. clones ($p \leq .01$) for both sugar content and acidity content. The 'Tămâioasă românească' 104 Dg. clone also reveals significant negative differences regarding the multiannual average of the sugar content in must ($p \leq .05$) and the average of the content in total acidity ($p \leq .01$) compared to 'Tămâioasă românească' cv. (Table 4).

CONCLUSIONS

Ampelographic and ampelometric descriptors can be useful tools in identifying many discriminating phenotypic characteristics between the grapevine variety and its clones.

Our partial results show that there is an important phenotypic variability within the population of the 'Tămâioasa românească' variety, but also of the two clones.

We consider it necessary to continue the investigation of intra-varietal and intra-vineyard variability in the 'Tămâioasa românească' variety both in the Dragasani vineyard and in other wine-growing areas in Romania, especially in the traditional ones (Cotnari, Pietroasa), in order to identify valuable clonal elites (with agrobiological and oenological performances, physiological and sanitary resistances), possible candidate clones, very useful in the context of current climate change and the growing and diversified demands of vine growers.

The use of polyclonal plantings of 'Tămâioasa românească' in the Dragasani vineyard can be a solution for vine growers, capitalizing in this way the bioproductive potential of the 'Tămâioasa românească' 104 Dg. clone, the high qualitative potential of the 'Tămâioasa românească' 36 Pt. clone and of the 'Tămâioasa românească' variety, thus creating the possibility of obtaining complex wines, with a high degree of typicality and specificity.

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ABUNDANCE AND DIVERSITY OF *AUCHENORRHYNCHA* SPECIES IN VINEYARDS FROM ROMANIA

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Abstract

The Auchenorrhyncha is one of the most abundant and species-rich insect groups present on grapevine. In our study there are presented the results on abundance, dominance, constancy and ecological significance as well as species diversity of Auchenorrhyncha group monitored in 2016-2018 and 2020-2021 in a network of ninety-five vine plots distributed in vineyards from Banat, Crişana and Maramureş Hills (Western Romania) and Moldova Hills (Eastern Romania). The collecting of insects was on yellow double sticky traps from May /June to October every year. Scaphoideus titanus, the vector for quarantine disease Flavescence dorée, was the most abundant species in vineyards from both zones, followed by Erasmoneura vulnerata, Empoasca spp., Neoaliturus fenestratus, Anoplotettix fuscovenosus and Fieberiella florii. Number of species varied between 31 and 49 species. Shannon-Wiener diversity indexes was 1.61 bits for insects in vineyards of Moldova Hills and 2.09 bits for insects in vineyards of Banat, Crişana and Maramureş Hills. Simson's diversity index was 0.68 for insects in vineyards of Moldova Hills and 0.83 for insects in vineyards of Banat, Crişana and Maramureş Hills. Sorensen' similarity coefficient was between 0.79 and 0.94 suggesting that the communities of Auchenorrhyncha in vineyards had a similar species composition.

Key words*: Auchenorrhyncha fauna, leafhoppers, planthoppers, Romanian grapevine.*

INTRODUCTION

The vineyard agroecosystem in Romania covered in 2020 an important area of about 190 thousand hectares (OIV, 2021). It is host of a wide range of insect species belonging to harmful and the useful entomofauna. The *Auchenorrhyncha* (Insecta: Hemiptera: Fulgoromorpha and Cicadomorpha) is one of the most abundant and species-rich pest insect groups associated with grapevine. There are two types of damage related to the presence of *Auchenorrhyncha*, direct damage as a result of their feeding, sting and suck the sap from plant tissues, and indirect damage generated by the ability of some species to act as vector for the economically important pathogens that multiply in the conductive tissues of the plants, like phytoplasma 'Candidatus Phytoplasma' (Ca. P.) (IRPCM 2004) causing devastating diseases known as the yellows of grapevine (Weintraub and Beanland, 2006) and bacteria *Xylella fastidiosa* responsible for the of Pierce's disease (Saponari et al., 2014, Cornara et al., 2016). In addition, the invasive species that have recently appeared in grapevine have considerably increased importance of the *Auchenorrhyncha* group. Therefore, knowing the status of plant and leafhoppers is essential in the economics of grapevine. Many studies have focused on the involvement of *Auchenorrhyncha* species in the epidemiology of phytoplasmic diseases (Mehle et al., 2010, Trivellone et al., 2015, Quaglino et al., 2019, Quiroga et al., 2020). Other studies assessed the diversity of species (Kunz et al., 2010, Saguez et al., 2014, Ramos et al., 2019).
However, plant and leafhoppers species in grapevine have not received an appropriate attention and have not represented themes of many studies in Romania. The studies have mainly focused on the North American

leafhopper *Scaphoideus titanus* Ball, the vector of Flavescence Dorée and were published by the authors of present study.

The aim of present study was to investigate the species belonging to *Auchenorrhyncha* group detected in vineyards located in Western and Eastern Romania, in term of ecological characteristics and diversity of species, in order to provide background knowledge with scientific and practical utility in this field.

MATERIALS AND METHODS

Samplings of plant and leafhoppers were carried out in two periods 2016-2018 and 2020-2021 in a network of ninety-five vineyard plots located in the western and eastern parts of Romania, within the framework of two research projects with financial support from Ministry of Agriculture and Rural Development.

Locations of collection areas during this study are presented on map in Figure 1. Of the ninety-five vineyard plots, eighty plots were surveyed in 2016-2018 and fifteen in 2020-2021, of which forty one in west and fifty-four in east of the country. The plots in the west were located in eleven wine centres from four vineyards, as follows: the wine centre Recaș (4) from Recaș-Tirol vineyard in the Viticulture Region of Banat (Banat Hills); the wine centres Păuliș (2), Miniș (5), Ghioroc (3), Cuvin (1), Covăsânț (3), Măderat (6), Pâncota (5) from Miniș - Măderat vineyard, the wine centres Biharia (1), Diosig (1) from Diosig vineyard from the Viticulture Region of Crișana (Crișana Hills); the wine centre Beltiug (1) from Rătești vineyard in the Viticulture Region of Maramureș (Maramureș Hills).

The plots in the east were located in eleven wine centres in four vineyards from the Viticulture Region of Moldova (Moldova Hills) as follows: the wine centres Odobești (18), Jaristea (2) from Odobești vineyard, the wine centres Panciu (5), Ţifeşti- Sârbi (4) from Panciu vineyard, the wine centres Vârteşcoiu (9), Cârligele (3), Dumbrăveni (2), Beciu (1), Faraoanele (1), Grebănu (2) from Coteşti vineyard, and the wine centre Huși (9) from Huși vineyard.

The plantations were of 3-11 years old, generated through the national program of reconversion and restructuring in Romanian viticulture started since 2014, excepting four plantations investigated in 2020 and 2021 (two in west and two in east of the country). All plots were for commercial production and were composed of national and international cultivars of *Vitis vinifera* L., nine in western and ten in eastern areas. Some of the vineyards showed symptoms typical to yellow diseases associated with phytoplasma. The vineyards were located on the slopes, mainly in the west part, or in the flatland. The plantations belonged to owners in the area and were protected differently with chemical products against pest and diseases. Generally, five to eight treatments for main pathogens and one to two against lepidepteras and mites were applied. None of the *Auchenorrhyncha* species has undergone any insecticides, not even *S. titanus*, which has shown high populations in some plantations. In practice, it is considered that this problem is solved by the insecticides applied against moths. In some plantations, every second interval between the vine rows was permanently covered with a mixture of plants, in others the inter-rows was partially covered or free of weeds that were controlled by tillage and standard herbicides. In the abandoned plots no control has been applied for several years.

The insect sampling was done using double-face sticky traps (Atraceras type), placed in a linear pattern on a row inside the plots. In each plot, four traps in 2016-2018 and six traps in 2020-2021 were used. Traps were changed every two weeks for the first three years and weekly for the last two years from May/June to October.

Adult insects from sticky traps were determined to genus and species level using a stereomicroscope, following the taxonomic keys in Biedermann and Niedringhaus (2009), Kunz et al. (2011) and Wilson et al (2015). Specimens are kept in entomological collection of the laboratory. The resulting data were used for calculating the indices of synecological analysis and estimating the diversity of species per zone and year of sampling.

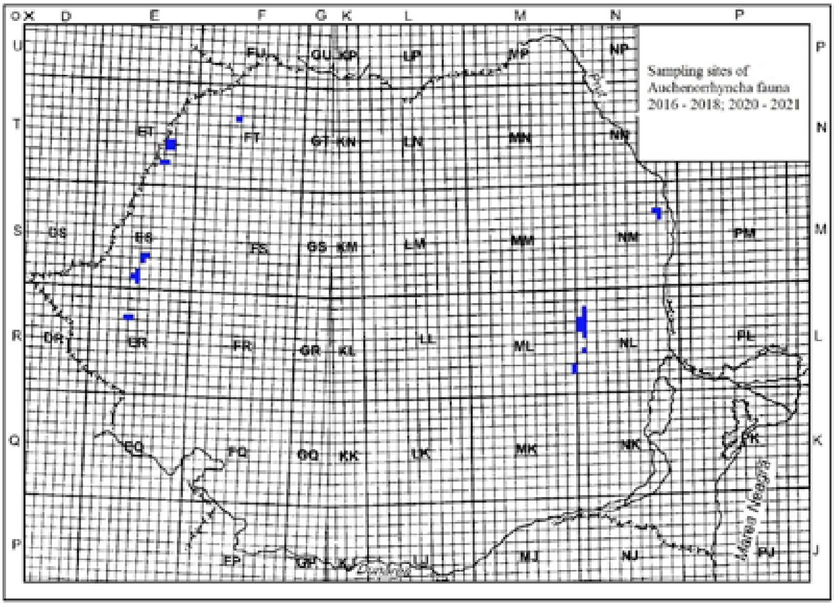

Figure 1. Map of sampling areas in west and east part of the country in periods 2016-2018 and 2020-2021

There were computed the analytic ecological indices, abundance (A), dominance (D%), constancy (C%) and synthetic indices of ecological significance (W%), following specific formulas (Stan, 1994).

D% = (A/n) x 100, where A is number of individuals of a species, n is total number of individuals of all species. Species were grouped as subrecedent (D_1 <1%), recedent (D_2 = 1.1-2%), subdominant (D_3 = 2.1-5%), dominant (D_4=5.1-10%) and eudominant (D_5 > 10%).

C% = (ns/n) x 100, where ns is number of samples with one species, n is total number of samples. Species were grouped as accidental (C_1 = 1-25%), accessory (C_2 = 25.1-50%), constant (C_3 = 50.1-75%) and euconstant (C_4 = 75.1-100%).

W% = (D x C) x 100. Species were grouped as subrecedent (W_1<0.1%), recedent (W_2 = 0.1-1%), subdominant (W_3=1.1-5%), dominant (W_4 = 5.1-10%) and eudominant (W_5 > 10%).

To measure the diversity of sampled species two widely used diversity indices were calculated: Simpson's index of diversity (Gini-Simpson's index) (Magurran, 2004) that take into account the relative abundance of each species, $D = 1- \Sigma p_i^2$ (Pielou, 1969), where p_i is the proportion of abundances belonging to species. It represents the probability that two individuals randomly drown from a sample belong to different species. According to this equation, the value of this index ranges between 0 and 1, the higher the index values, the more complex a biocenosis. Shannon-Wiener index considers both species richness and evenness, $H' = -\sum$ pi ln pi (Magurran, 1988). This ranges from 0 to 1 when all species in a sample have even abundances.

To measure species evenness describing the pattern of relative abundance of species in a community, the equitability derived from Shannon index and the other from Simson's index were assess, J and $E_{1\text{-}D}$ respectively. $J = H'/H_{max} = H'/\ln S$; $E_{1\text{-}D} = (1\text{-}D)/1\text{-}1/S)$, where S is species richness. Values for both indexes range between 0 and 1, high values indicating low variation between species.

To compare the species composition in the communities of *Auchenorrhyncha*, the Sorensen′ coefficient of similarity was calculated, $CC = 2C/(S_1+S_2)$, where C is the number of species common to the two communities, S_1 and S_2 represent the number of species found in community 1 and 2, respectively. Values of this coefficient between 0 and 1, high values indicate that the communities share similar species composition.

RESULTS AND DISCUSSIONS

A total of 229,514 specimens of leafhoppers and planthoppers were sampled in 95 vine plots located in vineyards in the eastern and western Romania (ER, WR) investigated in two periods, from 2016 to 2018 and from 2020 to 2021 (Table 1). Number of specimens differed between zones and years of sampling as well. During sampling periods, plant and leafhoppers collected in the ER grapevines were higher than in the WR grapevines. Of the total insects collected, 76% were recorded in the region Moldova Hills (ER) for 54 vine plots and 24% in the regions Banat, Crisana and Maramures Hills (WR) for 41 vine plots. The difference could be due to the higher number of plots as well as different management strategies of pest, disease and weeds conducted in each plot. The increase in number of insects from year to year was considerable in both regions although the number of sampled plots was equal to or less up to 5 times. In the region Moldova Hills (ER), in the first three-years of sampling, 16 vine plots were sampled each year and the insects collected were more numerous in the third year, 1.6 times than in the first and 1.2 times in the second sampling year. Numerical increase of specimens was also preserved in the second collection period, when for the same number of sampled plots, the number of detected specimens in 2021 was 1.3 times higher than 2020 (Table 1). A similar situation was found in the vineyards in the regions Banat, Crisana and Maramures Hills (WR). In both sampling periods, plant and leafhoppers catches were substantially higher from one year to the next, except in 2018 when slightly fewer insects were caught than in 2017. The highest increase was in the second sampling period, especially in the ER grapevines (Table 1).

Table 1. Number of individuals assigned on sampling vine plots, years 2016-2018 and 2020-2021

	Moldova Hills					Banat-Crisana-Maramures Hills				
	2016	2017	2018	2020	2021	2016	2017	2018	2020	2021
No of vine plot investigated	16	16	16	3	3	10	10	12	5	4
No of samples	128	160	160	69	69	80	90	84	110	88
No of samples (5yrs)	586					452				
No of specimens	9891	14578	16431	57735	75651	3265	8971	8615	14700	19677
Mean of specimens/vine plot	618	911	1027	19245	25217	327	897	718	2940	4919
Total No of specimens (5yrs)	174286					55228				

Our data in this study showed the presence of a consistent number of plant and leafhopper insects on grapevine in Romania. This is accordance with many results in literature presenting this agroecosystem as a suitable host for a large range of species of Auchenorrhyncha (Özgen & Karsavuran, 2009; Kunz et al., 2010; Saguez et al, 2014; Safarova et al. 2018, Ramos et al., 2019; Vizitiu et al, 2022). Overall, the collected specimens by us were assigned to 57 species (including the genus level), 11 families and 21 subfamilies. Per zone, the community of *Auchenorrhyncha* in the ER grapevines had the average of species richness of 56 assigned to 11 families and 20 subfamilies, while in WR grapevines, the average of species richness was 50 assigned to 9 families and 17 subfamilies. The species of plant and leafhoppers identified in the east and west zones are listed in Table 2 together with the values of ecological indices, abundance, dominance, constancy and ecological significance calculated for all specimens cumulated all the five years of sampling.

According to abundance and dominance indices obtained for the insects of each zone, the family Cicadellidae counted the most specimens, representing 98.15% in east and 92.77% in west vineyards. It included the most species, 37 (65% of total species) belonging to 8 subfamilies, Deltocephalinae, Typhlocybinae, Iassinae, Cicadellinae, Eupelicinae, Eurymelinae, Eurymelinae, Aphrodinae and Idiocerinae. Similar results were also reported for *Auchenorrhyncha* species in Italian vineyards (Kunz et al., 2010). Within subfamilies of Cicadellidae, Deltocephalinae was by far the most abundant in both zones, with 77.31% in WR and 64.82% in ER vineyards followed by Typhlocybinae with 33.23% in ER and 14.48% in ER vineyards. Cixidae and Aphrophoridae were the next two most abundant families collected in this study, with 3.58 and 1.96% in WR, and 0.77 and 0.24% in ER vineyards, respectively. The other families were numerically small, represented by only one or two species with few specimens. Of the species collected, the North American leafhopper *Scaphoideus titanus* (Deltocephalinae) was the most in both zones, reaching a level of 50.54% in ER and 28.68% in WR of total abundance of each zone. This result confirms the previous ones in other vineyards in the country (Chireceanu et al, 2011). *S. titanus* is natural vector of FD diseases on grapevine in Europa with high populations widely distributed on the continent (EFSA, 2020). Other species with more than ten percent, classified as eudominant species like *S. titanus*, were: *Erasmoneura vulnerata* (19.18%) and *Empoasca* spp. (11.85%) in ER and *Anoplotettix fuscovenosus* (17.50%), *Neoaliturus fenestratus* (17.22%) and *Empoasca spp.* (14.11%) in WR vineyards. Two dominant species were counted, *N. fenestratus* (9.35%) in ER and *Fieberiella florii* (9.12%) and WR. Only subdominant species was *Reptalus quinquecostatus* with a relative abundance of 3.28% in WR grapevine. As recedent species were *Platimetopius rostratus* for both zones, and *Phylaenus spumaris* in WR and *Arboridia kakogawana* in ER vineyards. The other species (89% in ER and 84% in WR) had low abundances, below 1%.

In samples from WR vineyards, *Fieberiella florii* was the most frequent (euconstant species) accounting for 81.64% of the samples, followed by *Neoaliturus fenestratus* (52.21%) (constant species) and eight accessory species *Reptalus quinquecostatus*, *Stictocephala bisonia, Philaenus spumarius, Scaphoideus titanus, Anoplotettix fuscovenosus Platymetopius rostratus, Allygidius atomarius* and *Empoasca* spp. with frequencies between 29.20 and 48.78%.

In samples from ER vineyards, *S. titanus, Platymetopius rostratus* and *Empoasca* spp. were the most common (between 49.78 and 42.92%) grouped as constant species, followed by *R. quinquecostatus*, *S. bisonia* and *P. spumarius, N. fenestratus* and *E. vulnerata* found between 29.52 and 45.05 of the samples and grouped as accessories species. The presence of a large number of species with low frequency in samples (<25%) was noted, 85.7% in ER and 80% in WR. These were classified as accidental species. Among the species with few individuals in ER vineyards is *Orientus ishidae*, too. This is confirmed as a new natural vector of FD to grapevine (Mehle et al., 210). Its presence on Romanian grapevine is reported for the first time in this study. There were species with low frequency in the samples although they were represented by a high number of individuals, e.g. *A. kakogawana* (2268 individuals) and *F. florii* (1585 individuals) in Moldova Hills and *Allygidius atomarius* and *Marosteles spp* (375 individuals each) in Banat-Crisana-Maramures Hills.

The results on the ecological significance index indicated that the most significant ecologically species were: *S. titanus* (eudominant species), *F. florii, A. fuscovenosus*, *N. fenestratus* and *Empoasca* spp. (dominant species), *R. quinquecostatus* (subdominant species) and *Laodelphax striatellus, S. bisonia, P. spumarius Macrosteles* sp., *P. rostratus, A. atomarius, Japananus hyalinus* (recedent species) for WR vineyards; *S. titanus* (eudominant species), *E. vulnerata* and *Empoasca spp.* (dominant species), *N. fenestratus* (subdominant species), *R. quinquecostatus*, *S. bisonia, P. spumarius, F. florii, Macrosteles* sp., *P. rostratus, Penthimia nigra, Zygina flamigera* and *A. kakogawana* (recedent species) for ER vineyards. The other species (76.78% in ER and 74% in WR) were grouped as subrecedent, their ecological significance index were below 0.1%. This group includes accidental species.

It is worth noting the invasive species *E. vulnerata* and *A. kakogawana* in the vineyards of Moldova Hills in ER with high populations. They were first captured in 2018 (Chireceanu et al., 2020) and in 2021 these reached abundances 25 and 6 times larger. It is also important to consider the consistent proportion of species vectors for pathogens responsible for grapevine phytoplasmoses, *S. titanus*, R. *quinquecostatus, A. fuscovenosus, N. fenestratus, F. florii, P. spumaris* and *Platymetopius rostratus* (Šafářová et al., 2018; Quaglino et al., 2019). In addition, *P. spumaris* is also a very important vector of the bacterium *Xylella fastidiosa* causing the Pierce's disease (Bodino et al., 2021).

Table 2. Values of and ecological indices and significance index of the *Auchenorrhyncha* species collected in Romanian vineyards

Family/Subfamily/Genus/Species	Moldova Hills							Banat-Crisana-Maramures Hills						
	A	D		C		W		A	D		C		W	
		(%)	Class	(%)	Class	(DxC)	Class		(%)	Class	(%)	Class	(DxC)	Class
Cixiidae/Cixinae														
Cixius wagneri (China)	58	0.03	D1	3.75	C1	<0.01	W1	57	0.10	D1	6.42	C1	0.01	W1
Hyalesthes obsoletus (Sing)	148	0.08	D1	11.26	C1	0.01	W1	103	0.19	D1	12.17	C1	0.02	W1
Reptalus quinquecostatus (Löw)	1094	0.63	D1	33.45	C2	0.21	W2	1814	3.28	D3	37.83	C2	1.24	W3
Reptalus cuspidatus (Fieber)	41	0.02	D1	1.54	C1	<0.01	W1	3	0.01	D1	0.66	C1	<0.01	W1
Issidae/Issinae														
Agalmatium bilobum (Fieber)	29	0.02	D1	3.41	C1	<0.01	W1							
Issus coleoptratus (Fabricius)	2	<0.01	D1	0.17	C1	<0.01	W1							
Delphacidae/Criomorphinae														
Laodelphax striatellus (Fallen)	108	0.06	D1	9.56	C1	0.01	W1	323	0.58	D1	21.46	C1	0.13	W2
Delphacidae/Delphacinae														
Javesella pellucida (Fabricius)	69	0.04	D1	5.46	C1	<0.01	W1	32	0.06	D1	4.20	C1	<0.01	W1
Dictyopharidae/Dictyopharinae														
Dictyophara europaea (L)	18	0.01	D1	2.39	C1	<0.01	W1	35	0.06	D1	3.98	C1	<0.01	W1
Membracidae/Smiliinae														
Stictocephala bisonia (K et Y)	518	0.30	D1	44.54	C2	0.13	W2	406	0.74	D1	32.74	C2	0.24	W2
Membracidae/Centrotinae														
Centrotus cornutus (L.)	18	0.01	D1	0.85	C1	<0.01	W1	51	0.09	D1	2.21	C1	<0.01	W1
Aphrophoridae/Aphrophorinae														
Philaenus spumarius (L)	696	0.39	D1	37.88	C2	0.15	W2	1038	1.88	D2	36.95	C2	0.69	W2
Neophilaenus campestris (Fallen)	34	0.02	D1	4.95	C1	<0.01	W1	42	0.08	D1	5.31	C1	<0.01	W1
Aphrophora salicina (Goeze)	4	<0.01	D1	0.68	C1	<0.01	W1	3	0.01	D1	0.66	C1	<0.01	W1
Lepyronia coleoptrata (L)	2	<0.01	D1	0.17	C1	<0.01	W1							
Cicadidae/Cicadettinae														
Cicadetta montana (Scopoli)								7	0.01	D1	1.11	C1	<0.01	W1
Cercopidae/Cercopinae														
Cercopis sanguinolenta (Scopoli)	6	<0.01	D1	0.68	C1	<0.01	W1	19	0.03	D1	0.88	C1	<0.01	W1
Tettigometridae/Tettigometrinae														
Tettigometra macrocephala (Fieber)	6	<0.01	D1	0.51	C1	<0.01	W1							
Tettigometra virescens (Panz.)	2	<0.01	D1	0.34	C1	<0.01	W1	7	0.01	D1	0.88	C1	<0.01	W1
Cicadellidae/Agalliinae														
Anaceratagallia ribauti (Ossiannilsson)	326	0.18	D1	19.62	C1	0.04	W1	161	0.29	D1	11.95	C1	0.03	W1
Cicadellidae/ Deltocephalinae														
Scaphoideus titanus (Ball)	88082	50.54	D5	67.92	C3	34.32	W5	15838	28.68	D5	49.78	C2	14.28	W5
Fieberiella florii (Stal)	1585	0.91	D1	20.14	C1	0.18	W2	5039	9.12	D4	81.64	C4	7.45	W4
Anoplotettix fuscovenosus (Ferrari)	205	0.12	D1	15.02	C1	0.02	W1	9665	17.50	D5	48.23	C2	8.44	W4
Neoaliturus fenestratus (H-S)	16289	9.34	D4	45.05	C2	4.21	W3	9509	17.22	D5	52.21	C3	8.99	W4
Macrosteles sp.	1380	0.79	D1	25.09	C1	0.20	W2	375	0.68	D1	14.16	C1	0.10	W2
Platymetopius rostratus (H-S)	2337	1.34	D2	60.75	C3	0.81	W2	733	1.33	D2	42.92	C2	0.57	W2
Platymetopius major (Kirschbaum)	149	0.08	D1	10.75	C1	0.01	W1	8	0.01	D1	1.77	C1	<0.01	W1
Platymetopius guttatus (Fieber)	5	<0.01	D1	0.68	C1	<0.01	W1							
Psammotettix spp.	199	0.11	D1	8.70	C1	0.01	W1	70	0.13	D1	6.64	C1	0.01	W1
Phlogotettix cyclops (M et R)	357	0.20	D1	19.62	C1	0.04	W1	93	0.17	D1	7.08	C1	0.01	W1
Allygus modestus (Scott)	301	0.17	D1	19.80	C1	0.03	W1	171	0.31	D1	21.24	C1	0.07	W1
Allygus mixtus (Fabricius)	46	0.02	D1	2.73	C1	<0.01	W1	24	0.04	D1	3.98	C1	<0.01	W1
Allygidius atomarius (Fabricius)	405	0.23	D1	20.99	C1	0.05	W1	375	0.68	D1	29.20	C2	0.20	W2
Japananus hyalinus (Osborn)	101	0.06	D1	9.56	C1	0.01	W1	360	0.65	D1	15.49	C1	0.10	W2
Selenocephalus obsoletus (Germar)	89	0.05	D1	4.78	C1	<0.01	W1	214	0.39	D1	11.50	C1	0.04	W1
Deltocephalus pulicarius (Fallen)	157	0.09	D1	6.83	C1	0.01	W1	22	0.04	D1	3.98	C1	<0.01	W1
Penthimia nigra (Goeze)	1098	0.60	D1	17.06	C1	0.11	W2	162	0.29	D1	11.95	C1	0.04	W1
Euscelidius variegatus (Kirschbaum)	191	0.11	D1	4.78	C1	0.01	W1	39	0.07	D1	2.43	C1	<0.01	W1
Orientus ishidae (Mats.)	2	<0.01	D1	0.34	C1	<0.01	W1							
Phlepsius ornatus (Perris)	2	<0.01	D1	0.34	C1	<0.01	W1							
Cicadellidae/Typhlocybinae														
Zygina flammigera (Fourcroy)	1309	0.75	D1	22.87	C1	0.17	W1	101	0.18	D1	5.09	C1	0.01	W1
Zyginella pulchra (Low)	6	<0.01	D1	0.85	C1	<0.01	W1	19	0.03	D1	2.43	C1	<0.01	W1
Erasmoneura vulnerata (Fitch)	33429	19.18	D5	29.52	C2	5.66	W4	11	0.02	D1	1.55	C1	<0.01	W1
Arboridia kakogawana (Matsumura)	2268	1.30	D2	12.46	C1	0.16	W2	32	0.06	D1	5.75	C1	<0.01	W1
Arboridia sp.	160	0.09	D1	6.48	C1	0.01	W1	4	0.01	D1	0.44	C1	<0.01	W1
Empoasca spp.	20651	11.85	D5	52.90	C3	6.27	W4	7793	14.11	D5	45.13	C2	6.37	W4
Eurhadina pulchella (Fallen)	15	0.01	D1	1.71	C1	<0.01	W1	15	0.03	D1	1.99	C1	<0.01	W1
Eupteryx sp.	73	0.04	D1	2.22	C1	<0.01	W1	23	0.04	D1	1.77	C1	<0.01	W1
Cicadellidae /Iassinae														
Iassus lanio (L)	4	<0.01	D1	0.34	C1	<0.01	W1	17	0.03	D1	1.99	C1	<0.01	W1
Cicadellidae/Cicadellinae														
Cicadella viridis (L)	79	0.04	D1	7.68	C1	<0.01	W1	318	0.58	D1	15.93	C1	0.09	W1
Oncopsis flavicollis (L)	2	<0.01	D1	0.34	C1	<0.01	W1	5	0.01	D1	1.11	C1	<0.01	W1
Cicadellidae/Eupelicinae														
Eupelix cuspidate (Fab)	2	<0.01	D1	0.34	C1	<0.01	W1	2	<0.01	D1	0.22	C1	<0.01	W1
Cicadellidae/Eurymelinae														
Macropsis fuscula (Zetterstedt)	54	0.03	D1	6.14	C1	<0.01	W1	23	0.04	D1	4.42	C1	<0.01	W1
Cicadellidae /Aphrodinae														
Aphrodes bicinctus (Schrank)	6	<0.01	D1	0.68	C1	<0.01	W1	6	0.01	D1	1.33	C1	<0.01	W1
Aphrodes makarovi (Curt.)	8	0.01	D1	0.51	C1	<0.01	W1	8	0.01	D1	1.33	C1	<0.01	W1
Cicadellidae/ Idiocerinae														
Metidiocerus rutilans (Kirschbaum)	1	<0.01	D1	0.17	C1	<0.01	W1	1	<0.01	D1	0.22	C1	<0.01	W1
Flatidae/Flatinae														
Metcalfa pruinosa (Say)	60	0.03	D1	5.46	C1	<0.01	W1	52	0.09	D1	3.76	C1	<0.01	W1

The data obtained in this study revealed a community of Auchenorrhyncha on Romanian grapevine with 57 species that gives it a satisfactory degree of stability (Table 3). The ER vineyards showed a species richness, total and per year, slightly higher than those in WR. Out of a total of 57 species identified, forty-nine species were common to both regions.

According to the values of Shannon diversity index (H′) in Table 3, the insect community in the WR vineyards was more diverse than community in the ER vineyards, both in each year and throughout the sampling period, indicating a better uniformity of species abundance in this zone. Value of H' for species collected in WR amounts to 2.47 bits (average 2.09) and for species collected in ER amounts to 2.05 bits (average 1.61). This result is in line with those of Kunz et al., (2010) and Saguez et al. (2014) for *Auchenorrhyncha* species found in Italian and Canadian vineyards, respectively. The lower diversity of species in ER vineyards can be attributed to the high abundance of the leafhopper *Scaphoideus titanus* with percentage of 50.54% out of total abundance of the zone, 1.7 times more than in WR. This together with other two species accounted for more than 81% of the specimens collected from ER zone. During the sampling period, there is a decrease in the value of this index from 2016 to 2021 in both zones.

The Shannon Equitability index (J) applied to the collected species also showed the *Auchenorrhyncha* community in WR with a more equitable distribution of abundances than in ER vineyards. The average value of J equitability was 0.53 (0.44 - 0.72) in WR vineyards and 0.40 (0.32-0.57) in ER vineyards, suggesting a less homogeneous distribution of collected specimens among the registered species over the years.

Referring to Simpson′s Diversity Index (D), this also indicated higher values for the insect's community in WR vineyards as did the Shannon diversity index (Table 3). The decrease of the values of this index from 2016 to 2021 was observed as in Shannon diversity index in both vineyards regions. The equitability E_{1-D} calculated for collected species reached average values of 0.33 (0.22-0.43) in ER and 0.17 (0.11-0.29) in WR vineyards. The uneven distribution of specimens among the recorded species is suggested as well. Lake in annual agriculture systems, diversity of arthropod species in grapevine is under influence of numerous agricultural practices. The ecological imbalance induced by harmful factors, such as pesticides can lead to changes in species richness and the distribution model of dominances, which in turn leads to a decrease in species diversity (Teodorescu & Cogalniceanu, 2005; Beketov et al., 2013). A strong correlation was found between the high pressure of insecticides and herbicides and negative effects on composition and behaviour of leafhopper communities sampled in vineyards in Southern Switzerland (Trivellone et al., 2012). A better plant cover of the soil between the rows can create a favorable microclimate with a good effect for organisms.

Table 3. Shannon-Wiener's and Simpson′s diversity indexes and Equitability indexes of leafhoppers collected in Romanian vineyards (2016-2018; 2020-2021)

	Moldova Hills					Banat-Crisana-MM Hills				
	2016	2017	2018	2020	2021	2016	2017	2018	2020	2021
No of species	37	31	40	38	49	31	33	38	38	43
Total No of species (5yrs)	55					50				
Shannon -Wiener′s Diversity Index (H)	2.05	1.38	1.89	1.35	1.24	2.47	2.20	2.12	1.71	1.65
Shannon-Wiener′s Diversity Index (H) 5 yrs	1.61					2.09				
Shannon′s Max Diversity Index (H_{max})	3.61	3.43	3.69	3.64	3.89	3.43	3.50	3.64	3.64	3.76
Shannon′s Max Diversity Index (H_{max})	4.01					3.91				
Equitability (J)	0.57	0.40	0.51	0.37	0.32	0.72	0.63	0.58	0.47	0.44
Equitability (J) 5 yrs	0.40					0.53				
Simpson′s Diversity Index (D)	0.79	0.58	0.73	0.65	0.59	0.89	0.85	0.84	0.76	0.72
Simpson′s Diversity Index (D) 5 yrs	0.68					0.83				
Equitability (E_{1-D})	0.22	0.43	0.28	0.36	0.42	0.11	0.15	0.16	0.25	0.29
Equitability (E_{1-D}) 5 yrs	0.33					0.17				

The high values of Sorensen′ similarity coefficient between 0.79 and 0.94 suggest that the communities of Auchenorrhyncha in WR and WR vineyards had a similar species composition (Table 4). The value of this coefficient calculated for total specimens collected entire sampling period was 0.92 reflecting the high number of species that are associated both WR and ER vineyards.

Table 4. Sorensen′ coefficient of similarity

		Moldova Hills					
		2016	2017	2018	2020	2021	2016-2021
Banat-Crisana-Maramures Hills	2016	0.91					
	2017		0.94				
	2018			0.85			
	2020				0.79		
	2021					0.89	
	2016-2021						0.92

CONCLUSIONS

The present study provides basic information about the presence, species richness and diversity of the *Auchenorrhyncha* fauna collected in vineyards located in Moldova Hills in Eastern Romania and Banat, Crisana and Maramures Hills in Western Romania in period 2016-2021. Management system of pest, diseases and weeds in vineyards was conventional and different. The data collected in this study showed the presence on grapevine in Romania of a consistent number of species belonging to *Auchenorrhyncha*. The *Auchenorrhyncha* community in vineyards from Moldova Hills displayed the largest number of species with the highest number of individuals. On the other hand, the highest diversity of *Auchenorrhyncha* species was in vineyards from Banat, Crisana and Maramures Hills. The communities of *Auchenorrhyncha* in vineyards had a similar species composition. The North American leafhopper *Scaphoideus titanus* (Deltocephalinae), the vector for quarantine disease *Flavescence dorée* of grapevine, was the most abundant in all vineyards. Other important species, vector or with potential to be vector of grapevine phytoplasmas, were *Reptalus quinquecostatus* and *Neoaliturus fenestratus, Anoplotettix fuscovenosus* and *Fieberiella florii.* Two invasive species, *Erasmoneura vulnerata* and *Arboridia kakogawana* recently recorded in Romania, were found in vineyards in Moldova Hills. *Orientus ishidae*, a new natural vector of FD, is reported for the first time in this study on grapevine in Romania.

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RESEARCH CONCERNING THE ECONOMIC EFFICIENCY OF STIMULANTS AND FOLIAR FERTILIZERS IN TABLE GRAPE VARIETIES

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Abstract

The research was carried out during 2019-2020 growing seasons in the Buzias-Silagiu vineyards. The aim of the study was to establish the efficiency of stimulants, biostimulants and foliar fertilizers, in two table grape varieties. In the experimental plots design with three replications, were studied eight treatments which were compared with the control plot in which was applied the conventional fertilization treatment with N_{80} P_{80} and K_{80}. The main investigation was focused on best inputs for increase the expenditure efficiency; stimulants, bio-stimulants and foliar fertilizers were tested in comparison with the chemical treatments applied in the vineyard in order to decrease the chemicals impact on the grapevine by-products and environment pollution. In the all experimental plots were recorded significant positive results compared with the control plot for grape yield, grape production, market value and income respectively. However, experimental plots (V_4 and V_8) had higher spending compared to the control plot. The most profitable experimental plots were V_7 and V_3 while V_8 provide the highest grape yields and market value. For higher profit and less environment and grape yield pollution, climate, soil and treatments must be carefully correlated in the future.

Key words: *grapes, foliar fertilizers, stimulants, yield, profit.*

INTRODUCTION

Current issues in viticulture like climate change, pollution, lack of labour force or costs, require continuous adjustment of crop technologies to improve mechanization, for complete wood-ripening, for better grapevine adaptation to climate variability, to reduce the number and the amount of chemical treatments, but without influence on the quality of grapevine by-products and economic performance (Dobrei et al., 2015; Vallad and Goodman, 2004). However, it is necessary to use more effective the inputs that represent most of the production costs (Dobrei et al, 2009; Sala and Dobrei, 2015). Depending the environment from each area and the grape variety, the farmers must found viable solutions to preserve the grape yield quality, typicity and the authenticity of wine and grapevine by-products (Nistor et al., 2018a). Vineyards managers have to decrease the useless fertilizers for the vine, which are a financial loss and, in the same time these chemicals are increasing the groundwater pollution and environment degradation (Nistor et al., 2018b).

Therefore, is advisable to decrease the conventional chemical fertilizers amounts, and conventional fertilizers to be replaced with small amounts of natural fertilizers, a also a different and gradual application of fertilizers in correlation with the plant growing stage (Ghiță et al., 2009).

Using stimulants and bio-stimulants can enable the release and better use of macro and microelements in the soil by increasing their accessibility for the vine (Halpern et al., 2015).

Biostimulants enable the regulation / changing of physical processes to increase plant growth and to limit the stress for increased grape yield (Sorrenti et al., 2012).

Biostimulants can be recycled from organic and food waste, composts or other agricultural wastes, providing new methods for avoiding undesirable disposals and for environmental safe solutions (Dobrei et al., 2018).

Seaweed extracts, humic and fulvic acids, microbial inoculants, amino acids, etc., and different microorganisms like rhizobacteria or growth-promoting fungi are biostimulants which have the property to increase crop yields by at least 5-10% and fertilizer efficiency by

5-25% (Adesemoye et al., 2009; Calvo et al., 2014).

However, only few farmers are ready to use new products like biostimulants and to replace conventional fertilizers in agriculture (Salvi et al., 2016). The scepticism about alternative methods for replacing chemical fertilizers relates to the variable efficacy of biostimulants used in the field, in contrast with the results from laboratory tests or application in greenhouses (Gozzo and Faoro, 2013). By applying biostimulants, not only the yield or soil properties are improved, but also the grapevine by-product quality. Salvi et al. (2016) found out that, by applying the natural biostimulants to Sangiovese grape variety was improved the balance between phenolic and technological maturity of grapes, by increasing polyphenols and anthocyanins content and by maintaining the sugar amount in berries.

The aim of the research was to evaluate the effect and influence of biostimulants on growth and yield of two grape varieties Victoria and Muscat Hamburg in two growing seasons field experiment and to assess the economic performance of both varieties.

MATERIALS AND METHODS

The research was carried out during 2019-2020 growing seasons, years with large climate variability, in a full maturity vineyard from the Buzias-Silagiu wine-growing region, Timis County. Two table grape varieties (Victoria and Muscat Hamburg) have been chosen for research because the grape production costs in both varieties are high; vineyards management and fertilization has to be done with major attention. Small amount of fertilizers, biostimulants and stimulants, in addition to conventional chemical fertilization were used in experimental plots. In the field experiment were tested the following biostimulants: V_1 Terra sorb complex; V_2 Terra sorb foliar; V_3 Atonik; V_4 Fertilpolina; V_5 Blak Jak; V_6 Blak Jak + Terra sorb foliar; V_7Atonik + Terra-Sorb Complex; V_8Atonik + Fertilpolina; V_9 Control-normal fertilization $N_{80}P_{80}K_{80}$.

In all experimental plots were observed several economic indicators such as: grape yield, grape production value, grape production costs, cost price, additional costs, production increase and gross profit. Data from experimental field were statistically estimated and correlations between different indicators were calculated by using the GraphPad Prism Vers. 7.04, software.

RESULTS AND DISCUSSIONS

Production costs represent for all vineyards an indicator that suppose continuous improving and has a major influence on grape production and especially on gross profit. In this research, the production costs were similar for both varieties. Data for several economic indicators are presented in Tables 1 and 2. In all experimental plots, by adding various stimulant and biostimulants fertilizers to the conventional fertilization, the grape production costs increased, with limits ranging between 1962.5 euro/hectare for the V_3 and 2251 euro/hectare for V_8. In both years of research, grape production per hectare has not increased proportionnally to production costs. V_4 was among the plots with the highest grape production costs, but without the grape highest production. Fertilizers and biostimulants had major influence both on the cost price (in Euros) per tons of grapes, and especially on the gross profit. In both varieties, the V_7 plot recorded both the lowest production cost (102 Euros/tonne for the Victoria variety and 117 Euros/tonne for the Muscat Hamburg variety) and the highest gross profit per hectare (19,574.5 Euros in the Victoria variety and 13,066 Euros for Muscat Hamburg, respectively).

Concerning the cost price of grape production for both varieties, the only plots that recorded a higher cost than control plot were V_4 and V_8. Therefore, the highest expenses have not been fully balanced by the value of the grape production. For the gross profit obtained, the only plot with a lower profit compared with the control plot was the V_4 plot from the Victoria variety. If only the gross profit is mentioned, the only plot with a lower profit than control was the V_4 plot from the Victoria variety.

In table 3 are presented data concerning the additional costs (Euro/ha), for each plot. Higher extra costs were recorded for V_8 and V_4 plots, of 307.9 and 288.4 Euros/ha respectively, while the lowest extra costs were recorded for V_3 and V_7 plots (19.2 and 40.5 Euro/ha, respectively).

There were no direct correlation between the additional costs and the grape production increase; some of the highest production expenditure did not provide appropriate production increases in both varieties. For example, V_4 plot has one of the highest extra costs, but had the lowest grape production increases for both varieties. On the contrary, the V_3 plot with the lowest additional costs recorded in both varieties had one of the largest grape production increases.

Higher extra costs were recorded in V_8 and V_4 plots of 307.9 and 288.4 Euros/ha respectively, and the lowest extra costs in V_3 and V_7 plots (19.2 and 40.5 Euros/ha, respectively).

Table 1. Production costs, yield, profit and costs in the Victoria variety

Experimental plot	Year	Production costs (Euro/ha)	Yield (kg/ha/year)	Production value (Euro/ha)	Yield costs (Euro/t)	Gross profit (Euro/ha)
V_1 Terra-sorb complex	2019	2188	18361	20491	119	18303
	2020	1762	19362	21414	91	19652
	Mean	1976.5	18862	20952.5	105	18977.5
V_2 Terra-sorb foliar	2019	2198	18356	20485	119	18287
	2020	1772	19316	21363	91	19591
	Mean	1985	18836	20919	105	18939
V_3 Atonik	2019	2175	18598	20755	116	18580
	2020	1750	19498	21565	89	19815
	Mean	1962.5	19048	21160	102	19197.5
V_4 Fertilpolina	2019	2402	18089	20187	132	17785
	2020	2062	19089	21112	108	19150
	Mean	2232	18589	20649.5	120	18417.5
V_5 Blak Jak	2019	2188	17998	20086	121	17898
	2020	1762	18999	21013	92	19251
	Mean	1975	18498	20549.5	106	18574.5
V_6 Blak Jak+ Terra-sorb foliar	2019	2216	18407	20542	120	18326
	2020	1790	19452	21514	92	19724
	Mean	2003	18929	21028	106	19025
V_7Atonik+Terra-Sorb Complex	2019	2196	18861	21049	116	18853
	2020	1771	19952	22067	88	20296
	Mean	1983.5	19407	21558	102	19574.5
V_8Atonik+Fertilpolina	2019	2421	18770	20947	128	18526
	2020	2081	19816	21916	105	19835
	Mean	2251	19293	21431.5	116	19180.5
V_9 Control-Conventional fertilization $N_{80}P_{80}K_{80}$	2019	2156	17907	19984	120	17828
	2020	1730	18861	20860	91	19130
	Mean	1943	18384	20422	106	18476

Table 2. Production costs, yield, profit and costs in Muscat Hamburg variety

Variant	Year	Production costs (Euro/ha)	Yield (kg/ha/year)	Production value (Euro/ha)	Yield costs (Euro/t)	Gross profit (Euro/ha)
V_1 Terra-sorb complex	2019	2188	15226	13582	143	11394
	2020	1762	17089	15107	103	13345
	Mean	1976.5	16157	14344.5	123	12369.5
V_2 Terra-sorb foliar	2019	2198	15271	13622	143	11428
	2020	1772	17135	15147	103	13375
	Mean	1985	16203	14453	123	12401
V_3 Atonik	2019	2175	15498	13824	143	11649
	2020	1750	17407	15388	103	13638
	Mean	1962.5	16452.5	14606	123	12643.5
V_4 Fertilpolina	2019	2402	15135	13500	158	11098
	2020	2062	16998	15026	121	12964
	Mean	2232	16453	14263	139	12031
V_5 Blak Jak	2019	2188	14953	13338	146	11150
	2020	1762	16816	14865	98	13103
	Mean	1975	15885	12102	122	12126
V_6 Blak Jak+ Terra-sorb foliar	2019	2216	15408	13744	146	11528
	2020	1790	17316	15307	103	13517
	Mean	2003	16362	14525	124	12522
V_7Atonik+Terra-Sorb Compex	2019	2196	15952	14229	137	12033
	2020	1771	17954	15871	98	14100
	Mean	1983.5	16953	15050	117	13066
V_8Atonik+Fertilpolina	2019	2421	15862	14149	152	11728
	2020	2081	17863	15791	116	13710
	Mean	2251	16862	14970	134	12719
V_9 Control-Conventional fertilization $N_{80}P_{80}K_{80}$	2019	2156	14635	13054	147	10898
	2020	1730	16407	14504	105	12774
	Mean	1943	15521	13779	126	11836

The experimental plot with the highest grape yield was V_7 with 1022.5 kg/ha for the Victoria variety and 1432 kg/ha for the Muscat Hamburg variety respectively. Analysing the ratio between the extra costs and the value of the grape production increase, the most efficient experimental plots proved to be V_7 and V_3, specifying that the value of the production increase is also influenced by the grapes price on the market (Wezel et al., 2014).

Table 3. Additional costs, production increase and value of production increase, in Victoria variety experimental variants

Variant	Year	Additional costs (Euro/ha)	Production increase (kg/ha)		Value of production increase (Euro)	
			Victoria	Muscat Hamburg	Victoria	Muscat Hamburg
V_1 Terra-sorb complex	2019	31.8	454	591	507	527
	2020	32.6	501	682	554	603
	Media	32.2	477.5	636.5	530.5	566
V_2 Terra-sorb foliar	2019	41.5	449	636	498	567
	2020	42.2	455	728	492	644
	Media	41.8	452	682	495	606
V_3 Atonik	2019	18.9	691	863	771	770
	2020	19.5	637	1000	704.5	884
	Media	19.2	664	931.5	738	827
V_4 Fertilpolina	2019	245.5	182	500	203	446
	2020	331.8	228	591	252	522
	Media	288.4	205	545.5	227.5	484
V_5 Blak Jak	2019	32.1	91	318	101.5	284
	2020	32.3	38	409	42	362
	Media	32.2	64.5	363.5	72	323
V_6 Blak Jak+ Terra-sorb foliar	2019	59.8	500	773	558	690
	2020	60.1	591	909	654	804
	Media	59.9	545.5	841	606	747
V_7 Atonik+Terra-Sorb Compex	2019	40.1	954	1317	1065	1175
	2020	41.0	1091	1547	1207	1368
	Media	40.5	1022.5	1432	1136	1272
V_8 Atonik+ Fertilpolina	2019	264.5	863	1227	963	1094
	2020	351.4	955	1456	1056	1287
	Media	307.9	909	1341.5	1009.5	1191

The highest grape production increase was registered in both Muscat Hamburg and Victoria varieties in V_7 and V_8 experimental plots in both 2019 and 2020 growing seasons.

In Figure 1 can be observed that in all experimental plots, the additional costs are low level, excepting V_4 and V_8, plots which recorded a very high increase of additional costs in both grape varieties and both experimental years.

Value of grape production increased to the highest level, over 1300 Euros, in V_7 Muscat Hamburg variety in both growing seasons. The same trend was observed in Victoria variety.

However, the value of grape production increased similar to the grape yield in both varieties in all plots during field trial.

The lowest grape production was recorded in both Muscat Hamburg and Victoria variety in V_5 plot (in both 2019 and 2020 growing seasons). Accordingly, the lowest value of production was registered by the same experimental plot. Gross profit (Euro/ ha) was quite uniform during field experiments in both grape varieties (Figure 2).

It cannot make the same affirmation about the value of grape production increase, which was very low level in V_4 and V_5 plots, while in V_7 plot was registered the highest level increase, in both grape varieties.

Grape production value was uniform over the years in all experimental plots in both grape varieties, but higher grape production was registered in Victoria variety.

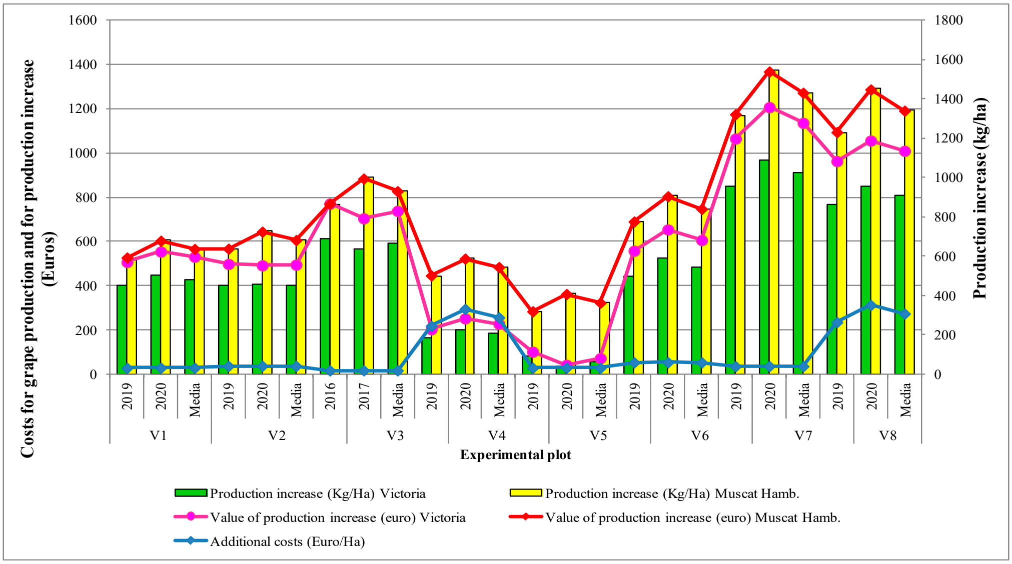

Figure 1. Additional costs, production increase and value of production increase, in experimental variants

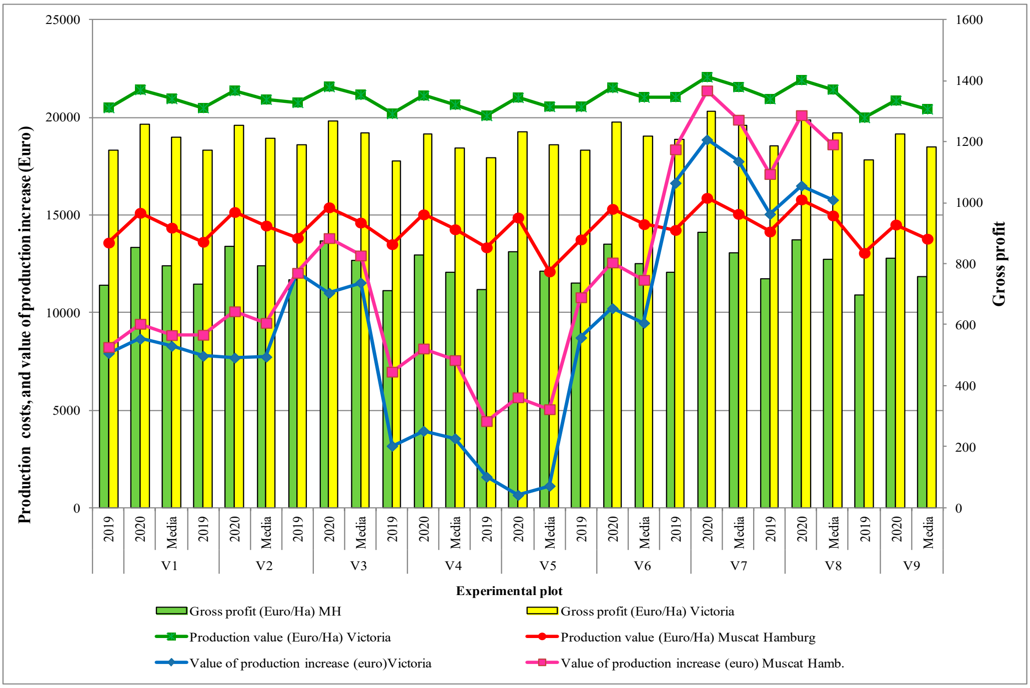

Figure 2. Value of production, value of production increase and gross profit in experimental variants

Colla et al. (2017), reported that after they use three biostimulants for testing on greenhouse tomato, although total production cost increased, but the nutrients status and tomato yield were improved up to a level of net economic benefits. The Pearson correlation (Table 4) between grape yield and gross profit is positive and very significant (r = 0.9809). Between production costs and grape yield selling price there is very strong and positive relationship (r = 0.9494) as well as between production value and grape yield (r = 0.8940) and between production value and gross profit (r = 0.8727). The relationship between grape yield and yield costs is negative (r = -0.8203). Very strong negative correlation there is between yield costs and gross profit (r = -0.9058).

Table 4. Correlation between economic indicators in 2019-2020

Variables	Production costs (Euro/ha)	Yield (kg/ha/year)	Production value (Euro/ha)	Yield costs (Euro/t)	Gross profit (Euro/ha)
Production costs (Euro/ha)	**1**	-0.6212	-0.5051	0.9494	-0.7490
Yield (kg/ha/year)	-0.6212	**1**	0.8940	-0.8203	0.9809
Production value (Euro/ha)	-0.5051	0.8940	**1**	-0.6838	0.8727
Yield costs (Euro/t)	0.9494	-0.8203	-0.6838	**1**	-0.9058
Gross profit (Euro/ha)	-0.7490	0.9809	0.8727	-0.9058	**1**

Table 5. Correlation between additional costs, production increase and value of production increase during 2019-2020

Variables	Additional costs (Euro/ha)	Production increase (kg/ha) Victoria	Production increase (kg/ha) Muscat Hamburg	Value of production increase (Euro) Victoria	Value of production increase (Euro) Muscat Hamburg
Additional costs (Euro/ha)	**1**	0.0650	0.2011	0.0654	0.2000
Production increase (kg/ha) Victoria	0.0650	**1**	0.9705	0.9999	0.9714
Value of production increase (Euro) Muscat Hamburg	0.2011	0.9705	**1**	0.9701	1.0000
Value of production increase (Euro) Victoria	0.0654	0.9999	0.9701	**1**	0.9710
Value of production increase (Euro) Muscat Hamburg	0.2000	0.9714	1.0000	0.9710	**1**

There were no negative correlations between economic indicators (grape production increase, additional costs and the value of production increase) during 2019 and 2020 growing seasons. Very strong positive correlation there was between grape production increases and the value of production in both grape varieties. The small negative correlation was recorded between grape production increase and additional costs (r = 0.0650) and between value of production increase in Victoria variety and additional costs (r = 0.0654). However, additional costs were very low correlated with grape production increase and the value of production for both table grape varieties. According to Mule (2015) research results, the use of bio-fertilizers has many benefits, like: reduced cost of crop cultivation, the net income of farmers increased steadily, saves water, products are sold with higher price, reduce the energy use, reduce pollution and establish higher compatibility between crop and environment.

CONCLUSIONS

On the background of the current viticulture issues outlined above, in order to be able to continue the grape growing in a competitive market, the viticultural holdings have to improve the crop technologies both by reducing the costs and the reasonable use of the inputs.

Therefore, the increasing need to reduce soil and environmental pollution, a modern fertilization concept is required to decrease the overall amount of fertilizers applied, but in the same time to ensure a higher level of fertilizers absorbed by the vines and, to lower the amount of fertilizers trapped in the soil. Low amount of fertilizers, stimulants and biostimulants are viable alternatives that, under reasonable additional costs, provide significant grape production increase and economic performance. These fertilizers are well assimilated by the vine and help to release the fertilizer components from the soil. Besides increasing production and maximizing profits, stimulants and biostimulants reduce soil and groundwater pollution. Among the investigated experimental plots, are noticed the V_7, V_3 and V_2 plots which, in the conditions of reduced additional expenses, ensure high grape production and high profits. There are experimental plots (V_8) that provide high grape yields and values of grape production, even do not provide the highest profit due to high extra costs. For the possibility of selecting the most suitable inputs concerning fertilization, besides the terroir conditions of each vineyard, a number of economic indicators like additional costs, grape production increase, grape production value or gross profit, must be carefully analysed, considering the financial situation of the vineyard, the grape wine by-product market and the selling price.

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FERMENTATIVE PROCESS FOR THE PRODUCTION OF GRAPE MARC ENRICHED YEAST - MICROPILOT LEVEL

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Abstract

Considering current food needs and environmental concerns worldwide, the full use of production and waste reduction is a practice that is increasingly present in our lives. Grape cultivation is one of the most widespread in the world, with more than 77 million tons of grapes harvested only in 2019. Main part of the production enters in the winemaking process. After the process completion, the main waste, grape pomace, is thrown in the field or, in the best case, used in composting processes, losing compounds with significant value. The aim of this paper was to obtain products based on yeast enrich with grape pomace as winemaking by-product (Fetească Neagră variety), rich in polyphenols, and with high antioxidant activity. The grape pomace used for fermentations was obtained following the winemaking processes carried out at the Didactic Research and Development for Viticulture and Pomiculture Pietroasa - Istrița, resort with a history of over 120 years in viticulture. The product thus obtained needs several more tests for the correct evaluation of its superior characteristics in terms of antioxidant properties.

Key words: *grape marc, yeast, fermentations, micro pilot, Pietroasa, Fetească Neagră.*

INTRODUCTION

Globally, grape cultivation has impressive values every year, reaching 73.5 mil. tons in 2017, going up to 80 mil. tons in 2018 and keeping almost the same level, 77.1 mil. tons, in 2019, values that make it one of the most widespread crops worldwide (FAO, 2020).

With current food needs and environmental concerns worldwide, the full use of production and waste reduction is a practice that is increasingly present in our lives.

Pomace is the main residue (a solid material) generated from the pressing of grape. (Munekata et al., 2021). Grapes are one of the most valued crops in the world. The average annual production worldwide exceeds 60 thousand tons. Around 80% of grapes are used for winemaking. (Ferreira and Santos, 2022). Grape pomace is the main residue associated with the antioxidant activity because of the polyphenols content and tannins.

It is well known that fermentation in a solid state is performed for obtaining potential feed additives for animal production. In the case of broiler chicks, the incorporation of fermented grape pomace in animal diets produced heavier animals with increased serum levels of catalase (a component of the antioxidant defense system) (Fang, J.; Cao et al., 2015). Grape pomace represents approximately 10-30 % of the mass of crushed grapes and contains unfermented sugars, alcohol, polyphenols, tannins, pigments, and other valuable products. (Muhlack et al., 2018). Due to the economic and environmental interest, this study aimed to demonstrate that grape pomace can be used as a fermentation substrate due to its high polyphenol content and antioxidant activity, adding value to the production of yeast biomass.

MATERIALS AND METHODS

For the fermentation processes, a yeast strain belonging to the Saccharomyces cerevisiae species was used, previously isolated and identified from Fetească Neagră grapes by specific methods described by Dumitrache et al., 2020.

Biological material

The grape pomace used for fermentations was obtained following the winemaking processes carried out at the Didactic Research and Development for Viticulture and Pomiculture Pietroasa - Istrița, resort with a history of over 120 years in viticulture (Dejeu, 2013).

In the research conducted by Borges and collab. (2020) described that grape pomace drying is considered to be an essential process for grape pomace conservation and stabilization because is susceptible to microbial degradation due to its high moisture content. According to this, the grape pomace of Fetească Neagră variety was dehydrated at 45°C and then ground with a laboratory mill to a granulation that allowed the fermentation in the bioreactor without any problems.

Fermentation at the micro-pilot level goes through the following stages, i.e: (*i*) obtaining static culture (pre-inoculum); (*ii*) obtaining the liquid inoculum; (*iii*) micro-pilot fermentation; (*iv*) post-fermentative processing.

Pre-inoculum preparation

Pre-inoculum was obtained by seeding a maintenance culture (YPG - agarized) on - tubes and incubating for 48 hours at 30°C. After the incubation it was analysed macroscopically to verify its viability and microscopically to determine the degree of development and its purity.

Inoculum preparation

The liquid inoculum was made by seeding the pre-inoculum culture in sterile liquid culture medium based on sucrose, yeast extract and peptone. Each raw material for the culture medium was weighed and dissolved in 400 mL of distilled water, evenly distributed in 4 Erlenmeyer flasks and sterilized at 121°C for 20 minutes. Then, each Erlenmeyer flasks with sterilized medium it was seeded with 2 pre-inoculum inclined tubes. The inoculum thus obtained was incubated for 20-22 hours at a temperature between 28-30°C and at a stirring rate between 170-240 rpm (Figure 1).

The obtained inoculum culture was analysed microscopically to determine its purity and the degree of budding of the yeasts (development stage) (Figure 2).

Figure 1. Aspects of yeast inoculum preparation by shacking in the incubator

Figure 2. Microscopic examination of the purity and budding of the yeast inoculum

Micro-pilot fermentation

For the micro-pilot level, the fermentation processes were performed in single batch system Biostat B PLUS micro fermenter (6 L total volume), at a working volume of 4 - 4.5 L. For micro-pilot fermentations, the used culture medium consisted of: yeast extract, $NH_4H_2PO_4$, KCl, $MgSO_4$, H_2O and sugar. These nutrients have been added to the bioreactor vessel and sterilized with it and its accessories at 121°C for 20 min. After completion of the sterilization process, the vessel with sterile medium was allowed to reach room temperature.

Before inoculating the culture medium with the previously obtained liquid inoculum, the apparatus was brought to the working parameters, respectively temperature of 30°C; stirring with a speed between 250-350 rpm; pH between 4-5.

The inoculation rate used for fermentation was 10-15% (Bărbulescu et al., 2021).

To keep a continuous fermentation, during the process, a sterile solution of 40 % sucrose was added depending on the evolution of the fermentations. To determine the favourable time for the addition of sucrose, samples were taken and the total sugar content was determined using a manual refractometer, model Kruss Handheld Refractometer HR10 (Figure 3).

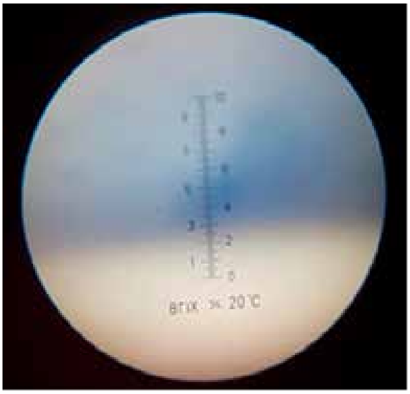

Figure 3. Aspects of the total sugar content reading on Kruss Handheld Refractometer HR10

When the situation required, one or two drops of anti-foam was added to break the foam and prevent entering in the device filters.

During fermentation the pH was maintained at a value between 4 - 5 with ammonia solution.

The grape pomace was added at different stages of fermentation and in different quantities (Figure 4).

Figure 4. Fermentation after 12 hours of cultivation with grape pomace added

Based on some previous tests at laboratory level, 2 methods of adding grape pomace were used: (*i*) a single addition of dry pomace (Table 1) and (*ii*) gradual addition of dry pomace, in several smaller doses (Tables 2 and 3). The total addition of grape pomace for a 4 L working volume of bioreactor was 50 g.

Table 1. Batch Fermentation with a single dose of 50 g of added dry grape pomace

Time of cultivation (h)	pH	Dry matter (%)	Remarks
0	4.8	10.5	
4	4.73	9	
6	4.73	7.5	
8	4.73	5.5	Addition of 50 g dry Fetească Neagră grape pomace
9	4.74	5	
10	4.74	4.5	
11	4.74	4	
12	4.75	3.5	Addition of sterile solution of sucrose
13	4.74	5.5	
14	4.74	5	
15	4.74	4	
17	4.74	3.5	Addition of sterile solution of sucrose
18	4.74	4.5	
19	4.75	4.5	STOP fermentation

Table 2. Batch Fermentation with two doses of 25 g of added dry grape pomace

Time of cultivation (h)	pH	Dry matter (%)	Remarks
0	5.3	10	
4	4.2	6	
6	4.74	4.5	
8	4.74	3.5	Addition of dry Fetească Neagră grape pomace - 25 g;
9	4.74	3	Addition of sterile solution of sucrose
10	4.75	4	Addition of dry Fetească Neagră grape pomace - 25 g;
11	4.75	3	Addition of sterile solution of sucrose
12	4.74	3.8	
13	4.74	3.5	
14	4.74	3	Addition of sterile solution of sucrose
15	4.74	4.5	
17	4.74	4	
18	4.74	3.5	
19	4.74	3.5	STOP fermentation

Table 3. Batch Fermentation with three doses of 16.7 g of added dry grape pomace

Time of cultivation (h)	*pH*	Dry matter (%)	Remarks
0	4.8	10	
4	4.74	8.5	
6	4.74	6.5	
8	4.74	4.5	Addition of dry Fetească Neagră grape pomace - 16.7 g
9	4.74	4	
10	4.74	3	Addition of dry Fetească Neagră grape pomace - 16.7 g
11	4.74	5	Addition of sterile solution of sucrose
12	4.73	4	Addition of dry Fetească Neagră grape pomace - 16.7 g
13	4.74	3.5	Addition of sterile solution of sucrose
14	4.74	5	
15	4.73	3.5	Addition of sterile solution of sucrose
17	4.74	4	
18	4.74	3.5	
19	4.74	3.5	STOP fermentation

Post-fermentative processing

After completion of the fermentation, the fermented medium was left to decant in the cold for 24 h, after which it was subjected to a mechanical separation by centrifugation to obtain the final product: a yeast cream with grape pomace (Figure 5).

Figure 5. Product obtained: yeast cream with added grape pomace

To purify the product, successive washes were performed with sterile distilled water at 3500-4500 rpm, for 5 - 10 minutes at each wash.

The wet cells weight (g/L) was measured after the post-fermentation process at the end of the cultivations (Bărbulescu et al., 2010).

Determination of total polyphenols content (TPC) and antioxidant activity.

The determination of the polyphenols content and the antioxidant activity was performed using the infrastructure from the Research Center for the Study of the Quality of Agri-Food Products within the University of Agronomic Sciences and Veterinary Medicine from Bucharest.

In order to determine the total concentration of polyphenols (TPC), the Folin - Ciocâlteu method used by Stan et al., in 2020, and in terms of antioxidant capacity, the DPPH method (2.2-diphenyl-1-picrylhydrazyl) was used, with subsequent modifications made by Ion et al. in 2020.

RESULTS AND DISCUSSIONS

In order to obtain the final product, previous laboratory experiences were used regarding the isolation, identification of the yeasts, as well as the optimization of the fermentative processes to yeast fermentation capacity (Dumitrache et al., 2020).

Following the experimental batches performed, it can be seen from Figures 6, 7 and Table 4 that the addition in 3 portions of 16.6 g of dry grape pomace to 4L working volume gave the best results, reaching a total of 77 g/L wet cell weight (WCW).

Table 4. WCW results

Batch	**WCW** g / L
Fermentation with a single dose of 50 g of added dry grape pomace	67.75
Fermentation with two doses of 25 g of added dry grape pomace	62.5
Fermentation with three doses of 16.7 g of added dry grape pomace	77

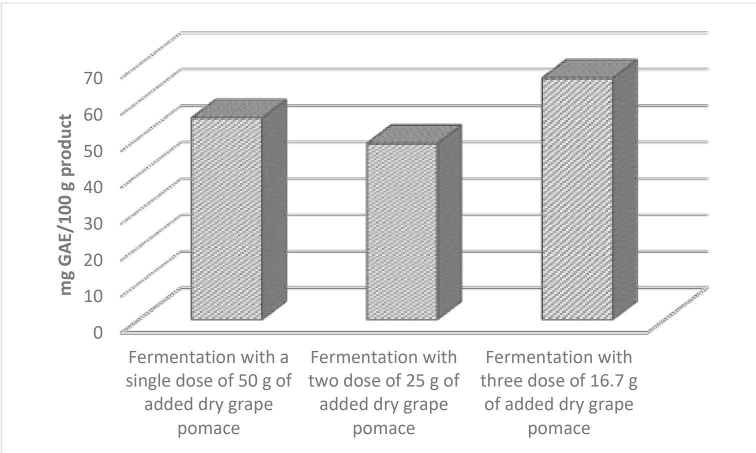

Figure 6. Total phenolic content of different samples of fermented dry grape pomace

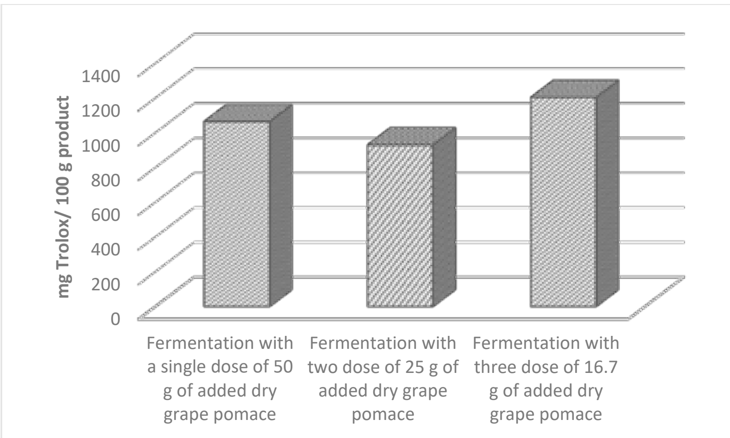

Figure 7. Antioxidant activity of different samples of fermented dry grape pomace

It is observed that the addition of the sugar source together with three portions of dried pomace positively influenced the fermentation improving the cellular concentration.

After the post-fermentation process was obtained 305 g/4 L (77 g/L WCW - wet cell biomass) compared with the addition of the dried pomace in two portions, when the amount of biomass was 250 g/4 L (62.5 g/L - WCW) and respectively in a single portion (271 g/4 L (67.75 g/L WCW). More specifically, fermentation with addition of three dried pomace portions had an increase of 12.78% WCW compared to fermentation with addition of one dried pomace portions, and 20.78% WCW compared to fermentation with additions of two dried pomace portions.

The inhibitory effect was observed in the second experiment (with the addition of pomace in two portions), respectively in the first experiment (with the addition of a single dose of pomace) by the growth rate of the fermentation.

The yield of biomass substrate is positively influenced by the way of addition of grape pomace as substrate source of fermentation.

For the *Saccharomyces cerevisiae,* strain PM1 isolated from Fetească Neagră grape variety and identified by RFLP analysis of 5.8S-ITS region by Dumitrache et al., 2020 were also pointed their capacity for polyphenols biosorption and the higher antioxidant capacity. The different behavior related to biosorption of polyphenols depends on the amount of addition of dried grape pomace and also by the time of cultivation.

The study performed by Rajha et al. (2014) proved that the accelerated solvent extraction (ASE) of phenolic compounds from wet and dried grape pomace, at 45°C, was conducted and the highest phenolic compounds yield (PCY) for wet (16.2 g GAE/100 g DM) and dry (7.28 g GAE/100 g DM) grape pomace extracts were obtained with 70% ethanol/water solvent at 140°C. Compared with this study, our content from our final product, yeast biomass enriched with grape pomace, show a higher content in polyphenols 66.3 mg/100 g dry pomace. In the research study performed by Negro et al. (2021) they investigated four grape pomace which shown different polyphenols and antioxidant activities. In our study content of polyphenols is corelated with antioxidant activity.

CONCLUSIONS

During the trial fermentations performed on laboratory level scale (working volume of 4-4.5 L) can be observed that the gradual addition of dried pomace had a positive effect on the final amount of wet yeast biomass enriched with grape pomace respectively polyphenols.

In the fermentations with a single addition of dry grape pomace, a slowdown of the growth phase is observed immediately after the addition (visible from the slowdown in carbon consumption), which may be due to the fact that the yeast culture needs a period of adaptation to new cultivation conditions. In fermentations with gradual addition of dried pomace, in three portions, no negative effect of the growth phase was observed.

The time of addition was established to be in full process of yeast growth - exponential phase (in the interval 12 hours from the start of fermentation), with a pH range between 3 and 4.5, pH range in which the grape pomace is located.

The methodology for adding the source of dry grape pomace during fermentation was established. The optimal variant for the development of biomass and for obtaining a high concentration of polyphenols and antioxidant activity was in three additional portions (16.6 g dry pomace/4 L working volume with fermentation medium). This interval was chosen so as not to make grape pomace an intruder in fermentation, but to be easily accepted. After a short period of accommodation of the fermentation, carbon source was added. For the fermentation with three additional portions, the grape pomace and carbon additions alternated, keeping between them a shorter period of accommodation than in the case of the other two batch.

The aim in the future is to evaluate different yeast biomass based on total polyphenol content in order to obtain valuable bio-based ingredient with potential to be used as nutritional supplements.

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RESEARCH INTO THE BEHAVIOR OF FRENCH CLONES USED UNDER THE SIMBURESTI VINEYARD

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Abstract

*The study was efectued in the Sâmbureşti vineyard, at S.C. Viti-Pomicola Sâmbureşti S.A. "Valvis Domains" and followed the behavior of vine clones brought from France, varieties recommended in the the CATALOGUE OF VINE VARIETIES ANS CLONES GROWN in FRANCE 2006 a *French Wine and Vine Institute Domaine de l'Espiquette from "Pépinières Duvigneau et Fils". In the study were Sauvignon varieties, clones 159/SO4, 108/SO4, 530/SO4, Cabernet Sauvignon variety, clones 685/SO4, 160/SO4, 341/SO4, Merlot clones 181/SO4, 343/SO4, 1058/SO4. These clones can be used in national and international genetic breeding programs. These clones can modernize and fundamentally develop viticulture in the vineyard and implicitly in Romania, offering prosperity on the human food market.*

Key words*: vineyard, climate, variety, clones, wine.*

INTRODUCTION

On the land of Olt the vine culture is so old that it is lost in the old days' black. The Sâmburești vineyard is located in the north of Olt county, on the left side of the river with the same name, about 60 km from the county municipality of Slatina and 25 km N-V from Drăgăşani. The territory is at the intersection of parallel 44° 48' with the meridian of 24° 24', which shows a position of the most favorable for the vine culture. Located in the transition area between the Getic hills and the long hills, which Carpathians, in relation to large relief units, the vineyard make up half of the Piedmont formed by the first part of the Quaternary, south of the Southern falls in the Getic Plateau more precisely in the Cotmeana Piedmont, located between the valleys of Olt to the west and Argeș to the east (Măcău I., 1994).

MATERIALS AND METHODS

In the study were Sauvignon varieties, clones 159/SO4, 108/SO4, 530/SO4, Cabernet Sauvignon variety, clones 685/ SO4, 160/SO4, 341/SO4, Merlot clones 181/ SO4, 343/SO4, 1058/SO4. The production and number of grapes on hub, the average weight of a grape and the average weight of 100 berries were determined. Analyzes were performed on grape harvesting by the Carl Zeiss hand refractometer method on sugar and by the titrimetric method on acidity (H_2SO_4), and on wine the following analyzes were performed: acquired alcohol, total and volatile acidity(Gheorghiţă M. et al., 2016).

SC Viti-Pomicola Sâmbureşti SA, and the technological study were carried out in the laboratory of the Research and Development Station for Viticulture and Vinification Drăgăşani, between the years 2013-2016. (Popa A, 2008).

RESULTS AND DISCUSSIONS

The climate of the Sâmburesti vineyard is favorable for the cultivation of vines, registering the following climatic data, between the years 2013-2016.

The average annual temperatures were normal for the studied period, with an average annual temperature in 2013 of 12.1°C, 11.7°C in 2014, 12.7°C in 2015 and 12.4°C in 2016.

The precipitations were good from a viticultural point of view during the 4 years analyzed, in the conditions in which in a year it must be 500-800 l/mp. Large amounts of precipitation were recorded in 2014, namely 1156.9 l/mp, in 2013 we have 735.2 l/mp, 705.1 l/mp in 2015 and 683.7 l/mp in 2016.

The geographical amplitude has the average value of 4760 compared to the normal minimum of 4600 required to obtain quality red wines (Măcău I., Condei Ghe., 2010).

Soil types-the Sâmburești vineyard has a great variety of soils, but the dominant ones are the weakly podzolite brown soils and the skeletal regosols.

In general, they are clay soils, with a high clay content, in the horizons AB and Bt_1, medium supplied with humus, nitrogen phosphorus and well supplied with potassium.

They are ferruginous soils, with high Fe_2O_3 content, ranging from 7 to 30 p.p.m. in Bt_3.

They are acidic or moderately acidic soils, with a pH value between 6.20 and 6.50 and decreasing in depth.

On regosol type soils located on the ridge of hills (Bolindețu) at a depth of 90-100 cm, the existence of $CaCO_3$ in the amount of 13.40-33.6% was found.

The acidity of the soils increases the durability of the color of quality red wines, and the CO_3Ca content ensures the specific softness of red wines, competing with French wines from the Bordeaux region (Măcău, 2006).

The French catalog for the Sauvignon, Chardonnay, Cabernet Sauvignon, Merlot, Pinot Noir varieties presents, at 117 clones, the qualitative and quantitative parameters of grape and wine production made in France. Of these, 24 clones representing 20%, achieved very high productions, 75 clones representing 65%, achieved high productions and 18 clones representing 15%, achieved quality records.

The S.C. Viti-Pomicola Sâmbureşti S.A. "Valvis domains" were planted in Câmpu - Mare and Bolindețu 35 clones (29 clones belonging to the French catalog and 6 new clones Cabernet Sauvignon 160, 214, 327 and Merlot 169, 338, 1058 also of French origin).

At Sauvignon - Câmpu - Mare, 4 clones are planted in 5 biosystems. At Chardonnay - Câmpu - Mare, 4 clones are planted in 6 biosystems.

At Cabernet Sauvignon - Sâmburești, 14 clones are planted in 22 biosystems.

At Merlot - Sâmburești, 10 clones are planted in 17 biosystems.

At Pinot Noir- Sâmburești, 3 clones are planted in 3 biosystems.The total number of biosystems is 53.At Sauvignon Câmpu - Mare are planted 3 quantity clones and one quality clone.

At Chardonnay Câmpu - Mare are planted 3 quantity clones and one quality clone. At Cabernet Sauvignon - Sâmburești, 10 quantity clones and 4 quality clones were planted.

At Merlot - Sâmburești are planted 9 quantity clones and one quality clone.

At Pinot Noir - Sâmburești are planted 2 quantity clones and one quality clone Of the 35 clones planted in total, only 8 clones are of quality, representing about 23% (Măcău I. and Gorjan S.Ş., 2016).

Sauvignon varieties, clones 159/SO4, 108/ SO4, 530/SO4; Cabernet Sauvignon variety 685/SO4, 160/SO4, 341/SO4; Merlot clones 181/SO4, 343/SO4, 1058/SO4 were studied, they are very suitable for the eco-pedological conditions of the vineyard,

both quantitatively and qualitatively, offering quality grapes for obtaining wines, high quality white and red. It is observed that these clones are of some value in obtaining high quality white and red wines. The alcohol content obtained from Sauvignon clones is between 12.9 and 13.24

vol%,with a very good alcohol concentration in obtaining DOC and IG type wines.

For red wines we have an alcohol concentration of 12.58-13.02 vol%, for Cabernet Sauvignon clones and 12.02-12.80 vol % for Merlot clones. Therefore, the red wines of the studied clones have a typical alcoholic concentration in obtaining dry and semi-dry wines. The total acidity of these clones is between 3.87-4.60 g/l (H_2SO_4)

In Tables 1 and 2 we present the analyzes for these clones brought from France.

Table 1. Field delimitations of intraclonal selection

Variety	Clone type	Grapes production kg/hub (average)	Number of grapes/ hub (average)	The average weight of a grape (g) (average)	Average weight a 100 grains (gr) (average)
Sauvignon	159/SO_4	0.800	10	80	110
	108/SO_4	0.884	11	80.4	108
	530/SO_4	0.906	11.3	80.2	109
Cabernet Sauvignon	685/SO_4	1.032	13	79.4	98.2
	160/SO_4	1.121	14	80.1	97.8
	341/SO_4	1.037	13	79.8	98.1
Merlot	181/SO_4	1.471	14	108	114
	343/SO_4	1.325	12	115	113.4
	1058/SO_4	1.480	13	113	113.7

Table 2. Physico-chemical analyzes at grape harvesting and wine

Laboratory analyzes	Sauvignon			Cabernet Sauvignon			Merlot		
	159/SO_4	108/SO_4	530/SO_4	685/SO_4	160/SO_4	341/SO_4	181/SO_4	343/SO_4	1058/SO_4
Grapes:									
Sugar (g/l)	232.2	221.3	228.8	225.6	226.6	224.5	220.3	221.2	219.2
Acidity (g/l)	3.07	3.05	3.08	3.26	3.16	3.20	5.20	5.30	5.25
Wines:									
Alcohol, vol %	13.02	12.90	13.24	12.58	13.02	12.74	12.02	12.30	12.80
Total acidity g/l (H_2SO_4)	3.87	4.01	4.08	4.40	4.55	4.60	4.20	4.30	4.27
Volatile acidity g/l (H_2SO_4)	0.32	0.30	0.34	0.32	0.35	0.38	0.30	0.42	0.38

CONCLUSIONS

The clones of the planted varieties have in their structure high quality traits in a percentage of over 23% (according to the French catalog of observations).

They will contribute decisively to increasing the proportion of high quality wines, provided that a modern viticultural agrotechnics is applied (compliance with production load, phytosanitary treatments at warning, fertilization based on soil analysis, harvesting at CMD and quality stages: CSB, CT .

High quality white and red wines can be obtained from these clones in order to obtain DOC and IG type wines.

These clones can be used in national and international genetic breeding programs.

These clones can modernize and fundamentally develop viticulture in the vineyard and implicitly in Romania, offering prosperity on the human food market.

During this period, high quality white and red wines were obtained from these varieties, offering freshness to the Sâmbureşti domains.

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***Catalogue des variéte et clones de vigne cultives en France – 2006.

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THE BEHAVIOUR OF SOME VINE VARIETIES FOR TABLE GRAPES CREATED AT SCDVV IAȘI TO THE MAIN PATHOGENS AND PESTS ATTACK IN THE CONTEXT OF DIFFERENT CLIMATIC CONDITIONS

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Abstract

Climate change more evident in recent years has been cause changes in life cycle of pathogens and pests, as well as has been increase aggression attack on vineyards. In this sense, in 2020-2021 period was followed the behavior of some vine varieties for table grapes newly created at SCDVV Iași, 'Mara', 'Paula' and 'Aromat de Iași' to the attack of the main pathogens (Plasmopara viticola, Uncinula necator, Botrytis cinerea) and pests (Lobesia botrana, Calepitrimerus vitis and Colomerus vitis). The observation of intensity and frequency on the leaves and grape attack highlight a different behaviour of the studied varieties. The results of the study confirm that the temperature and rainfall from the vegetation period influenced the pathogens attack and pests occur, attack degree and the economic damage threshold, as well as the quantitative and qualitative level of grape production. Also, varieties resistance in specific climatic conditions of the analyzed period under the application of anticryptogamic treatments was appreciated with notes on the OIV scale from 7-9.

***Key words**: climate change, pathogens, pests, resistance, table grape.*

INTRODUCTION

Climatic factors, especially temperature, light and precipitation, have a decisive role in the vines growth and development. Over time studies on how climate change will affect viticulture in the future shows that it is producing changes in growing phenophases (Urhausem et al., 2011; Biasi et al., 2019), on the wine quality (Cunha et al., 2016) and implicitly on the vineyards profitability (Ashenfelter & Storchmann, 2010; Deschenes & Kolstad, 2011). Estimates of how climatic conditions will evolve suggest an increase of temperatures and a decrease of rainfall in all regions of the world (Santillán et al., 2018), including our country (Rotaru & Colibaba, 2011; Zaldea et al., 2021). It has been shown that changes in temperature and precipitation can directly affect the pathogens development and survival (Calonnec et al., 2004; Tomoiagă, 2006; Carisse, 2016). For example, heat and drought during the growing season can reduce the incidence of pathogens that require high humidity conditions for the onset of infection (Caffarra et al., 2012).

Another important aspect in the vines cultivation is the diseases and pests control by applying phytosanitary treatments that must be carried out according to climatic conditions (Innerebner et al., 2020). Climate change can affect the biology and populations of parasites, and therefore plant protection is expected to evolve in response to increasing and/or decreasing the development of pests or diseases in vineyards (Salinari et al., 2006). Understanding and anticipating the impact of biotic factors on plants is necessary to assess how diseases and pests may affect agriculture in the future, so that adaptations of cultivation technologies in line with plantation requirements can be suggested to minimize crop losses (Duso & Lilo, 1996; Caubel et al., 2014; Altimira et al., 2019).

MATERIALS AND METHODS

The study consisted in evaluating the behavior of newly created vine varieties at SCDVV Iași, 'Aromat de Iași', 'Paula' and 'Mara' (interspecific hybrid with biological resistance)

(Damian, 2007; Damian et al., 2012), compared to the control variety ‘Chasselas doré’, to the attack of the main pathogens (*Plasmopara viticola, Uncinula necato*r and *Botrytis cinerea*) and pests (*Lobesia botrana, Colomerus vitis* and *Calepitrimerus vitis*) of vines in the context of different climatic conditions, over two years, 2020 and 2021. The tolerance of the vine variety to the pathogens attack was assessed by analyze the severity of symptoms on leaves and grapes in the case of downy mildew and powdery mildew and the analysis of symptoms on grapes in the case of the gray mold attack, expressed as degree of attack, based on the frequency and intensity, according to the OIV standards. The observations were made during the vegetation period of each year, examining a number of 100 stumps, followed by the collection of plant material and microscopic determinations. The resistance of the studied varieties to biotic stress factors was assessed by assigning marks from 1 (very poor) to 9 (very good) according to the OIV scale and establishing the degree of attack. The monitoring of the appearance and evolution of *Lobesia botrana* pest was performed using atraBOT pheromone traps for capturing males, produced at the “Raluca Ripan” Institute for Research in Chemistry, Cluj-Napoca, Romania. The number of captured males was noted at 3-4 days, and the change of rubber capsules impregnated with synthetic sex pheromones was made after about a month of use. The presence and evolution of the population of eriophyid mites from the *Calepitrimerus vitis* species was followed by repeated surveys to highlight the evolution of pests. The first survey consisted of microscopic examination of winter buds and identification of the number of mites present in the buds. For this observation 100 buds were analyzed for each variety studied, harvested from stumps with different positions on the plot. The second survey was conducted according to the evolution of the attack, at 100 leaves per variety. The presence of the population of *Colomerus vitis* mites was monitored by observations of 100 leaves for each variety and the assessment of the attack characteristic symptoms.

RESULTS AND DISCUSSIONS

The thermal regime of the two years of study, according to the data recorded by the weather station AgroExpert corroborated with the data from the Regional Meteorological Center Moldova Iasi, was different, 2020 warmer, with average monthly temperatures during the vegetation period, higher than the normal values and the year 2021, cooler, with lower temperatures in April, May and September, close to normal in June, August and slightly higher in July. The absolute maximum temperatures were 36.2°C in 2020 and 34.5°C in 2021, they did not exceed the multiannual values. The absolute minimum recorded in the air were within normal limits, so that they did not affect the vines (Table 1).

The pluviometric regime was deficient in 2020, being considered a dry year, the precipitations were unevenly distributed and quantitatively reduced. In 2021, the amounts of precipitation recorded were sufficient, in some places higher than normal, only September was more deficient by only 10.4 mm (Table 2). Under these conditions, the rainfall regime during the active vegetation period (April-September) was 408.8 mm compared to the normal 398.1 mm. Air hygroscopicity values were lower than normal in both years of study.

Table 1. The thermal regime during the vegetation period

Month	Average temperatures, °C					Absolute maximum temperatures, °C					Absolute minimum temperatures, °C				
	Normal	2020	Dif.	2021	Dif.	Normal	2020	Dif.	2021	Dif.	Normal	2020	Dif.	2021	Dif.
IV	10.3	11.3	+1.0	8.1	-2.2	29.8	25.8	-4.0	23.1	-6.7	-4.8	-5.0	-0.2	-1.3	+3.5
V	16.3	14.0	-2.3	15.4	-0.9	34.5	29.0	-5.5	28.1	-6.4	-0.6	3.2	+3.8	3.6	+4.2
VI	19.5	20.9	+1.4	19.7	+0.2	37.0	32.7	-4.3	33.3	-3.7	5.5	6.6	+1.1	9.5	+4.0
VII	21.3	22.7	+1.4	23.4	+2.1	42.3	35.4	-6.9	34.5	-7.8	8.5	10.8	+2.3	13.7	+5.2
VIII	20.6	23.5	+2.9	20.9	+0.3	40.7	36.2	-4.5	33.5	-7.2	6.7	12.8	+6.1	12.1	+5.4
IX	15.6	19.6	+4.0	14.7	-0.9	33.4	34.7	+1.3	27.2	-6.2	1.3	7.4	+6.1	4.2	+2.9

Table 2. The rainfall regime during the vegetation period

Month	Rainfall (mm)					Days with rainfall >10		Hygroscopicity %				
	Normal	2020	Dif.	2021	Dif.	2020	2021	Normal	2020	Dif.	2021	Dif.
IV	46.6	8.4	-38.2	53.2	+6.6	0	1	68	35	-33	63	-5
V	61.4	102.2	+40.8	68.6	+7.2	4	1	66	64	-2	64	-2
VI	82.5	108.4	+25.9	93.6	+11.1	4	4	70	67	-3	73	+3
VII	83.8	42.0	-41.8	87.6	+3.8	2	4	71	57	-14	66	-5
VIII	62.7	9.2	-53.5	95.4	+32.7	0	3	70	48	-22	67	-3
IX	61.1	29.8	-31.3	10.4	-50.7	1	-	74	53	-21	65	-9
Total	398.1	300.0	-98.1	408.8	+10.7	11	13	70	54	-16	66	-4

The application of phytosanitary treatments to prevent and control diseases and pests on vines was made according to the climatic conditions and the economic damage threshold of each pathogen and pest studied, using contact and systemic products. In 2021, it was necessary to apply two additional treatments compared to 2020, imposed by the heavier rainfall since the entering in ripening and ripening of the grapes (Table 3).

Table 3. Phytosanitary treatments applied in 2020-2021

Phenological stage	Pathogen or pest agent	2020		2021	
		The product used	Dose (kg, L/ ha)	The product used	Dose (kg, L/ ha)
Sprout 3-5 cm	Powdery mildew Mites	Sulfocalcic gravy	20 L	Sulfocalcic gravy	20 L
Sprout 10-25 cm	Downy mildew Powdery mildew Mites	Folpan Kumulus Envidor	1.5 kg 3.0 kg 0.4 L	Dithane Kumulus	2.5 kg 3.0 kg
Before flowering	Downy mildew Powdery mildew Mites	Profiler Topas	2.5 kg 0.25 L	Zorvec Talendo Vertimec	0.2 L 0.2 L 1.0 L
End of flowering	Downy mildew Powdery mildew	Forum Gold Vivando	1.5 kg 0.2 L	Profiler Topas	2.5 kg 0.25 L
Growing grains	Downy mildew Powdery mildew Grey mold	Ridomil Vivando Cantus	2.5 kg 0.2 L 1.2 kg	Ridomil Sercadis -	2.5 kg 0.15 L -
Compaction of bunche	Downy mildew Powdery mildew Grey mold	Dithane Kumulus Cantus	2.0 kg 3.0 kg 1.2 kg	Delan Pro Kumulus Cantus	4.0 L 3.0 kg 1.2 kg
Entering in ripening	Grey mold	-	-	Cantus	1.2 kg
Ripening	Downy mildew	-	-	Bouillie bordelaise	5.0 kg

In 2020, the downy mildew attack on the leaves registered the highest values for ‘Aromat de Iași’ variety, with an intensity of 23.2% and a frequency of 11.0% which generated a degree of attack of 2.2%. In 2021, the most affected was the control variety ‘Chasselas doré’, with an intensity of 12.5%, a frequency of 15.1% and a degree of attack of 1.9%. On grapes, the most affected by the downy mildew was ‘Aromat de Iași’ in both years of study, the attack being visibly higher in 2021, when the degree of attack was 7.5% compared to 2020, when the degree of attack was 2.7%. At the other part, ‘Mara’ was the least affected by the downy mildew attack. On leaves, the symptoms manifested themselves with a degree of attack of 0.03% in 2020 and 0.2% in 2021. On the grapes, the attack symptoms were not present in 2020, and in 2021 the degree of attack had the 0.2% value. During the two years of study, the presence of *Uncinula necator* was reported only in 2020, at ‘Paula’ variety, where it had quite low values, on the leaves the intensity was 3.4%, the frequency 5.3% and the degree of attack 1.8%, and on grape intensity was 7.5%, frequency 7.9% and degree of attack 0.5%.

The most affected by the *Botrytis cinerea* attack was ‘Aromat de Iași’, in which the attack manifested itself with the values of the degree of attack of 0.3% in 2020 and 3.1% in 2021. The least affected variety by grey mold attack was ‘Mara’, which was attacked only in 2020, with a degree of attack of 0.1% (Table 4). Following the evaluation of the degree of resistance to the main pathogens of the vine, the varieties created at SCDVV Iași distinguish itself with a good and very good resistance, having values close to those of the control variety, with notes on the OIV scale between 7- 9.

Table 4. Manifestation of the attack of the main pathogens of the vine

Variety	Pathogen agent	Leaves						Grapes						OIV score of resistance level	
		2020			2021			2020			2021				
		I%	F%	GA%	I%	F%	GA%	I%	F%	GA%	I%	F%	GA%	2020	2021
Mara	*Plasmopara viticola*	1.35	2.5	0.03	7.9	3.0	0.2	0	0	0	3.7	6.7	0.2	9	9
	Uncinula necator	0	0	0	0	0	0	0	0	0	0	0	0	9	9
	Botrytis cinerea	-	-	-	-	-	-	3.0	5.2	0.1	0	0	0	9	9
Aromat de Iași	*Plasmopara viticola*	23.2	11.0	2.2	3.0	1.7	0.04	16.5	16.4	2.7	7.3	73.7	7.5	8	7/9
	Uncinula necator	0	0	0	0	0	0	0	0	0	0	0	0	9	9
	Botrytis cinerea	-	-	-	-	-	-	4.4	6.8	0.3	5.6	55.6	3.1	9	8
Paula	*Plasmopara viticola*	10.3	9.7	1.0	19.7	7.0	1.3	17.7	12.2	2.1	9.9	28.8	2.8	8	8
	Uncinula necator	3.4	5.3	1.8	0	0	0	7.5	7.9	0.5	0	0	0	8/9	9
	Botrytis cinerea	-	-	-	-	-	-	3.6	5.5	0.2	4.5	11.9	0.5	9	9
Chasselas doré	*Plasmopara viticola*	4.27	5.5	0.2	12.5	15.1	1.9	3.0	4.5	0.1	5.3	7.7	0.4	9	8/9
	Uncinula necator	0	0	0	0	0	0	0	0	0	0	0	0	9	9
	Botrytis cinerea	-	-	-	-	-	-	3.7	3.1	0.1	5.3	4.3	0.2	9	9

In 2020, the first generation flight of *Lobesia botrana* began on April 10, when the sum of the effective temperatures has the value of 27.3ºC. The second generation flight began on June 23, when the sum of the effective temperatures was 434.9ºC. In 2021, the first generation males start flight later compared to the previous year, on May 5, when the sum of the effective temperatures was 49.8ºC. The second generation began its flight on July 2, when the sum of the effective temperatures was 498.3ºC, and the third generation in 2021 began its flight on August 2, when the sum of the effective temperatures was 923.6ºC. From these data it result that an effective temperature of approximately 400ºC is required for a generation. The results presented in Table 5 correspond to the information in the literature according to which the flight of grapevine moths is influenced by high temperatures, which reduce the number of individuals in the crop (Popa, 2012).

By comparing the two years of the study, it is observed that there are significant differences in the moths flight for each generation. In 2020, the first generation flight occured over a period of 39 days, there was a delay in the appearance of adult moths on varieties located on flat ground or those with north-western exposure (‘Paula’ and ‘Mara’). The maximum flight curve for the first generation of moths of 2020 was reached at different times depending on the variety, so for the varieties where the flight started earlier (‘Aromat de Iași’ and ‘Chasselas doré’) the maximum flight curve took place at the end in April and early May, while in the ‘Paula’ and ‘Mara’ varieties it was grown in mid-May (Figure 1). The second generation flight started at the end of June, with a maximum of the flight curve recorded in early July. Comparing the two generations, it is found that during the second flight the number of individuals captured was lower than in the first generation, which confirms the general laws of insect development according to which the population density decreases from the egg stage to adulthood (Ranca, 2001).

Table 5. Biology of *Lobesia botrana* grape moth at SCDVV Iași

Year	Generation	Start fligh	Tm-12°C	Top flight	End of the flight	Male caches average
2020	G1	10 April	27.3	20 April	18 May	59.25
	G2	23 June	434.9	6 June	31 July	35.5
	G3	-	-	-	-	-
2021	G1	5 May	49.8	17 May	9 June	26.0
	G2	2 July	498.3	23 June	2 August	24.5
	G3	2 August	923.6	31 August	22 September	54.5

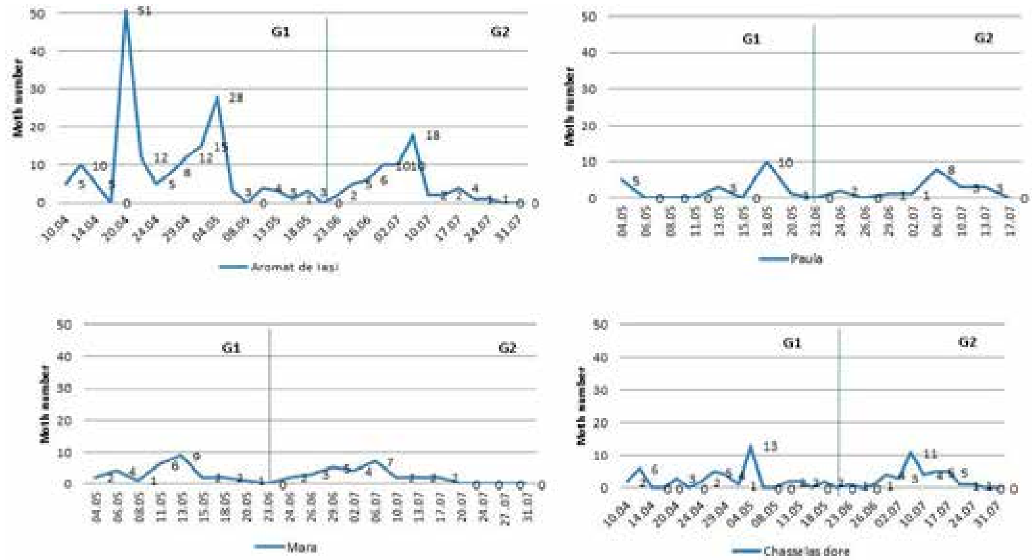

Figure 1. *Lobesia botrana* flight curves in varieties studied in 2020

In 2021, the first generation flight began in early May and occurs over 36 days. The maximum flight curve was recorded in mid-May for all varieties studied (Figure 2). The second generation of european grapevine moth began its flight in early July, and as in the previous year, there was a decrease in the number of individuals caught during the second flight. Due to the favorable climatic conditions, high temperatures and high biological reserves of previous years, in 2021 the flight of the third generation of moths occur, which intersected with the second generation, the adults continuing their flight from August to mid-September for 51 days. Due to the emergence of the third generation, in 2021 there were a higher number of moths in culture, resulting a stronger attack of grey mold on all varieties studied compared to the previous year.

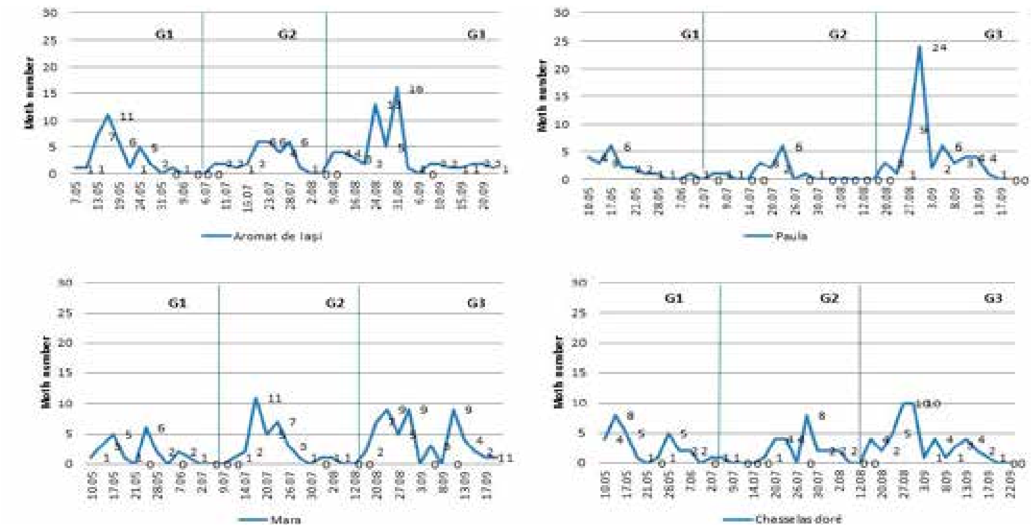

Figure 2. *Lobesia botrana* flight curves in varieties studied in 2021

The largest number of moths was caught in 'Aromat de Iași', a variety sensitive to pest attack, in both years of study, followed by 'Paula' and 'Mara', in which the number of individuals was close to the control. With the exception of 'Aromat de Iași', for all the varieties analized, the number of individuals captured was higher in 2021, compared to 2020, due to the appearance of the third generation (Figure 3).

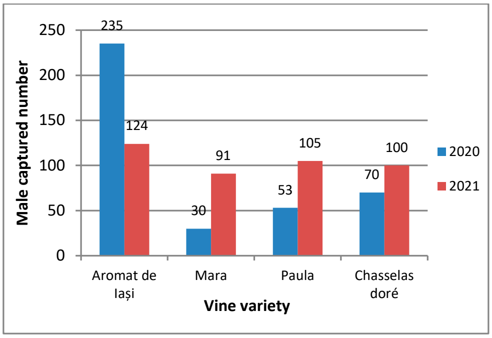

Figure 3. Cumulative male trap catches of *Lobesia botrana* during 2020-2021 at SCDVV Iași

The biological reserve of mites remained below the economic damage threshold in both years of study, for all varieties analyzed. For *Calepitrimerus vitis* species, on the first survey, the most affected variety was 'Chasselas doré', in its buds were 129 individuals in 2020, and 178 individuals in 2021. At the opposite pole is the 'Mara' variety, in the buds of which no pests of the species *Calepitrimerus vitis* have been found in any of the years studied. In the 'Paula' variety, 34 individuals were found in 2020 and 113 in 2021, and in 'Aromat de Iași', following the first survey, 28 individuals were identified in the first year and 49 mites in the second year (Table 6).

The second survey of leaf symptoms caused by vine rust mite of grapevine shows that the most affected was the 'Chasselas doré' variety, which in 2020 had 24% of leaves with symptoms of attack, and in 2021, 21% leaves with attack symptoms. The least affected variety was 'Mara', in which the attack in 2020 had a percentage of 9% attacked leaves, and in 2021 there were 10% attacked leaves. The 'Paula' and 'Aromat de Iași' varieties obtained relatively low values at the leaf attack of the pest, between 12 and 20%.
Grape erineum mite (*Colomerus vitis*) in 2020 manifested itself with greater intensity in the 'Aromat de Iași' variety, where it had a value of 22% symptoms on the leaves. The lowest value was recorded by the 'Mara' variety, with leaf attack symptoms of only 9%, and 'Paula' and 'Chasselas doré' varieties had a value of 19%. In 2021, the most affected variety of mite attack from the *Colomerus vitis* was 'Chasselas doré', with a percentage of 25%, followed by 'Aromat de Iași', which obtained the value of 24%, 'Paula' with 21% and the least affected by the gall mites attack was 'Mara' , with 11%. symptoms. By comparing the two years, the eriophyes mites attack was stronger in 2021 compared to the previous year, and symptoms of the attack were also identified on the inflorescences.
The results regarding the production capacity of the studied varieties showed that in the climatic conditions of 2020 and 2021 and the proper application of the cultivation technology, they reached the biological potential specific to the mass varieties. Among the varieties analyzed, the most productive was 'Paula', with 6.40 kg / hub, respectively 6.85 kg/hub, and from the point of view of marketed production, the resistant 'Mara' variety stood out (Table 7).

Table 6. Eriophydes mites attack in 2020-2021

Variety	Total buds/ leaves analized	*Calepitrimerus vitis*						*Colomerus vitis*	
		No. mites/ sample		No. mites/bud		Leaves with attack symptoms (%)		Leaves with attack symptoms (%)	
		2020	2021	2020	2021	2020	2021	2020	2021
Aromat de Iași	100	28	49	0	0	16	18	22	24
Paula	100	34	113	0	1	12	20	19	21
Mara	100	0	0	0	0	9	10	9	11
Chasselas doré	100	129	178	1	2	24	21	19	25

Table 7. The production components of the varieties studied

Variety	The average weight of a grape, g		Effective production, kg/stump		Calculated production, t/ha		Marketed production, %	
	2020	2021	2020	2021	2020	2021	2020	2021
Aromat de Iași	200	170	5.40	5.78	20.41	21.85	62	60
Paula	310	298	6.40	6.85	24.19	25.89	79	80
Mara	175	256	4.30	6.14	16.25	23.27	94	95
Chasselas doré	135	154	3.24	4.31	12.27	16.32	89	95

CONCLUSSIONS

From the analysis of the climatic data recorded in 2020-2021 it can be seen the 2020 year was drier, the rainfall regime recorded during the vegetation period was deficient, with unevenly distributed rainfall, while the 2021 year was a normal meteorological one. This has resulted in the cryptogamic occur diseases whose development is dependent on humidity with higher intensity, frequency and degree of attack in 2021, compared to the previous year.

Among the analyzed varieties, 'Mara' was distinguished by its resistance to the pathogens attack, at the opposite pole being the 'Aromat de Iași' variety, which was the most affected by the attack of vine diseases.

Another consequence of the temperature differences between the two years was the need to apply two phytosanitary treatments to control grey mold and downy mildew in 2021, compared to 2020, which were stronger due to the conditions optimal development during the growing season.

Regarding the grapevine pests, the treatments applied in culture for protection against mites from *Calepitrimerus vitis* and *Colomerus vitis* species have proved their effectiveness, the number of populations being below the economic damage threshold both in 2020 and in 2021.

There are differences in the development of *Lobesia botrana* populations from one year to another and from one variety to another, depending on the recorded temperatures and the altitude or location of the plantation. Thus, for the varieties with north-western exposure ('Mara', 'Paula') there was a delay in the beginning of the flight for each generation compared to the control variety. The delay in the start of the flight can also be associated with temperature differences, so in the year with higher temperatures the flight started much earlier compared to 2021, when the rainfall was more frequent and the temperatures lower.

The most receptive variety to the *Lobesia botrana* attack was 'Aromat de Iași', and at the opposite pole, the least affected by the pest attack was the 'Mara' variety.

Under the conditions of application of anticryptogamic treatments to the studied varieties, grape productions were specific to their biological potential, the differences from one year to another being insignificant, which shows that they have acquired genetic stability and have the adaptability to the conditions of the viticultural ecosystem where they were created.

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ANALYSES OF THE INFLUENCE OF CROP LOAD ON BIOLOGICAL AND PRODUCTIVE CHARACTERISTICS OF SOME TABLE GRAPE VARIETIES GROWN IN THE SEVERIN VINEYARD

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Abstract

The paper presents the results regarding the influence of the regulation of inflorescences on the vegetative phases of the Cardinal, Alphonse Lavalleé, Afuz-Ali and Victoria varieties. In parallel, the influence on some technological properties was studied, such as: the production of grapes, extra quality class, and first quality, second quality of grapes, carbohydrate content and acidity. The study was carried out over a period of three years, within the Severin vineyard, in the Severin - Dealul viilor wine center. The results showed that the number of inflorescences on the stem influences the moment of the initiation of the vegetative phases but also the productive properties; the crop load has been identified and ensures optimal results for each studied variety.

Key words: *grape, quality, vegetative phases, crop load.*

INTRODUCTION

Studies conducted on climate change in recent years have shown favourable ecological conditions for cultivating table or wine grape varieties but also the need to adapt viticulture technologies to new ecological challenges (Buzatu & Mărăcineanu, 2015; Mărăcineanu et al., 2020, 2021). Therefore the conclusion of a recent study (Chevet et al., 2011) was that the unfavourable influence of climate on the yield decreased over time due to the technological improvement.

The most favourable conditions for the cultivation of table grape varieties are in Romania, in the wine centers located in the south of the country, because the light and temperature resources are at a high level. This favours the creation of competitive varieties consisting of varieties with early, medium and late maturation, so that the market can be supplied for a long period of time. However, in the last 30 years, in Romania, the production of table grapes has decreased by 63% and the cultivated area has decreased by 73% while consumption is increasing after 2010 and is supplied by imports (Cichi et al., 2019). Another current issue refers to the insufficient promotion and cultivation of the newly created varieties, with proven superior productive features (Stroe, 2016).

Facing ecological and economic challenges, studies in recent years have focused on how the climate and the applied technology influence the vegetation phases and the quality of production in order to ensure the profitability of this crop.

A study conducted in France, over a long period of time, showed that the warm years ensure precocity in flowering, ripening and maturation, while the cold years extend the growing season. Also, after 1985, the trend of advancing the data for the onset of vegetation phases was noticed (Chevet et al., 2011).

The study was also confirmed in terms of the influence of extreme weather conditions on production by other researchers (Fontes et al., 2016).

The author also demonstrated the existence of climate variability within the vineyards, which requires a local climate assessment. Other researchers (Ruml et al., 2013; Jiang et al., 2020) show that temperature has higher influence on the onset of vegetation phases than the genetic factor and the difference between varieties increases in the years with extreme climate. There are also differences between clones, the longest period of time between flowering and ripening being of 16 days between early and late clones (Houten et al., 2020). The inconsistency between the onset of phenophases for the same variety has also been reported (Reis et al., 2020). In Turkey, current climatic conditions lead to an increase in vegetative growth and the amount of wood removed during pruning (Demirkeser & Kamiloglu, 2020), while a solution to improve the carbohydrate/acidity ratio would be to set up plantations at a higher altitude.

The plantation maintenance system also proved to have an influence on the vegetation phases. Thus, full maturity for some wine grape varieties was recorded one or two days earlier, in the case of organic viticulture, compared to conventional viticulture (Stroe et al., 2020).

For table grape varieties, the summer pruning is important, as it ensures an increase in the quality of the grape harvest. A remarkable review of these works, with the description of the effects and the way of working was made by Lorenzo et al., 2011. However, the application of summer pruning remains an expensive technology because it is done manually. Good results were recorded for the Cardinal variety in the shoots thinning but also in the inflorescences thinning and shortening (Costea D.C. et al., 2017). Studies on the King Ruby variety have shown that it reacts well to shortening internodes, simultaneously with defoliation and ringing (Belal et al., 2016).

The influence of defoliation was also monitored for wine grape varieties and its effects are influenced by the characteristics of the variety and the time of execution. Thus, defoliation performed before flowering ensured the increase of grape berries skin thickness, maintaining the acidity of the stum and improving the taste qualities, leading to a better resistance to thermal stress but to a lower production (Poni & Bernizzoni, 2010).

The improvement of the grapes taste qualities, by defoliation at the beginning of ripening and its repetition after 15 days, was also confirmed by Pessenti et al., 2020. On the other hand, defoliation carried out 2-3 weeks after the end of flowering is able to reduce the attack of diseases but reduces the production by decreasing the weight of the grapes. In this case, the effects of defoliation are reduced if the variety has a long period of vegetation and high vigour (Nicolosi et al., 2012). In terms of crop load, it influences both the yield and the biological characteristics of the varieties. Basically, the high crop load leads to an increase in production but also to a decrease in the fertility of the respective variety and the weight of the grapes. The leaf area is larger in this case and is correlated with improved pollination (Lorenzo & Pisciotta, 2019). On the other hand, the low crop load favours the fertility of the varieties and the growth of the grapes (Abo-Elwafa, 2018). According to the author, it ensures a higher growth of leaves, shoots and chlorophyll content when distributed on shorter canes.

Studies carried out in Romania on Victoria and Coarnă neagră varieties have reached similar results. Thus, the high crop load is correlated with the decrease of the varieties fertility, with the decrease of the grape weight, the increase of the acidity and the grape yield and the decrease of the carbohydrate content (Popescu C., 2012).

MATERIALS AND METHODS

The study was located in a private plantation in the Severin vineyard and was carried out during of three years. The influence of the crop load, expressed by the number of inflorescences per plant, on the vegetation phases and the recording of the effect on the quantity and quality of production was observed. Four table grape varieties (Cardinal, Alphonse Lavalleé, Afuz-Ali and Victoria) and 6, 8, 10, 12 and 14 inflorescences per plant were used. For each variant, the date of ripening, the date of full maturation, the carbohydrate content, the acidity content and the grape production were noted. The organoleptic properties of the production were evaluated through scoring by a tasting committee composed of three specialists. The appearance, consistency, colour, aroma and taste were evaluated.

RESULTS AND DISCUSSIONS

From a climatic point of view, the average of the last 20 years indicates: the average annual temperature - 12.8ºC, the average temperature in July - 24.8ºC, the annual amount of precipitation - 693 l/m^2, the annual insolation -

2335 hours. In this context, the oenoclimate aptitude index (IAOe) was calculated in order to establish the favourability of the vineyard from this point of view. The high value of this indicator (5241) shows a favourable area for obtaining high quality white and red wines but also for cultivating table grapes varieties, with early, medium and late maturation.

The phenological observations made on the four varieties showed that the date of the onset of the vegetation phases depends on the climatic conditions. For example, in 2016, the colder climate prolonged the vegetation period, compared to the following year, when lower rainfall and higher temperature led to an advance of vegetation (Table 1). Considering the De Martonne aridity index, the climate in the first year is characterized as humid (32.6) and the following year as semi-humid (20.3).

Table 1. The data on the vegetation phases

Variety	year	Number of inflorescences/plant									
		6		8		10		12		14	
		BBCH 81	BBCH 89	BBCH 81	BBCH 89	BBCH 81	BBCH 89	BBCH 81	BBCH 89	BBCH 81	BBCH 89
		Number of day in the year									
Cardinal	2016	197	210	197	214	197	214	197	216	197	216
	2017	195	209	196	213	196	213	196	213	196	216
	2018	198	207	193	213	196	213	196	216	196	216
	average	194	209	195	213	196	213	196	215	196	216
Victoria	2016	202	222	204	223	207	223	210	228	214	233
	2017	204	220	205	224	207	227	210	233	213	236
	2018	204	221	203	222	206	222	208	233	210	232
	average	203	221	203	223	207	224	209	231	212	234
Alphonse Lavalée	2016	225	243	227	250	228	254	228	255	233	264
	2017	224	242	224	246	227	251	227	253	229	263
	2018	226	244	226	246	227	253	227	254	230	261
	average	225	244	226	247	227	253	227	254	231	263
Afuz-Ali	2016	226	243	228	245	233	249	231	254	233	258
	2017	227	248	232	253	232	253	232	258	232	258
	2018	227	248	232	251	232	253	232	255	232	255
	average	227	247	231	250	232	252	232	256	232	257

Regarding the influence of the number of inflorescences per plant, on the passage of the vegetation phases, there is a delay in the onset of the ripening and full maturity phases as the number of inflorescences per plant increases.

The vegetation phases we are referring to started earlier, regardless of the existing climatic conditions, in the case of the variant with 6 inflorescences per plant and started the latest in the case of the variant with 14 inflorescences (Figure 1).

The recorded differences ranged between 2 days (Cardinal variety) and 9 days (Victoria variety), the average being 5.5 days for the ripening phase. For the maturity phase of the grapes, the same influence was observed on the number of inflorescences per plant, with the difference that the delay was more accentuated. Thus, the differences ranged from 7 days (Cardinal variety) to 19 days (Alphonse Lavaleé variety). The average value of the varieties is 12.2 days.

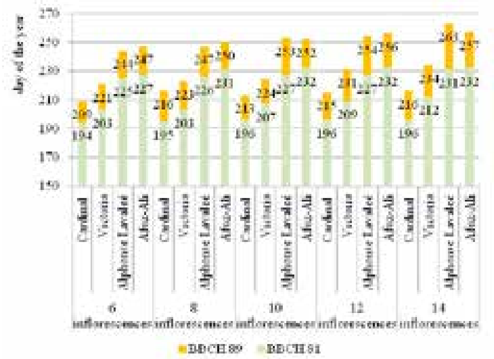

Figure 1. The date of the vegetation phases onset (average values)

For all four varieties, the tendency to delay the BBCH 81 and BBCH 89 vegetation phases was highlighted as the number of inflorescences per plant increased (Figure 2). The correlation is positive and the values of the correlation and determination coefficient have high values, the dependence between the two variables being close.

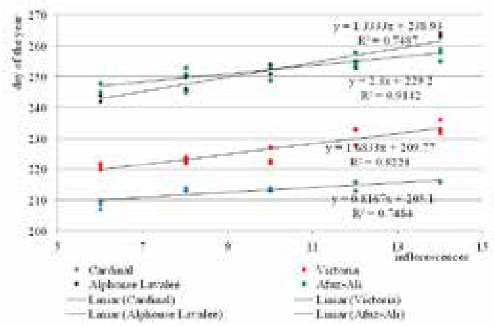

Figure 2. The correlations between the number of inflorescences per plant and the date of phenophases onset

Regarding the grape maturation period, this delay does not create problems in this area, because the Severin vineyard is located in the southern part of the country, where the special climatic conditions also ensure the maturation of the late varieties.

In terms of quantity of production, its increase was observed simultaneously with the increase of the number of inflorescences (Figure 3).

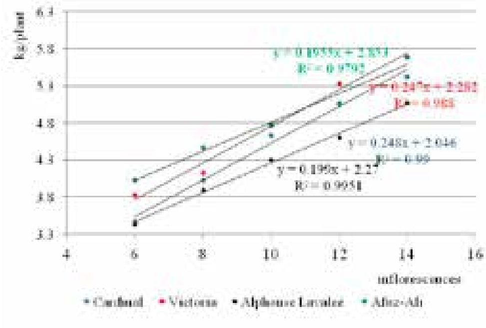

Figure 3. The correlations between the number of inflorescences per plant and the yield rate

Also in this case, the correlation is positive, the correlation and the determination coefficient recording high values, which indicates a close dependence between the two variables. The difference was between 1.64 kg/stem, in the Alphonse Lavalleé variety, and 1.96 kg/stem, in the Cardinal variety, and it was in favour of the variants with 14 inflorescences per stem.

Acidity followed the same trend (Figure 4). It has increased as the number of inflorescences per plant has increased, so it has evolved in the same direction as the production obtained on the stem and in the opposite direction, if we relate to the amount of accumulated carbohydrates (Figure 5).

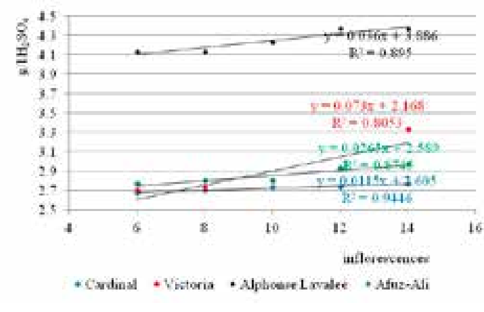

Figure 4. The correlations between the number of inflorescences per plant and acidity

In this case we find a decrease in the amount of carbohydrates that accumulate simultaneously with the increase in the number of inflorescences per plant. The correlation is negative for all four varieties and has similar values, according to the determined coefficients. The lowest difference, of only 9 g, is recorded by the Cardinal variety and the highest, of 30 g, by the Victoria variety. Also this variety recorded the highest difference in acidity (0.63 g/l H_2SO_4) between the variant with 14 inflorescences and the one with 6 inflorescences/plant and the lowest value was recorded by the Cardinal variety.

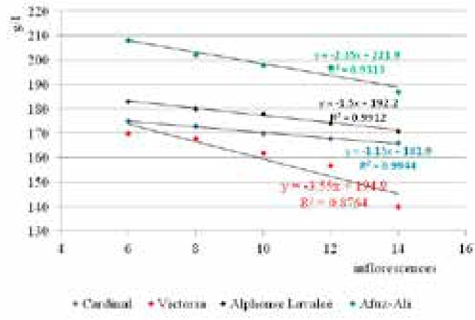

Figure 5. The correlations between the number of inflorescences per plant and the amount of accumulated carbohydrates

In this respect, Victoria is the variety that reacts the most to the regulation of inflorescences, which is why we consider that we must pay attention to the crop load to find the optimal value depending on the area and the applied technology. For the table grape varieties, an important quality parameter is the maturation index, which must have a value that ensures the freshness and fruitfulness characteristic. The recommended value is 2.5-4.5. The values for the studied varieties of this indicator are presented in Table 2.

Table 2. The evolution of the gluco-acidimetric index depending on the number of inflorescences per plant

Variety / No. of inflorescences	Cardinal	Victoria	Alphonse Lavaleé	Afuz-Ali
6	6.6	6.3	4.4	7.5
8	6.4	6.2	4.4	7.2
10	6.2	5.8	4.2	7.1
12	6.2	5.4	4.0	6.7
14	6.0	4.2	3.9	6.3

From its analysis, it can be concluded that Alphonse Lavaleé is the variety that maintains an optimal value of the gluco-acidimetric index regardless of how the number of inflorescences per stem evolves. This can be explained by the greater ability to maintain acidity in the conditions of the Severin vineyard. For the other varieties, the gluco-acidimetric index

decreases as the number of inflorescences per plant increases. In many cases it remains above the optimal range. This means high levels of accumulated carbohydrates, due to the lower acidity, specific to the southern areas.
However, the taste characteristic of table grapes is not the only quality parameter; when released for consumption, these varieties must meet other characteristics which may prevail in the purchasing decision. Therefore, the grapes were sensorial evaluated taking into account the appearance, consistency, colour, aroma and taste through the scoring scale method (Figure 6).

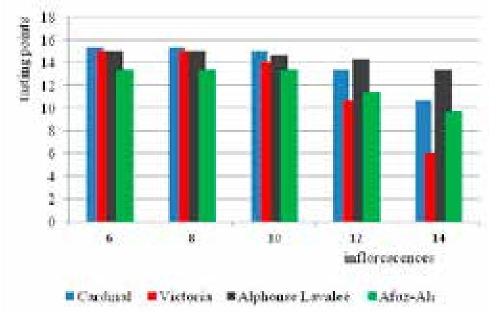

Figure 6. The evolution of tasting notes in relation to the number of inflorescences per plant

Taking these aspects into account, the analysis of the tasting sheets showed that the organoleptic properties of the grapes decrease as the number of inflorescences per plant increases. The most pronounced decline is in the Victoria variety, while the least affected is the Alphonse Lavaleé variety. The score for each analysed characteristics is shown in Figure 7 for the two varieties.
We note that in the case of a small number of inflorescences per plant, both varieties are characterized by maximum organoleptic characteristics. The alteration of the organoleptic qualities is manifested in the consistency of the grape and the taste, for the Victoria variety, starting with a crop load of 10 inflorescences per plant. Starting with 12 inflorescences per plant, the organoleptic characteristics are further diminished, and affect all parameters.
An impairment of organoleptic properties for the Alphonse Lavaleé variety was observed, starting with 12 inflorescences per plant, but at a lower level compared to the Victoria variety.

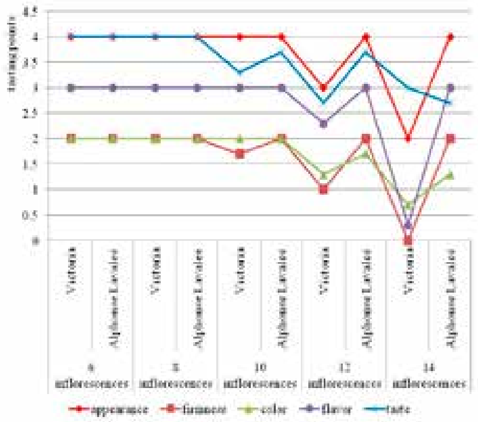

Figure 7. The evolution of organoleptic properties in Victoria and Alphonse Lavaleé varieties

One consequence of this development is the fact that, as organoleptic properties deteriorate, the production in the extra class and quality class I decreases and the production in the quality class II increases.

CONCLUSIONS

The study showed that the start date of the vegetation phases is influenced by the number of inflorescences per stem; the higher their number, the higher the delay.
From a productive point of view, the large number of inflorescences per stem is correlated with the increase in grape production, the increase in acidity and the decrease in carbohydrate content. For this reason, an optimal value has been identified for each variety. For example, a maximum of 10 inflorescences per stem for the Victoria variety is recommended in this area; for the others, higher loads can be used, but not maximum of 14 inflorescences per plant.

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STUDY OF THE BEHAVIOR OF THE CENTENIAL SEEDLESS 48MF CLONE IN THE CONDITIONS OF THE MURFATLAR VINEYARD

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Abstract

A study was performed on the behavior of the 48/9/6 clonal elite selected from a population of the Centennial seedless variety at Research Station for Viticulture Murfatlar, in the period 2016-2020. During the 5 years of observations and determinations, the clonal elite 48/9/6 was noticed which, following the testing at ISTIS (State Institute for Testing and Registration of Varieties), was approved under the name of Centennial seedless 48 Mf in 2021.
From climartic point of view, the research period is characterized by average annual temperatures 2-4°C higher and less rains (267.9 mm compared to normal 436 mm).
The agrobiological properties, the technological and agroeconomic characteristics were compared to those of the population. The clonal elite is distinguished by a shorter vegetation period (full maturity occurs in the first half of September) and higher fertility. The productivity of the clone (the average weight of a grape bunch) is 527 g compared to 423 g in the population.

Key words: *clonal elites, grapevine, parthenocarpy, seedless grapes, table grapes.*

INTRODUCTION

The ecoclimate of the Murfatlar vineyard is characterized by a relatively short period of vegetation, during which a large amount of global radiation and a large heliothermal reserve accumulate, which represent favorable conditions for the growth and development of the grapevine.
The grape breeding achievements obtained thus far, such as clones extracted from populations of older varieties, natural mutants and newly created varieties with superior traits, have ensured increased grape production, of superior and relatively constant quality from one year to another (Căzăceanu et al., 1982).
In viticulture, research to improve and modernize the structure of assortments must be prospective, based on a careful estimate of socio-economic developments in general, as well as the progress of science and technical and material possibilities. As a method of vegetative grapevine breeding, mass selection does not create anything completely new, the main objective being restoring the initial state for older varieties, which by accumulating invaluable forms and poorly productive genotypes due to repeated vegetative propagation, have been eroded (Oprea & Moldovan, 2007).
In ampelographic collections, in addition to the conservation of genetic resources, the aim is to carry out detailed research to describe existing genotypes and prospective elites in the population. Their properties make them suitable for testing in order to obtain valuable clones, after which they may be tested and approved by ISTIS, so they may be multiplied and viticultural plantations can be established. One of promising variety from this collection is Centenial seedless variety.
The parthenocarpy of the Centennial seedless variety is of the "Sultanin type" or stenospermocarpy. Apparently, fertilization proceeds normally: the oosphere is fertilized, the zygote is formed, but the secondary nucleus degenerates just before fertilization and no longer forms albumen, which leads, after a few weeks, to ovary abortion. As a result, the seeds remain dry, with a soft skin (Irimia, 2012).

MATERIALS AND METHODS

The Murfatlar research team is undertaking studies and research for the creation of varieties and the identification of clonal elites in the table grape population of the Centennial seedless variety, which, along good quality and productivity, also possess resistance properties to environmental conditions, diseases and pests, which eliminate or require only a minimum of phytosanitary treatments, considerably reducing pollution in the crop ecosystem and in the finished products.

At Research Station for Viticulture Murfatlar, in the period 2016-2020, the selection activity was carried out on several biotypes choosed from a population of the Centennial seedless variety, a grape variety for the table, aiming to establish the botanical, agrobiological, technological and agroeconomic characteristics.

The choice of elite vines was made in two stages: in the first stage, field observations were made regarding the vigor, the phytosanitary condition, some morphological components and the production capacity; in the second stage, determinations were made regarding the quantity and quality of grapes, the size of the bunches, the number of berries per bunch and the quantity of must obtained.

Following the observations, trunks were marked, from which the elite were chosen to meet the selection criteria chosen by breeders: consistency in production and quality, as well as resistance to diseases and pests.

In the selection process within the Centennial seedless table grape population, observations and determinations were made regarding the morphological, agrobiological and technological descriptors, based on which the ampelographic description sheet was elaborated.

In order to evaluate the authenticity of the genotypes maintained in the ampelographic collection from Murfatlar, the following methodology was applied: within the table grape variety population, 10 vines of the same vigor but with some superior characteristics to those specific to the population of the variety were marked, called elites and numbered (vine number on the row/row number/interval number). From these, 5 vines with traits superior to the other elites were chosen and studied in the following year.

RESULTS AND DISCUSSIONS

Grapevine fertility represents the ability of the plant to form fruiting organs every year as the initial basis for the grape harvest and can be appreciated under two aspects: potential and actual fertility. In the climatic conditions of the last year of observations, the fertility values recorded for each elite were close to normal or even higher than the population (Table 1).

Table 1. Observations and determinations in the population of the variety

Clonal elites/ population	No. of buds/ trunk	Dead buds/ trunk	Total shoots/trunk	Fertile shoots	Sterile shoots	Inflorescence primordia/trunk	Fertility %
Clonal elite 48/9/6	38	13	25	18	7	22	72
Clonal elite 48/3/2	30	11	19	12	7	12	63
Clonal elite 42/7/3	32	14	18	10	8	10	55
Clonal elite 46/5/4	38	15	19	10	9	10	53
Clonal elite 40/1/6	31	7	24	10	14	11	42
Average	**33**	**12**	**21**	**12**	**9**	**13**	**57**
Centennial seedless population	30	12	18	10	8	8	40-50

The total amount of annual and multiannual wood that is removed after pruning is based on the planned production, the load of fruit on the trunk and the growth vigor of each elite in the previous year. The elite that has greater annual and multiannual wood quantities than the population is 48/9/6 (Table 2).

Table 2. The amount of annual and multiannual wood removed after pruning

Clonal elite/ population	Annual wood amount g/trunk	Multi-annual wood amount g/trunk	Total amount of wood g/trunk
Clonal elite 48/9/6	917	543	1,4600
Clonal elite 48/3/2	882	487	1,3690
Clonal elite 42/7/3	890	509	1,3990
Clonal elite 46/5/4	931	524	1,4550
Clonal elite 40/1/6	865	502	1,3670
Average	**897**	**513**	**1,4100**
Centennial seedless population	815	436	1,2510

All elites have vigorous shoot growth in relation to the population, with an average of 58.6 cm for clone 48/9/6 (Table 3).

Table 3. Vegetative growth measurements*

Clonal elite/ population	Total shoots/ trunk	Shoot length cm		Average shoot length/trunk, cm
		Min.	Max.	
Clonal elite 48/9/6	25	28	104,5	58,6
Clonal elite 48/3/2	19	12	36	17,6
Clonal elite 42/7/3	18	12	67,5	37,6
Clonal elite 46/5/4	19	28	92,5	56,6
Clonal elite 40/1/6	24	13	60	33,4
Average	**21**	**18,6**	**72,1**	**40,76**
Centennial seedless population	18	10	70,6	40,3

* When the grapes enter veraison, shoot growth stops.

Analyzing the data in Table 4, an increase in thermal balances: global, active and useful, can be seen in comparison to the multiannual average. The sum of real insolation during the growing season (1520 hours) is lower than the multi-annual average (1708.4 hours).

A decreasing trend when comparing values from the recent years to the multi-annual values can also be seen in the case of annual rainfall (450.2 mm compared to 559.7 mm), growing season rainfall (264.7 mm compared to 367.6 mm).

When comparing the average values from the 5 studied years to the multiannual values, an increase can be observed for the night cooling index (14.5, compared to 12.8), the Huglin heliothermal index (3976.9 in comparison with the average of 3130.1) and the length of the vegetation period (204 days compared to 192.3 days).

Due to climate change, the number of days with maximum temperatures above 30°C has also increased: 82 days, compared to the multiannual average of 51 days (Table 4).

Table 4. Climatic indicators for the Murfatlar Wine Center

Analyzed climatic elements	**Multi-Anual averasge**	**2016**	**2017**	**2018**	**2019**	**2020**
Global thermal balance, Σt° g, °C	5203,5	5.757,8	5303,8	5.379,6	5534,4	4999,6
Active thermal balance, Σt° g, °C	4689,8	5.216,1	4826,9	4.815,5	5061,8	4500,5
Useful thermal balance, Σt° g, °C	2538,7	2.676,1	2515	2.427,2	2811,8	2270,5
Absolute minimum air temperature, °C	-15,6	-15,0	**-15**	-12,8	-10,5	-13,9
Average annual temperature °C	12,3	16,4	14,5	14,6	14,8	14,9
Maximum air temperature, °C	37,8	28,2	40,5	37,4	39	33,9
Σ annual precipitation, mm	559,7	492,0	483,6	696,2	311,4	267,9
Σ active period precipitation, mm	367,6	283,8	333,2	364,3	180,4	161,7
Σ real annual insolation, (h)	2096,7	2095,9	2142,2	1946,0	2125,5	2502,0
Σ real active period insolation, (h)	1708,4	1.714,8	1527,2	1.453,9	1329,7	1574,6
No. of days with max. temperatures >30°C	51	94	84	78	98	57
Active period, days	192,3	201	200	202	206	213
Real heliothermic index (IHr)	4,5	4,4	3,7	3,4	4,5	5,7
Hydrothermic coefficient (CH)	0,9	0,6	0,7	0,8	0,4	0,4
Bioclimatic index (Ibcv)	12,8	15,1	11,1	8,7	22,6	20,3
Oenoclimatic aptitude index (IAOe)	5492,7	5.836,6	5332,8	5.125,0	5847,5	5457
Huglin heliothermic index (IH)	3130,1	3800	3637	3280	4583,7	4584
Cool night index (IF)	12,8	13,8	15,6	14,1	14,4	14,5
Annual aridity index Martonne $I_{ar\text{-}M}$	22,7	23,2	18,6	19,7	11,9	10,7

During the 5 years of observations, the clonal elite 48/9/6 was remarked from the population (Figure 1), which after being tested by ISTIS was approved as Centennial seedless 48 Mf in 2021 (Figure 2). The budburst is early, the rosette is reddish, with rare hairs. The clonal elite has a fertility rate of 59% and a very high vigor. The young shoot is reddish in color, the appearance of the tip is completely open, hairs are absent or very rare, the position is semi-erect, the tendrils are long. The cane is reddish brown, darker at the nodes.

Figure 1. Centennial Seedless population

Figure 2. Centennial Seedless 48 MF Clone

The color of the adaxial side of the young leaf is light copper red, with soft hairs between the main ribs, which are very rare or absent on the abaxial side. The color of the dorsal part of the internode and the ventral part of the node of the shoots are green and red.

The adult leaf has a slightly shorter petiole than the median rib, a very large green to red cordiform blade, slightly embossed on the superior side, sparse hairs on the veins from both sides. The upper lateral sinuses are very deep, with seven slightly overlapping lobes. The teeth are long, with a small tooth length/width ratio and a rectilinear shape and rare hairs. The petiolar sinus has semi-overlapping lobes.

The flower has fully developed stamens and incompletely developed gynoecium. The variety is seedless, manifesting the phenomenon of parthenocarpy. The bunch has a cylindrical-conical shape, is long, yellow, large, with a very long green peduncle with reddish hues, medium compactness and early ripening.

The berry is narrow, elliptical, large and uneven, yellow, with a fairly easy pedicel detachment. The berry is also characterized by medium epidermal thickness, semi-crunchy pulp, moderately firm skin, which adheres to the fleshy core, absent or very weak anthocyanin pigmentation, with a different taste and absent seeds.

The development of vegetation phenophases is specific to the ecoclimate of the Murfatlar vineyard, and differs depending on the variety. The active vegetation period begins with the budburst and ends in autumn when the leaves fall. Centennial seedless 48 Mf buds in the first half of April, blooms in late May to early June, starts the veraison in the second half of July and ripening in the second decade of August. The vegetation period sums 198 days, a lower value in comparison with the population - 206 days (Table 5, Figure 3).

Table 5. Phenological data of Centennial Seedless 48 Mf clone, compared to the population

Variety	Year	Calendaristic dates						No. Of days vegetation period
		Budburst	Flowering	Veraison	Full maturity	Harvest	Leaf fall	
Centennial seedless 48/9/6	**2016**	10-15IV	9-12VI	18VIII	4IX	4IX	24X	197
	2017	14-20IV	30V-5VI	14VIII	6IX	6IX	2XI	201
	2018	9-14IV	4-11VI	16VIII	12IX	12IX	30X	198
	2019	12-18IV	8-12VI	12VIII	10IX	10IX	26X	199
	2020	10-15IV	2-7VI	8VIII	8IX	8IX	11XI	197
Average Centennial seedless 48 Mf		**12.04**	**2.06**	**10.08**	**6.09**	**6.09**	**2.11**	**198**
Centennial seedless population		16.04	13.06	24.08	16.09	16.09	7.11	206

In table grapes, winter buds are affected from a harmful temperatures of -18 ± 3°C, depending on the biological characteristics of the varieties. The Centennial Seedless 48 Mf clone, as the population, has a poor frost resistance (Table 6). The Centennial seedless 48 Mf clone is superior to the population in terms of quantity of grapes reported per trunk/hectare. In terms of quality, in comparison with the population, the clonal selection possesses superior traits: ripens earlier, has a medium epidermal thickness, and large and long grapes (Table 7).

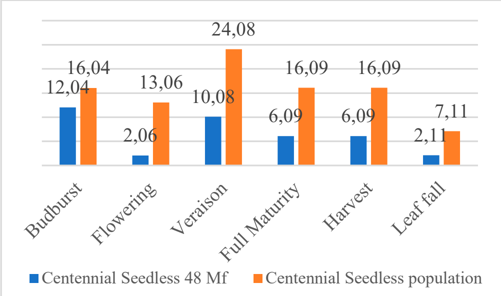

Figure 3. Phenological data of the Centennial Seedless 48 Mf clone, compared to the population

Table 6. Agrobiological characterization of the Centennial seedless clone 48 Mf compared to the population

	Year	Fertility, fertile shoots %	Absolute fertility coefficient	Relative fertility coefficient	Absolute production index	Relative production index	Growth vigour	Cold resistance, %viable buds
Centennial seedless 48/9/6	2016	72	1,0	0,9	527	474	Great	Weak/87%
	2017	63	1,2	0,7	632	369	Great	Weak/85%
	2018	55	1,0	0,8	527	422	Great	Weak/80%
	2019	53	1,1	0,8	580	422	Great	Weak/90%
	2020	52	1,1	0,6	580	316	Great	Weak/75%
	Avg.	**59**	**1,1**	**0,7**	**569**	**369**	Great	Weak/**85%**
Centennial seedless population		40-50	1,0	0,6	403	319	Great	Weak/70%

Table 7. Technological characterization Centennial seedless clone 48 Mf compared to the population

	Year	Average bunch weight, g	Weight of 100 berries, g	Average berry weight, g	Must sugar content g/L	Must total acidity g/L H_2SO_4	Grape production kg/trunk and t/ha	Production %
Centennial seedless 48/9/6	2016	548	206	3,2	196,4	3,95	11,32/19	75-80
	2017	452	170	2,5	193,2	3,92	8,86/18	75-80
	2018	369	138	2,8	181,7	4,15	8,14/18	75-80
	2019	769	289	3,5	214,5	3,00	12,7/27	75-80
	2020	497	187	3,0	194,2	3,98	9,03/18	75-80
	Avg.	**527**	**198**	**3,0**	**196,0**	**3,80**	**10,01/20**	**75-80**
Centennial seedless population		423	167	2,0	172,3	4,84	8,02/15	75-80

The grapes and berries of the Centennial Seedless 48 Mf clone weigh more than the population (Figure 4).

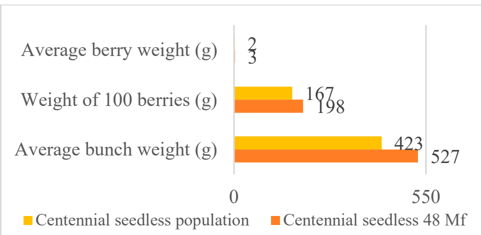

Figure 4. Average berry and grape weights of the Centennial Seedless 48 Mf clone, compared to the population

Regarding the sugar content, the Centennial Seedless 48 Mf clone differed significantly from the population, presenting values of 196 g/l compared to 172.3 g/l (Figure 5).

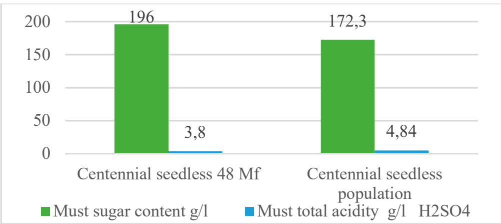

Figure 5. Total acidity and sugar content of the Centennial Seedless 48 Mf clone, compared to the population

Grape yield raported as kg/trunk and t/ha is clearly higher for the clone (Figure 6).

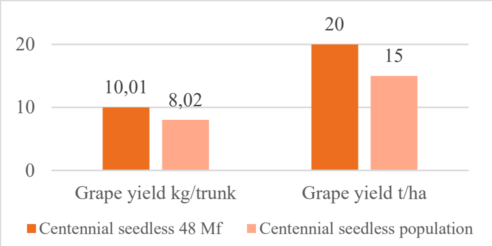

Figure 6. Average grape yields kg/trunk and t/ha of the Centennial Seedless 48 Mf clone, compared to the population

The technological indices for the Centennial seedless 48 Mf clone are clearly higher, compared to the population, the productive potential of the clone is highlighted and leads to the improvement of the assortment of varieties for table grapes in the Dobrogea area (Table 8).

Early ripening, skin resistance, core consistency allow the grapes to stay on the vine for 18-25 days, it is recommended for fresh consumption and for raisins (Table 9). It is advisable that for an even ripening, a terrain with southern exposure should be chosen.

Table 8. Centennial seedless 48 Mf clone technological indices compared to the population

	Year	Berry index	Bunch structure index	Berry composition index	Yield index	No. berries/bunch	Peduncle length (cm)
Centennial seedless 48/9/6	2016	46	2,3	5,6	10,2	178	6,5
	2017	62	2,3	5,7	10,3	169	5,4
	2018	50	2,4	6,6	10,7	161	5,05
	2019	42	2,2	5,1	9,7	190	6,8
	2020	50	2,4	6,2	10,6	175	6,4
	Avg.	**50**	**2,3**	**5,8**	**10,3**	**174,6**	**6,03**
Centennial seedless population		40	1,9	5,8	10,2	161	5,21

Table 9. Technological characteristics regarding commercial value, suitability for transport and storage

Clonal selection	Bunch size	Berry size and uniformity	Compact-ness	Shape and color	Pruine layer thickness	Skin adherence and elasticity	Pulp consistency	Presence of seeds	Taste	Aroma
Centennial seedless 48 Mf	Large	Large and uneven	Medium	Narrow eliptical, yellow	Thin	Moderately ferm	Semi-crispy	Absent	Other	No

CONCLUSIONS

The research work was carried out to identify and select valuable elites in the table grape population of the Centennial seedless variety, which, together with good quality and productivity, also possess resistance to environmental conditions, diseases and pests. During the 5 years of observations and studies, the clonal elite 48/9/6 was noticed, which after being tested by ISTIS was approved as Centennial seedless 48 Mf in 2021. This table grape clone is early fruiting, with a very high growth vigor and medium fertility (59% fertile shoots). The Centennial seedless 48 Mf clone is superior to the population in terms of quality and quantity of grapes per trunk and hectare, and has medium epidermal thickness, large and long grapes, compared to the population in which the epidermis is thin and the grapes are medium and loose.

The technological indices for the Centennial seedless 48 Mf clone are clearly superior, compared to the population, and the productive potential of the clone leads to the improvement of the assortment of varieties for table grapes, which is deficient in the Dobrogea area.

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CLIMATE VARIABILITY AND MAIN NUTRIENTS IN LEAF BLADE AND PETIOLE FOR DIAGNOSE THE GRAPEVINE NUTRITIONAL STATUS

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Abstract

For monitoring the Cabernet Sauvignon nutritional status, leaf blade and petiole tissues collected in flowering and veraison from 2018-2019 growing seasons, were analysed for main macronutrients. Sampling in flowering or veraison, do not show significant differences in variables response. Rather than otherwise, the differences were observed between growing seasons, and the climate variability from 2019 with heavy rain in the spring correlated with low temperatures and the summer and autumn draught associated with hot weather, influenced the absorption of macronutrients, canopy development, and the relationship between leaf blade and petiole macronutrients concentration. Results indicate that leaf blade prevails over petiole for nitrogen (veraison) potassium (flowering) calcium (flowering) and magnesium (flowering) in predicting vine nutrients status while petiole is better indicator for phosphorus (flowering stage). Grape berry mineral main compounds are influence by dry weather and the degree of dehydration. Research results can be used as guide in main macronutrients nutritional status in Cabernet Sauvignon, for regions with similar terroir conditions.

Key words: *climate, Cabernet Sauvignon, juice, petiole, nutrients, quality.*

INTRODUCTION

The soil, a huge sink of nutrients, plays a major role in interaction with plants and grape production, amid continuous climate variability (Santos et al., 2020). Some studies from last decade, show that in summer growing season (June–August) in the soil first 50 cm, temperature was higher with about 6ºC than in autumn season (September-November) (Biasi et al., 2019). Heat stress is shortening the phenological stages, some starts earlier, other are delayed and grape berries composition is changed (Koufos et al., 2020). Higher temperature has large impact on organic matter mineralization, microbial communities and water availability (Pareek, 2017). Soil and climate influence the growth and fruiting processes, the quantity and quality of the wine, vineyard life, the resistance to diseases and pests (Celette & Gary, 2013). As grapevine develops large canopy and grape yield, the amount of nutrients absorbed from soil increase, with an inclined balance towards macronutrients (N, P, K, Ca, Mg) compared to micronutrients (Fe, Cu, Mg, Mn, etc.) (Fogaça et al., 2007). Therefore, the evaluation of soil nutrient supply is useful to establish the nutritional diagnoses for fertilization (Dobrei et al., 2016). However, testing soil samples to assess the nutrient supply is not always sufficient, especially for the grapevine which has many varieties and clones or rootstocks, with different growing stages and high consumption of nutrients (Benito et al., 2015). The supply of vines with nutrients from the soil can be assessed qualitatively depending on the symptoms from the leaves (especially on the older leaves for macronutrients and the younger leaves for micronutrients) and the vigour of the vine, or quantitatively by analyzing plant tissues (Costa et al., 2019). Samples composition is variable depending on the soil fertility and fertilization, on climate variability during the growing season, the time of day when samples were collected or growing stages (Dominguez et al., 2015).
Different fertilizers and the ratio achieved between the main macroelements (N, P, K, Ca, Mg) have great influence on grape production

with high impact on wine quality (Domagała-Świątkiewicz et al., 2019). The disruption of balance among macro and micro nutrients limits the performance of the vine (Dobrei et al., 2016). Climate variability and heat stress contribute to the increase of potassium levels accumulation according to de Orduña (2010).
In recent decades, abiotic stress has been associated with micronutrient deficiency that decreases production efficiency due to limited metabolism or biosynthesis (Bencke-Malato et al., 2019). The antagonism magnesium-potassium is aggravated by high soil pH.
The aim of the study was to diagnose the nutrient content of the leaf blade and petiole tissue, in two reliable growing stages - at flowering and veraison when diagnose is quite similar, in two different growing seasons (2018-2019) to can establish better fertilization management correlated with the soil nutrients supply and climate variability, and other complement observations including berry main minerals, in the Cabernet Sauvignon variety.

MATERIALS AND METHODS

Experimental site and climate

The research was carried on in Recas Vineyards, Timis County, east-side of Romania, region located on the same latitude as Bordeaux, which benefits from microclimate with gentle Mediterranean influence, mild winters, short springs, warm summers, long autumns and sudden transitions from winter to summer. During the last decade the annual average temperature in the area was 12.52°C and annual precipitation 547 mm. In last decade, summers were very hot (June-August, average 20.69 - 23.57°C). Climate data (Figure 1) were registered and collected from the Wireless Weather Station Wi-Fi Connection Solar Charging (Davis 6250) installed in the vineyard.

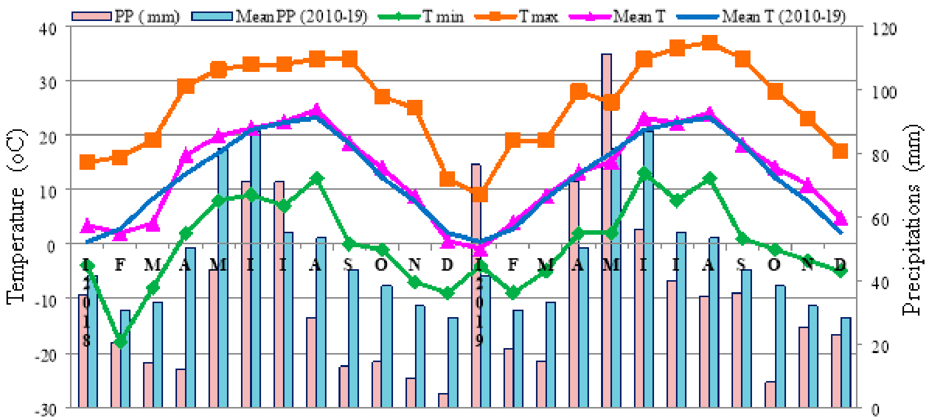

Figure 1. Climate variability during 2018-2019 growing seasons
(mean temperature and precipitations over 2010-2019 decade)

Precipitations show high variability during both the growing and dormant seasons. The weather in 2018 was versatile; in the last decade of April there was a late frost, next period, the rainfall prevented the timely accomplishment of some works in the vineyard, followed by a warm summer with high temperatures and a warm autumn during the day and cool nights. With large variability from month to month, in 2018 precipitations significantly decreased (336 mm), but the hailstorms and heavy rains from June decreased the grape production. In 2019 total precipitations accumulated 514.3 mm, close to the annual mean value in the area. The first growth stages, bud break and beginning of flowering were normal developed in 2019. From the last decade of May, throughout April, the last decade of June, as well as July, due to heavy long term rainfall, phytosanitary treatments could not be optimally applied. The temperature values were variable from one year to another. In 2018, weather favoured the fast grapes ripening, registering the earliest grape harvest in the last 40 years, considering that the harvesting period started 14-21 days earlier. However, the vines were not affected by the low temperatures of winter 2018-2019 or by late spring frosts. Warm nights and days with over 28-30 ºC, but also the draught greatly favoured the sugar and tannins accumulation.

Soil

The slope gradient for vineyard rows rank between 10 and 12% and altitude from 120 to 170 m. Distance was 1.2 m between vines and 2.2 interrows. Vines were double Guyot trained and shoots were cut back at 35 cm above the third/top trellis wire. In the fertilization programme was applied 100 kg nitrogen early in the spring before budburst, 40 kg phosphorus and 120 kg potassium for one hectare area in the dormant season (autumn).

The wine grape varieties were planted 12 years ago on sandy loam, and loam clay soils, medium supply of phosphorus, good in potassium, nitrogen, and organic matter, with pH average value around 6.28 (0-110 cm). Soil samples were analysed for total nitrogen phosphorus, potassium, calcium and magnesium supply level by spectrophotometer analysis, using different colour reagents. The analysis of the exchangeable bases (SB) was done by the Kappen method and the humus by the oxidimetric method.

Leaf blade and petiole sampling and analysis

The leaf blades with petiole were harvested from the shoot in position opposite to the bunch, at 80% flowering and during the veraison. Each year 100 leaf blades were harvested, but were selected only 40 for analyses. The samples were collected between 8 to 10 o'clock when the leaves had maximum turgidity and the soil moisture was optimal. The leaves on the same vine were placed in the same bag and then labelled for transfer to the laboratory. Samples were minimum handling to avoid the risk of contamination and transport in insulated and cool container. Leaf blade and petiole were washed, draught, milled, ashed, then dilute and main components analysed. The total plant nitrogen (N%) was determined by near infra-red spectroscopy (NIR) with wavelengths ranked between 780–2,500 nm, after calibration. Phosphorus and potassium concentration from petiole and leaf blades were measured by inductively couple plasma - optical emission spectrometry (ICP-OES), after was calibrated with standard solutions of a different concentration. No foliar spray or drip irrigation fertilizers were applied during research to avoid samples contamination. Copper fungicides and other treatments for fungal foliar diseases control were performed after berry set stage. Direct monitoring at flowering stage has the advantage of nutritional correction problems for the current crop. For juice sampling 100 berries were crushed and pressed. The minerals were determined by laboratory analysis spectrophotometry.

Statistical analysis

The data were statistically analysed by XLSTAT software version 2019.1.3 (Addinsoft, Inc. New York) for: the simple linear regression based on Ordinary Least Squares (OLS), R^2 (coefficient of determination), confidence interval (CI) and the analysis of variance. CV%, p-value and t-test (one-tailed) were calculated in GraphPad Prism Version 7.04.

RESULTS AND DISCUSSIONS

The analysis of leaf and petiole tissue allows the estimation of the nutritional status of the vine, and can provide information on the absorption and supply of soil with nutrients. The results from leaf blade and petiole samples analyses for macronutrients (N, P, K, Ca, Mg) during flowering and veraison, in 2018 and 2019 growing seasons are summarized in Figures 2, 3, 4, 5 and 6, respectively. Usually, leaf blade is a better indicator of nitrogen (N) status, while petiole gives more indications for potassium (K) deficiencies or toxicity. The significance level was established to $\alpha = .05$. Climate variability, grape variety, and soil type can influence the concentration of main macronutrients in petiole and leaf blade, which reflect the absorption of nutrients by the vines. The confidence interval (CI) is quite narrow, which reflect a good accuracy ($p < .0001$) of variables relationship for all macronutrients analysed (Figures 2, 3, 4, 5, 6). P-value is extremely low ($p < .0001$) compared to the $\alpha = .05$ risk threshold, and the differences between variables mean are extremely significant, conclusion also confirmed by the CI limits.

Nitrogen

Climate variability, grape variety, and soil type can influence the concentration of main macronutrients in petiole and leaf blade, which reflect the absorption of nutrients by the vines.

In grapevine, spring growing stages mainly depends on reserves stored in roots and wood in the previous growing seasons (Costa et al., 2019). Nitrogen has two main incorporation phases: before "pea-size" and during veraison. Cabernet Sauvignon is ranking as medium petiole nitrogen concentration during bloom-time, among grape varieties (Schreiner et al., 2013). In present study, leaf blade show in both flowering and veraison stages higher concentrations of nitrogen than petiole. However, petioles had higher concentrations in phosphorus and potassium regardless of growing season and stage of sampling.

Around 52% in 2018 and by 64% in 2019 growing seasons from leaf blade nitrogen variability is explained by the petiole nitrogen concentration. In veraison stage the nitrogen concentration in petiole explains nearly in the same rates (59.64% and 54.96%) the leaf blade nitrogen variability in 2018 and 2019 (Figure 2).

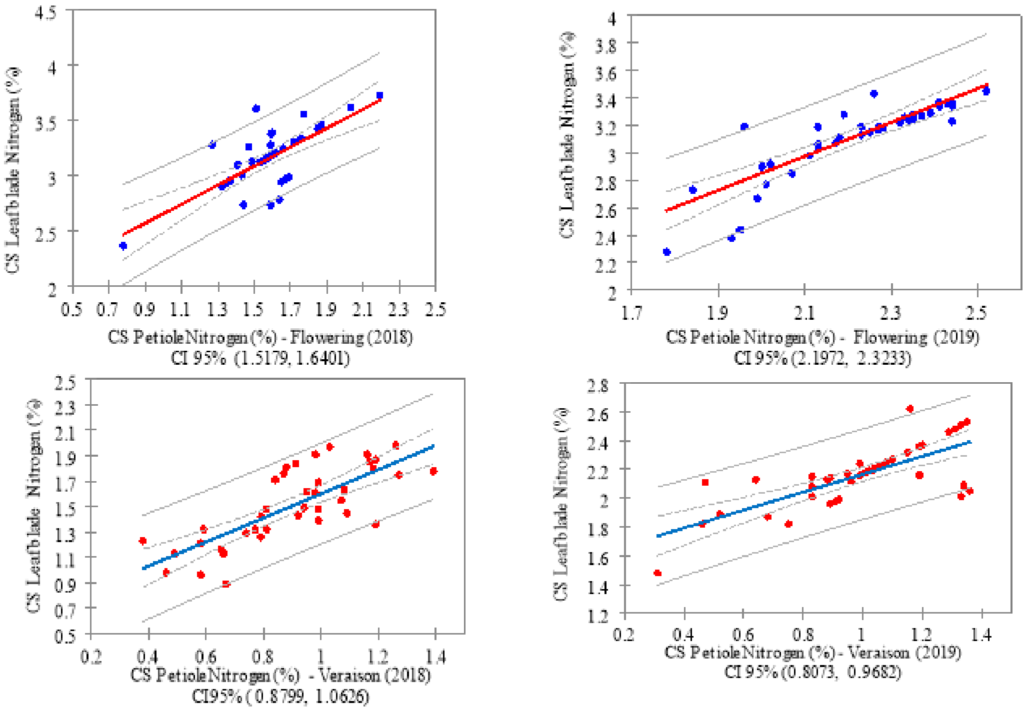

Figure 2. Nitrogen concentration in leaf blade and petiole, at flowering and veraison, for Cabernet Sauvignon (CS) during 2017-2018 growing seasons (CI = confidence interval; CV% = coefficient of variation; R^2 = coefficient of determination)

Phosphorus

During flowering stage in both growing seasons, phosphorus linear regression model explain in moderate rate the phosphorus from leaf blade (Figure 3). In the veraison stage the relationship between the variables is changed: the phosphorus concentration in the petiole explains moderately the phosphorus in the leaf (62.81%) in the first growing season and largely (78.54%) in the second. In 2018 growing season (flowering and veraison) the phosphorus concentration was lower compared with 2019 (leaf blade show lower variability than petiole). In 2019, excepting the veraison stage the variability of phosphorus concentration was higher in petiole than in leaf blade. Leaf blade and petiole phosphorus concentration is variable from flowering to veraison and higher in the first growing season than the second. Soil moisture due to heavy rainfall, correlated with lower temperatures from spring of second growing season (2019) decreased the ability of vines roots to uptake the phosphorus. Leaf blade phosphorus concentration was higher compared to petiole in veraison than in flowering stage.

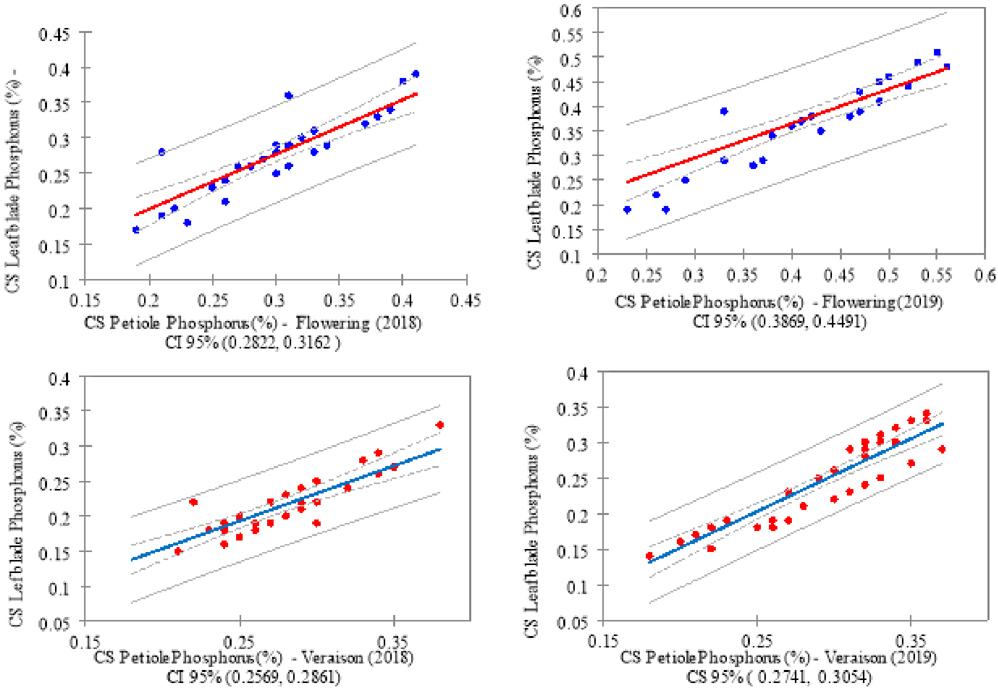

Figure 3. Phosphorus concentration in leaf blade and petiole, at flowering and veraison, for Cabernet Sauvignon (CS) during 2017-2018 growing seasons (CI = confidence interval; CV% = coefficient of variation; R^2 = coefficient of determination)

Potassium

According to Conradie (1981) potassium is the main mineral accumulated before and after veraison, with the highest rate during ripening, in grape berries. Potassium cation (K^+) accumulation is linked with the water availability and correlated with berry flesh weight and dry matter, and also contributes to the sap flow. In the present research, there were no differences between seasons in petiole and leaf blade potassium concentration relationship (Figure 4). The resulting variability rate accounting for petiole potassium was slightly larger in 2019 than in 2018 veraison stage. Potassium doesn't combine in organic compounds and therefore is easily leached from leaves to the soil, but has high mobility in plants tissues and the amount removed in grapes can be replaced from the soil sink.

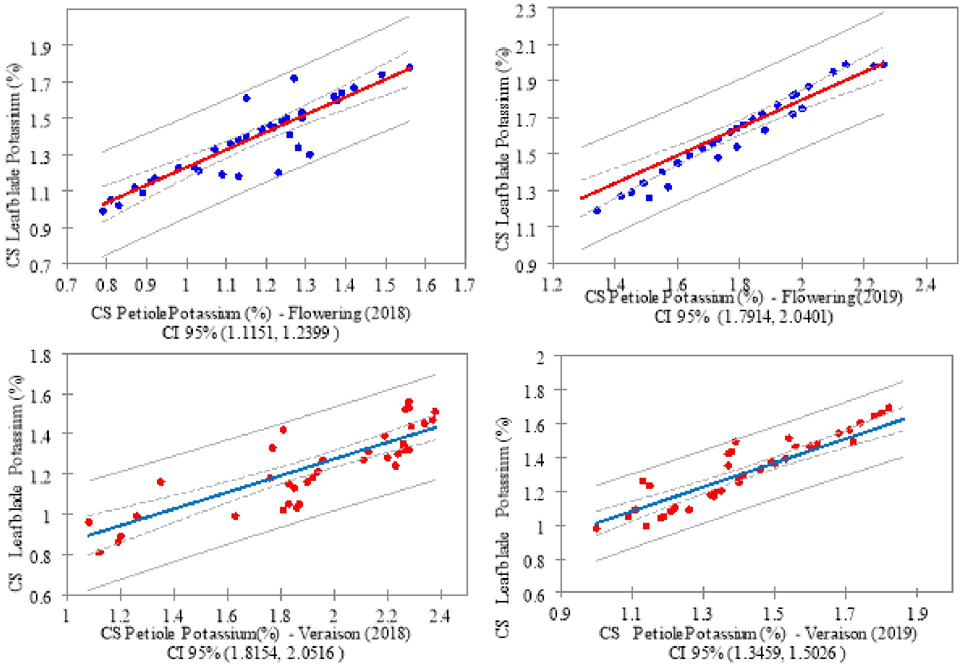

Figure 4. Potassium concentration in leaf blade and petiole, at flowering and veraison, for Cabernet Sauvignon during 2017-2018 growing season (CI= confidence interval; CV% = coefficient of variation; R^2 =coefficient of determination)

Calcium

In both years petiole calcium of Cabernet Sauvignon variety, explained in large ratios (81.98% and 83.12% respectively) the variability of these macronutrients in the leaf blade during flowering stage. Quite different was the situation for veraison, when higher calcium variability from petiole compared to leaf blade was found. In flowering stage (in both growing seasons), the calcium concentration variability was higher in petiole than leaf blade, while in veraison stage the limits of variability were narrow in both seasons (Figure 5). Calcium is presumably in antagonism with potassium and magnesium; therefore interactions between calcium and previous macronutrients have to be considered when nutrient programs are applied (Benito et al., 2013).

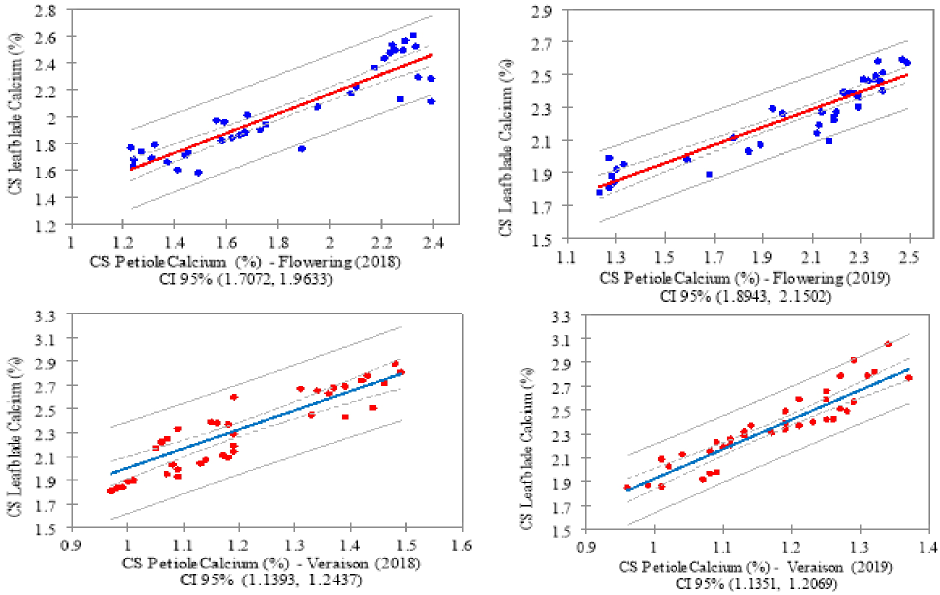

Figure 5. Calcium concentration in leaf blade and petiole, at flowering and veraison, for Cabernet Sauvignon during 2017-2018 growing seasons (CI = confidence interval; CV% = coefficient of variation; R^2 = coefficient of determination)

Magnesium

Medium influence of magnesium concentration from petiole was found in both growing seasons in samples from flowering stage. During veraison, linear regressions indicated that petiole magnesium had larger influence on leaf blade, and was quite similar across years. Regarding the variability of the magnesium concentration, was higher in the petiole compared to the leaf blade (Figure 6).

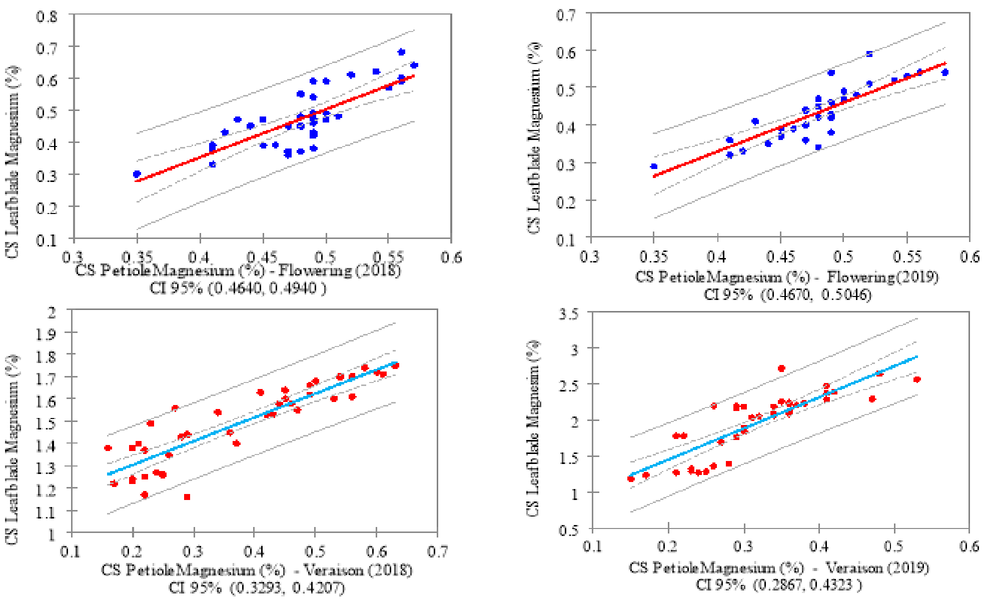

Figure 6. Magnesium concentration in leaf blade and petiole, at flowering and veraison, for Cabernet Sauvignon during 2017-2018 growing seasons (CI = confidence interval; CV% = coefficient of variation; R^2 = coefficient of determination)

Climate variability correlated with grapevine nutrition influence on grape yield and berry composition

Berry composition is important for both viticulturists and winemakers. Mineral status is influenced by many factors as soil, translocation from roots to canes and shoots, crop load, number of berries on cluster, cultural practices or vine vigour, climate variability and microclimate.

At ripening stage, berry composition was different during two growing seasons; berries from 2018 had higher level of anthocyanins than those from 2019.

Climate variability correlated with grapevine nutrition influenced the berry composition and yield. Rainfalls from the 2019 flowering season have positive influence on titratable acidity (TA) level (Table 1).

By contrary, soil moisture had little impact on TA level at grape maturity. In both growing seasons, TA level was negatively correlated with the temperature (variables involved diurnal and nocturnal temperature) from early growing season to ripening stage.

However, the higher pH berry juice at ripening was found in the warmer 2019 growing season, and was correlated with higher sugars concentration (Table 1).

Nitrogen

In 2018, the nitrogen in petiole and leaf blade show higher concentration in veraison compared to flowering stage, and was opposite in 2019. The variability of nitrogen concentration in petiole and leaf ranked to a large extent, being generally higher in petiole.

Table 1. Grape yield and main production parameters for Cabernet Sauvignon variety during 2018-2019 growing seasons

	Yield (kg/vine)	Cluster weight (g)	Berry weight (g)	Pruning weight (kg/vine)	ºBrix	pH	TA (g/l)	Total anthocyanins mg/l
2018	4.8	106.55	1.30	1.38	22.14	3.54	7.76	66.75
2019	5.6	128.30	1.42	1.25	23.50	3.72	7.42	54.20

Table 2. Results for macro - and trace elements in Cabernet Sauvignon grape berries during 2018-2019 growing seasons

	N	K	P	Ca	Mg	B	Fe	Cu	Mn	Zn
					μg $berry^{-1}$					
2018	1472± 86.4	3842± 103.17	288 ± 39	471 ± 13	142 ± 6	14 ± 1.2	5.2 ± 0.4	2.6 ± 0.18	2.2 ± 0.18	1.1 ± 0.19
2019	1524 ±92.6	3925± 112.05	295 ± 42	475 ± 44	149 ± 8	12 ± 0.9	5.7 ± 0.6	2.9 ± 0.21	2.4 ± 0.3	1.4 ± 0.21

According to Vrignon-Brenas et al., (2019), the post harvest application has the same influence on petiole nitrogen concentration like that applied at flowering; in the present research, the first nitrogen application was before budburst. According to Costa et al. (2019) results, usually fertilization is positive correlated with nitrogen concentration in the petiole. Lower temperature and heavy rainfed in flowering stage in 2019 growing season, are correlated with the higher influence of petiole on leaf blade nitrogen concentration. The higher vigour of Cabernet Sauvignon facilitates more nitrogen accumulation, because large canopy need more nitrogen during budbrake and flowering stages.

More favourable climate during veraison stage from 2018 (Figure 1) and the availability of nitrogen from soil, facilitate the increase of nitrogen concentration in petiole and leaf blade; lower petiole and leaf blade nitrogen concentration during second growing season in veraison stage (2019) is correlated with less humidity from June and July.

Several researches demonstrated that draught can decrease the nutrients uptake from soil (due to nutrients mineralization or less nutrient diffusion in the soil) and the nitrogen concentration and phosphorus in tissues (Parker et al., 2011; Fila et al., 2014). Year to year variability of nitrogen concentration in petiole and leaf blade was reported by Benito et al. (2013) in Garnacha Tinta variety, Schreiner et al. (2013) in Pinot Noir or Romero et al. (2014) in Tempranillo variety. In present study, overall lower variability of nitrogen concentration recommends both leaf blade and petiole from veraison stage, for nitrogen status of the vine. Similar results were found in Garnacha Tinta (Benito et al., 2013) and Tempranillo (Romero et al., 2014). However, Conradie (1981) reported that petiole is better indicator of nitrogen status in vines than leaf blade, because is more sensitive to variation. Large doses of nitrogen applied in vineyards can influence the absorption and interact with other nutrients (Helwi et al., 2015).

Phosphorus

Phosphorus concentration in petiole was found to be higher than in leaf blade according to Klein et al. (2000) founding. Large variability in petiole phosphorus concentration in Red Grenache variety (Benito et al., 2013) indicate leaf blade to be more useful for phosphorus diagnose in wine grapes varieties. On contrary, phosphorus was the less abundant macronutrient in leaf petiole (4.0 g kg^{-1}) found by Arrobas et al. (2014) in cv. Viosinho Blanc. In present research, the higher phosphorus concentration in petiole compared to leaf blade and less variable in leaf blade flowering stage in the first growing season, recommends this macronutrient as phosphorus status in Cabernet Sauvignon.

Potassium

Cabernet sauvignon show lower variability from flowering to veraison and from year to year, compared with nitrogen. Lower variability is contrary to the observations of Romero et al. (2014) and Benito et al. (2013) which suggest that petioles are better indicators of potassium status from vines compared to leaf blade, due to better response after potassium supply. In present study, the regression model for potassium concentration indicates that petiole had medium or lower influence on leaf blade potassium. The draught from the last growing seasons and over cropping, can explain the lower levels of potassium in vines analysed tissues. According to Abbasi et al. (2016) less humidity in soil decrease potassium uptake due to lower mobility, transpiration rate and declined of roots membrane transporters. Potassium level has been found to be variable from one year to another by Christensen (2000) and his research results confirm limits variability from 30 to 50% in the same vines from the same vineyard, depending on rootstock, variety and soil moisture. Therefore, Cabernet Sauvignon leaf blade (flowering stage) lower variability during both growing seasons can be a better predictor of potassium status.

Calcium

Calcium commonly range between 1.0-2.0% and can increase to 3.5% in petioles when potassium and magnesium are lower, according to Christensen (2000) findings. In preset study, some calcium uptake over 2% in leaf blade and petiole can be partly explained by the new roots which usually start to grow after bloom stage and the balanced soil pH.

This statement is according with de Herralde et al. (2010) findings, which indicates that roots main functions are nutrient and water uptake, and those with Ø < 2 mm, are often mycorrhized. Higher calcium concentration (25.7 g kg^{-1}) in petiole samples collected at veraison was found by Arrobas et al. (2014) in cv. Viosinho Blanc from Portugal.

Conradie (1981) research results in Chenin Blanc show that little amount of calcium is accumulated before and after budburst due to the reserves used for new growth from vines roots. However, Cabanne and Doneche (2003) after studying the calcium accumulation in Cabernet Sauvignon, Merlot, Semillon and Sauvignon Blanc varieties from Bordeaux vineyards concluded that calcium concentration increased from flowering to veraison.

Overall low variability of potassium from one year to another in both leaf blade and petiole (flowering and veraison) from Cabernet Sauvignon, recommends this macronutrient for wine grape varieties in study nutrient status.

Magnesium

The negative correlation between magnesium and potassium concentration was documented by other researchers (Gransee & Führs 2013; Domagała-Świątkiewicz et al., 2019). However, magnesium concentration is positive influenced by phosphorus application according to Skinner and Matthews (1990). Leaf blade in both flowering and veraison in first growing season show less variability, therefore can be used for magnesium status for future nutrition with the macronutrient in the vineyard. In Shiraz variety from Riverina (Australia), while phosphorus concentration decline from spring to the end of growing season similar to nitrogen, calcium and magnesium increased in substantially amounts in leaves, with the highest calcium concentration in flowering stage and magnesium at berry set stage (Benito et al., 2013). Conradie (1981) research results show that in Chenin Blanc variety, magnesium absorption was not significant three weeks before budburst and after one month; instead, magnesium increased in leaves during flowering stage till veraison.

Climate variability correlated with grapevine nutrition

Nitrogen berries content at harvest can offer the plant status for this macronutrient for the whole season; nitrogen accumulates during berries development with a considerable dropping in the first stage of veraison (50% in the skin and seeds), but higher amounts of nitrogen decrease the grape production (Amiri & Fallahi, 2007). According to Satyanarayana (1972) the nitrogen level from leaves is a major indicator in grape yield. The more dry vintage 2018 decreased the nitrogen content in Cabernet Sauvignon berries at harvest and was less altered in fruits the next vintage year (Table 2). Potassium is one of the most necessary nutrient in grapevine and large amounts are removed with grape yield (Kodur, 2011). It is absorbed by vines during all stages, but mainly after veraison, with a sharp increase during ripening and has greatly influence on grape juice pH. Leaf potassium concentration was not significantly different between the two growing seasons but less humidity during veraison and ripening in 2018 season, decreased potassium uptake by roots; the content in berries was lower compared to 2019 season. On contrary, Etchebarne et al. (2009) doesn't found in ripened berries potassium concentration modified by water availability for plants. Component of the nucleic acids and ATP, phosphorus accumulate in grape berries during the whole growing season but mainly after veraison; translocation from leaves increase phosphorus into bunches to almost 300 μg $berry^{-1}$ in both growing seasons. Structural role of the calcium in berries cell wall and membranes increases until veraison (decreased after). The calcium accumulation depends of vine water status and was lower in 2018 growing season. Magnesium which is involved in photosynthesis and can be found mainly in leaves is absorbed in small quantities in grape berries especially in seeds (before, during and after veraison) was also influenced by vine water status; magnesium concentration in berries reached 142 in the first growing season and 149 μg $berry^{-1}$ respectively in the second; the magnesium from petiole doesn't show any link with berry quality (Table 2). Research results of Panceri et al. (2013) for calcium and magnesium content in grape berries during dry weather, confirm the concentration increase of both minerals in grape juice. Boron deficiency - which is associated with draught stress, decrease pollen tube growth and pollen germination is limited, resulting hen and chick syndrome – was observed in June-July 2019 vintage. No significant differences were found in grape juice between growing seasons for Fe, Mn and Zn which accumulates mainly in berry pulp and skin, and their concentration is associated with the migration from the skin to pulp and positively influence by dehydration; Galgano et al. (2008) found similar results. The presence of Cu in grape juice is influenced mainly by copper fungicides treatments and was higher in more dehydrated berries during 2019 vintage (Table 2).

CONCLUSIONS

The comparison with other results is difficult because terroir is different within the same region; however, most of the petiole and leaf blade concentrations found ranked around those reported in the literature which signifies that the vineyard area nutritional status is adequate for Cabernet Sauvignon growing. The higher temperature from 2019 growing season (the highest from 1960), negatively influenced the macronutrients accumulation in leaf blade and petiole both in flowering and veraison stages, comparing to more balance growing season of 2018. Leaf blade macronutrients concentration was more uniform excepting phosphorus which is better predicted by petiole. Flowering stage was more reliable for predicting nutritional status of the vines excepting nitrogen. Results can be useful as guide for Cabernet Sauvignon grape growers in similar terroir. Climate change but especially climate variability influence on grapevine nutrition requires further research not only on this variety but also on others, as well as for the essential microelements for vines. Dry weather, lower water activity and water loss from berry during development, veraison and ripening, induces changes in the soluble solids and mineral composition and is related with their concentration which is positive correlated with the degree of dehydration.

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THE INFLUENCES DEGREE OF VARIOUS FACTORS ON THE DEVELOPMENT OF ENTERPRISES IN THE GRAPES AND WINE SECTOR

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Abstract

The grapes and wine sector in the Republic of Moldova is a strategic one for the national economy. During the 2020-2021 year, a study was conducted on the factors influencing the development of enterprises in the grapes and wine sector in the Republic of Moldova.
The research was carried out within the project: "Impact of macromedia and geographical factors on bankruptcy and business performance of economic entities in the agri-food sector in the Republic of Moldova", project code 20.80009.0807.26, according to contract between SAUM and NARD. The study was conducted by interviewing companies.
As a result of the study it was established: economic factors/risks obtained an average rating of 4.3 points on the scale of 5 pt.; technical and technological factors/risks obtained an average rating of 4.2 points on the scale of 5 pt.; ecological factors/risks obtained an average rating of 4.2 points on the scale of 5 pt.; legislative-legal factors/risks obtained an average rating of 4.1 points on the scale of 5 pt.; information factors / risks obtained an average rating of 4.2 points on the scale of 5 pt.; moral factors/risks obtained an average rating of 4.3 points on the scale of 5 pt.

Key words: *agriculture, development, enterprise, grapes, horticulture, influencing, factors, wine.*

INTRODUCTION

The agri-food sector in the Republic of Moldova, especially the wine sector is a strategic one. The wines produced, wineries, vineyards and gastronomy, along with other tourist objects in Moldova are the business card of our republic.
Risk management involves the identification and assessment of risks, the identification and establishment of the risk response in order to reduce the possibility of risks, as well as the reduction of the consequences, as a result of the materialization of risks (POCA, 2018).
Risk is the likelihood or threat of damage, injury, loss or any other adverse situation caused by external or internal vulnerabilities and which can be avoided by certain preventive measures (BNM, 2022).
Risks that accompany the entity's activity and that focus on obtaining unfavourable results, in which the entity loses or does not lose part of its income, profit, capital, etc., as a result of a situation of uncertainty regarding its activity are grouped in financial risks (Paladi et al., 2018).
The agri-food sector is exposed to environmental risks, especially in recent years in connection with global climate change (Oprea et al., 2014; Balan et al., 2021).
There are more classifications of risks and business success factors worldwide.

Risk exposure is a probabilistic concept, being directly related to the probability of materialization of the risk. It has significance only before the onset of risk. Risk exposure operates with an implicit hierarchy of identified risks (POCA, 2018).
The aim of the research is to evaluate the wine sector, in terms of different risks / factors.

MATERIALS AND METHODS

The research was conducted based on the project "Impact of macro-environmental and geographical factors on bankruptcy and business performance of economic entities in the agri-food sector in the Republic of Moldova" under the State Program (2020-2024) with the code 20.80009.0807.26 and is summarized as:
- studying the specialized literature;
- highlighting the entities in the agri-food sector for conducting surveys;
- assessment of risk factors of entities in the agri-food sector;
- analysis of survey results;
- processing survey results.

In the 2020-2021 years, 638 entities from the Republic of Moldova, which carry out one or more activities in the agri-food sector, were interviewed based on the questionnaire developed by the research team. In regional profile, they represented - Northern Region - 220 entities or 34.48%; Central Region - 343 entities or 53.76% and Southern Region - 75 entities, or 11.76%.
The study was attended by 160 entities in the wine sector out of the 638 entities that participated in the survey, for which a survey was conducted on a number of performance or risk factors for the activity.

RESULTS AND DISCUSSIONS

All risks are grouped into several groups:
C. Risk group by origin main factors:
- subgroup C.1. Economic risks - Market, Price of production factors, Financial assets, Exchange rate, Inflation, Subsidies, Tax system, Other risks;
- subgroup C.2. Political risks - Political affiliation, President, Parliament, Government, Ministry, Local public administration, Food Safety National Agency, Other risks;
- subgroup C.3. Technical-technological risks, - Application of technical progress in the production process, Modernization / Renewal of technique, equipment, apparatus, etc. necessary for the activity, Modernization of the applied technology, Know-how, Land consolidation, Leasing of the goods necessary for the activity, Property in property, Other risks;
- subgroup C.4. Ecological risks - Climate change, Natural disasters (frost, hail, drought, torrential rains, etc.), Landslides, Deforestation, Drainage of water basins (lakes, rivers), Other risks;
- subgroup C.5. Legislative-legal risks - National legislation, International legislation, Codes - land, water, fiscal, etc., Laws, GD, Sector development programs, Technical regulations, Standards, Other risks;
- subgroup C.6. Information Risks - Delayed Information Release, False Information, Disclosure of Production Secrets and Confidential Information, Other Risks;
- subgroup C.7. Moral risks - Sale of expired products, goods and services, which may cause damage to the consumer, Purchase of expired products, goods and services - by false update to the seller, which may cause indirect damage to the consumer, Theft of products, goods, Other risks;

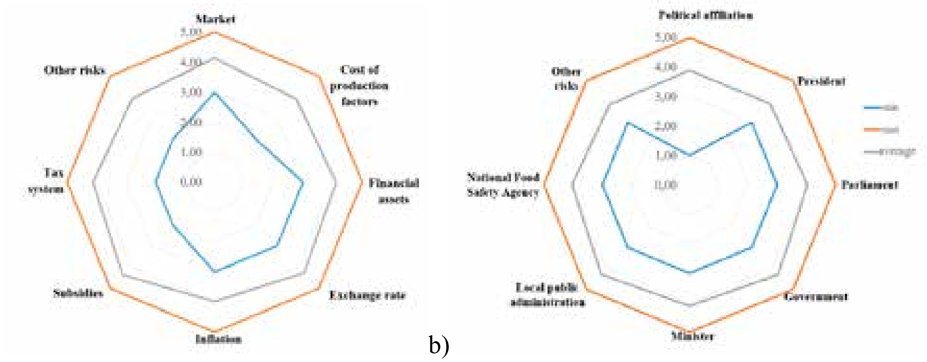
D. The group of risks after their occurrence:
- subgroup D.1. Retrospective risks - Mistakes in the production process, Mistakes in the promotion process, Mistakes in the marketing process, Other risks;
- subgroup D.2. Current risks - Temporary interruption of the production process, Temporary equipment failures, Inability of staff to work, Other risks;
- subgroup D.3. Prospective risks - Business plan, Development strategies and policies, Other risks;

E. The group of risks according to the nature of the record:
- subgroup E.1. External risks - Inflation, Currency, Nature, Other risks;
- subgroup E.2. Internal risks - Partners, Suppliers, Consumers, Marketing strategy, Activity policy, Production potential, Technical

endowment, Level of specialization, Workforce qualification, Safety technique, Other risks;

F. The group of risks according to the sphere of appearance on which the fields of entrepreneurial activity are based:

- subgroup F.1. Risks in the field of production - Application of technical progress in the production process, Modernization / Renewal of equipment, machinery, equipment, etc. Needs for business, Modernization of applied technology, Know-how, Land consolidation, leasing of goods needed for business, Property, Marketing strategy, Activity policy, Production potential, Technical endowment, Level of specialization, Qualification of the workforce, Security technology, Other risks;
- subgroup F.2. Risks in financial activity - Banks, Financial institutions, Other risks;
- subgroup F.3. Commercial risks - Auction, Wholesale, Retail, Insurance, Other risks;
- subgroup F.4. Intermediate risks - Notary, Broker, Lawyers, Other risks.

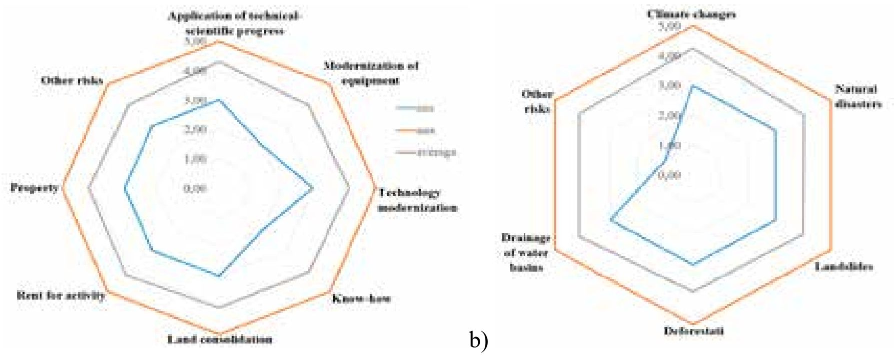


a) b)

Figure 1. Diagram of the economic (a) and the political (b) risks assessment

Analysing the risks group C. The risk group according to the main factors of occurrence we can mention that:

- in subgroup C.1. Economic risks, the market has a contribution as a risk factor between 3 and 5. The average value obtaining 4.14 points. The price of the factors of production has a contribution as a risk factor between 2 and 5. The average value obtaining 3.92 points. The financial assets have a contribution as a risk factor between 3 and 5. The average value is 4.12 points. The exchange rate has a contribution as a risk factor between 3 and 5. The average value is 4.29 points. Inflation has a contribution as a risk factor between 3 and 5. The average value is 3.97 points. Grants have a contribution as a risk factor between 2 and 5. The average value is 4.39 points. The system of taxes and fees has a contribution as a risk factor between 2 and 5. The average value obtaining 4.12 points. Other risks of subgroup C.1. Economic risks have a contribution as a risk factor between 2 and 5. The average value is 3.93 points.
- subgroup C.1. Economic risks obtained an average rating of 4.11 points. This is easy to see from the risk assessment chart (Figure 1 a).
- in subgroup C.2. Political risks, Political affiliation has a contribution as a risk factor between 1 and 5. The average value is 3.88 points. The president has a contribution as a risk factor between 3 and 5. The average value is 3.89 points. Parliament has a contribution as a risk factor between 3 and 5. The average value is 4.03 points. The government has a contribution as a risk factor between 3 and 5. The average value is 4.29 points. The Ministry has a contribution as a risk factor between 3 and 5. The average value is 4.11 points. The local public administration has a contribution as a risk factor between 3 and 5. The average value obtaining 4.28 points. ANSA has a contribution as a risk factor between 3 and 5. The average value obtaining 4.03 points. Other risks of subgroup C.2. Political risks have a contribution as a risk factor between 3 and 5. The average value is 3.88 points.
- subgroup C.2. Political Risk obtained an average rating of 4.05 points. This is easy to see from the risk assessment chart (Figure 1 b).



a) b)

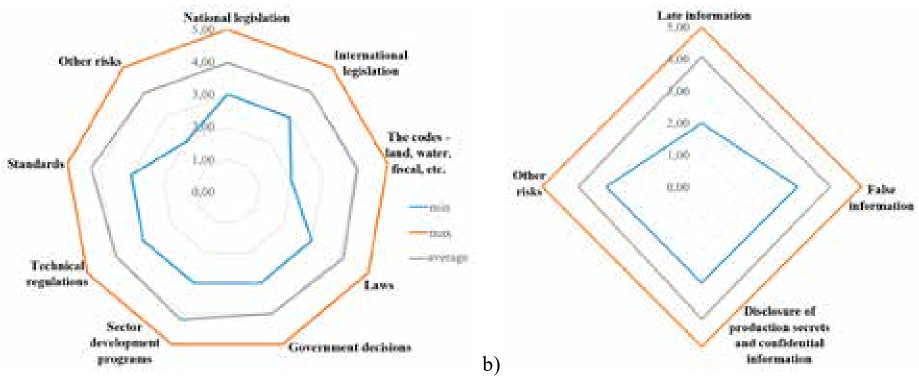
Figure 2. Diagram of the technic and technological (a) and the ecological (b) risks assessment

- in subgroup C.3. Technical and technological risks, the application of PTȘ in the production process has a contribution as a risk factor between 3 and 5. The average value obtaining 4.31 points. Modernization / Renewal of equipment, machinery, equipment, etc. required for the activity has a contribution as a risk factor between 2 and 5. The average value obtaining 4.02 points. The modernization of the applied technology has a contribution as a risk factor between 3 and 5. The average value obtaining 4.14 points. The know-how has a contribution as a risk factor between 2 and 5. The average value obtaining 4.05 points. Land consolidation has a contribution as a risk factor between 3 and 5. The average value is 4.09 points. The lease of the goods necessary for the activity has a contribution as a risk factor between 3 and 5. The average value obtaining 4.19 points. The property has a contribution as a risk factor between 3 and 5. The average value is 4.14 points. Other risks of subgroup C.3. Technical-technological risks have a contribution as a risk factor between 3 and 5. The average value obtaining 4.04 points.

- subgroup C.3. Technical and technological risks obtained an average rating of 4.12 points. This is easy to see from the risk assessment chart (Figure 2 a).

- in subgroup C.4. Ecological risks, Climate change has a contribution as a risk factor between 3 and 5. The average value is 4.24 points. Natural disasters (frost, hail, drought, torrential rains, etc.) have a contribution as a risk factor between 3 and 5. The average value is 4.02 points. Landslides have a contribution as a risk factor between 3 and 5. The average value is 3.98 points. Deforestation has a contribution as a risk factor between 3 and 5. The average value is 3.89 points. The drainage of water basins (lakes, rivers) has a contribution as a risk factor between 3 and 5. The average value is 4.11 points. Other risks of subgroup C.4. Ecological risks have a contribution as a risk factor between 1 and 5. The average value is 4.11 points.

- subgroup C.4. Ecological risks obtained an average rating of 4.06 points. This is easy to see from the risk assessment chart (Figure 2 b)



a) b)

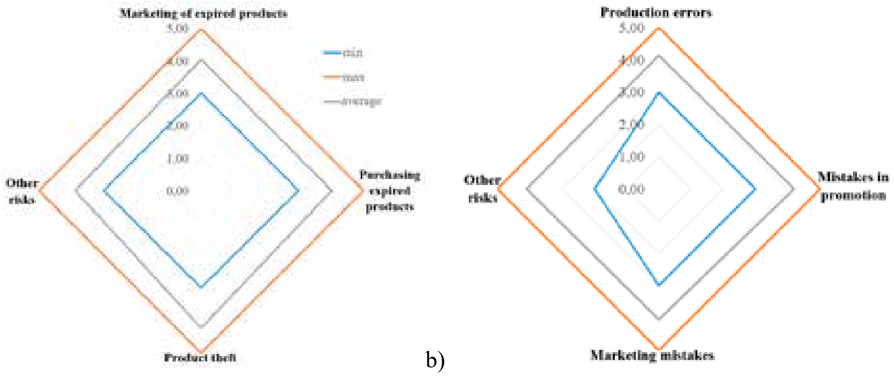
Figure 3. Diagram of the legal (a) and the informational (b) risks assessment

- in subgroup C.5. Legislative-legal risks, the national legislation has a contribution as a risk factor between 3 and 5. The average value obtaining 3.97 points. International law has a contribution as a risk factor between 3 and 5. The average value is 4.02 points. Codes - land, water, tax, etc. has a contribution as a risk factor between 2 and 5. The average value is 4.06 points. Laws have a contribution as a risk factor between 3 and 5. The average value is 4.11 points. GD has a contribution as a risk factor between 3 and 5. The average value is 3.99 points. The development programs of the sector have a contribution as a risk factor between 3 and 5. The average value obtaining 4.2 points. Technical regulations have a contribution as a risk factor between 3 and 5. The average value obtaining 3.98 points. The standards have a contribution as a risk factor between 3 and 5. The average value is 4.26 points. Other risks of subgroup C.5. Legislative-legal risks have a contribution as a risk factor between 2 and 5. The average value obtaining 3.98 points.

- subgroup C.5. Legislative-legal risks obtained an average rating of 4.06 points. This is easy to see from the risk assessment chart (Figure 3 a).

- in subgroup C.6. Information risks, Delayed information has a contribution as a risk factor between 2 and 5. The average value is 4.08 points. False information has a contribution as a risk factor between 3 and 5. The average value is 4.01 points. Disclosure of production secrets and confidential information has a contribution as a risk factor between 3 and 5. The average value is 4.14 points. Other risks of subgroup C.6. Information risks have a contribution as a risk factor between 3 and 5. The average value is 3.86 points.

- subgroup C.6. Information Risk obtained an average rating of 4.02 points. This is easy to see from the risk assessment chart (Figure 3 b).



a) b)

Figure 4. Diagram of the moral (a) and the retrospective (b) risks assessment

- in subgroup C.7. Moral risks, The sale of products, goods and services with expired expiration date, which may cause harm to the consumer has a contribution as a risk factor between 3 and 5. The average value is 4.04 points. Procurement of expired products, goods and services - by false update to the seller, which can cause indirect damage to the consumer has a contribution as a risk factor between 3 and 5. The average value is 4.02 points. Theft of products, goods has a contribution as a risk factor between 3 and 5. The average value obtaining 4.24 points. Other risks of subgroup C.7. Moral risks have a contribution as a risk factor between 3 and 5. The average value is 3.88 points.

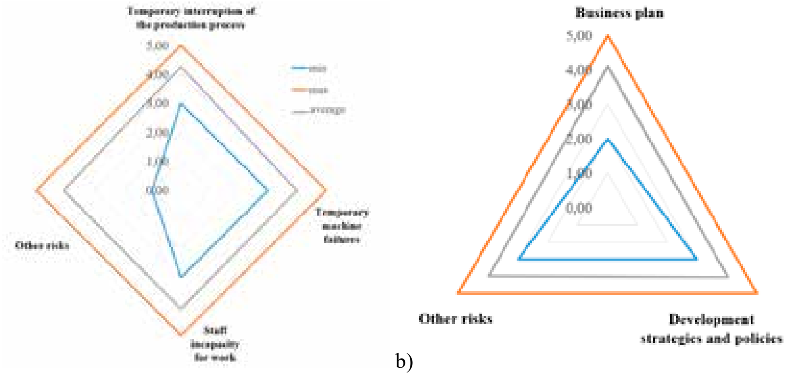
- subgroup C.7. Moral risks obtained an average rating of 4.05 points. This is easy to see from the risk assessment chart (Figure 4 a).

Analyzing the risk group D. The risk group after their occurrence we can mention that:

- in subgroup D.1. Retrospective risks, Mistakes that occurred in the production process - for their analysis has a contribution as a risk factor between 3 and 5. The average value obtaining 4.14 points. Mistakes that occurred in the promotion process - for their analysis has a contribution as a risk factor between 3 and 5. The average value obtaining 4.18 points. Mistakes that occurred in the trading process - for their analysis has a contribution as a risk factor between 3 and 5.

The average value obtaining 4.05 points. Other risks of subgroup D.1. Retrospective risks have a contribution as a risk factor between 2 and 5. The average value obtaining 4.11 points.

- subgroup D.1. Retrospective Risks obtained an average rating of 4.12 points. This is easy to see from the risk assessment chart (Figure 4 b).

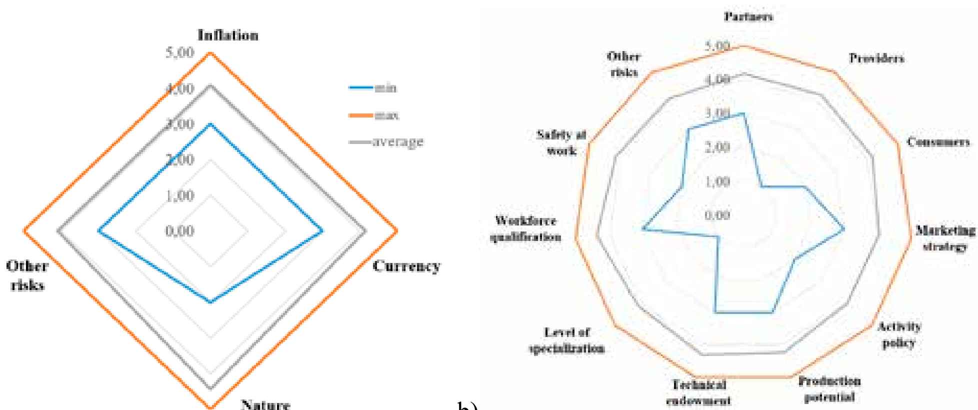


a) b)

Figure 5. Diagram of the current (a) and the prospective (b) risks assessment

- in subgroup D.2. Current risks, The temporary interruption of the production process has a contribution as a risk factor between 3 and 5. The average value obtaining 4.25 points. Temporary damage to the machine has a contribution as a risk factor between 3 and 5. The average value is 3.99 points. The incapacity for work of the staff has a contribution as a risk factor between 3 and 5. The average value obtaining 4.09 points. Other risks of subgroup D.2. Current risks have a contribution as a risk factor between 1 and 5. The average value is 4.05 points.
- subgroup D.2. Current Risks scored an average rating of 4.1 points. This is easy to see from the risk assessment chart (Figure 5 a).

- in subgroup D.3. Prospective risks, The business plan has a contribution as a risk factor between 2 and 5. The average value obtaining 4.11 points. Development strategies and policies have a contribution as a risk factor between 3 and 5. The average value obtaining 4.05 points. Other risks of subgroup D.3. Prospective risks have a contribution as a risk factor between 3 and 5. The average value is 3.99 points.
- subgroup D.3. Perspective Risk obtained an average rating of 4.05 points. This is easy to see from the risk assessment chart (Figure 5 b).



a) b)

Figure 6. Diagram of the external (a) and the internal (b) risks assessment

Analyzing the risk group E. The risk group according to the nature of the record we can mention that:

- in subgroup E.1. External risks, Inflation has a contribution as a risk factor between 3 and 5. The average value is 4.09 points. The currency has a contribution as a risk factor between 3 and 5. The average value is 4.14 points. Nature has a contribution as a risk factor between 2 and 5. The average value is 4.43 points. Other risks of subgroup E.1. External risks have a contribution as a risk factor between 3 and 5. The average value obtaining 4.09 points.
- subgroup E.1. External risks obtained an average rating of 4.19 points. This is easy to see from the risk assessment chart (Figure 6 a).
- in subgroup E.2. Internal risks, The partners have a contribution as a risk factor between 3 and 5. The average value obtaining 4.17 points. The suppliers have a contribution as a risk factor between 1 and 5. The average value is 4.21 points. Consumers have a contribution as a risk factor between 2 and 5. The average value is 4.16 points. The marketing strategy has a contribution as a risk factor between 3 and 5. The average value obtaining 4.04 points. The activity policy has a contribution as a risk factor between 2 and 5. The average value obtaining 4.03 points. The production potential has a contribution as a risk factor between 3 and 5. The average value is 4.23 points. The technical endowment has a contribution as a risk factor between 3 and 5. The average value obtaining 4.31 points. The level of specialization has a contribution as a risk factor between 1 and 5. The average value obtaining 4.1 points. The qualification of the labor force has a contribution as a risk factor between 3 and 5. The average value obtaining 4.38 points. The security technique has a contribution as a risk factor between 2 and 5. The average value obtaining 4.15 points. Other risks of subgroup E.2. Internal risks have a contribution as a risk factor between 3 and 5. The average value is 4.08 points.
- subgroup E.2. Internal Risk obtained an average rating of 4.17 points. This is easy to see from the risk assessment chart (Figure 6 b).

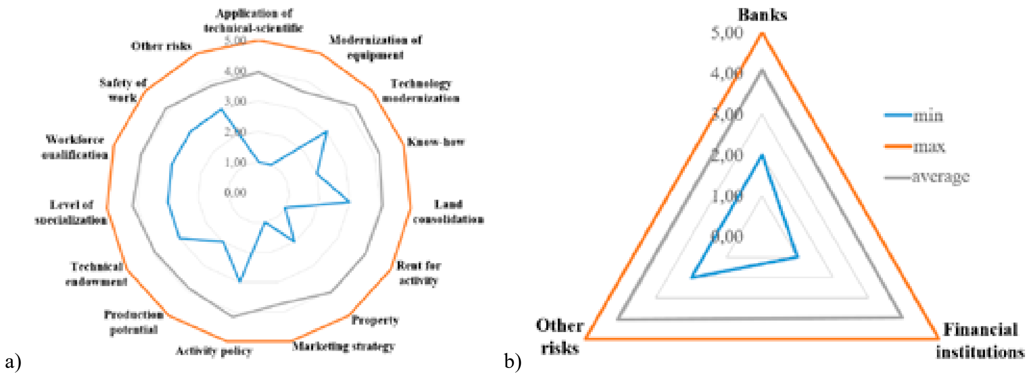


Figure 7. Diagram of the production (a) and the financial activity (b) risks assessment

Analyzing the risk group F. The risk group by the sphere of appearance on which the fields of entrepreneurial activity are based, we can mention that:

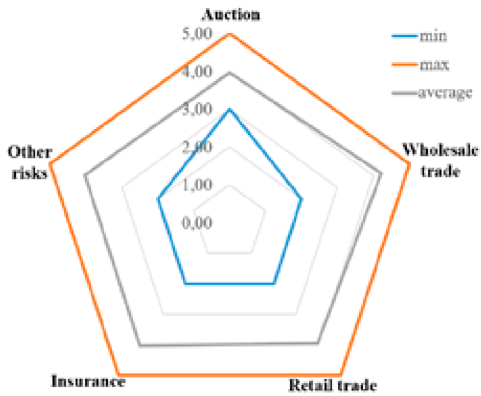
- in subgroup F.1. Risks in the field of production, The application of PTȘ in the production process has a contribution as a risk factor between 1 and 5. The average value obtaining 3.94 points. Modernization / Renewal of equipment, machinery, equipment, etc. required for the activity has a contribution as a risk factor between 1 and 5. The average value obtaining 3.62 points. The modernization of the applied technology has a contribution as a risk factor between 3 and 5. The average value obtaining 4.27 points. The know-how has a contribution as a risk factor between 2 and 5. The average value is 4.15 points. Land consolidation has a contribution as a risk factor between 3 and 5. The average value is 4.08 points. The lease of the necessary assets for the activity has a contribution as a risk factor between 1 and 5. The average value obtaining 4.04 points. The property has a contribution as a risk factor between 2 and 5. The average value is 4.06 points. The marketing strategy has a contribution as a risk factor between 1 and 5. The average value is 3.72 points. The activity policy has a contribution as a risk factor between 3 and 5. The average value obtaining 4.17 points. The production potential has a contribution as a risk factor between 2 and 5. The average value is 3.89 points. The technical endowment has a contribution as a risk factor between 3 and 5. The average value obtaining 3.93 points. The level of specialization has a contribution as a risk factor between 3 and 5. The average value obtaining 4.15 points. The qualification of the labor force has a contribution as a risk factor between 3 and 5. The average value obtaining 4.05 points. The security technique has a contribution as a risk factor between 3 and 5. The average value obtaining 4.12 points. Other risks of the subgroup have a contribution as a risk factor between 3 and 5. The average value is 3.84 points.

- subgroup F.1. Risks in the field of production obtained an average rating of 4 points. This is easy to see from the risk assessment chart (Figure 7 a).

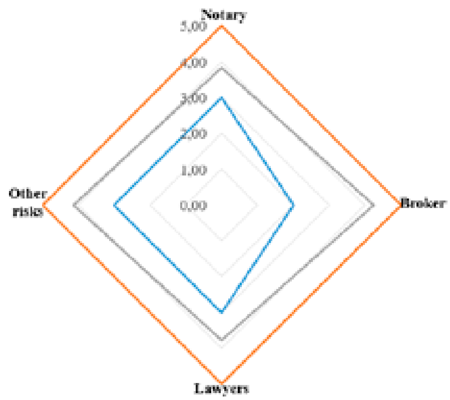
A special situation is if we compare the above results. Most agri-food entities mention that there are different classification criteria, even if it is the same indicator. Therefore, are different results.

- in subgroup F.2. Risks in financial activity, Banks have a contribution as a risk factor between 2 and 5. The average value obtaining 4.08 points. Financial institutions have a contribution as a risk factor between 1 and 5. The average value is 3.96 points. Other risks of the subgroup have a contribution as a risk factor between 2 and 5. The average value is 4.08 points.

- subgroup F.2. Risks from financial activity obtained an average rating of 4.04 points. This is easy to see from the risk assessment chart (Figure 7 b).



a)



b)

Figure 8. Diagram of the commercial sphere (a) and the intermediate activity (b) risks assessment

- in subgroup F.3. Risks in the commercial sphere. The auction has a contribution as a risk factor between 3 and 5. The average value obtaining 3.97 points. The wholesale trade has a contribution as a risk factor between 2 and 5. The average value is 4.22 points. Retail trade has a contribution as a risk factor between 2 and 5. The average value is 3.96 points. The insurance has a contribution as a risk factor between 2 and 5. The average value obtaining 4.04 points. Other risks of the subgroup have a contribution as a risk factor between 2 and 5. The average value is 4.04 points.

- subgroup F.3. Risks in the commercial sphere obtained an average rating of 4.05 points. This is easy to see from the risk assessment chart (Figure 8 a).

- in subgroup F.4. Risks from the intermediate activity, Notary has a contribution as a risk factor between 3 and 5. The average value obtaining 3.83 points. The broker has a contribution as a risk factor between 2 and 5. The average value is 4.24 points. Lawyers have a contribution as a risk factor between 3 and 5. The average value is 3.78 points. Other risks of subgroup F.4. Risks in the intermediate activity

have a contribution as a risk factor between 3 and 5. The average value obtaining 4.14 points.
- subgroup F.4. Risks from the intermediate activity obtained an average rating of 4 points. This is easy to see from the risk assessment chart (Figure 8 b).

CONCLUSIONS

The agri-food sector is a difficult one. Its development has recently been influenced by a number of factors - objective and subjective.

The predominantly foreign agri-food trade is oriented towards Russia, especially grapes and wine production. Since 2006, it has been influenced by global political relations, creating a negative impact through the embargo on Moldovan production.

The COVID-19 pandemic had a particularly negative influence. At the same time, it has favored the food industry and trade in favor of online activities.

The study allowed us to identify the weaknesses and strengths in the activity of entities in the agri-food sector, especially the wine sector.

This study is a pretext to propose some changes in legislation, tax system, subsidies, etc. in order to stimulate the successful activity of the wine-producing entities, and to reduce the risk of bankruptcy.

ACKNOWLEDGEMENTS

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MATHEMATICAL APPROACH TO EVALUATION OF THE INFLUENCE OF CLIMATE INDICATORS ON QUALITY OF GRAPES IN SYRAH CLONES

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Abstract

The aim of the present study is to apply a mathematical approach (correlation and factor analysis) to assess the similarity and remoteness of the impact of climate indicators in some clones of Syrah variety. Their grouping is based on phenological, and technological indicators. Temperatures during the individual experimental years have a dominant influence on the quality of grapes in the individual clones included in the study. As a result of analysis, correlations were established between phenological indicators like follow: sap, bud burst, first leaf separation, flowering, fruit set, veraison, ripeness and technological ones - average mass per bunch, normal and undeveloped berries, percent of clusters and damaged berries, average bunch size (width and length). The phenological indicators - sap flow, flowering and fruit set "pea size" and technological - undeveloped berries, damaged berries have high factor weights in the first component, which is a summary of these indicators, with the highest relative weight in the vines grouping.

Key words*: Syrah clones, phenology, quality components, correlation, factor analysis.*

INTRODUCTION

Climate represents one of the main inputs necessary for plants to complete their growing cycle, having a direct impact on the set and duration of phenological stages and development of crops.

The effects of climate on crop phenology (bud burst, flowering, fruit set and harvest time) were investigated by means of regression analysis.

Meteorological information has a significant effect on the set of the grapevine phenological stages (Marta et al., 2010; Kizildeniz et al., 2015).

An accurate model for estimating the timing of seasonal phenological stages of the grapevine (*Vitis vinifera* L.) would be a valuable tool for crop management (Schrader et al., 2020).

Unfavourable trends have been identified in the evolution of climate factors (temperatures, precipitation, etc.) over the past years, with a direct impact on the vegetative and productive potential of the vine. This calls for a reassessment of climate resources and adaptation of the cultivation technologies to the new conditions.

The tendency for increasing the average annual temperature and decreasing precipitation amounts is a point to a marked warming of the vineyard climate, especially after 2000.

High temperatures, corroborated with soil water deficit, determined an intensification of the atmospheric and pedological drought, a shift in vegetation stages, shortened development periods and a forced berry ripening, with a negative impact on yields, which fluctuated from one year to another (Zaldea et al., 2021).

Grapevine yield in is determined by the cluster numbers and their weight. Number of clusters per vine, is determined by the number of canes, and cane productivity is measured by the cluster-cane ratio. Earlier studies have revealed that increase in the number of canes does not result in proportionate increase in cluster number per vine (Shikhamany et al., 2015).

Ambient radiation and temperature are global factors of grapevine growth, yield and composition, and wine quality. Knowledge of the implications of vineyard row orientation, microclimate is required for taking a decision in current and future macro and meso climate conditions (Hunter et al., 2021).

In particular, temperature is a main factor in controlling grapevine phenological development and ripening. Phenology models have been developed for a wide range of

species, including grapevines, and using observational data from many different countries and regions.
Several previous studies have been performed in order to create a model for the phenological stages, e.g., enabling a classification of varieties for technical purposes, to predict phenology or to assess the impact of climate change on it (Costa et al., 2019).
The global climate change consequences have appeared during the last decades, with increasing weather variability in many world regions. The use of aerobiological and meteorological studies for crop yield prediction has been widely used in different crops that are important engines for economy development. This enables growers to adapt their crop management and adjust the spent resources (González-Fernández et al., 2020).
The aim of the present study is to establish the correlation between the studied indicators, using the possibilities of factor analysis, to reduce their number by combining those that correlate with each other in new factors.

MATERIALS AND METHODS

The field experiment was conducted in the training and experimental station of the Agricultural University - Plovdiv, using two-year data (2020, 2021).
The scheme of the experimental work includes the following variants of research with four branches of the Syrah variety (Figures 1-4):

Figure 1. V1 - Syrah variety, clone 100

Figure 2. V2 - Syrah variety, clone 174

Figure 3. V3 - Syrah variety, clone 470

Figure 4. V4 - Syrah variety, clone 524

Sixty vines (4 repetitions x 15 vines) are included in each variant. All Syrah clones are grafted on Berlandieri x Riparia SO4 rootstock. The bud loading of the vines in all variants is ensured by a short pruning system, using spurs with two buds, a total of 6 spurs (12 buds per vine).
The experiment is based on the grouping of phenological indicators (sap movement - x_1, bud burst - x_2, first leaf - x_3, flowering - x_4, fruit set to "pea size" -x_5, veraison -x_6 and technological ripeness-x_7), and technological indicators of grapes (normal berries-%, bunches-%, undeveloped berries-%, damaged berries%, average sizes of one grape bunch, length and width in cm).
Phenological observations were made for each of the four clones numbered 100, 174, 470, and 524 of the Syrah variety for each development stage. For this purpose, normally developed vines that have entered full fruiting have been selected. The beginning of the stage is the day when 5% of the vines entered it, the mass entry - 50% of the vines, and the end when 95% of the vines entered the separate stage (Braykov et al., 2005; Roytchev 2012).
The duration in days of the main phenological periods was reported: from sap movement to bud burst, from budding to the appearance of the first leaf, from the first leaf to flowering, from flowering to the "pea" phase, from "pea" to layering, from layering to technological maturity and from bud burst to technological ripeness.
The air temperature data were taken from the meteorological station located in the experimental vineyard of the Agricultural University. The evaluation of the tested regimes is based on the phenological and technological indicators of the grape performed by correlation analysis (Lakin, 1990), aiming to establish the presence of statistically significant correlations between the studied indicators. In the next stage, the study was continued, applying the technique of factor analysis (Kline, 1994) in order to reduce the number of initially included indicators.
Factor analysis was performed by the principal components method (PCA). The number of principal components is determined by the number of eigenvalues of the correlation matrix that are greater than 1 (Kaiser's criteria). Eigenvalues show the contribution of the eigenfactor in explaining the total variance in the variables.
Adequacy assessment of the factor analysis was performed by using the Kaiser – Mayer – Olkin (CMO – test) and Bartlett tests.
A similar approach has been used in the evaluation of wheat, cotton and tomatoes (Ganchev, et al., 2019; Gospodinova, et al., 2020; Stoyanova, et al., 2019). The statistical program SPSS 26.0 was used for processing the experimental data.

RESULTS AND DISCUSSIONS

The agro-meteorological conditions for the considered years are quite close (Figures 5, 6 and 7).

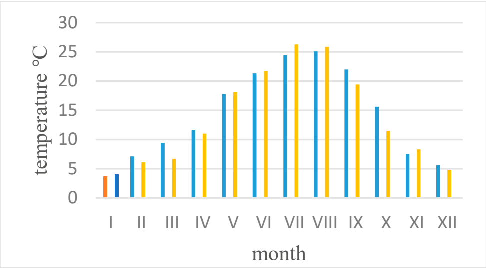

Figure 5. Average monthly air temperature in ˚C

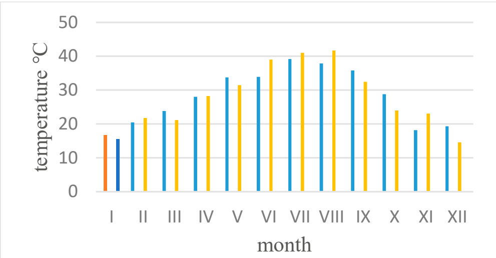

Figure 6. Average monthly maximum air temperature in ˚C

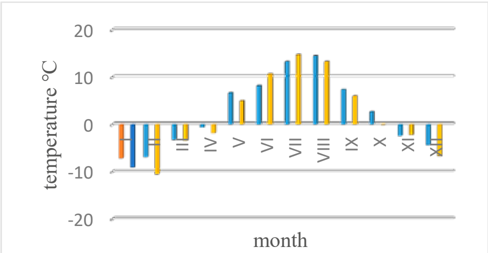

Figure 7. Average monthly minimum air temperature in ˚C

Correlation analysis are used to describe the strength and direction of dependence between the indicators under consideration. The correlation coefficients expressing the relationship between the studied phenological indicators, which are indicated in the correlation matrix (Table 1).

Table 1. Correlation matrix of the phenological indicators

	x1	x2	x3	x4	x5	x6	x7
x1	1	-0,223	0,445	-0,884**	0,942**	0,501	0,534
x2		1	0,748*	-0,228	0,118	0,729*	0,705
x3			1	-0,734*	0,713*	0,957**	0,980**
x4				1	-0,977**	-0,830*	-0,832*
x5					1	0,761*	0,787*
x6						1	0,993**
x7							1

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

A strong positive correlation was found between first leaf separation (x_3) and veraison (x_6), technological ripeness (x_7) with correlation coefficients (r = 0.957, r = 0.980), as well as between the indicators: sap (x_1) and fruit set “pea size” (x_5) - r = 0.942.
The strongest is the relationship between the indicators veraison (x_6) and technological ripeness (x_7) with a correlation coefficient r = 0.993. Negative correlation is reported between the indicators of flowering (x_4) and fruit set "pea size" (x_5), veraison (x6) and technological ripeness (x_7), respectively, with coefficients r = - 0.977; r = - 0.830; r = - 0.832.
All these correlation are statistically proven at the level of significance $\alpha = 0.001$.
The performed correlation analysis and the established high, statistically proven values of ‘r’ give us a reason to apply the methodology of factor analysis.
When performing factor analysis, applying the method of principal components, it turns out that two factors have values of eigenvectors greater than 1, which determined the choice of two principal components (Figures 8 and 9).
Table 2 shows the factor weights and the distribution of variation between the main components of phenological indicators.
They explain 98.9% of the total variance of the sample. The first main component (the first factor) explains 74.45% of the variance and the second - 24.45% of it.

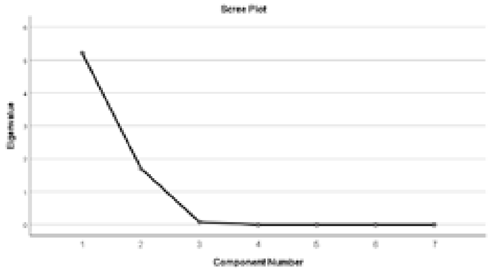

Figure 8. Values of eigenvectors of phenological indicators

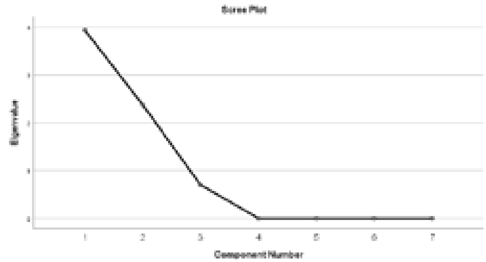

Figure 9. Values of eigenvectors of technological indicators

Table 2. Factor matrix of phenological indicators obtained by the principal components method

N	Indicators	Main components		Signes
		1	2	
1	SAP	0.999	-0.021	x_1
2	Bud burst	-0.200	0.978	x_2
3	First leaf	0.455	0.869	x_3
4	Flowering	-0.900	-0.408	x_4
5	Fruit set „PEA SIZE“	0.948	0.316	x_5
6	Veraison	0.523	0.847	x_6
7	Technological ripeness	0.551	0.834	x_7
Percentage of the total variation, %		74.45	24.45	
Cumulative percentage of the total variation, %		74.45	98.90	

The indicators of SAP, flowering and fruit set "pea size" have high factor weights in the first component. We could define this factor as a summary for those indicators that have the greatest relative weighting grouping.
The second component is mainly related to budding, the appearance of the first leaf, veraison and technological ripeness.
Depending on the technological indicators for grape structure in the studied Syrah clones, the

correlation relationships are less pronounced than in the phenological ones (Table 3).
A positive correlation was found between the indicators of damaged berries and the width with a correlation coefficient r = 0.999; average mass and length, r = 0.954.
Strong negative dependence is observed between the indicators normal berries and bunches r = -0.999, as well as between the indicators undeveloped berris and cluster length r = -0.965.

Table 3. Factor matrix of technological indicators, obtained by the method of the main components

	Average mass, g	Normal berries, numbers	Bunches numbers	Undeve-loped berries, numbers	Damaged berries, numbers	Width, cm	Length, cm
Average mass	1	0,477	-0,505	-0,847	-0,556	0,954*	-0,565
Normal berries		1	-0,999**	0,029	0,201	0,231	0,172
Clusters			1	0,006	-0,189	-0,265	-0,160
Undeve-loped berries				1	0,607	-0,965*	0,598
Damaged berries					1	-0,565	0,999**
Width						1	-0,565
Length							1

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

Table 4 shows the factor weights and the distribution of variation between the main components of the technological indicators.

Table 4. Correlation matrix of technological indicators

N	Indicators	Main components	
		1	2
1	Average mass per bunch, g	-0.847	0.524
2	Normal berries, %	0.018	0.976
3	Clusters, %	0.009	-0.982
4	Undeveloped berries, %	0.910	-0.077
5	Damaged berries, %	0.871	0.279
6	Average bunch size, (width) cm	-0.895	0.321
7	Average bunch size, (length) cm	0.868	0.254
Percentage of the total variation,%		56.20	33.86
Cumulative percentage of the total variation, %		56.20	90.05

They explain 90.05% of the total variance of the sample. The first main component (the first factor) explains 56.20% of the variance, and the second, respectively, 33.86% of it.
Indicators underdeveloped berries, damaged berries have high positive factor weights in the first component.
We could define this factor as a summary for those indicators that have the greatest relative weight in the vineyards grouping. The second component is mainly related to normal grains and bunches.

CONCLUSIONS

The conducted correlation and factor analysis give us a reason to determine the presence of two main factors in the grouping of vineyards - phenology and technological indicators characterizing the grape quality. The strong positive correlation between the stages of separation of the first leaf, veraison and technological ripeness, respectively, with correlation coefficients r = 0.957; r = 0.980; r = 0.993, gives us a reason to say that even the slightest change in environmental factors would affect the quality of grapes. The phenological indicators of sap flow, flowering and fruit set "pea size" and technological - undeveloped berries, damaged berries have high factor weights in the first component, which is a summary of these indicators, with the highest relative weight in the grouping of vines. The classification of the included options allows to increase the objectivity in assessing the impact of the studied indicators. The results of the factor analysis allow more efficient planning of the experiment.

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RESEARCH ON THE AGROBIOLOGICAL AND TECHNOLOGICAL POTENTIAL OF SOME HYBRID ELITE WITH BIOLOGICAL RESISTANCE OBTAINED AT R.D.S.V.V. ODOBEŞTI

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Abstract

The practice of sustainable viticulture by reducing the quantities of pesticides applied by phytosanitary treatments and capitalizing on the ecopedoclimatic conditions specific to each wine-growing area, involves obtaining and promoting vine varieties with complex biological resistance, with high potential for adaptation to changes caused by climate change and valuable agrobiological and technological potential. In response to this, at R.D.S.V.V. Odobeşti were studied in order to evaluate the agrobiological and technological potential three hybrid elites with biological resistance: hybrid elite 10-1-6 (Traminer roz x Isabella), hybrid elite 2-5 (Galbenă de Odobeşti x Lydia) and hybrid elite 10-18 (Riesling italian x Siebel 6720). This paper presents the ampelographic description and the agrobiological and technological potential of the elites studied during the years 2020-2021. The results obtained from this study showed that these hybrid elites have high productive and qualitative potential, show high biological resistance to the main diseases of the vine, and can be proposed for approval in order to improve the national assortment, in the context of sustainable viticulture

Key words: *agrobiological and technological potential, biological resistance, hybrid elite.*

INTRODUCTION

Practicing sustainable viticulture in tandem with environmental and health concerns is currently one of the most important goals of the wine world. That also involves the diversification of genetic resources by creating new varieties has an important role in protecting the environment, being known that native varieties have genes with tolerance and resistance to disease and pests, ensuring its sustainable development at national, regional and local level (Fregoni, 1998, Mestre et al., 2018, De Nardi et al., 2019, Giacomelli et al., 2019). Community regulations only recognize grape varieties of the European species *Vitis vinifera* L. for the production of wines with a name, while grape varieties resistant to disease come from crosses between *Vitis vinifera* L. and other *Vitis* species (American and/or Asian, which carries resistance genes). In this context, the research programs in renowned wine research centers in Europe and America have resulted in the creation of new varieties to control disease and weather challenges (Ilnitskaya et al., 2019, Ollat et al., 2019, Guimier et al., 2019). The varieties that emerge from these programs are typically crosses between so-called European vinifera, and others native to North America and Asia, like *V. riparia*, *V. labrusca* and *V. rotondifolia.*
Moreover, various professional associations of winegrowers and winemakers in both Europe and America have also considered the so-called "return of hybrids".
To control the cryptogamic diseases in vines is currently based on the application of chemical treatments. The most promising option to reduce the need for fungicides in viticulture is the use of resistant cultivars (Riaz et al., 2019, Bavaresco, 2019). This is why a new breeding programme called INRA-ResDur was launched in 2000 to create cultivars with durable resistance to downy and powdery mildews and with berry quality suitable for the production of high-quality wines (Merdinoglu D., 2018, Schneider C. et al., 2019). Thus, developed by the French National Research Institute for

Agriculture and the Environment (INRAE), the first four French vine varieties resistant to fungal diseases (Artaban, Floreal, Vidoc and Voltis) have been affiliated to the *Vitis vinifera* L. botanical taxon by the Community Plant Variety Office (CPVO).
In this context, the research conducted in the last four decades in our country has led to the creation of several genotypes of vines with increased tolerance to disease and resistance to stressors (frost, drought), and the results were expressed by creating a base with genetic material valuable and homologation of many varieties (Damian et al., 2012, Culcea et al., 2004, Pușcalău et al., 2018, Bosoi and Pușcalău, 2020).
Thus, this article presents a study on the agrobiological and technological potential of three hybrid elites with biological resistance obtained at SCDVV Odobești.

MATERIALS AND METHODS

The research was carried out in a plantation of over 30 years established on a leached chernozem type soil, located in the biological field of the Research and Development Station for Viticulture and Vinification (RDSVO) Odobești, with geographical coordinates 45º45′ north latitude, 27º06′ east length, and an altitude of 150 m. The study was conducted during the years 2020-2021.
The biological material was represented by three hybrid elites with biological resistance: H.E. 10-1-6 (Traminer roz x Isabelle), H.E. 2-5 (Galbenă de Odobeşti x Lydia) and H.E. 10-18 (Riesling italian x Siebel 6720). Each genotype was represented in this study by three replications with 5 vines each. The hibrid elites studied were grafted on to the rootstock Kobber 5 BB. The pruning system practiced was the Dr. Guyot system, with a fruit load between 38-44 buds/vine, distributed on 8-9 buds per fruiting cane with 2 buds per spur, and a semi-high driving shape. For control the pathogens (*Plasmopara viticola, Uncinula necator, Botrytis cinerea*, etc.), were applied six phytosanitary treatments.
The three hybrid elites studied were characterized ampelographically and the phenological spectrum was monitored. In order to establish the agrobiological potential, observations and determinations were made regarding: the vigor of growth by measurements of the shoots during the period of intense growth; elements of fertility and productivity by calculating the percentage of fertile shoots (FS%), fertility coefficients (Cfa and Cfr) and productivity indices (Ipa and Ipr); the behavior to the main diseases of the vine and stressors (frost, drought) by grading from 1 to 9 depending on the scale of resistance developed by the OIV (2009). To determine the technological potential, determinations were made regarding the quantity (kg/vine, t/ha) and the quality of grape production (g/l sugars, g/l H_2SO_4 acidity). The results were statistically interpreted using the FoxPro Monofactorial 2.0 software, by analyzing the variance, using the average of the three hybrid elites as control.
Climatic data for the study period were provided by the AgroExpert weather station and the multiannual climate database of the R.D.S.V.V. Odobești.

RESULTS AND DISCUSSIONS

Climatic conditions. The evolution of the thermal regime and of the precipitations in the viticultural ecosystem of the Odobești vineyard during the study is presented in Table 1. Global warming, a phenomenon that has strongly characterized the last decades, has considerably influenced the evolution of the annual thermal regime and during the growing season. From a thermal point of view, the two years of study were different, 2020 being considered one of the driest years. The values recorded in 2020 for the average air temperature during the vegetation period (19.7°C), respectively the active thermal balance (3543.5°C) and the useful thermal balance (1793.4°C) were much higher than the multiannual values for this period. The values recorded for the minimum average (13.3°C) and the maximum average (27.0°C) air temperatures were much higher than the multiannual values for these elements (8.2°C, respectively 24.5°C).
Precipitation regime. The precipitation regime was deficient during the growing season, the amount of precipitations recorded during this period, respectively 218.6 mm in 2020 and 289.6 mm in 2021 representing 56%, respectively 74% of the multiannual value for

this period (391.3 mm). In the two years of study, except for June, which recorded an amount of precipitation higher than the multiannual value, the months of April, May, July, August and September had values lower than the multiannual values for these months.

Table 1. The climatic conditions during the growing season (SCDVV Odobeşti, 2020 - 2021)

<table>
<tr><th rowspan="3">Month</th><th colspan="3">Average temperatures (ºC)</th><th colspan="4">Extreme temperatures (ºC)</th><th colspan="4">Temperatures sum -Σºt (ºC)</th><th colspan="3">Rainfall sum (mm)</th></tr>
<tr><th rowspan="2">multi-annual</th><th rowspan="2">2020</th><th rowspan="2">2021</th><th colspan="2">2020</th><th colspan="2">2021</th><th colspan="2">2020</th><th colspan="2">2021</th><th rowspan="2">multi-annual</th><th rowspan="2">2020</th><th rowspan="2">2021</th></tr>
<tr><th>min.</th><th>max.</th><th>min.</th><th>max.</th><th>active (Σºta)</th><th>useful (Σºtu)</th><th>active (Σºta)</th><th>useful (Σºtu)</th></tr>
<tr><td>April</td><td>11.2</td><td>12.2</td><td>8.9</td><td>5.3</td><td>19.2</td><td>3.8</td><td>14.5</td><td>302.2</td><td>82.1</td><td>105.3</td><td>15.3</td><td>49.1</td><td>5.2</td><td>41.0</td></tr>
<tr><td>May</td><td>16.8</td><td>15.8</td><td>16.5</td><td>10.0</td><td>22.7</td><td>10.7</td><td>22.7</td><td>489.6</td><td>179.6</td><td>510.5</td><td>200.5</td><td>73.4</td><td>55.6</td><td>22.8</td></tr>
<tr><td>June</td><td>20.2</td><td>21.8</td><td>20.3</td><td>15.3</td><td>29.4</td><td>15.6</td><td>26.2</td><td>653.3</td><td>353.3</td><td>609.0</td><td>309.0</td><td>85.2</td><td>85.2</td><td>134.6</td></tr>
<tr><td>July</td><td>22.1</td><td>23.5</td><td>24.3</td><td>17.0</td><td>31.2</td><td>18.4</td><td>31.3</td><td>729.1</td><td>419.1</td><td>753.5</td><td>443.5</td><td>77.9</td><td>28.2</td><td>40.0</td></tr>
<tr><td>Aug.</td><td>21.7</td><td>24.3</td><td>22.9</td><td>17.6</td><td>31.8</td><td>16.6</td><td>30.4</td><td>751.8</td><td>441.8</td><td>708.6</td><td>398.6</td><td>59.7</td><td>13.0</td><td>45.0</td></tr>
<tr><td>Sept.</td><td>17.1</td><td>20.6</td><td>16.7</td><td>14.6</td><td>27.7</td><td>11.0</td><td>23.9</td><td>617.5</td><td>317.5</td><td>499.8</td><td>199.8</td><td>45.7</td><td>31.4</td><td>6.2</td></tr>
<tr><td>Average/ sum</td><td>18.2</td><td>19.7</td><td>18.3</td><td>13.3</td><td>27.0</td><td>12.7</td><td>24.8</td><td>3543.5</td><td>1793.4</td><td>3186.7</td><td>1566.7</td><td>391.3</td><td>218.6</td><td>289.6</td></tr>
</table>

Ampelograpfic characterization.

Hybrid elite 10-1-6. At budding, the rosette is whitish to fluffy, whitish green (Figure 1). The shoot is glabrous and has a slightly intense anthocyanin coloration on the sunny side.

The adult leaf is medium in size, intensely green, pentagonal to orbicular, pentalobate (Figure 2). The upper lateral sinuses are closed with slightly overlapping lobes, rarely open, in the shape of a lyre with a rounded base, and the lower sinuses completely open. The petiolar sinus is closed, V-shaped. The flower is a normal hermaphrodite, on type 5.

The grapes are of medium to high size (254 g), have a conical shape, rarely cylindrical, with an average of two wings, with dense berry, very little mobile (Figure 3). The berries are medium in size, spherical in shape, with a more intense pink skin on the sunny side. The pulp is not anthocyanin in color, it is juicy, with low firmnes.

a) b) c)

Figure 1. Hybrid elite 10-1-6:
a) budding; b) rosette; c) shoot tip

a) b) c)

Figure 2. Hybrid elite 10-1-6: a) mature leaf - upper side; b) mature leaf - lower side; c) inflorescence

Figure 3. Hybrid elite 10-1-6 (grape, berry)

Hybrid elite 10-18. At budding, the rosette is with prostrate hairs, green with a slight anthocyanin coloration (Figure 4). The shoot is glabrous, green, with a faint anthocyanin coloration on the sunny side. The mature leaf is medium in size, wedge-shaped to pentagonal, pentalobate (Figure 5). The upper lateral sinuses are closed with slightly overlapping, lyre-shaped lobes with a sharp base, and the lower sinuses completely open. The petiolar sinus is open, sometimes slightly closed, V-shaped. The flower is a normal hermaphrodite, on type 5. The grapes are of medium size (189 g), have a conical shape, rarely cylindrical with dense and semi-mobile berry. The grains are medium in size, spherical in shape, with greenish-yellow skin, with rust spots on the sunny side. The pulp does not show anthocyanin coloration, it is juicy, soft to slightly firm (Figure 6).

a) b) c)

Figure 4. Hybrid elite 10-18: a) budding: b) rosette; c) shoot tip

a) b) c)

Figure 5. Hybrid elite 10-18: a) mature leaf - upper side; b) mature leaf - lower side; c) inflorescence

Figure 6. Hybrid elite 10-18 (grape, berry)

Hybrid elite 2-5. At budding, the rosette is with prostrate hairs, white-green with a slight anthocyanin coloration. The shoot is green with red streaks on the sunny side, with slight traces of lint (Figure 7).

The adult leaf is medium to large, dark green, wedge-shaped to pentagonal, trilobate, rarely pentalobate, with high perosity on the underside. The upper lateral sinuses are closed with slightly overlapping, lyre-shaped lobes with a slightly rounded base, and the lower sinuses are slightly sketched. The petiolar sinus is closed, V-shaped, with overlapping lobes. The flower is a normal hermaphrodite, on type 5 (Figure 8).

The grapes are of medium size (192 g), have a conical or cylindrical-conical shape, with dense and semi-mobile berry. The berries are medium in size, spherical in shape, with yellow-green skin, more intense on the sunny side. The pulp is not anthocyanin in color, it is juicy and soft to slightly firm (Figure 9).

a) b) c)

Figure 7. Hybrid elite 2-5: a) budding; b) rosette; c) shoot tip

a) b) c)

Figure 8. Hybrid elite 2-5: a) mature leaf - upper side; b) mature leaf - lower side; c) inflorescence

Figure 9. Hybrid elite 2-5 (grape, berry)

Phenological spectrum. Under the climatic conditions of the growing season 2020, the hybrid elites taken into the study the buds break has begun April 13-14 (Table 2). In 2021, due to the low temperatures recorded in April, the start of vegetation of the vine was delayed by more than two weeks, the phenophase of the buds break being recorded between April 29 and May 2. The earliest proved to be the 10-1-6 hybrid elite.

The flowering phase took place between June 3-5, in 2021, and about a week later, in 2021. The earliest proved to be the 2-5 hybrid elite. Determined by the climatic conditions during the vegetation period in 2020, the veraison was recorded early (between 2 and 6 August) and more than a week later in 2021 (between 9 and 15 August). The earliest proved to be the 10-18 hybrid elite.

The full ripening of the grapes took place in the second decade of September (10-5 IX) in 2020 and later 5-7 days in 2021 (16-20 IX). The hybrid elite 10-18 proved to be the earliest, and the hybrid elite 2-5 the latest.

Table 2. The phenological spectrum (Odobeşti, 2020-2021)

Hybrid elite		H.E. 10-1-6		H.E. 10-18		H.E. 2-5	
		2020	2021	2020	2021	2020	2021
Phenological phase	Disbudding	13. IV	29. IV	14. IV	01. V	13. IV	02. V
	Flowering	04. VI	11. VI	03. VI	10. VI	05. VI	15. VI
	Veraison	06. VIII	15. VIII	02. VIII	09. VIII	04. VIII	12. VIII
	Physiological maturity	11. IX	18. IX	10. IX	16. IX	15. IX	20. IX
Vegetation period active		IV-IX	IV-IX	IV-IX	V-IX	IV-IX	V-IX

Fertility and productivity characteristics. The fertility and productivity characteristics of the studied hybrid elites were assessed by the percentage of fertile shoots, fertility coefficients (absolute and relative) and productivity indices (absolute and relative). The average values recorded for the percentage of fertile shoots varied between 65.8% in the hybrid elite 10-1-6 and 74.6%, respectively 74% in the hybrid elite 10-18, respectively the hybrid elite 2-5 (Table 3). The fertility coefficients (absolute and relative) recorded higher values in the hybrid elite 10-18 (Cfa - 1.08; Cfr - 1.46) and lower in the hybrid elite 10-1-6 (Cfa 0.82; Cfr 1.24). Determined by the average weight of the grapes (245.3 g), the hybrid elite 10-1-6 recorded the highest values of the productivity indices.

Table 3. The fertility and productivity characteristics (Odobeşti, 2020-2021)

Hybrid elite		Fertile shoots (%)	Fertility coefficients		Average weight of a bunch (g)	Productivity indices	
			Cfa	Cfr		Ipr	Ipa
H.E.10-1-6	2020	61.7	0.75	1.20	254.0	191	310
	2021	69.9	0.89	1.27	236.5	210	300
Average		65.8	0.82	1.24	245.3	201	305
H.E. 10-18	2020	66.4	1.05	1.58	192.3	202	303
	2021	82.7	1.11	1.34	185.7	206	249
Average		74.6	1.08	1.46	189.0	204	276
H.E. 2-5	2020	66.5	1.07	1.60	172.4	184	276
	2021	81.5	0.97	1.18	211.7	205	250
Average		74.0	1.02	1.39	192.1	195	263

Statistical interpretation of the data obtained shows that the hybrid elite 10-1-6 differs distinctly significantly lower for the percentage of fertile shoots and the relative fertility coefficient, and the elite 10-18 differs significantly positively for the relative fertility coefficient compared to the control - average of the three hybrid elites (Table 4).

Table 4. The statistical interpretation of fertility elements

Hybrid elite	Fertile shoots (%)		Relative fertility coefficients (Cfr)	
	2020-2021	Signifi-cance	2020-2021	Signifi-cance
H.E. 10-1-6	65.8	00	1.24	00
H.E. 10-18	74.6	-	1.46	*
H.E. 2-5	74.0	-	1.39	-
Average -control	71.5	-	1.36	-
	DL 5%=3.55 DL 1%=5.38 DL 0.1%=8.64		DL 5%=0.10 DL 1%=0.15 DL 0.1%=0.24	

Vegetative growth of shoots. Climate conditions in 2020 have been less favorable, negatively affecting the physiological and metabolic processes that condition the growth and development of shoots. The analysis of the length of the shoots in 2020 showed for all three studied hybrid elites increases smaller by 28-60 cm compared to 2021, a year with values of climatic parameters close to the multiannual values (Table 5). According to the analysis of the growth of shoots during the vegetation period (2020–2021), the highest value was recorded in the hybrid elite 2-5 with the average length of the main shoots of 156.9 cm/shoot, followed by the hybrid elite 10-1-6 with 153.8 cm/shoot.

Table 5. Length of vegetative growth (Odobeşti, 2020-2021)

Hybrid elite	Total shoots / vine			Shoot length, cm					
				minimum			maximum		
	2020	2021	average	2020	2021	average	2020	2021	average
H.E. 10-1-6	43.1	42.3	42.7	57.5	39.6	48.6	222.0	296.4	259.2
H. E. 10-18	42.3	41.7	42.0	33.8	37.6	35.7	135.4	215.8	175.6
H.E. 2-5	30.4	41.3	35.8	42.9	75.8	59.3	210.8	298.3	254.5
Average (control)	38.6	41.7	40.1	44.7	51.0	47.8	189.4	270.1	229.7

These hybrid elites registering significant positive differences compared to the control - the average of the hybrid elites (Table 6).

Table 6. Average shoot length

Hybrid elite	Average shoot length			
	2020 – 2021 (cm)	%	Difference (+/-cm)	Signifi-cance
H.E. 10-1-6	153.83	110.84	15.05	*
H.E. 10-18	105.63	76.11	-33.15	000
H.E. 2-5	156.93	113.08	18.15	*
Average (control)	138.78	100.00	0.00	-
DL 5% DL 1% DL 0.1%			13.11 19.85 31.88	

The hybrid elite 10-18 recorded the smallest increases (105.63 cm), which caused very significant negative differences compared to the average of the three elites - control.

Biological resistance to major fungal diseases was determined by calculating the degree of attack on leaves and grapes (Table 7).

Under the influence of climatic conditions during the growing season recorded in the two years of study, the values obtained for the degree of attack were subunit for the main pathogens of the vine. Determined by the different climatic conditions of the two years of study, higher values were recorded in 2021.

Table 7. Behavior at the main diseases of the vine (Odobeşti,2020 - 2021)

Hybrid elite	Part of the plant	Degree of attack of the fungal disease (DA)					
		Downy mildew (*Plasmopara viticola*)		Powdery mildew (*Uncinula necator*)		Black rot (*Botritis cinerea*)	
		2020	2021	2020	2021	2020	2021
H.E. 10-1-6	Leaf	0.00	0.19	0.00	0.04	0.00	0.00
	Grape	0.03	0.21	0.03	0.13	0.14	0.03
H.E. 10-18	Leaf	0.00	0.23	0.00	0.10	0.00	0.00
	Grape	0.10	0.25	0.07	0.22	0.22	0.15
H.E. 2-5	Leaf	0.00	0.26	0.00	0.07	0.00	0.00
	Grape	0.03	0.19	0.00	0.19	0.27	0.03

Depending on the degree of attack of the main vine pathogens, the resistance of the hybrid elites studied was evaluated according to the resistance scale developed by the O.I.V. (Table 8). The three hybrid elites studied demonstrated during the two years of study the high and very high resistance to the main diseases of the vine.

Table 8. Classification of hybrid elites studied for disease resistance, according to OIV descriptors

Hybrid elite	Downy mildew (*Plasmopara viticola)*		Powdery mildew *(Uncinula necator)*		Black rot *(Botrytis cinerea)*	
	Leaf OIV 452	Grape OIV 453	Leaf OIV 455	Grape OIV 456	Leaf OIV 458	Grape OIV 459
H.E. 10-1-6	7-9	7-9	9	9	9	9
H.E. 10-18	9	7-9	9	7-9	9	7-9
H.E. 2-5	7-9	7-9	9	9	9	7-9

Drought resistance (OIV 403) - According to the OIV descriptor list for grape varieties and Vitis species, 2nd edition, 2009.

In the conditions of growing season 2020 considered based on climatic data recorded, one of the driest years in the Odobeşti vineyard, the hybrid elites 10-1-6 and 2-5 showed the high to very high tolerance to the phenomenon of atmospheric and pedological drought (Table 9). The hybrid elites studied did not show specific manifestations of thermal and water stress (withering of the tops of the shoots, yellowed leaves at the base of the trunk, withering of the grapes etc.).

Table 9. Behavior of hybrid elites studied at drought according to OIV descriptors (Odobești, 2020)

Hybrid elite	OIV 403	Expression level
H.E. 10-1-6	7-9	High-Very high
H.E. 10-18	5	Medium
H.E. 2-5	7-9	High-Very high

Quantity and quality of production. The study of the technological characteristics of the grape production completed the knowledge elements for the hybrid elites studied (Table 10). The main quantitative characteristics of grape production refer to the average weight of the grapes and the average production per vine and per hectare.

The average weight of the grapes in the two years of study was 189.0 g in the hybrid elite 10-18, 192.1 g in the hybrid elite 2-5 and 245.3 g in the hybrid elite 10-1-6.

Table 10. Quantitative characteristics of grape production (Odobeşti, 2020-2021)

Hybrid elite		Average weight of a bunch (g)	Grape production	
			kg/vine	t/ha
H.E. 10-1-6	2020	254.0	7.11	26.93
	2021	236.5	7.12	26.97
Average		245.3	7.12	26.95
H.E. 10-18	2020	192.3	6.73	25.49
	2021	185.7	6.85	25.95
Average		189.0	6.79	25.72
H.E. 2-5	2020	172.4	5.17	19.58
	2021	211.7	6.08	23.03
Average		192.1	5.63	21.30

The average grape production on the vine had higher values in 2021 compared to 2020, with 0.01 kg (H.E. 10-1-6) to 0.91 kg (H.E. 2-5).

The statistical interpretation of the data obtained for the quantitative characteristics of grape production shows that the 10-1-6 hybrid elite differs significantly positively for the average weight of the grape and distinctly significantly positive for the average grape production per vine compared to the control - average hybrid elites (Table 11). A distinctly significant difference low compared to the control demonstrated the hybrid elite 2-5 for the average production of grapes per vine.

Table 11. The statistical interpretation for the quantitative characteristics of grape production

Hybrid elite	Average weight of a bunch (g)		Grape production (kg/vine)	
	2020-2021	Significance	2020-2021	Significance
H.E. 10-1-6	245.3	*	7.12	**
H.E. 10-18	189.0	-	6.79	-
H.E. 2-5	192.1	-	5.63	00
Average - control	208.8	-	6.51	-
	DL 5%=24.62 DL 1%=37.28 DL 0.1%=59.88		DL 5%=0.40 DL 1%=0.60 DL 0.1%=0.97	

The technological potential of a genotype is complemented by the quality of grape production, represented by the sugar content (g/l), the total acidity (g/l H_2SO_4) and the maturation index of the must (Table 12).

Table 12. Qualitative characteristics of grape production (Odobeşti, 2020 - 2021)

Hybrid elite		Quality of the juice		
		Sugars g/l	Total acidity g/lH_2SO_4	Maturation index
H.E. 10-1-6	2020	235	3.92	60
	2021	214	5.08	42
Average		225	4.50	51
H.E. 10-18	2020	206	4.14	50
	2021	222	4.85	46
Average		214	4,49	48
H.E. 2-5	2020	183	4.51	41
	2021	193	4.55	42
Average		188	4.53	42

In 2020, the lack of precipitation and the maximum temperatures higher than 30 ° C recorded for 55 days during the ripening, influenced the optimal development of the biochemical processes of accumulation of sugar in the berries.

Under these conditions, the three genotypes accumulated between 183 g/l sugars (H.E. 2-5) and 235 g/l sugars (H.E. 10-1-6), under conditions of a total acidity of 4.14 g/l H_2SO_4, respectively 3.92 g/l H_2SO_4. The maturation index had values between 41 in the 2-5 hybrid elite and 60 in the 10-1-6 hybrid elite.

In the climatic conditions of 2021, with a thermal regime close to the multi-annual values, but with a poor precipitation regime during the growing season, the hybrid elites studied accumulated between 193 g/l sugar (H.E. 2-5) and 222 g/l sugar (H.E. 10-18), under conditions of a total acidity of 4.55 g/l H_2SO_4 (H.E. 2-5) and 5.08 g/l H_2SO_4 (H.E. 10-1-6). The maturation index had values between 42 (H.E. 10-1-6 and H.E. 2-5) and 46 (H.E. 10-18).

Statistical interpretation of the data obtained for the sugar content of grapes (g/) shows that the hybrid elite 10-18 differs significantly low compared to the control - the average of the hybrid elites (Table 13).

Table 13. The statistical interpretation for the qualitative characteristics of grape production

Hybrid elite	Sugar content (g/l)			
	2020-2021 (cm)	%	Difference (+/-cm)	Significance
H.E. 10-1-6	225.50	107.51	15.68	-
H.E. 10-18	214.00	102.48	5.18	-
H.E. 2-5	188.00	90.03	-20.82	0
Average (control)	208.82	100.00	0.00	-
DL 5% DL 1% DL 0.1%			13.11 19.85 31.88	

The agrobiological potential and the quantitative and qualitative characteristics of the grape production recommend the three genotypes studied for registration in the approval process, in order to be introduced into the culture to complete the assortment of varieties with biological resistance to major fungal diseases and stressors.

CONCLUSIONS

The values of the main climatic parameters during the vegetation period varied significantly during the 2 years of study, with 2021 having values close to the multiannual average, while 2020 was extremely dry.

The hybrid elites studied showed a high fertility potential, the percentage of fertile shoots varies between 65.8% in the 10-1-6 hybrid elite and 74.6% in the 10-18 hybrid elite.

According to the analysis of the growth of shoots during the vegetation period (2020-2021), the most vigorous proved to be the hybrid elite 2-5 with the average length of the main shoots of 156.9 cm/shoot, followed closely by the hybrid elite 10-1- 6 with 153.8 cm/shoot.

Depending on the degree of attack of the main pathogens of the vine, the studied hybrid elites demonstrated during the two years of study the high and very high resistance to the main diseases of the vine.

In the conditions of 2020, considered one of the driest years in the Odobeşti vineyard, the hybrid elites 10-1-6 and 2-5 showed great tolerance to very high tolerance to the phenomenon of atmospheric and pedological drought. The studied hybrid elites demonstrated a superior technological potential, achieving average grape yields between 5.63 kg/vine. in the hybrid elite 2-5 and 7.12 kg/vine in the hybrid elite 10-1-6, with a sugar content ranging from 188 g/l in the hybrid elite 2-5 to 225 g/l in the hybrid elite 10-1-6, under conditions of a total acidity between 4.49 g/l H_2SO_4 and 4.53 g/l H2SO4.

The evaluation of the agrobiological and agroproductive potential of the hybrid elites 10-1-6, 10-18 and 2-5 is necessary for the promotion in cultivation and diversification of the assortment of varieties with biological resistance to the main diseases of the vine and tolerant to climate change.

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THE GRAPEVINE PHENOLOGY AND THE CLIMATE CHANGES IN TARNAVE VINEYARD

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Abstract

In the context of climate change, the paper presents an analysis of the evolution of grapevine phenophases, in the period 1991-2021, for four grapevine cultivars Selena, Blasius, Rubin and Radames, and two clones Sauvignon blanc 9 Bl, Feteasca alba 29 Bl homologated at SCDVV Blaj. The study is based on the climatic and phenological data from SCDVV Blaj. The climatic data have been processed into fourteen parameters and for the phenological periods the starting date was analyzed. Regarding the average annual temperature, during 2010-2021 there is an increase of 1.1 ℃ compared to the multiannual average reference temperature (calculated as the annual average of the years 1975-2010), which means that in the studied area, during the period 2010-2021 there was a warming compared to reference years. The average duration of the vegetation period from 2010-2021 is shorter than the reference period by 3.2 days. In 2011 the annual aridity index denotes a semi-arid dry climate, and in 2012, 2015, 2017 and 2019 a moderately humid climate. The hydrothermal coefficient was higher in the studied period compared to the reference period, registering values between 1.0 (2011) and 2.8 (2016), which has a positive influence on grape production. The Huglin index for the studied period is between 2237.6 in 2021 and 2928.5 in 2012, with an average of 2542.6 which places Tarnave vineyard area in a warm climate zone. Concerning the precipitation, a slight increase of 34.7 mm/m^2 compared to the multiannual reference amount was monitored. In these conditions the warming of the climate induced an earlier budburst with an average of 8 days, correlated with an earlier maturation with 9-11 days in average. So far for the blooming and veraison phenophases starting date, no differences of the outrunning were clearly observed.

Key words: *grapevine phenophases, Tarnave vineyard, climatic parameters, climate change.*

INTRODUCTION

The culture of the grapevine in Transylvania has been known since the Iron Age, and there is sufficient evidence of this since the 1st century BC. to this day. Viticulture, entrenched in Transylvania, since antiquity, has been perpetuated during the migration of peoples, developing during the feudal period, both in terms of cultivation and preparation and preservation of wines. The colonization of the Saxons by the Arpadian kings (12th century) in the regions of Sibiu, Alba and Bistrita, led to the development of viticulture in the territory between Tarnave and Mures, an area that appeared on time maps under the name Weinland (Wine Country) (Iliescu et al. 2010; Calugar et al. 2018; Macici 1996).

The first clones of noble vine, of German origin, were brought to Transylvania in the 12th century in the Alba wine center, on the territory of the current localities Alba Iulia, Ighiu and Cricau. Until the invasion of phylloxera, the varieties Grasa, Iordană, Fetească alba, Bakator, Furmint, Pinot gris, Muscat Ottonel, Traminer roz, Riesling Italian were cultivated in Transylvania.

In 1950 a new phase started in the Romanian and also Transylvanian viticulture, by the establishment of effective technical measures for its restoration and development, by the elimination of direct producing hybrids, replanting and expansion of areas planted with noble cultivars, especially with valuable selections and new cultivars (Iliescu et al. 2010; https://www.scvblaj.ro/articole/istoricul-viticulturii-din-transilvania).

Tarnave vineyard is part of the viticultural zone 1 of Romania and is positioned at the intersection of the geographical coordinates of

46°-47° Northern latitude and 23°-24° Eastern longitude, on the Transylvanian Plateau (Iliescu et al., 2010; Calugar et al., 2018; Cudur et al., 2014; Donici et al., 2019; Chedea et al., 2021). The most significant viticultural area of Transylvania, the prestigious Tarnave vineyard, is known and appreciated for its quality wines with a specific flavor and a good sugar/acidity balance mainly from the cultivars Fetească albă, Fetească regală, Traminer roz, Pinot gris, Sauvignon blanc, Neuburger, Riesling Italian, Muscat Ottonel (Iliescu et al., 2010; Cudur et al., 2014; Donici et al., 2019). In Târnave vineyard, from the environmental point of view, there are appropriate conditions for grapevine growing (Cudur et al., 2014; Cotea, 2003). These condition might be unbalanced by the climate change (Santos et al., 2020; Fraga 2021; Chedea et al., 2021, Bălăceanu et al., 2021), the forecasting of the seasonal weather factors being difficult in this context (Sillmann et al., 2017; Cyr et al., 2010; Webb et al., 2010; Chedea et al. 2021, Bălăceanu et al., 2021). The environmental dynamic, with late spring freezes, flooding, heatwaves, droughts, area wildfires, early fall frosts, and pest infestations on the background of new and irregular temperature and humidity regimes, require its fast and appropriate mitigation from the viticulturists (Jones & Webb, 2010; Chedea et al., 2021). The final point of this process is the maintaining of the economic feasibility of grape, grape juice and wine production (Jones et al., 2011; Chedea et al., 2021).

In this context, the paper presents an analysis of the evolution of grapevine phenophases, in the period 1991-2021, for Selena, Blasius, Rubin and Radames, grapevine cultivars: and two clones homologated at SCDVV Blaj: Sauvignon blanc 9 Bl and Feteasca alba 29 Bl.

MATERIALS AND METHODS

Monitoring of climate factors with major impact (temperature and rainfall) on SCDVV Blaj vineyards was carried out by recording and processing the daily climate data. The following indices were calculated: the global thermal balance (∑tºg,ºC - sum of positive average daily temperatures), active thermal balance (∑tºa,ºC - sum of average daily temperatures >10°C), useful thermal balance (∑tºu,ºC - sum of differences between daily average temperature > 10°C and biological threshold for starting the vine vegetation (10ºC), annual average temperature ºC, average temperature during the vegetation period, ºC, absolute minimum temperature, ºC, absolute maximum temperature, ºC, the annual precipitation amount (∑ of the annual precipitations - mm), the precipitations amount during the vegetation period (∑ of the precipitations during the vegetation period - mm), precipitation coefficient (Pc), (∑ of precipitations/no. of days - the amount of precipitation related to the number of days in the growing season), the length of the vegetation period, (number of days), hydrothermal coefficient (HTC), (∑ annual precipitations/∑ precipitations during the vegetation period)*10 De Martonne's aridity index (Iar-DM) and Hughlin Index (Iliescu et al. 2019; Jarvis et al., 2017). De Martonne's aridity index (Iar-DM) (mm $°C^{-1}$) expresses the connection between climate and soil and is and is given by the relation Iar-DM = P/(Ta+10) where P is the annual amount of rainfall in mm and Ta is the mean value of the annual air temperature in ºC (De Martonne, 1926; Pellicone et al., 2019).

The Huglin Heliothermal Index (Huglin 1978) was developed in northern Europe and utilizes growing temperatures as well as including the maximum temperature. The combination of daily maximum temperature helps take into account also the warm afternoons from April 1st to September 30th (in Romania and northern hemisphere) (Jarvis et al., 2017).

Huglin index (HI)=$\Sigma([(Tdmed - 10) + (Tdmax - 10)]/2 \times k)$ from April 1st to September 30th where Tdmax is the daily maximal temperature, Tdmed is the daily average temperature and k is the latitude coefficient which for Tarnave vineyard is 1.05 (46º.2 lat N) (https://www.onvpv.ro/sites/default/files/20210429_huglin_index_21_apr_2021_ro.pdf).

For reporting the starting of the phenological phases: budburst, flowering, veraison (change of color of the grape berries) and ripening-maturation we have taken the data from the SCDVV Blaj database for the studied cultivars Selena, Blasius, Rubin, Radames, Sauvignon blanc 9 Bl, Feteasca alba 29 Bl (Figure 1).

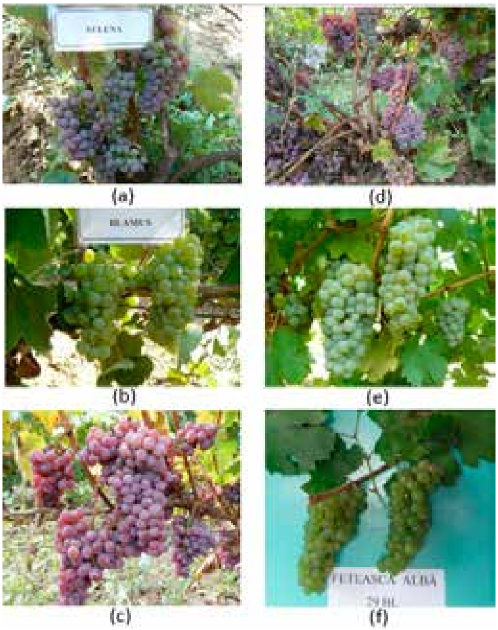

Figure 1. Grapevine cultivars Selena (a), Blasius (b), Rubin (c), Radames (d), Sauvignon blanc 9 Bl (e), Feteasca alba 29 Bl (f) homologated a SCDVV Blaj-grapes in the phenological phase of maturation

The period of time analyzed in this study was divided as follow: for the climatic data 2010-2021 with 1975-2009 taken as reference period, and for the phenological observations 1991-2021 with 1991-1999 considered as comparison reference period

RESULTS AND DISCUSSIONS

Tarnave vineyard is the largest vineyard of all the vineyards in Transylvania, with an area of approximately 2,250 km² and extends over a length of several tens of km, from Craciunelul de Jos (7 km west of Blaj) to the upper course of Tarnave rivers, reaching beyond Hartibaci, being located on the territory of 3 counties (Alba, Mureş, Sibiu) (Coros et al., 2019; Chedea et al., 2021). The vineyards are located on the southern slopes of the heights of this area, starting with the altitude of 250-270 m and up to 400-450 m, the slope of these lands being between 15-35%. The highest slopes are located on the river Tarnava Mare and decrease on Tarnava Mica, Murea and inland valleys (Iliescu et al., 2010; Calugar et al., 2018; Cudur et al., 2014; Donici et al., 2019).

The climate characteristics of the Transylvanian Plateau, under the influence of which are the Tarnave vineyard, is of the moderate temperate-continental type (Iliescu et al., 2019; Chedea et al., 2021). This climate is characterized by a lower level of heliothermal resources, accentuated by the location of the plateau inside the intra-Carpathian area, but it is very favorable for the cultivation of grapevines for wine grapes as the autumns are long, hot and sunny (Iliescu et al., 2010; Calugar et al., 2018; Donici et al., 2019; Chedea et al., 2021).

This allows the accumulation of sugars in grapes, while the acidity remains quite high, favoring a good balance for obtaining high quality wines (POD) (Iliescu et al., 2010; Calugar et al., 2018; Donici et al., 2019; Chedea et al., 2021). The viticultural area of Târnave vineyard is also characterized by the boreal climate, with cold and humid winters, but the grapevine development is not affected if the technological recommendation of partial mechanized protection of the vines at the base of the slope are followed (Iliescu et al., 2010).

Climate of the cultivation area, like the choice of an appropriate cultivar, is one of the most important elements influencing grape production as well as the type and quality of the wine produced (Koźmiński et al., 2020). The most essential climate parameter influencing grapevine growth and development is air temperature (Tonietto & Carbonneau, 2004; Coombe, 1987; Jones & Alves, 2012; Lisek, 2008; White et al., 2006; Santos et al., 2013; Koźmiński et al., 2020). The temperature determines when grapes ripen, which varies by cultivar (Jones & Davis, 2000; Malheiro et al. 2013; Karvonen, 2015; Koźmiński et al., 2020).

As Table 1 shows the most climatic parameters are presented and calculated based on the air temperature. For this study all the reference values were calculated for the period 1975-2009 and the comparison of the parameters of the period 2010-2021 was done with the above reference period. The average monthly temperatures show an increase compared to the reference value in all the months of the studied period. The highest increase was recorded in February with a positive deviation of 2°C compared to the multiannual average temperature (Table 1). Regarding the average annual temperature, for the period 2010-2021 there is an average increase of 1.1°C compared to the average multiannual reference temperature (calculated as the annual average of the years 1975-2009), which means that in the studied area, during the period 2010-2021 there was a warming of the air temperature compared to previous years (1975-2009).

Every year there have been increases in the multiannual temperature, the highest value being 11.9°C (in 2019), and the lowest being 9.7°C (in 2011), a value identical to the reference temperature (Table 1).

Regarding the global thermal balance and the active thermal balance, no differences were found compared to the reference values. The lowest value of the global thermal balance was recorded in 2021 (3027.1°C), the highest being recorded in 2015 (3682.5°C). The lowest value of the active thermal balance was recorded in 2010 (2950.0°C) and the highest being recorded in 2012 (3473.3°C) (Table 1).

Santos et al. (2013), Jones et al. (2005), Keller (2016), Clingeleffer (2010), and Koźmiński et al. (2020) stated that the air temperature during the vegetative part of the culture year expressed as active thermal balance has a higher impact on a specific wine vintage (grape composition, wine quality) than cultivation technology. When it comes to thermal resources, it should be noted that excessive heat during the vegetative period can cause grapes to mature earlier, have higher sugar content, and have lower acidity (De Orduna, 2010; Koźmiński et al., 2020). In contrast, insufficient active heat may result in delayed or limited maturation, and as a result, the produced wine will have a low alcohol concentration and a poor palatability (Jackson & Lombard, 1993; Koźmiński et al., 2020).

The useful thermal balance, in the studied period (2010-2021), on average, had an increase of 80.8°C compared to the reference value (1975-2009) (Table 1). The maximum value is observed in 2012 (1733.3°C), and the minimum value in 2010 (1320.0°C) (Table 1).

In the period 2010-2021, the average temperature during the vegetation of the vine is 17.8°C, thus registering a positive deviation from the reference value of 0.6°C (Table 1).

The absolute minimum temperature was recorded in 2017 (-24.7°C), a lower value than the absolute minimum reference (-24.0°C). The absolute minimum temperatures with the highest values are 2019 and 2020 (-12.5°C and -12.9°C) (Table 1). The absolute maximum temperature was recorded in 2012 (41.6°C), a much higher value than the absolute maximum reference (38.0°C). The absolute maximum temperatures with the highest values are 41.6°C for the year 2012 and 38.8°C for 2013 and 2015 (Table 1).

The precipitations are also an important climatic factor for grapevine cultivation. In this study, a slight increase of 34.7 mm of the annual precipitation amount was observed compared to the multiannual reference amount. The highest amount of precipitation was recorded in 2016 (1006.6 mm). The lowest amount of precipitation was recorded in 2011 (mm). The amount of precipitation during the vegetation period of the vine, during the studied period follows the same trend, registering a slight increase (by 15.3 mm) compared to the reference value, which is also found in the increase observed for the precipitation coefficient.

During the studied period, the number of days in the vegetation period varied from 200 days in 2012 (the warmest and driest year) to 160 in 2021. The average duration of the vegetation period in 2010-2021 is shorter than the reference period with 3.2 days.

The hydrothermal coefficient was higher in the studied period compared to the reference period, registering values between 1.0 (2011) and 3.1 (2016), which has a positive influence on the grape production. The average value (31) of the annual De Martonne's aridity index places the studied period in the lower limit of the humid climate category, similar and in the same climate as the reference period (Iar-DM = 31.1) . However, in 2011 the annual aridity index denotes a semi-arid dry climate, and in 2012, 2015, 2017 and 2019 a moderately humid climate (De Martonne, 1926; Pellicone et al., 2019).

The Huglin index for the studied period ranges between 2237.6 in 2021 and 2928.5 in 2012, with an average of 2542.6 which places the Tarnave vineyard in an area with a warm climate (Jones et al., 2010; ONPV, 2021 https: //www.onvpv.ro/sites/default/files/20210429_huglin_index_21_apr_2021_en.pdf)

Table 1. Climatic parameters for Tarnave vineyard as measured in Blaj wine center for the period 2010-2021 with references values calculated for the period 1975-2009.

	Reference value (1975-2009)	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Average 2010-2021
January	-2.0	-2.5	-3.4	-2.0	-1.2	0.8	-0.4	-1.8	-6.9	0.9	-0.3	-2.2	0.1	-1.6
February	-0.4	1.8	-2.1	-6.8	3.4	3.5	1.3	6.3	1.7	2.0	2.2	3.7	2.7	1.6
March	5.0	4.9	5.6	4.7	5.1	9.1	6.3	6.9	9.0	5.2	8.0	6.9	3.7	6.3
April	10.5	10.6	11.3	12.1	12.6	12.2	10.1	13.5	10.4	16.7	12.2	11.2	8.1	11.8
May	15.6	15.8	15.6	16.1	17.7	15.8	16.5	14.8	15.7	19.5	14.1	13.8	14.5	15.8
June	18.8	19.2	19.5	21.1	20.1	19.1	19.5	20.2	20.2	20.0	21.0	18.8	19.5	19.9
July	20.4	21.1	21.2	25.2	21.5	21.6	22.5	21.5	20.7	19.7	20.3	20.1	22.6	21.5
August	19.8	21.1	21.0	22.4	22.8	21.3	22.4	20.3	22.2	21.8	22.3	21.2	20.3	21.6
September	15.5	14.6	18.6	18.6	14.7	17.4	18.4	16.9	16.1	16.9	17.7	18.2	15.0	16.9
October	9.9	7.1	8.1	11.5	11.1	12.0	11.0	9.1	10.5	12.1	12.9	11.8	8.9	10.5
November	3.9	5.5	-0.3	4.6	7.6	5.5	5.4	2.8	5.2	5.7	10.0	3.1	4.8	5.0
December	-0.7	-1.6	1.1	-1.9	-1.8	2.6	1.2	-2.1	1.8	0.5	1.8	3.7	2.1	0.6
Annual average temperature, ºC	9.7	9.8	9.7	10.5	11.1	11.7	11.2	10.7	10.6	11.8	11.9	10.9	10.2	10.8
Global thermal balance, ∑tºg, ºC	3366.4	3127.7	3274.6	3534.0	3302.0	3551.1	3682.5	3273.0	3197.3	3482.4	3293.3	3418.3	3027.1	3346.9
Active thermal balance, ∑tºa, ºC	3249.2	2950.0	3167.7	3473.3	3302.0	3440.6	3412.9	3220.5	3017.2	3441.7	3214.9	3329.2	3016.9	3248.9
Useful thermal balance, ∑tºu, ºC	1410.7	1320.0	1477.7	1733.3	1522.0	1470.6	1592.9	1450.5	1429.2	1670.7	1474.9	1429.2	1326.9	1491.5
Average temperature during the vegetation period, ºC	17.2	17.1	17.9	19.3	18.2	17.4	18.1	15.2	17.6	19.5	17.9	17.2	15.57	17.8
Absolute minimum temperature, ºC	-24.0	-17.4	-17.6	-21.6	-13.7	-21.7	-23.5	-18.4	-24.7	-17.6	-12.5	-12.9	-13.1	-24.7
Absolute maximum temperature, ºC	38.0	32.5	35.4	41.6	38.8	37.3	38.8	37.3	37.9	34.9	35.1	33.5	35.7	41.6
∑ annual precipitations (mm/ m^2)	611.8	698.0	303.9	519.8	677.0	668.0	598.6	1006.6	583.4	832.4	500.6	699.4	670.2	637.0
∑ precipitations during the vegetation period (mm/ m^2)	435.9	487.2	201.8	335.8	441.2	464.2	427.4	647.0	401.2	577.0	328.0	568.2	535.4	451.2
Precipitation coefficient, Pc	3.3	3.8	1.8	2.6	4.1	3.5	3.2	4.7	3.1	4.5	2.7	3.5	4.2	3.5
The length of the vegetation period (number of days)	187.0	182.0	173.0	200.0	166.0	192.0	188.0	190.0	190.0	183.0	183.0	199.0	160.0	183.8
Hydrothermal coefficient, HTC	1.9	2.4	1.0	1.5	2.1	1.9	1.8	3.1	1.9	2.4	1.6	2.1	2.2	2.0
De Martonne's aridity index Iar-DM	31.1	35.1	15.4	25.4	32.1	30.8	28.2	48.6	28.4	38.2	22.9	33.5	33.2	31
Huglin Index		2352.5	2280.2	2928.5	2802.7	2556.8	2653.6	2597.9	2429.3	2883.6	2520.6	2268.4	2237.6	2542.6

According to Table 2, the budburst phenophase, in the Tarnave vineyard, takes place between April 12 and May 10. Most of the studied cultivars buddbursted most frequently between April 14 and 20 (Table 2). Late budding, can be seen mainly between

1990 and 1998 (April 24-May 10) (Table 2). Selena, in most years, budbursted on April 16-17th (Table 2). Blasius, in most years, budbursted on April 18, the earliest buddburst being on April 12 (2018), and the latest on May 10 (2005) (Table 2). Rubin, in most years, budbursted on April 19, the earliest on April 16 (2018, 2019), and at the latest on April 22 (2013) (Table 2). Radames, in most years, budbursted on April 19, the earliest on April 14 (2001, 2003 and 2019), and at the latest on May 9 (1997 and 1998) (Table 2). Sauvignon blanc 9 Bl, in most years it budbursted on April 20, the earliest on April 15 (2003), and at the latest on May 10 (2005) (Table 2). Feteasca alba 29 Bl, in most years budbursted on April 15-16, the earliest on April 12 (2018), and at the latest on April 19 (2013) (Table 2).

Table 2. Budburst phenophase for the studied Tarnave vineyard cultivars for the period 1991-2021

Budburst Date	Cultivars – homologation year					
	Selena - 1995	Blasius - 1994	Rubin - 2007	Radames - 1993	Sauvignon blanc 9 Bl - 1975	Feteasca alba 29 Bl - 2006
12.04	2001; 2003; 2019	2018				2018
13.04	2016					2016
14.04	2018	2003		2001; 2003; 2019		2019
15.04	0	2016			2003	2006; 2009; 2010; 2011
16.04	2000; 2004; 2008; 2009; 2010	2001; 2019	2018; 2019	2018		2012; 2015; 2017
17.04	2006; 2014; 2015; 2017	2009	2016	2016	2018; 2019	2014
18.04	2007; 2011	1994; 2008; 2014; 2015; 2017	2008; 2014	2000; 2006; 2008	1994; 2016	2007; 2008
19.04	2012	2000; 2004; 2012	2007; 2010; 2011; 2012; 2015; 2017	2004; 2007; 2010; 2011; 2012; 2014	2012; 2014; 2015	2013
20.04		2006; 2007; 2010	2009	2009; 2015; 2017	2000; 2004; 2007; 2009; 2017	
21.04	2013	2011		1994	2006	
22.04			2013		2010; 2011	
23.04		2013		2013	2013	
24.04	1996				2008	
25.04	1995	1995			1991; 1995	
26.04		1996			1996	
28.04				1995		
30.04				1996		
1.05					1993	
2.05	2002					
3.05					2002	
4.05	2005			1993; 2005		
5.05		1997; 1998; 2002			1997; 1998	
6.05				2002		
7.05	1997; 1998					
9.05				1997; 1998		
10.05		2005			2005	

As Table 3 shows, the flowering phenophase, in the Tarnave vineyard, takes place between May 26 and June 23. In most years, the studied cultivars bloomed between June 5 and 10 (Table 3). Due to the climatic conditions manifested in June (capricious and rainy month) the flowering phenophase includes a fairly wide range of dates in which it takes place, therefore notable differences between the studied period and the reference period were not observed.

Selena cultivar, in most years, bloomed on June 8, the earliest on May 26 (2000, 2019), and the latest on June 17 (1997, 1998 and 2005) (Table 3). Blasius, in most years, bloomed on June 10, the earliest on May 26 (2019), and the latest on June 23 (2005) (Table 3). The Rubin cultivar bloomed between May 27 and June 22 and Radames between May 27 and June 21 (Table 3). Sauvignon blanc 9 Bl, in most years it bloomed on June 10-11, the earliest on May 28 (the years 2000 and 2019), and at the latest on June 22 (the years 2005 and 2006) (Table 3). Feteasca alba 29 Bl, in most years it bloomed on June 5-6, the earliest on May 26 (2000 and 2019), and at the latest on June 19 (2006) (Table 3).

Table 3. Flowering phenophase for the studied Tarnave vineyard cultivars for the period 1991-2021

Flowering Date	Cultivars – homologation year					
	Selena - 1995	Blasius - 1994	Rubin - 2007	Radames - 1993	Sauvignon blanc 9 Bl - 1975	Feteasca alba 29 Bl - 2006
26.05	2000; 2019	2019				2000; 2019
27.05			2000; 2019	2000; 2020		2013
28.05					2000; 2019	2000
29.05		2000				
30.05	2013		2013	2013	2013	
1.06	2018	2018				2018
2.06	2002	2002; 2013	2002	2002	2002	
4.06	2012;	2012;				2012
5.06	2015; 2017		2018	2018	2018	2008; 2011; 2015; 2017
6.06	2016	2015; 2016; 2017	2001	2001	2015; 2016	2001; 2003; 2016
7.06			2016	2016		2009; 2010
8.06	2001; 2003; 2009; 0210; 2011	2001	2015; 2017	2015; 2017	2017	
9.06		2011	2009; 2011	2009; 2011; 2012	2011; 2013	2004
10.06	1996	1996; 2003; 2009; 2010	2010	2010	1991; 1993; 1996; 2009	
11.06				1993	2001; 2003; 2010	
12.06	2014					2014
13.06		2014	2014	1996; 2014	2008; 2014	
14.06	1995; 2004	1995			1995	
15.06		1997; 1998		1995	1997; 1998	
16.06	2006; 2007; 2008	2008	2003	2003	2004	
17.06	1997; 1998; 2005		2018	1997; 1998		2005
18.06		2004	2004	2004		2007
19.06			2007			2006
20.06		1994; 2007	2005	1994; 2005; 2007	1994; 2007	
21.06		2006		2006		
22.06			2006		2005; 2006	
23.06		2005				

According to the analyzed data, the grapevine phenophase of veraison (change of color of the grape berries), in Tarnave viticultural area, takes place between July 25 and August 27 (Table 4). In most years, the varieties studied started to change the color of the grapes between August 14 and 20 (Table 4). Selena, in most years, entered veraison on August 14-16, the earliest veraison phenophase was on July 25 (2002), and at the latest on August 22 (2014) (Table 4). Blasius, in most years, started the veraison phenophase on August 18, the earliest on July 28 (2002), and at the latest on August 23 (2005) (Table 4). Rubin, in most years, started the veraison on August 20, the earliest date of veraison was observed on

August 8 (2012), and at the latest on August 22 (2014) (Table 4). Radames, in most years, started the veraison phenophase on August 16 and 20, the earliest on July 31 (2002), and at the latest on August 27 (1997 and 1998) (Table 4). Sauvignon blanc, in most years, started the veraison on August 20, the earliest veraison was on July 30 (2002), and at the latest on August 25 (1991) (Table 4). For the Feteasca alba 29 Bl cultivar, no consistency was observed in terms of veraison phenophase, which took place between July 29 and August 20 (Table 4).

It is noticeable that during the years 1991-2000, the veraison phenophase took place mainly in the second half of August and the period 2000-2010 stands out with an early entry into this phenophase compared to the rest of the studied years.

Table 4. Veraison (change of color of the grape berries) phenophase for the studied Tarnave vineyard cultivars for the period 1991-2021

Veraison Date	Cultivars – homologation year					
	Selena - 1995	Blasius - 1994	Rubin - 2007	Radames - 1993	Sauvignon blanc 9 Bl - 1975	Feteasca alba 29 Bl - 2006
25.07	2002					
28.07		2002				
29.07						2009
30.07					2002	
31.07				2002		
1.08	2000; 2012					
3.08		2009				2010; 2012
4.08		2000				2008; 2011
5.08		2008; 2012				
7.08	2008				2009	
8.08	2009; 2011	2011	2012	2012	2012	
9.08	2010	2010	2008	2008		2007
10.08	2007	2007	2007; 2009	2009	2000	
11.08				2000; 2007	2008; 2011	
12.08			2010		2010	
13.08			2011	2010; 2011	2007	
14.08	1995; 2001; 2003; 2016; 2019	1995		1995	1995	2016; 2019
15.08		2016				2015; 2017
16.08	1996; 2015; 2017; 2018	1996; 2001; 2019	2016	2001; 2016; 2019	1996	
17.08	2006		2019	2004		
18.08	2004; 2005	1994; 2003; 2006;2015; 2018			1994; 2016	2013; 2018
19.08		2004		1996; 2005	2004	2006
20.08	1997; 1998	1997;1998; 2013	2013; 2015; 2017; 2018	2003; 2006; 2013	1993; 2001; 2003; 2013; 2015; 2019	2014
21.08	2013			2015; 2017	2017	
22.08	2014	2014	2014	2018	1997; 1998; 2018	
23.08		2005		2014	2014	
24.08				1993; 1994	2005; 2006	
25.08					1991	
27.08				1997; 1998		

Table 5 presents the ripening-maturation starting date for all the studied cultivars According to it, the phenophase of grape ripening, in the Tarnave vineyard, takes place between September 2 and October 1 (Table 5). In most years, the varieties studied reached maturity between September 10-18 (Table 5).

Selena, in most years, entered the grape ripening phenophase on September 10-16, the earliest ripening was observed on September 2 (year 2002), and at the latest on September 30 (the year 2014) (Table 5). Blasius, in most years, entered the grape ripening phenophase on September 10-16, the earliest start was on September 3 (2002), and at the latest on September 30 (the year 2014) (Table 5). Rubin, in most years, entered the grape ripening phenophase on September 16-18, the earliest maturation was on September 10 (2002), and the latest on September 30 (the year 2014) (Table 5). Radames, in most years, started to ripe on September 15-18, the earliest maturation was observed on September 10 (2002), and the latest on October 1 (the years 1997; 1998) (Table 5). Sauvignon blanc, in most years, entered the grape ripening phenophase on September 16-18, the earliest ripening date was observed on September 10 (2002), and the latest on October 1 (year 1991) (Table 5). Feteasca alba, in most years, started the grape ripening phenophase on September 5, the earliest maturation date was observed on September 2 (2002), and the latest on September 24 (2013) (Table 5).

It can be easily observed that during the years 1991-1999, the ripening phenophase of the grapes took place mainly at the end of September (after September 20).

Table 5. Ripening-maturation phenophase for the studied Tarnave vineyard cultivars for the period 1991-2021

Harvest Date	Cultivars – homologation year					
	Selena - 1995	Blasius - 1994	Rubin - 2007	Radames - 1993	Sauvignon blanc 9 Bl - 1975	Feteasca alba 29 Bl - 2006
2.09	2002					2002
3.09		2002				
5.09		2000				2000; 2001; 2003; 2004; 2019
6.09	2000					2010
8.09						2009; 2012
10.09	2001; 2003; 2012; 2019	2001; 2009; 2012; 2019	2002	2002	2002	2006; 2008; 2011
11.09	2010					
12.09	2004; 2009; 2018	2003; 2008; 2010	2001; 2019	2001; 2019	2001; 2003; 2019	
13.09	2015					2015; 2017
14.09	2008; 2011	2006		2003		
15.09	2016; 2017	2004; 2011; 2015; 2018	2000; 2012	2000; 2004; 2012	2000; 2012; 2016	2016; 2018
16.09	2005; 2006; 2007	2005; 2007; 2016	2003; 2004; 2008; 2009; 2010	2008; 2009; 2010; 2011	2004; 2008; 2009; 2010; 2011	2005; 2007
17.09			2011			
18.09		2017	2005; 2006; 2007; 2016; 2018	2005; 2006; 2007; 2016; 2018	2005; 2006; 2007; 2016; 2018	2014
20.09	1995	1995	2017	2017	1995	
22.09	1996	1996		1995	1996	
24.09		2013				2013
25.09			2015	1996; 2015	2015; 2017	
26.09	2013	1994			1994	
28.09	1997; 1998	1997; 1998	2013	1994; 2013	1993; 2013	
29.09					1997; 1998	
30.09	2014	2014	2014	1993; 2014	2014	
1.10				1997; 1998	1991	

CONCLUSIONS

The climatic data for Tarnave vineyard as collected at SCDVV Blaj have been processed into fourteen parameters, and for the phenological periods the starting date was analyzed. The average annual temperature, during 2010-2021 shows a warming with 1.1 ºC compared to the multiannual average reference temperature. The average duration of the vegetation period from 2010-2021 is shorter than the reference period by 3.2 days.

Acording to the climate indexes observations, our study places Tarnave vineyard, during the years 2010-2021, in a humid (given by the Martonne aridity index), warm climate (given by the Hughlin Index) with an increase of precipitations (given by the sum of annual precipitations and also the precipitation coefficient).

In the conditions above presented, most of the studied varieties budbursted frequently between April 14 and 20, bloomed between 5 and 10 June, started the veraison phenophase between August 14 and 20 and reached maturity between September 10-18.

In conclusion the warming of the climate induced an earlier budburst with an average of eight days and an advance regarding the maturation phenophase with an average of 9-11 days. So far for the blooming and veraison phenophases starting dates, the outrunning was not clearly observed.

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PRODUCTION COSTS FOR THE CULTIVATION AND HARVESTING OF TABLE GRAPES UNDER CONDITIONS OF SOUTH-CENTRAL BULGARIA

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Abstract

The production and supply of table grapes is becoming an increasing challenge for grape growers and processors. The reasons are complex - from increased competitive pressure and requirements of retail market in terms of environmental production and certification of products according GLOBAL GAP criteria, to intense climate changes, which is a major factor during the growing season. This gives grounds to deepen research in the field of economic analysis in connection with use of good practices in the choice of technology for growing vines and refining work processes during the growing season, reducing pesticide usage and increasing the investment efficiency and sustainability.

Key words: *Bulgaria, economic efficiency, global gap, production costs, table grapes.*

INTRODUCTION

Globally, the production of table grapes is developing dynamically, reaching 27 million tons in 2014, which is an increase of nearly 71% based on the amount produced in 2000 (FAO-OIV, 2016). Against the background of the general trend of reducing the harvested areas of table grapes vineyards in Europe, countries such as Italy, Spain, Greece and Bulgaria occupy a position on the world map of table grape production with a share of 27.2% of total production in 2014. Exports of table grapes globally reached 4.2 million tons in 2014, which is an increase of about 50% compared to the level reported in 2000 - 2.8 million tons. The main factors determining the framework of global trade are mainly related to changing consumer tastes, the entry of new producers and exporters (Dimitrova, D., 2018). This requires a change in wholesale and retail trade, improvement of cultivation technologies (Hooker et al., 1999), improvement of storage, packaging and transport of products, the use of information and communication technologies (Seccia et al., 2015, Seccia et al., 2017).

Although most food safety standards are designed to pursue legitimate objectives for the protection of human, plant and animal health, they can also serve as technical barriers to trade at random (David E., 1999). One of the brightest examples of this is an experiment conducted with table grapes in the Republic of Turkey. The experiment reported 172 pesticides as residues in table grapes. A total of 280 samples of table grapes were collected from supermarkets, farm markets and grocery shops located in four provinces of Turkey from August to October 2016. The samples were analysed by liquid chromatography combined with tandem mass spectrometry. The limit of quantification varies from 0.002 to 0.010 mg/kg^{-1}. The validation data revealed good repeatability and reproducibility and met the other requirements of the European guidelines SANTE/ 12682/2019 (Implemented by 01.01.2020). One or more pesticide residues were found in 59.6% of table grapes. Residues above the EU maximum levels are 20.4% of the samples. The most common pesticide residues are azoxystrobin, chlorpyrifos, boscalid and cyprodinil. In the worst case scenario, the hazard index (HI) is 9.42% for adults and 3.37% for children. Chlorpyrifos has a major contribution (65%) to HI (Ozgur & Kabak, 2018). In the harmonization of food safety measures, the EU, guided by the precautionary principle, requires the adoption of the strictest standards previously applied by individual Member States. Although European Union

legislation is the minimum access to the EU market, many wholesalers and retailers require their suppliers to demonstrate compliance with independently verifiable private standards, such as GLOBAL GAP.
This is an internationally recognized standard for agricultural production. Although many of the standards are seemingly voluntary, they become de facto standards for acquiring or maintaining access to certain buyers or market segments, having an important impact on the international competitiveness of individual market participants, industry or country.
Exporters from countries without a certification system may be forced to use the services of an accredited body in another country at significant costs, so it is important that each country builds certification capacity and parallel institutions for accreditation of certification bodies (Semerdzhieva, T., 2018).
The production of table grapes on the territory of Bulgaria is complicated by the fact that the average age of workers in the sector is increasing. This increases the hourly rates needed to grow and harvest table grapes. This is essential for the sustainable development of table grape production, as well as issues of its economic efficiency.

MATERIALS AND METHODS

The research was conducted in the period 2020-2021 in vineyards planted with table variety 'Victoria'. The soil surface is covered with natural grass cover between the rows. The vineyards were planted in 2013 on the land of the village of Iskra on an area of 6.5 ha.
The scheme of the experimental work includes two variants with an area of 1 ha each. The cultivation of variant I is in the open outdoor cultivation, and variant II grown under a safety net (Figure 1).


Figure 1. Variant I - outdoor cultivation (left), Variant II - grown under a safety net (right)

The economic efficiency of the production of table grapes from fruit-bearing vineyard, in both variants are established by taking into account the following indicators:
- Product per unit area - kg/ha;
- Value of total revenue per unit area - euro/ha;
- Production costs (total) - euro/ha;
- Material costs - euro/ha;
- Labour costs - euro/ha;
- Own value - euro/kg;
- Profitability rate (based on production costs) - %;
- Net income (profit) per unit area - euro/ha
- Qualification of grape production according to the standard "GLOBAL GAP" - Regulation EU-396/2005 and Regulation 1107/2009.

RESULTS AND DISCUSSIONS

For the production of table grapes it is very important to know what is the economic result of cultivation, as well as very carefully to take into account the change in net income from the application of one or another cultivation technology.

The application of various means of protection against the intensity of sunshine in its various spectrum and hail, creates real prerequisites for changing the berries epidermal structure. Differences are observed in the size of the total production, collected from the two variants.

The second variant shell 36,560 kg of First class grapes. The production of grapes with this technology allows us to form a larger quantity and better quality grapes, unlike the first variant, which produces 32,940 kg of First class. This variant forms twice the amount of

Nevertheless, the higher selling price gives an advantage in financial results. Comparative analysis shows the level of key economic indicators vary. In both variants the yields are high due to the fact that the experiment meets the agronomic requirements for cultivation. They provide maximum manifestation of the biological capabilities of the vine and the potential capabilities of the applied waste - 7220 kg, which is unsuitable for subsequent use and remains between the rows, as a siderant (Table 1). This is due to one important limiting factor - the lack of anti-hail net. High temperatures during the months June, July, according to the quality of the grapes and their presence on the commodity exchanges and retail shops. Respectively, this is determined by the fact related to the load of vines with grapes.

The growth rates of the average selling price depend on cultivation technology and quality of grapes in accordance with the standard "GLOBAL GAP" (Table 2).

This has a positive effect on the overall cost and cost of production. As a result, the profitability rate on cultivation for both technologies is respectively 92.30% for Variant I and 94.87% for Variant II (Table 1).

August (Table 3), in combination with the small amount of precipitation that took place in the same period (Table 4), also contribute to the large amount of grapes falling out during the grape harvest.

This set of environmental factors significantly affects the level of average yields.

The values of total revenues are influenced by the level of purchase prices of end products. The average purchase price fluctuates technologies. The production price is formed on the basis of tracking the dynamics and sales from the beginning to the end of the supply in the retail shops. The value of the incurred material costs is determined on the basis of the amounts actually paid for their purchase and the quantities according to accounting documents. Labor costs are set and realized on the actual wages. They are reduced due to the use of less manual labour in various operations. The lower production cost of 0.18 EUR in Variant I is due to the reduced costs of harvesting and sorting the final product. High yield, average sales price and relatively optimal production costs are factors that determine high

profit achieved during the study period. The profit per unit area in the open-air variant is 10887.56 EUR, and profitability rate 92.30%.

In the net covered variant, the profit margin is 15721.54 EUR and the profitability rate is 94.87%. One of the reasons for this is the higher sales prices, the better commercial appearance and the lower loss of production during the grape harvest. It should be noted that the increase in yield in table grape production has a limit in terms of product quality.

The GLOBAL GAP standard must be applied when we offer table grapes in retail market.

Table 1. Economic efficiency in the production of 'Victoria' grapes

№	Indicators	Measure	Variant I outdoor cultivation (1 ha)	Variant II grown under a safety net (1 ha)
1	Product per unit area	kg	40160	40160
2	First class grapes	kg	32940	36560
	Secondary product (in variant I it is eliminated in the rows)	kg	7220	3600
3	Average sales price	Euro/kg	0.50	0.64
4	Total value of production (Total revenues)	Euro/ha	17156.25	25665.64
5	Material costs	Euro/ha	2750.77	4618.46
	Labour costs	Euro/ha	3267.82	5325.64
	Total costs	Euro/ha	6018.59	9944.10
6	Own value	Euro/kg	0.18	0.25
7	Net income	Euro/ha	10887.56	15721.54
8	Profitability rate	%	92.30	94.87

Table 2. Qualification of table grapes according to GLOBAL GAP standard

Variant	First class, %	Secondary product, %
I outdoor cultivation	82.02	0.00
II grown under a safety net	91.11	8.89

Table 3. Air temperatures for the study period, ^{0}C

Measurement point Iskra village	Period	MONTHS										
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Maximum	2021	25.8	23.9	22.8	31.1	34.1	41.2	44.4	43.2	34.6	25.2	23.7
Average	2021	4.2	5.2	5.9	10.6	17.8	21.8	26.2	25.7	19.2	11.2	7.7
Minimum	2021	-6.8	.14.2	-8.1	-3.6	-1.6	6.5	11.2	12.1	4.8	-1.4	-5

Table 4. Precipitation for the study period, l/m^2

Measurement point Iskra village	Period	MONTHS											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Total	2021	0.25	50.5	85	109	47.5	42.75	35	77.75	3.25	74	16.5	46

Until recently, it was known as Eurep GAP and has been gaining ground worldwide since the beginning of the 21st century. By 2002, 5,000 certificates had been issued, and at the end of 2007 their number exceeded to 100,000 (Petrov, 2009). This guarantees control over production and packaging, as well as higher purchase prices (Figure 2).

From a production point of view, we can conclude that the second technology allows us to reach the amount of First class to 91.11%, and in the first case it is 82.02% (Table 2). The formation of the so-called secondary product was also established during the experiment. The grapes that remain at 8.89% in Variant II provide an opportunity to generate additional

Figure 2. Grapes, classified and packed according to the GLOBAL GAP standard

income on agricultural holdings (Figure 3). The market for table grapes is very demanding as the competition is fierce, which is why producers need to focus on the introduction of systems to combat climate change, such as high temperatures and hail.

Figure 3. Grapes from Variant I - outdoor cultivation (left), Variant II - grown under a safety net (right)

The sale of table grape products on the country territory can be guaranteed in practice on three grapes should be done in the presence of anti-hail systems. This will lead to the provision of:
1. Higher labour productivity by mechanizing technological processes.
2. Reduction of operating costs for growing vines.
3. Production of larger batches of high-quality products to compete in the domestic and main lines, and they are in retail market, exchanges and through exports within the EU and third countries. Nevertheless, the main distributor and consumer remains the Romanian trader and consumer.
4. Taking into account the overproduction of large countries, which produce and export more and more often and on a larger scale, as a production unit part of the EU we must select grape harvest. It should be noted that the increase in yield in table grape production has a limit in terms of product quality.

CONCLUSIONS

The economic analysis shows that there are prerequisites for growing the 'Victoria' variety on the proposed technological solutions and on larger areas. The increase of the production and economic size of the farms growing table foreign markets.

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CURRENT PEDOLOGICAL STUDIES IN THE VINEYARD DRĂGĂŞANI IN RELATION TO THE EXISTING CLIMATE CONDITIONS

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Abstract

In the year 2020, a soil study was carried out in the Drăgăşani vineyard, following the natural fertility of the soil, humus, content in mobile forms of phosphorus and content in mobile forms of potassium, in order to behave some varieties of vines at the moment, in parallel to the influence of climatic conditions. The rational system of soil fertilization of vineyards is largely determined by the existing soil and climatic conditions in the area. Organic fertilizers, green manures, so necessary for heavy soils, will be promoted. Among the chemical fertilizers, slow-acting fertilizers will be preferred to reduce the risk of leaching and pollution. The soil samples collected were analyzed at the Agrochemistry Laboratory of IC-DVV Valea Călugărească, in accordance with the methods provided in the "Methodology for elaboration of agropedological studies" elaborated by IC-DVV Valea Călugărească.

Key words: *fertility, fertilizers, samples, soil, chemical analyzed.*

INTRODUCTION

In the Drăgăşani vineyard, soil studies were carried out in 2021, focusing on establishing the doses of organic and green fertilizers, in accordance with the type of soil currently existing in the vineyard, in order to behave some varieties of vines from qualitatively and quantitatively.
The soils of Dragăşani vineyard are generally clay-illuvial, occupying within the area 42.2% of the surface; followed by cambic soils with 23.5%, lithomorphic soils 20.8%, undeveloped soils 8.8%, hydromorphic soils 2.8% and atropic soils 1.9% (Condei et al., 1986).
In this context, a proper fertilization with phosphorus and potassium fertilizers will be required, so that the agrochemical values of the soil reach the optimal parameters (Davidescu D., Davidescu Felicia, 1992).

MATERIALS AND METHODS

The Drăgăşani vineyard is located between the parallels 44º30' - 44º55 'north latitude and the meridians 23º55'-24º15'east longitude (Condei, 1982).
The study was carried out in Dragasani vineyard in 2021, on the existing soils on top of the hills at an altitude of 290 m altitudine.
There were presented 3 soil samples, numbered with numbers from 1 (CF), 2 (SB) and 3 (M). The soil samples were collected from the studied field at depths of 0-30 cm and 30-60 cm, the samples being coded according to their own coding system as follows: 83.1, 83.2, 84.1, 84.2, 85.1 and 85.2.
The soil samples collected were analyzed at the Agrochemistry Laboratory of IC-DVV Valea Călugărească, in accordance with the methods provided in the "Methodology for elaboration of agropedological studies" elaborated by ICDVV.
The mobile phosphorus contents, the mobile potassium contents, the nitric nitrogen content, the humus content, the total calcium content were analyzed in order to fertilize according to the fertilization of the soil in the vineyards.

RESULTS AND DISCUSSIONS

The results of these mobile contents are analyzed and studied in Tables 1-10, the interpretation limits indicating average indices of soil supply with mineral fertilizers.

Table 1. Mobile phosphorus content

Test number	Test number register	Depth cm	P ppm	Supply level
0	1.	2.	3.	4.
1	83.1	0 - 30	72	middle
	83.2	30-60	64	middle
2	84.1	0 - 30	68	middle
	84.2	30-60	56	middle
3	85.1	0 - 30	64	middle
	85.2	30-60	48	middle

Table 2. Mobile phosphorus content

Limit values (phosphorus ppm)	Appreciation of the degree of supply
< 8.0	very weak
8.1-18.0	weak
18.1-36.0 37.0-72.0	middle
72.1-108.0 108.1-144.0	good
> 144.1	very good

The content in mobile forms of phosphorus is medium, the values oscillating between 64-72 ppm in the horizon 0-30 cm and medium with values between 48-64 ppm in the horizon 30-60 cm (the optimal level for the cultivation of quality varieties is 108-144 ppm).

Table 3. Mobile potassium content

Test number	Test number register	Depth cm	K ppm	Supply level
0.	1.	2.	3.	4.
1	83.1	0 - 30	160	good
	83.2	30-60	144	good
2	84.1	0 - 30	140	good
	84.2	30-60	124	middle
3	85.1	0 - 30	152	good
	85.2	30-60	124	middle

Table 4. Mobile potassium content

Limit values (potassium ppm)	Appreciation of the degree of supply
< 66.0	weak
66.1-132.0	middle
132.1-200.0	good
200.1-265.0 265.1-400.0	very good

The content in mobile potassium varies from medium to good, the values oscillating between 140-160 ppm in the horizon 0-30 cm and between 124-144 ppm in the horizon 30-60 cm (the optimal values for the cultivation of quality varieties are between 200-265 ppm).

Table 5. Nitric nitrogen content

Test number	Test number register	Depth cm	Nitric nitrogen ppm	Supply level
0.	1.	2.	3.	4.
1	83.1	0 - 30	1.07	weak
	83.2	30-60	0.91	very weak
2	84.1	0 - 30	1.00	weak
	84.2	30-60	0.86	very weak
3	85.1	0 - 30	1.05	weak
	85.2	30-60	0.90	very weak

Table 6. Nitric nitrogen content

Limit values (Nitric nitrogen ppm)	Appreciation of the degree of supply
< 0.5	extremely small
0.6-1.0	very small
1.1-2.0	small
2.1-3.0	middle
3.1-6.0	big
6.1-25.0	very big
> 25.1	extremely large

Regarding the mobile forms of nitrogen (ammoniacal-NH_4 and nitric-NO_3) it is found that they register low values. However, the content of mobile nitrogen forms does not allow a correct interpretation of the soil supply of nitrogen, because it is very fluctuating during the vegetation period of the vine, depending on the thermal and pluviometric regime, the mobile forms of nitrogen can be easily washed on soil profile in conditions of heavy rainfall.

Table 7. Humus content

Test number	Test number register	Depth cm	Humus %	Supply level
0.	1.	2.	3.	4.
1	83.1	0 - 30	1.83	middle
	83.2	30-60	1.72	middle
2	84.1	0 - 30	1.78	middle
	84.2	30-60	1.66	middle
3	85.1	0 - 30	1.89	middle
	85.2	30-60	1.72	middle

Table 8. Humus content

Limit values (humus %)	Appreciation of the degree of supply
< 1.0	weak
1.1-2.0	middle
2.1-3.0 3.1-4.0	good
4.1-5.0 5.1-8.0	very good

The humus content is medium, the values oscillating between 1.78-1.89% in the horizon 0-30 cm and between 1.66-1.72% in the horizon 30-60 cm.

Table 9. Total calcium content

Test number	Test number register	Depth cm	Total calcium %	Appreciation
0	1.	2.	3.	4.
1	83.1	0 - 30	3.27	middle
	83.2	30-60	3.37	middle
2	84.1	0 - 30	2.73	middle
	84.2	30-60	3.55	middle
3	85.1	0 - 30	2.68	middle
	85.2	30-60	3.18	middle

Table 10. Total calcium content

Limit values	Appreciation of the presence of total calcium	Limits
OO	it's not necessary	absent
MC	small	< 1
MO	middle	2-4 5-8 9-12
MR	big	13-15 16-20 21-25
FR	very large (marly)	26-40
ER	extremely high (marl-limestone)	> 40

The total calcium content is medium, registering values that oscillate between 2.68 and 3.27% in the horizon 0-30 cm and between 3.18 and 3.55%.
(***Limits of according to instructions developed by ICDPA, 1976).
(*** ICDVV- Methodology for elaboration of agropedological studies, 2021).

CONCLUSIONS

From the obtained data it results that the studied land has an average natural fertility, with an “average” supply in humus, an “average” content in mobile forms of phosphorus and an “average to good” content in mobile forms of potassium.
From this point of view, a proper fertilization with phosphorus and potassium fertilizers is required, so that the agrochemical values of the soil reach the optimal parameters.

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THE INFLUENCE OF WATER STRESS GRAPEVINE UNDERSTANDING THE PLANT'S RESPONSE FROM LEAF TO WHOLE PLANT

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Abstract

Of all the fruit crops of horticultural importance, the grapevine (Vitis vinifera L.) stands out as the most tolerant drought. However, there is relatively little information on grapevine responses to water stress tolerance. Climate change is probably the most discussed issue today. Climate change includes uneven distribution of regional water, extreme weather events (heat waves, heavy rains, hail, frost, and strong winds), and increasing droughts. This analysis summarizes the latest results on grapevine drought responses, the impact of water scarcity on the physiology of the grapevine and its fruit, and highlight some potential solutions in brief and medium-term in grapevine plantations.

Key words: *water deficit, Vitis vinifera L, viticulture, drought.*

INTRODUCTION

Climate change scenarios predict that Central Europe, including Romania, will be affected by water constraints, especially in southern Europe, changing rainfall patterns, and will suffer from summer drought (Serra et al., 2014). Drought indicates the state of a biological system in which the water requirement is below the optimal values and works in terms of absorbing a considerable variable power depending on the growth phase and the stage of development. This phenomenon can be considered meteorological, hydrological, agricultural and economic. Many scientists report a rise in temperature in Europe, e.g. Guedon &Legave (2008) with about 1.1-1.3°C in France, Blanke & Kunz (2017) with 0.6°C and Waldau & Chmielewski (2018) with 1.9°C in different regions of Germany.

Adaptation of fruit crops grown in temperate areas in many places will be endangered in the future due to climate change, with warmer winters and earlier springs (Wenden et al., 2017, Florea et al., 2020). Climate data for the investigated region were first reported in Romania about two decades ago (Păltineanu et al., 2000, Florea et al., 2020), and the warming trend was observed by Păltineanu et al. (2011, 2012), later by Florea et al. (2020). Studies reported by Busuioc et al. (2015) highlight a trend of increasing air temperature in the period 1962-2010 in Romania, while Chitu et al. (2015) and Florea et al. (2020) highlighted the increased variability of seasonal and annual extreme temperature trends over the last three decades in the study region. Chmielewski (2004) mentions that the phenological stages of German crops have shown an advanced trend due to heating

In other parts of Europe, such as France, an advance in flowering tree species has been reported for some fruit tree species due to rising temperatures from January to April. Also in Germany, Chmielewski et al. (2011) showed an advance of the beginning of apple blossoming from 1989 to 2011, caused by climate change in the studied areas, and Rivero et al. (2017) in Scandinavia. Similar studies have been performed by Slavko Bernáth et al. (2021) which analyze the period 1985 to 2018 for the vine and report an earlier budding by five to seven days, the earlier onset of flowering by 7 to 10 days, the earlier softening of the berries by 18 days and dates of advanced harvesting with 8 to 10 days on average.

In the many Mediterranean and south-eastern areas of Europe, with low rainfall, often below 250 mm per year (Williams & Matthiews, 1990), and the negative effects of climate change could be exacerbated, water scarcity has become a problem. Recent studies show that limiting water availability in vine cultivation can affect productivity (Chai et al., 2015; Pérez-Pastor et. al., 2014, 2016), moderate water scarcity can reduce yield, but with benefits some aspects of fruit quality; Severe water shortages lead to low yields and lower fruit quality, while the absence of water exacerbates these negative aspects, thus harming the proper production of crops. Consequently, water storage has become a major environmental challenge to limit the expansion of irrigated agriculture (Williams et al., 2003). Some drought tolerance studies of cultivated vine genotypes have focused on key agronomic indicators, such as grape yield and physico-chemical composition (fruit quality indicators), while others have focused on the physiology of grapes to the finer scale of plants, such as stomatal regulation, carbon assimilation, etc. However, it remains an open and critical question for water management in vineyards and how different varieties respond to drought and water needs, fruit growth, yield, and quality. Although the yield is affected by drought, a recent meta-analysis reported by Dayer et al. (2020) suggests that this decrease may be specific to the variety. Recent work on plant physiological indicators suggests that all genotypes can regulate the use of vine water (ie stomatal conductance) to protect against more severe damage, through physiological indicators provided by the petiole cavity or leaf, even fall (Hochberg et al., 2017b; Dayer et al., 2020). Despite studies to date, it is unclear to what extent differences in the regulation of vine water use between varieties result from innate genotypic differences, or environmental (Hochberg et al., 2018).

In addition, although the exact mortality rates for grapes are not known, vines appear to almost always operate within a "safe" margin of water potential in which stem cavity is extremely rare (Charrier et al., 2018). Thus, many gaps remain in understanding what constitutes a drought-adapted vine variety, which makes it even more difficult to address future climate challenges.

Therefore, in this review, we summarize the current state of research on techniques for the physiology of drought stress on the vines, the impact on the whole plant and leaves, and fruit composition to make the best decisions on vine irrigation, time, the timing of irrigation.

Visual observation

The first way to assess the condition of a vine's water is to look directly at the field of view. One of the earliest responses of a plant that has a water supply limit is the loss of turgidity first, followed by a slowdown in vegetative growth (Markus Rienth &Thibaut Scholasch (2019). Such an assessment can be made systematically, in which 30-50 peaks per plot are observed visually and then classified into three groups, as follows: a straight-growing apex, where the first expanded leave is small and well beneath the apex; then a slowing down of growth with the first expanded leave covering the apex; until where the apex has dropped and shoot growth has completely ceased (Rodriguez-Lovelle B. et al., 2018)

However, this method cannot be applied for the necessary irrigation after the growth of the shoots ends once the meristem is cut or dried (Keller, 2010), moderate water deficit leads to wilting and even their subsequent abscission when the water deficit becomes severe. (Keller, 2010; Rodriguez-Lovelle et al., 2018).

Intelligent methods for monitoring, early diagnosis of water stress in viticulture based on multisensory soil-plant-air techniques

Classical methods of monitoring crop water stress include "in situ" measurements of soil water content, taking into account plant properties and meteorological variables, to estimate the amount of water lost from the plant-soil system over some time. These methods are time-consuming and produce timely information that provides inaccurate indications of the general state of the system in question (Jackson, 1982) unless a very large number of samples are processed.

Precision agriculture is doing everything possible to increase efficiency, productivity, and profitability in many agricultural production systems while minimizing the unwanted impact on biocenosis and natural biotope. Real-time crop status information will provide a solid basis for farmers to adapt to strategies at all times. Instead of making decisions based on a hypothetical state of the environment, an approach in precision agriculture recognizes the differences and regulates management actions. Phyto-monitoring is an information management technology that provides fruit plants with real-time, invaluable information about both the dynamic physiological state of the crop and continuous analysis of plant growth trends, real-time information on moisture dynamics, soil, and microclimate conditions. Precision agriculture is doing everything possible to increase efficiency, productivity, and profitability in many agricultural production systems, while minimizing the unwanted impact on biocenosis and natural biotope. Real-time crop status information will provide a solid basis for farmers to adapt to strategies at all times. Instead of making decisions based on a hypothetical state of the environment, an approach in precision agriculture recognizes the differences and regulates management actions. Phyto-monitoring is an information management technology that provides fruit plants with real-time, invaluable information about both the dynamic physiological state of the crop and continuous analysis of plant growth trends, real-time information on moisture dynamics, soil, and microclimate conditions.

Both gas exchange and fluorescence can be used to detect water stress in C4 photosynthetic plants. However, in C3 photosynthetic plants, the exchange of gas from the leaves will measure water stress in the very early stages, while standard methods based on chlorophyll fluorescence will only detect moderate or severe water stress. There is a fluorescence test for measuring water stress very early, but it involves the use of several leaves, and the combination of thermal and water stress (Burke, 2007).

The Fs indicator of chlorophyll fluorescence, a component of "Fv / Fm" (also known as F / FM, or Fms - FS / SMF), has been reported as the most sensitive moderate water stress test (Flexas 1999). and (Flexas, 2000).

PI, or "performance index", has been shown to detect water stress after about seven days (Zivcak et al., 2008), and Fv / Fm is insensitive to water stress.

Another method of investigation to identify water stress in time is by measuring the turgor pressure in the leaves.

This can be done using a newly developed magnetic clamp leaf pressure probe (LPCP), which provides information on the relative pressure changes (PC) due to turgidity in monitored plants at PC> 50 kPa. PCP probe

Is an instrument that allows relative measurements changes in leaf turgidity pressure or - after calibration against the cell turbidity pressure probe (Zimmermann et al. 2004, Rüger et al., 2010) - absolute changes in turbidity pressure. Records on tall vines in greenhouse conditions and the first measurements on vines showed that the changes in turgidity pressure in response to environmental changes and / or watering was reflected in the LPCP probe patch outlet pressure.

The theory shows (Zimmermann et al., 2008) that the outlet pressure (Pp) sensed by the probe is inversely proportional to the PC. Under a turgid pressure of approx. 50 kPa, the Pp indicator collects valuable information about the interaction between air and water supply to the leaves. The probe is non-invasive and able to operate automatically and continuously in real field conditions (Fernandez et al., 2011).

The probe signals are sent over the mobile network to an Internet server, in which case the data is stored and viewed graphically or as tables in real-time.

Export functions allow data to be retrieved from personal assessment software (Zimmermann et al., 2008). The evaluation of the time constants in the turgescence pressure recovery phase can give some information about the dynamics of water supply to the trees, in extremely variable field conditions.

SPAC (soil-plant-atmosphere continuum) is the system that allows water to move from the soil through plants to the atmosphere.

This system characterizes the state of the water in its various components, as expressions of the energy level or water potential of each. Modeling water transport between SPAC components is similar to studies on water potential gradients between segments. The concept of the soil-plant-atmosphere continuum (SPAC) was first proposed by Phillip in 1966.

Since then, the fundamentals of soil physics have been enriched, for example, by Hillel (1980). Allen et al. (1998) presented and updated the methodology for calculating culture evapotranspiration (ETc). This plant parameter depends on both the reference evapotranspiration (ETo) and the stage of development of the plants for each species. Sweating is a process that unites the flow of water in the SPAC system (Allen et al., 1998).

Grapevine drought responses

Stomatal regulation of water use

Stomata are those tiny pores on the surface of the leaves that control the exchange of gases between the plant and the atmosphere (Darwin, 1898). Stomata are key players in a plant's response to drought. The xylem makes it possible for water to flow from the ground into the atmosphere under tension (that negative tension (Tyree, 1997), pulled by the more negative water potentials (Ψ) in the leaf tissues where water is transpired into the atmosphere through stomata (Zimmermann, 2002), Figure 1.

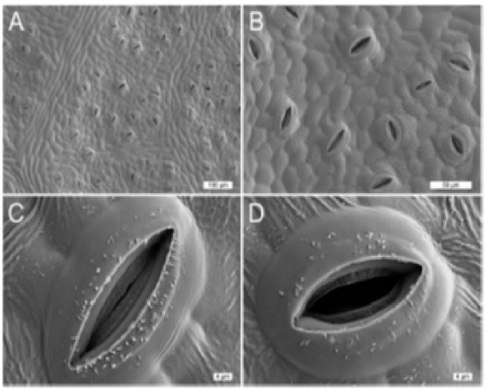

Figure 1. Cryo-scanning electron microscopy images of the underside of a grapevine (Vitis rupestris) leaf at various magnifications. Islands of stomata (A) are visible in between leaf vascular traces, and there is ample aperture diversity (B) of stomata in more closed (C) or open (D) states. Scale bars are shown in each panel

Darwin (1898) suggests avoiding water potential (Ψ) critically and emphasizing water conservation, the stomata being the first to feel prolonged drought, the phenomenon of transpiration being intense during this period. Jordan et al., (1975), later Patakas et al. (1997) indicated that mature leaves close stomata below Ψ lower compared to younger leaves, as mature leaves have more lignified cell walls and a potential for more negatively dissolved, allowing them to maintain perspiration while sustaining the low strain (Hsiao et al., 1976; Patakas and Noitsakis, 2001). The response of the stomatal conductance (gs) to the water potential (Ψ) is considered a basic physiological characteristic of a species (Brown et al., 1976; O'Toole & Cruz, 1980), these (gs) being frequently reduced to the water potential. water, up to 50% or 75% stomatal closure (Ψgs50 or Ψgs25, respectively; Klein, 2014) Transpiration is controlled by the stomata, being abundant on the lower part of the leaves, the stomata open and close actively to allow or restrict the exchange of gases. The stomata are often closed at night, photosynthesis being slowed down because there is no sunlight. With humidity during the day, however, sunlight stimulates them to open up to allow the vine to take up carbon (CO2) for photosynthesis and release oxygen (a byproduct of photosynthesis) and water vapor (Tim Martinson & Alan Lakso, 2018).

Without this reduction in transpiration, the rapid flow could lead to large disturbances of the plant, more precisely to large decreases in pressure between the soil and the leaves (ie, Ψ more and more negative). Irrigated vineyards typically operate in a safe range of water potential (Figure 2, Ψ stem> -1.5 MPa, adapted from Charrier et al., 2016) that do not lead to cavitation or turgor loss in vineyards non-irrigated rarely exceed these values (Charrier et al., 2018). When poor irrigation strategies are used, especially in vineyards grown for the production of red wine grapes, they normally target water deficit levels (Ψ stem -1.2 to -1.4 MPa) which are sufficient. large to decrease stomatal conductance (gs), that are certainly great enough to decrease stomatal conductance (gs), transpiration, photosynthesis, and ultimately fruit yield (Figure 2).

More severe water deficiency (Ψ stem <−1.6 MPa) can lead to loss of turgidity and xylem cavity, which could lead to leaf loss and eventually vine decline.

Relatively recent studies report that vine genotypes are different, their stomata reacting differently to water deficiency (Lavoie-Lamoureux et al., 2017; Charrier et al., 2018; Levin et al., 2019; Dayer et al., 2020). Thus, some varieties tend to close their stomata earlier than others. Regardless of these differences in vine varieties, all close their stomata in response to water deficit in a relatively narrow range of water potential compared to the width of plant taxa in general (Lavoie-Lamoureux et al., 2017; Martin-StPaul et al., 2017). Understanding the basic mechanisms that determine the regulation of stomata in drought conditions allows the identification of targets that could be used to grow more drought-tolerant varieties and / or rootstocks.

Extreme drought

McDowell et al., (2008) state that high levels of embolism in perennial organs, also known as "hydraulic failures", lead to plant decline, as vines can die from prolonged drought and extreme water deficits 2, stem Ψ <−2 MPa) leading to the loss of a large or the entire roof and the crop in the current season.

In potted-plant experiments even when vines are stressed to levels that result in nearly complete defoliation and 100% loss of conductivity due to embolism in stems, a large percentage of vines still regrow the following season (Tombesi et al., 2018; Charrier et al., 2018).

Knipfer et al., (2015), later Nardini et al., (2017) highlight in their studies the ability of vines to recover from and/or repair extensive embolism over winter may involve their ability to refill embolized xylem vessels in the stem except for leaves and petioles that do not appear to be recovering from embolism (Hochberg et al., 2016; Hochberg et al., 2017a). One concern is cavitation fatigue, in which previous cavitation events lead to an increase in cavitation vulnerability (Hochberg et al., 2017b). In terms of season-to-season transfer effects on fruit production, water deficits can sometimes lead to a decrease in production in the following season (Williams & Matthews, 1990; Dayer et al., 2013), but so far, few studies have investigated the deferral effects of more severe drought events (Tombesi et al., 2018).

Pressure chamber

The xylem potential of the vine (Ψ) is the negative pressure or pressure under which water flows from the roots to the interface with the leaf air through the xylem and is then vaporized. Thus, the xylem potential can be measured at the petiole to reflect the potential of water from leaves or stems.

To maintain a continuous flow of water from the roots to the leaves, where it is transpired through the stomata, the tension of the water column inside the vine gradually increases from the soil-root interface to the leaf-air interface. When the tension of the xylem becomes too high, air bubbles form inside the xylem vessels, thus leading to the gradual disconnection of the petioles of shoot leaves. This phenomenon is called cavitation and is measured as a loss of hydraulic conductivity (Hochberg et al., 2017b; Charrier et al., 2016).

Figure 2 shows the ximel's vulnerability curves, which show that as the xylem potential decreases, the hydraulic disconnect between the petiole and the shoots increases.

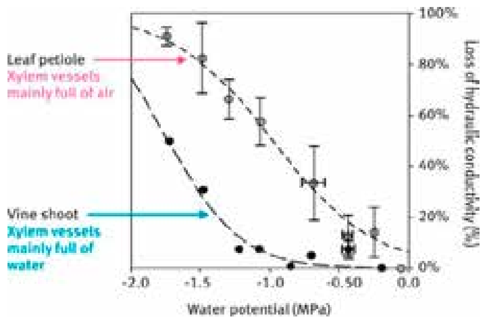

Figure 2. Petiole and shoot xylem vulnerability curve (adapted from Charrier et al., 2016)

When the xylem potential is close to -12 bar at the petiole, only 50% of the xylem vessels remain filled with water, and the remaining 50% no longer contribute to the conductivity of the water between the leaf and the shoot (Figure 2).

Based on measurements using a hydraulic apparatus, the water potential leading to 50% loss of conductance (Ψ_{50}) of petioles was −1.56 MPa for grapevine and −2.77 MPa for red oak (Holbrook, 2017). Sperry (1998) evaluates the potential of water leading to a 50% loss of conductivity (Ψ50; MPa) using three different methods: bench dehydration in combination with hydraulic measurements (Sperry et al., 1988) of petioles, optical measurement of dehydrating leaves, or gas injection in combination with optical measurement.

Leaf water potential (Ψleaf)

This assessment is usually done at noon, on the forehead of an adult well exposed to the sun, being a fairly quick measurement. The disadvantage of this assessment is that homeostasis between the water potential of the leaves and the water potential of the soil is the basis of rapid temporal fluctuations depending on environmental conditions (such as passing clouds).

Leaf Ψ is a convenient measurement with the use of a leaf pressure chamber. Jones (1990) a suggested that the leaf may be an erroneous indicator of the state of the plant's water, because the homeostasis of the leaf can occur under different soil and environmental conditions.

Vine varieties have been shown to vary in their homeostasis in declining leaves. The differential response between varieties is considered to be related to the abscisic acid of the leaves and xylem and the hydraulic regulation (Dayer et al., 2020). Schutz (2003) found in a study of vine varieties for wines that the Syrah variety (syn. Shiraz) proved to be relatively anizohydric compared to Grenache, which was almost isohydric. Based on these physiological responses, the use of Ψleaf for irrigation scheduling of relatively isohydric plants may underestimate their true water stress and therefore irrigation requirements potentially leading to a vicious cycle. (Dayer, et al., 2020);

Stem water potential (Ψstem)

It is determined by enclosing a leaf in an aluminum foil bag for 30- 120 min before the measurement (Delloire et al., 2020). This way, the leaf reduces its transpiration and equilibrates its water potential to the stem water potential (but not necessarily to the shoot water potential as this varies with petiole loss of hydraulic conductivity). Stem water potential is sensitive to vapor pressure deficit and integrates the combined effect of soil and tissue water availability on the one hand and climatic demand on the other hand. The water potential of the strain is sensitive to the vapor pressure deficit and integrates the combined effect of soil and tissue water availability, on the one hand, and climate demand, on the other (Delloire et al., 2020). Chone (2001) states that there is a correlation between the water potential of the stem and the climatic conditions and the transpiration of the plant.

Pre-dawn leaf water potential (ΨPD)

The measurement is made just before sunrise on the adult leaves, when the state of the living water is at its maximum (Deloire, 2020). The advantage of pre-dawn water potential measurements is that they are stable, regardless of climatic conditions, and are closely related to the condition of the groundwater in the vicinity of the roots (Deloire, 2020).

Table 1. Pre-dawn leaf water potential and grapevine water status (Carbonneau, 1998; Lovisolo et al., 2010, 2016 adapted after Deloire et al., 2020)

Classes	Predawn leaf water potential ($\Psi_{plwp,}$ MPa)	Level of water constraint or stress
1	0 MPa ≥ ψ_{plwp} ≥ -0.3 MPa	No water deficit
2	-0.3 MPa > ψ_{plwp} ≥ -0.5 MPa	Mild to moderate water deficit
3	-0.5MPa > ψ_{plwp} ≥ -0.8 MPa	Moderate to severe water deficit
4	< -0.8 MPa	Severe to high water deficit (= stress)

However, ΨPD remains affected by night transpiration, water transfers between organs, and vapor pressure deficit (VPD), (Coupel-Ledru et al., 2014; Rogiers et al., 2012).

Carbonneau (1998), quoted by Deloire et al. (2020) assesses the PLWP threshold for the degree of water deficit following a study of over 20 years, conducted in several vineyards. Table 1 provides answers on vine physiology and grain maturation as PLWP decreases (Carbonneau, 1998).

Sap flow-based measurement

One way to measure crop water status, which is essential for optimized irrigation scheduling, is to manually measure water from leaves or stems (Shackel, 2011; Williams & Baeza, 2007), using a Scholander pressure chamber invented in the 1960s. (Scholander et al., 1965). In precision agriculture these limitations given by manual technique are used precision electronic sensors inserted on plants to continuously measure the state of the culture water developed on the basis of various detection methods. These sensors applied to fruit crops include either sap flow sensors

(Ginestar et al., 1998), thermal diffusivity sensors (Pagay & Skinner, 2018), dendrometers (Corell et al., 2014), or thermal or infrared sensors. (Jones, 1999; Pagay, 2021).

Sap flow is the movement of water inside the xylem from the roots to the leaves, where it transpired especially through the stomata. Sap flow directly measures the amount of water used at the whole vine level. Two methods of measurement exist.

1. The thermal dissipation probe method

The sap flow is the movement of water inside the xylem from the roots to the leaves, where it has transpired especially through the stomata. The sap flow measures the amount of water used in the whole vine. Vergeynst et al. (2014) showed that the circumferential and radial variation of the sap flow density may be due to the overestimation of the sap flow. In addition, the sap flow density may be underestimated when the heated needle is in contact with non-conductive tissues. Therefore, this method is little used, not being able to be used in commercial use.

2. The stem heat balance method

In this process, the sap flow sensor design consists of a heated sleeve wrapped around the stem (Lascano et al., 2016). By this method the heat is supplied uniformly and radially on the section of the rod; the sleeve is flexible and maintains a perfect fit between the stem and the thermocouple during the diurnal contractions of the stem (Figure 3, after Ginestar et al., 1998; Scholasch, 2018).

Figure 3. Close-up on a sap flow sensor placed on a lateral vine branch The heating tab surrounds the entire section of the branch. Temperature sensors measure the amount of heat displaced by the sap (after Ginestar et al., 1998; Scholasch, 2018)

The sensors are easy to apply, either over slightly bent stems or even when they are partially necrotic, as is sometimes seen in response to cutting lesions. Because the entire section of the stem is heated, the method of heat balancing can be applied even if the trajectory of the sap flow through the stem is sinuous. Zhang et al. (2011) show that the thermal balance of the stem can be a reliable method for using vine water.

Figure 4. Close up view of an individual microtensiomete. MT data of trunk water potential (Ψtrunk) (Vinay Pagay, 2021)

The potential consequences of drought on vegetative growth and yield

Many studies have shown that stress caused by prolonged drought can lead to decreased vigor, yield, poor fruit quality (Romero et al., 2004; Alves et al., 2013; Gerós et al., 2015), and may even induce changes in key metabolic pathways (Roby et al., 2004; Romero et al, 2010), changes in the abundance of transcripts and metabolites involved. in the metabolism of phenylpropanoid, isoprenoid, carotenoid, amino acids and fatty acids (Oliveira et al., 2003; Deluc et al., 2009; Savoi et al., 2016) . All these responses of the vine plant depend on several factors such as varieties, crop load, vineyard age, soil type, phenological stage or canopy development (Cook et al., 2015, Ojeda et al., 2002). Other researchers Keller et al., 2008; Castellarin et al., 2007) mention that water stress can have a positive impact on the composition of berries by improving sugars, flavors and color. Under these conditions, irrigation can have a great influence on the yield of the vine, the quality of the grains and the sensory characteristics of the wine (Matthews , et al., 1990; Keller et al, 2006; Keller et al., 2008).

- Reduction in vegetative growth and hence in the exposed leaf area.
- Reduction in the growth of lateral shoots, with positive or negative consequences depending on the climatic conditions (increased exposure of the bunches to sunshine, reduced activity of carbon sinks or sources depending on the stage).
- In the case of severe and early water deficit, uneven budburst and a reduction in the fertility of latent buds on the growing primary shoots (Guilpart, 2014);
- Deterioration in the growth of inflorescences and flowers in year N + 1 (for which the primordia were formed in the latent buds in year N), which may lead to fertilization defects including coulure (poor fruit set) and millerandage (uneven berry development and ripening), (Deloire &Pellegrino, 2021).
- Severe water deficiencies during a growing season can lead to both inhibition of the biosynthesis of primary metabolites (organic acids, sugars) (Wang et. al., 2003), and disruption of berry ripening dynamics (Antalick et al., 2021)
- Severe water deficiencies can lead to inhibition of the biosynthesis of secondary metabolites (phenols, flavor precursors) before and after the test, although it is known that the content of anthocyanins in berries is favored at moderate levels of water deficiency.
- Change in the aromatic profile of the wine when more than 20% of the berries are wrinkled (Šuklje et al., 2016).
- Water deficiencies during fruit formation can lead to uneven growth of berries which can affect wine style (Antalick et al., 2021).
- An earlier harvest, which is mainly due to the overall concentration of sugar in berries (ie Brix or potential alcohol) which is used to determine the date of harvest, rather than the daily accumulation of sugar per grain, resulting in more sugar per grain (mg/berry).

CONCLUSIONS

This analysis summarizes the latest results on the responses to drought on vines, the impact of water shortages on the plant, and fruit production and composition. Based on the results provided by us in this review, the following conclusions can be drawn**:** Water potential measurements as an index for irrigation control are challenging due to the effect of environmental fluctuations on hydraulic conductivity between the leaf and the shoot. This methodological drawback is particularly acute in a context of temperature warming and increased aridity where irrigation must be managed with greater precision.

As heatwaves become more frequent, the irrigation strategy must take into account the effect of variations in the vapor pressure deficit for optimal use of vine water and, ideally, should be taken into account depending on the variety and rootstock (Scholasch, 2019). Therefore severe water deficiencies should be avoided (water stress from the beginning of the bud until after harvest.Water potential is considered the best indicator of plant water status, being the integrated result of above-ground and subterranean environmental conditions, promising as an essential variable of the model to which other plant processes respond. In this review we wanted to highlight some of the physiological processes that are mechanically related to the potential of water.

The following aspects should be considered when analyzing the impact of water status on the functioning of the vine (vegetative growth; fertility/yield of the vine; fruit growth and composition; categories and aromatic profiles of wines (Deloire &Pellegrino, 2021):

- The period of onset of water deficit during the phenological stages (van Leeuwen et al., 2009).
- The intensity of water deficit (deficit versus stress).

Irrigation management is crucial for grape quality. Therefore, excessive irrigation can cause loss of quality due to high yields, vigorous tree growth and sparse grains (van Leeuwen et al., 2009)

Quality production on irrigated vines can only be achieved when a moderate water deficit is mentioned (van Leeuwen et al., 2009).

The condition of the vine water is most accurately assessed by the use of physiological indicators. This analysis highlighted research showing that the water potential of the phloem , stomatal conductance, leaf are ways to assess the state of water in plants and can be precise tools for irrigation management. van Leeuwen et. al, 2009)

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THE DIVERSITY OF INSECT SPECIES AT GROUND LEVEL IN THE ȘTEFĂNEȘTI VITICULTURAL CENTER

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Abstract

The paper aimed to present the evolution of the insect that were active on the ground surface during the 2020-2021 period collected from 3 plantations from the Stefanesti viticultural center. By the end of the study, 37 species of insects caught in pitfall traps had been identified. Of these, in 2020, 20 species with a total of 2429 individuals were collected and in 2021, 28 species with a total of 395 individuals. Of all the species identified, only 11 are repeated in both years. The data obtained were processed using the BioDiversity Professional 2 program. Thus, were calculated: Rarefaction, Simpson index, Shannon index, SHE analysis and Bray - Curtis analysis. The insect species identified in the period 2021-2022 did not cause damage in the monitored vineyards.

Key words*: biodiversity, grapevine, pests, arthropod, Lasius.*

INTRODUCTION

Control pathogens and pests measures needed to increase production and income can have negative effects on biodiversity from viticultural ecosystems (Tabaranu G. et al., 2018).
Biodiversity management in the face of climate change is recommended to be achieved by monitoring the evolution of species, expanding and maintaining monitoring programs (Ernst et al., 2015). Therefore, choosing the most efficient sampling method is crucial (Csaszar et al., 2018). Pitfall traps are most often used for environmental studies and monitoring programs (Southwood & Henderson, 2000; Babin-Fenske & Anand, 2010; Da Silva et al., 2011; Isaia et al., 2015; Brown & Matthews, 2016; Boetzl et al., 2018). Originally described by Barber (1931), pitfall traps are inexpensive, easy to use and operate round-the-clock, resulting in large, species-rich samples (Schmidt et al., 2006).

MATERIALS AND METHODS

Pitfall traps, filled 2/3 with formalin solution 4%, have been installed on the vineyard rows (on the intervals between the grapevine rows the soil was kept grass cover) in order to establish the structure of entomofauna from the soil surface.
These were placed in 3 vineyards and collected once every two weeks. 14 collections were made and the monitoring has been done between July-October for 2020 and 2021, as follows:
06.07.2020-17.07.2020,17.07.2020-03.08.2020, 03.08.2020-17.08.2020,17.08.2020-31.08.2020, 31.08.2020-14.09.2020,14.09.2020-30.09.2020, 30.09.2020-12.10.2020,07.07.2021-21.07.2021, 21.07.2021-04.08.2021,04.08.2021-18.08.2021, 18.08.2021-01.09.2021,01.09.2021-16.09.2021, 16.09.2021-29.09.2021,29.09.2021-13.10.2021.
The collected insects were transported to the laboratory and identified using the IPM-scope digital microscope (Spectrum Technologies, USA) and an Optika SZM-2 trinocular stereomicroscope equipped with an Optika CP-8 photo camera (Optika, Italy).
The obtained results have been processed with the BioDiversity Professional 2 program, thus being calculated: Rarefaction, Shannon index, Simpson index, SHE analysis and Bray - Curtis analysis.

RESULTS AND DISCUSSIONS

Biodiversity loss it has become an international problem (Thukral, 2017). Thus, the biodiversity monitoring is important because helps with restoration and conservation.

In the July - October 2020 period, 2429 individuals were collected that are belonging to 20 species: *Gnaphosidae* sp., *Heliophanus* sp., *Hydrotaea diabolus*, *Metcalfa pruinosa, Graphosoma lineatum, Apis mellifera, Camponotus* sp., *Cardiocondyla* sp., *Formica* sp., *Lasius* sp., *Vespula germanica, Vespula vulgaris, Opilio* sp., *Opiliones* sp., *Calliptamus italicus, Calliptamus* sp., *Acheta domesticus, Melanogryllus desertus, Modicogryllus frontalis, Modicogryllus truncatus*. *Lasius* sp. (1255), *Formica* sp. (597), *Camponotus* sp. (244) and *Melanogryllus desertus* (124) recorded the highest number of individuals (Table 1).

In the same period, but in 2021, 28 species were identified (with a total of 395 individuals): *Acheta domesticus, Anthomyia procellaris, Caliptamus* sp., *Calliptamus italicus, Camponotus* sp., *Carabus coriaceus, Cardiocondyla* sp., *Chorthippus brunneus, Corythucha* sp., *Cyphophthalmus duricorius, Gryllus campestris, Ichneumon gracilentus, Lasius sp., Melanogryllus desertus, Micrommata virescens, Modicogryllus frontalis, Modicogryllus truncatus, Opilio* sp., *Opiliones sp., Oxycarenus lavaterae, Pezotettix giornae, Phaneroptera falcata, Platycleis albopunctata albopunctata, Pterostichus melanarius, Raglius alboacuminatus, Scaphoideus titanus, Scymnus* sp., *Tomoxia bucephala*. Of these, the highest number of individuals was in the case of *Modicogryllus* truncatus (125), *Opilio* sp. (71), *Lasius* sp. (56) (Table 1).

The largest number of individuals during the entire study period were registered in the case of ant from *Formica* and *Lasius* genres.

So far, at the international level have been discovered over 17,500 ant specimens belonging to nearly 220 extinct species from 73 genera (34 extinct and 39 extant) and 12 subfamilie
s (Radchenko, 2021).

Table 1. Species richness collected in pitfall traps

No	Species	Year	
		2020	2021
1.	*Acheta domesticus*	9	31
2.	*Anthomyia procellaris*	0	1
3.	*Apis mellifera*	3	0
4.	*Calliptamus italicus*	4	1
5.	*Calliptamus* sp.	1	1
6.	*Camponotus* sp.	244	11
7.	*Carabus coriaceus*	0	5
8.	*Cardiocondyla* sp.	69	4
9.	*Chorthippus brunneus*	0	1
10.	*Corythucha* sp.	0	2
11.	*Cyphophthalmus duricorius*	0	2
12.	*Formica* sp.	597	0
13.	*Gnaphosidae*	1	0
14.	*Graphosoma lineatum*	1	0
15.	*Gryllus campestris*	0	1
16.	*Heliophanus* sp.	1	0
17.	*Hydrotaea diabolus*	1	0
18.	*Ichneumon gracilentus*	0	1
19.	*Lasius* sp.	1255	56
20.	*Melanogryllus desertus*	124	36
21.	*Metcalfa pruinosa*	1	0
22.	*Micrommata virescens*	0	1
23.	*Modicogryllus frontalis*	21	3
24.	*Modicogryllus truncatus*	48	125
25.	*Opilio* sp.	2	71
26.	*Opiliones* sp.	7	5
27.	*Oxycarenus lavaterae*	0	3
28.	*Pezotettix giornae*	0	24
29.	*Phaneroptera falcata*	0	1
30.	*Platycleis albopunctata albopunctata*	0	1
31.	*Pterostichus melanarius*	0	2
32.	*Raglius alboacuminatus*	0	2
33.	*Scaphoideus titanus*	0	1
34.	*Scymnus* sp.	0	1
35.	*Tomoxia bucephala*	0	2
36.	*Vespula germanica*	9	0
37.	*Vespula vulgaris*	31	0
	Total	**2429**	**395**

Lasius species bring benefits to the soil changing its profile and texture, favoring drainage, being able to handle well in unstable and disturbed habitats (Quque & Bles, 2020). This explains the large number of individuals in 2020 when periods of drought alternated with periods of excess rainfall.

The genus *Formica* includes about 180 species, distributed almost exclusively in the Holarctic Region (Radchenko, 2021). Half of the species of the genus *Formica* are confirmed or suspected social parasites (Borowiec et al., 2021).

The ants from the genus *Formica* have effect on soil qualities and the presence of some flora, also strongly influences the surrounding zoocenosis. The apparition of ants is dependent on the quantity of light, the structure and quantity of vegetation and the quantity of food supplies relating to it. Also, air and soil temperatures are important for the activity of workers (Véle et al., 2009). This may explain the lack of individuals in 2021, when lower temperature values were recorded in January, February, March, April, September and December compared to 2020 (Figure 1).
Five treatments were applied each year (Table 2), of which only sulfur, abamectin and spirodiclofen (first treatment of the year) were applied to control mites. Therefore, no insecticides were applied and the evolution of the insects was not influenced by them.

Table 2. Pesticides applied in the two years of study

Nt*	Phytosanitary treatment 2020	2021
1	Metiram 70 %	Metiram 70 %
	Sulf 80 %	Sulf 80 %
	Abamectin 18 g/l	Spirodiclofen 240g/l
2	Fosetil de aluminiu 50 % + folpet 25 %	Penconazol 100 g/l
	Penconazol	Oxatiapiprolin 100 g/l
	Fluopicolid 4,44 % + fosetil-Al 66,7 %	Mancozeb 64 % + Mefenoxam 4 %
		Miclobutanil 240 g/l
4	Azoxistrobin 93,5 gr/litru + folpet 500 gr/litru	Folpet 250 g/kg + Fosetil de aluminiu 500 g/kg
		Tebuconazol 500 g/kg + Trifloxistrobin 250/kg
5	Sulfat de cupru 20 %	Cupru 200 g/kg
	Sulf 80 %	Sulf 80 %

*Nt = Number of treatments

The insect species identified in the 2021-2022 years did not cause damage in the monitored vineyards. Of all the species identified, only 11 are found in both years. The others were probably affected by the non-uniformity of temperatures and precipitation recorded in 2020 and 2021.
Compared to the multiannual averages for the period 1979-2011, in 2020 there were higher increases in average temperatures in January (+1.81°C), February (+3.51°C), March (+3.20), April (+1.53°C), July (+1.89), August (+3.06°C), September (+3.92°C), October (+2.09°C) and December (+2.08°C). In 2021, there were temperature increases of more than 1.5°C compared to the multiannual average only in February (+2.60°C), July (+3.5°C), August (+2.64°C), November (+2.24°C) and a decrease of 1.85°C in April (Figure 1).

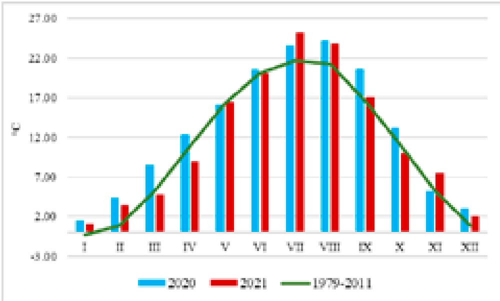

Figure 1 The temperature evolution in 2020 and 2021

The vineyards water consumption it is between 500 and 700 mm (Dejeu, 2010; Medrano et. al., 2015) of which at least 250-300 mm evenly distributed during the growing period of the vine (Dejeu, 2010).
The multiannual amount of rainfall for the period 1979-2011 was 665.89 mm with 8.69 mm more than that recorded in 2020 (657.2 mm) and with 104.91 mm less than the amount recorded in 2021 (770.8 mm) (Figure 2).

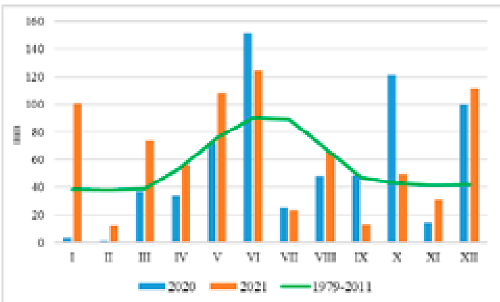

Figure 2 The precipitation evolution in 2020 and 2021

The relationship between the total number of insects counted (as individuals) from 2020 and 2021 and the total number of different species of which they belong is presented in Figure 3. It can be seen that the largest number of individuals was collected during the 17.07.-31.07.2020, 6.07.-17.07.2020, 03.08.-17.08.2020, 17.08.-31.08.2020 periods.

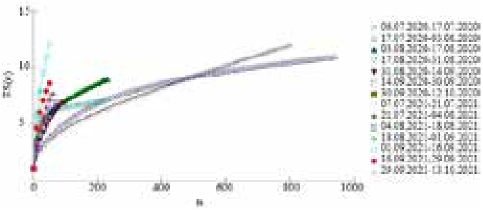

Figure 3. Rarefaction plot

The Simpson and Shannon indices measure species diversity on the basis of species richness and evenness in abundance (Santini et al., 2017).

One difference between the Shannon and Simpson indices is their relative sensitivity to rare species. The Simpson Index calculate the weighted dominance over common species, while the Shannon Index is an equally weighted informational statistic for rare and ordinary species (Magurran 1988).

The Shannon Index is the most preferred of all diversity indices. Its values are between 0.0 and 0.5 (Kocatas, 1992). The values above 3.0 suggest that the habitat structure is stable and balanced and the values under 1.0 indicate that there is a degradation of habitat structure (Türkmen and Kazanci, 2010). Also, 1.0 means a low diversity and 5.0 a high diversity (Gering et. al, 2003). For both years (2020 and 2021) the Shannon index shows values below 1 (Figure 4), the highest value (0.910) being registered in the 01.09.2021-16.09.2021 period and the lowest (0.509) in the 06.07.2020-17.07.2020 period. Therefore, the diversity of insects at ground level was reduced over the entire study.

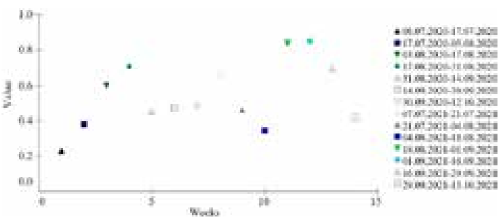

Figure 4. Shannon Index Results

Simpson index (D) measures the dominance strength and varies inversely with species diversity (Sagar & Sharma, 2012). The Simpson index always has values less than 1 (Pielou, 1969, Gering et al, 2003). Thus, the species diversity for this index, varies from 0-1; where, zero means no diversity and 1, maximum diversity (Sagar and Sharma, 2012). Therefore, during the 2 years of study the maximum diversity was recorded in the 06-17.07.2020 and 4-18.08.2021 periods and the lowest in 18 -01.09.2021 and 01-16.09.2021 periods (Figure 5).

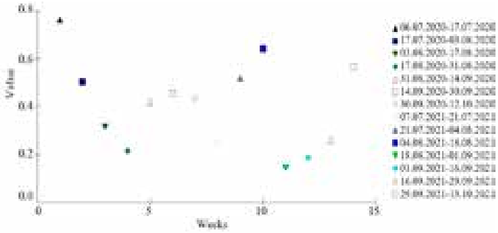

Figure 5. Simpson index

Examination of the relationship between S (species richness), H (information), and E (evenness) was performed through SHE analysis. When in the SHE analysis the Ln(E)/Ln(S) fraction is the most constant, it means that there is a balance between species richness and evenness (Zamfirescu & Zamfirescu, 2005). During insect monitoring was observed that the Ln(E)/Ln(S) fraction has become constant from the fourth collecting and lasted until the end of the monitoring (Figure 6). Therefore, in the 17.08.2020-31.08.2020, 31.08.2020-14.09.2020,14.09.2020-30.09.2020,30.09.2020-12.10.2020,07.07.2021-21.07.2021,21.07.2021-04.08.2021,04.08.2021-18.08.2021,18.08.2021-01.09.2021,01.09.2021-16.09.2021,16.09.2021-29.09.2021,29.09.2021-13.10.2021 periods was a balance between species richness and evenness.

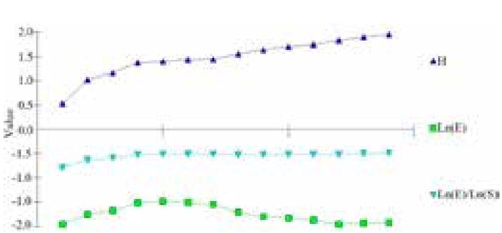

Figure 6. She analysis

Bray-Curtis index was used to determine the degree of similarity in species composition between 14 insects collecting periods. As you can see in Figure 7, there is no similarity between the two years in which the insects were monitored. There were similarities only between the periods of the same year. Thus, for the 2020 year the highest similarity was between the 17.08.2020-31.08.2020 and 31.08.2020-14.09.2020 periods (56%); for 2021, 21.07.2021-04.08.2021 and 4.08.2021-18.08.2021 periods (80%).

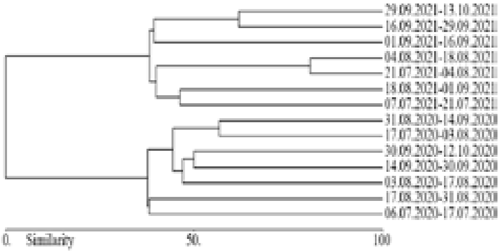

Figure 7. Bray-Curtis Cluster Analysis between insect collection periods

The insects compose the biggest group of organisms on earth about 66% of all animal species. They form an important part of ecosystems, provides valuable ecosystem services and can be found almost everywhere. Herbivorous insects damage 18% of worldwide agricultural production. Despite these damages fewer than 0.5 % are considered pests (Jankielsohn A., 2018).

CONCLUSIONS

In 2020, 20 species of insects were identified (a total of 2429 individuals) and in 2021, 28 species (a total of 395 individuals).

According to the Simpson Index, the greatest diversity took place in the 06.-17.07.2020 and 04.-18.08.2021 periods.

From 17.08.2020 until 13.10.2021 there was a balance between species richness and evenness.

There was similarity in species composition only between the periods of the same year, not between years.

The insect species identified in the 2021-2022 years did not cause damage in the monitored vineyards.

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VEGETABLE GROWING

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SURVEY ON CONSUMERS PREFERENCE IN THE NEW ACCLIMATIZED SPECIE IN ROMANIA: *BENINCASA HISPIDA*

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Abstract

In the climate change scenario, growing adequate food is a major challenge for food security. To provide food to the population, introduction of new vegetable crops and extending newer assortments are important tasks of breeding programs. One of the recently acclimatized species in Romania by VRDS Buzau is Benincasa hispida, a multi-purpose and nutritious vegetable. In this study, a consumers preference survey was conducted to inform local smallholder farmers, traders and other stakeholders on opportunities for expanding production and commercialization of Benincasa hispida. Three genotypes of wax guard, G1, G2, G3 were taken into study. Data was collected from 57 participants aged between 16 and over 45 years from urban and rural areas. Results indicated that 43 participants were interested to eat the fruit due to its nutritional properties. Consumers are also interested in growing winter melon because this crop is easy to grow. Of the three genotypes tasted, G1 was preferred by participants, followed by G3 and G2. G1 variety was registered in the Official Catalogue of Romanian Crop Plants, under the name of Zefir.

Key words: *breeding, climate change, winter melon.*

INTRODUCTION

In last years, the agriculture sector has been under growing pressure to produce sufficient food, biofuel and feed on insufficient land for the planet's predicted 9 billion people by 2050 (Dhaliwal et al., 2020). At the same time, climate change affects agriculture and food production in complex ways. (Schmidhuber and Tubiello, 2007). So, in the developing world, climate change is a significant driver of change for food security, because it threatens food production and its stability as well as other aspects of food systems such as food access, storage, and utilization (Vervoort et al., 2014. In the climate change scenario, growing adequate food is a major challenge for food security (Dhaliwal et al., 2020).

To provide food to the population, introduction of new vegetable crops and extending newer assortments are important tasks of breeding programs (Dahlia et al., 2012). The Vegetable Research Development Station (VRDS) Buzău is an important research centre for acclimation and breeding vegetable species in Romania (Vînătoru et al., 2021). One of the recently acclimatized species by VRDS Buzau is *Benincasa hispida*, a multi-purpose and nutritious vegetable (Nimbal et al., 2011; Zaini et al., 2011; Vînătoru et al., 2021). It provides a good source for natural sugars, organic acids, aminoacids, vitamins and mineral elements (Zaini et al., 2011).

The fruit of *Benincasa hispida* (Thunb.) Cogn., commonly called as ash gourd, wax gourd, white gourd, winter melon, fuzzy melon, hairy melon (Doharey et al., 2021), belongs to the Cucurbitaceae family. This is employed as a main ingredient in kusmanda lehyam, a product which is used in numerous nervous disorders in the Ayurvedic medicine (Sabale et al., 2011).

A number of medicinal properties such as anti-diarrheal (Bhyrapur et al., 2005), anti-obesity (Kumar and Vimalavathini, 2004), anti-inflamatory (Shetty et al., 2008: Park et al., 2009; Gill et al., 2010), anti-ulcer (Grover et al., 2001; Rachchh and Jain, 2008: Rachchh, 2011), anti-compulsive (Girdhar et al., 2010), anti-histaminic, anti-ageing (Sabale et al., 2011), antioxidant and diuretic (Jayasree et al., 2011; Mandana, 2012; Samad et al., 2013) have been ascribed to winter melon pulp. Also, it prove to have beneficial effects in allergic inflammation, epilepsy and insanity (Bhalodia et al., 2009), preventive and curative effects in nervous disorder, diabetic, leucorrhoea (Lee et al. 2005), intestinal worms, jaundice, stomach and bile problems, potential uses as, laxative, aphrodisiac, diuretic, clearing heat and detoxificant, used for Alzheimer disease treatment (Roy et al., 2008), facial eruption, inhibition of angiotensin converting enzyme (ACE) (Huang et al., 2004) and nootropic effects (Zaini et al., 2011.

Usually, deep pharmacological activities are performed on the entire wax gourd plant, including flower, leaves, fruit peel, and seed (Huang et al., 2004; Doharey et al., 2021).

Sreenivas et al., 2011 showed that winter melon peel is a good source of edible wax having a potential application in shelf life improvement of fruits. After wax extraction, these defatted peels can be utilized for adsorption of Cr (VI) from water (Sreenivas et al., 2014).

Benincasa hispida is a particularly valuable species in term of its use as a rootstock. The specie is compatible with most cucumber and melon cultivars. At the same time, the plant has genetic resistance to specific soil pests and insects (Vînătoru et al., 2019).

The aim of this study was to acknowledged the consumer preference about the wax gourd fruit benefits and to inform local smallholder farmers, traders or other stakeholders on opportunities for expanding production and commercialization of *Benincasa hispida*.

MATERIALS AND METHODS

The Breeding and Biodiversity Laboratory from Vegetable Research Development Station Buzău has taken into study this species after year 1996. The research began with purchase of genetic material from various area of origin, followed by acclimatization of the species. After year 2005, valuable genotypes have been subject to intensive breeding work and the research completed so far with obtaining of three genotypes noted G1, G2 and G3 (Figure 1).

These three genotypes of wax guard, with distinct phenotypic expressiveness, especially in terms of fruit shape and size, were taken into study.

The breeding method used was repeated individual selection. The seedlings were produced in alveolar pallets with 70 cubes with a volume of 50 cm^3 in a mixture a peat and sand. After 60 days, the seedlings were planted in the greenhouse. The planting scheme used was 70 cm between plants and 300 cm between rows. The special care works were the one specific to Cucurbitaceae species. The plants were vertically support. A particular attention has been paid to isolation distance between genotypes in order to prevent their impurity, knowing that the species is allogamous and preferred by the insect.

Figure 1. Crop detail of G2 (left) and G3 (right) genotypes

The fruits were harvested at physiological maturity and were analysed by our colleagues from Department of Chemistry, Physics and Environment, "Dunărea de Jos" University of Galati. The biochemical results (Busuioc et al., 2020) have shown that *B. hispida* fruit has a high content in vitamin C and is rich in gallic acids which are linked with its ability to reduce Type II diabetes.

Our survey included 20 questions, in the first part was described the demographic profile of the respondents, followed by the main characteristics of the fruits and a comparison between them. The last section focused on the nutritional and medicinal properties and on the price of fruit.

The tasting panel was formed by 57 respondents with ages from 18 to over 45 years old, both males and females, from rural and urban area. The resulted data were analysed with descriptive statistic methods.

RESULTS AND DISCUSSIONS

Benincasa hispida is a monoecious vine and is easy to grow in the greenhouse condition.

Mature fruits of *Benincasa hispida* are covered with a waxy coat which allows them long-term storage. One of the unique characteristics of winter melon, if there is no injury to the fruits, in that it can be stored for many months, in dry and cool atmospheres (Vînătoru et al., 2019).
The fruits of the three genotypes of wax guard, G1, G2 and G3 (Figure 2), were harvested at technological maturity and were share within our respondents.

Figure 2. Mature fruit of G1 (left), G2 (middle) and G3 (right) genotypes

For a better understanding of respondents opinion and preferences on the Table 1 is presented their socio-demographic profile. The interviewed respondents were from 18 to more than 45 years old. From our study, 47.4% were women and 52.6% men. In 50.8% of the cases people were from the urban area and 49.2% of them from the rural area. Mainly are people with college (38.6%) and high school (35.1%) studies.
Sensorial analyses were organized in September 2021 for the three genotypes and the results are presented in the Table 2 for each criterion: fruit smell, taste and texture.
So, 54.38% consumers think that winter melon has a cucumber-like smell, while others (24.56%) assimilate the smell to squash or watermelon (14.05%) and for 7.01% the smell remind of fresh cut grass. The fruit taste was sweet-sour for 26.7% consumers, while others found it neutral (39.2%), aromatic (10.5%) or sour (13.72). Concern to fruit taste, 9.8% participants found taste bitter or easy spicy.

Table 1. The socio-demographic profile of respondents

Variable	Consumers	
	No.	%
Sex		
- Men	30	52.6
- Women	27	47.4
Area		
- Urban	29	50.8
- Rural	28	49.2
Age		
- 18-24	21	36.8
- 25-35	9	15.8
- 35-45	6	10.6
- Over 45	21	36.8
Studies		
- Elementary school	15	26.3
- High school	20	35.1
- College	22	38.6

The fruit texture was meaty (40.3%) crispy (26.6%) and fleshy 19.2%. The fruit texture was watery or spongy for 7.6% respondents. The fruit taste reminds of cucumber to 48.07% of respondents while others assimilate with squash (24%) or watermelon (20%). For 7.9% consumers, the fruit taste reminds of Galia watermelon.

Table 2. Sensorial analyses of the fruits

Variable	Consumers					
	G1		G2		G3	
	No	%	No	%	No	%
Fruit smell						
- Squash	8	14	8	14	12	21.5
- Cucumber	40	70.2	37	65	21	36.8
- Watermelon	8	14	12	21	21	36.8
- Other	1	1.8			3	5.3
Fruit taste						
- Aromatic					4	7.1
- Sweet-sour	45	78.9	32	56.1	4	7.1
- Sour	8	14	4	7.1	8	14
- Neutral	4	7.1	31	36.8	32	56.1
- Other					9	15.7
Fruit texture						
- Crispy	4	7.1	8	14	4	7.1
- Fleshy	20	35.7	13	22.8	17	29.7
- Meaty	25	43.7	32	56.1	32	56.1
- Other	8	14	4	7.1	4	7.1
What does the fruit taste remind you of?						
- Watermelon	13	22.8	4	7.1	13	22.8
- Squash	9	15.8	17	29.7	17	29.7
- Cucumber	35	61.4	35	61.4	20	47.5
- Other			1	1.8		

Fruit mass was different for each genotype. The value ranges from 2.39 kg to 4.39 kg for G1, from 8.21 kg to 9.33 kg for G2 and between 12-16.84 kg for G3 variety (Figure 2) (Vînătoru și col., 2021).
Considering this aspect, 38.59% of people are tempted to buy G1 variety, 24.57% are tempted to buy G2 variety and 22.8 % G3 variety, while 14.03% would not be willing to buy any variety.
Regarding the commercial aspect of the fruit, wax guard was tempted to buy for 22.8% of respondents, while for 36.6% participants were in between 15.9% of respondents people were not interested in buying the fruit and 24.5% where barely inclined to purchase the fruit. However, 75.5% of participants would be tempted to buy winter melon due to its nutritional and medicinal proprieties.

Table 3. Potential utilization of *B. hispida* fruit

Variable	**Consumers** No.	%
Do you find the fruit easy to consume?		
- No	14	24.6
- Yes	43	75.4
If you knew that kiwano has a lot of vitamins, and other medicinal properties, will you consume it daily?	23	40.3
- No	34	59.7
- Yes		
If you knew that winter squash has a lot of nutritional proprieties, will consume it daily?		
- No	26	45.6
- Yes	31	54.4
Will you recommend the fruit to other people?		
- No	13	22.8
- Yes	44	77.2
Which one of the variants did you like most?		
- G1	24	42.1
- G2	13	22.8
- G3	20	35.1
Winter melon is an easy crop to grow. Would you cultivate it?		
- No	20	35.1
- Yes	37	64.9

After a short presentation of the winter melon fruit benefits, we discussed about the introduction of winter melon fruit in the daily diet of consumers for its nutritional and medicinal proprieties. Also, we had questions about how easy is to consume *B. hispida* fruit and if they will recommend it to other people (Table 3). In this case, 75.4% of respondents find the fruit easy to consume while 77.2% of consumers will recommend it to other people.
Winter melon is an easy crop to maintain and during vegetation period, no serious insects or disease problems were recorded, as a result, 64.9% respondents would like to grow it.
After the sensorial analyses, G1 variety was the most preferred by consumers (42.1%), followed by G3 (35.1) and G2 (22.8%) cultivar. G1 variety was registered in the Official Catalogue of Romanian Crop Plants, under the name of Zefir (Figure 3).

Figure 3. Crop details of Zefir

In the near future, genotypes G2 and G3 will be proposed for registration. The Vegetable Research Development Station Buzau has promoted the species with its health benefits and the demand for seeds and seedlings had increased significantly from year to year among growers and also the consumers demand (Vînătoru et al., 2021).
In the last part of our survey, it was an open-ended question about how much money are the consumer able to spend per kilogram. The mean results of the respondents were about 50 cent/fruit kg.
As yet, in Romanian fruit of wax gourd has not been sold in specialized stores, even if in the survey made by Dobre and Toma (2013), regarding the perception of *Benincasa* fruit it

was shown that with a proper promotion the vegetable can be introduced in Romanian consumer's behaviour.

CONCLUSIONS

From our research it can be concluded that *Benincasa hispida* has commercial potential due to its nutritional and medicinal properties.

After the sensorial analyses, Zefir was the most preferred by consumers followed by G3 and G2 cultivar. In the near future, genotypes G2 and G3 will be proposed for registration.

We recommend winter melon crop to be grown also in organic farming.

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REVIEW ON THE POSITIVE INFLUENCE OF INTERCROPPING SYSTEMS FOR ORGANIC VEGETABLE GROWING

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Abstract

Intercropping is considered to be a fundamental tool for ensuring agricultural sustainability and productivity, a matter of major importance in the specific context of the last decades and, mainly, the last two years.
Within European agriculture, conventional advantages of intercropping system following laborious experiments were disregarded by farmers because of the justified goal of maximizig profits using affordable pesticides on the market. This determines farmers to focus on increasing the size of their farms, replacing manual labor with a mechanized one, resulting a technological specialization of a few crops at the expense of biodiversity.
However, nowadays, following the Covid-19 pandemic and the entire chain of effects it generated, agriculture was directly affected due to the limitation of worldwide transport amplitude and the scarcity of products and raw materials that arose, the price for some of them becoming trully prohibitive (to be seen the case of chemical fertilizers at the end of year 2021).
The present paper aims to highligh some paramount matters of using intercropping systems in vegetable crop practice, regarding the perspective of soil, environment, ecosystem biodiversity and economical sustainability.

Key words*: intercropping, organic farming, environmental sustainability, GHG emissions, cover crops.*

INTRODUCTION

Probably never before in the history of mankind emerged the need of revolutionizing the way we practice agriculture, adopting a new perspective in contrast with the conventional one, which is based on an impressive number of inputs, intensely promoted after the Second World War, alongside the far advertised "Green Revolution". Nowadays, on the background of increasing climate changes, it becomes imperative to find solutions to minimize the impact that agriculture has on the environment. One cannot argue about sustainability in the true sense of the word from an environmental point of view if we refer strictly to the management of intensive farming systems because the enhancement of agriculture has, among other things, several unfavorable effects, such as: soil erosion, decreased biodiversity, nutrient loss and reduced soil fertility (Islam et al., 2016, cited by Diacono et al., 2021).

Therefore, the environmental challenges attributed to agriculture are primarily related to the reduction of soil, water and air quality, which are often resulting from the application of inappropriate nutrient management strategies. Farmers are typically using intensive chemicalization practices to maintain soil productivity, alongside management, which reduces organic soil matter (SOM) and at the same time increases erosion, acidification and salinisation (Dumanski et al., 1986, cited by Chapagain and Riseman, 2014).

Organic agriculture becomes more relevant than ever, the example in this respect being the policies adopted at the European Community level, which aims that 25% of all agricultural operations should respect the rigors of this type of agriculture by 2030.

One of the main aspects that ensures the sustainability of organic farming is related to the judicious use of land. If we refer strictly to plant production, this goal is mainly achieved by adopting intercropping systems. According to Vandermeer (1989), they involve "the cultivation of two or more plant species in a way that enables them interact agronomically". Intercropping is considered to be a fundamental means of ensuring agricultural sustainability and productivity (Brooker et al., 2015). Regarding organic vegetable production, Shanmugam et al., (2021) shows that intercropping systems can improve both yield and the efficiency of the nitrogen use by mixing complementary species in terms of resource use. Overall, the benefits identified from intercropping two or more plant species include higher productivity and high profitability per unit area (Yildirim and Guvence, 2005), improved soil fertility by nitrogen fixing (Hauggaard-Nielsen et al., 2001, 2009), increased resource efficiency (Knudsen et al., 2004), limited damage caused by disease and pest attack (Banik et al., 2006; Sekamatte et al., 2003), improved fodder quality (Bingol et al., 2007; Ross et al., 2004), as well as improving the carbon and nitrogen dynamics (Oelbermann and Echarte, 2011; Dyer et al., 2012).

MATERIALS AND METHODS

Data source and selection criteria

Data have been collected from an impressive number of scientific studies, mostly up-to-date, especially from the last 15 years. However, given the importance of the topic and the relevance of the studies conducted by the promoters of this vegetable growing system in the second half of the last century, some of the results of their research were briefly presented.

The main advantages of adopting the intercropping system for vegetable growing have been identified, classified and rated.

The database from Google Academic, ScienceDirect, Springer.com has been reviewed using keywords such as "intercropping", "organic farming", "environmental and soil sustainability".

A number of 397 scientific papers were identified that we considered to be paramount and, consequently, were analyzed.

The studies selected for this synthesis met the following criteria: 1. many of them are relatively recent and, as such, the conclusions drawn may be immediately applicable; 2. they present in detail the advantages of using the intercropping system in organic farming; 3. the results drawn from researches are relevant or based on a sufficient number of scientific papers.

RESULTS AND DISCUSSIONS

Intercropping implies the cultivation of two or more species simultaneously, on the same area of land, during a growing season (Ofori and Stern, 1987) and is considered to be an important strategy in the development of sustainable production systems, especially those aimed at limiting the use of raw materials of an external nature (Adesogan et al., 2002).

Embracing these organic farming practices increases the diversity and complexity of the agro-ecosystem, providing it long-term sustainability (Montemurro et al., 2018; Altieri and Koohafkan, 2013).

The use of agri-environmental practices also enhances the ability (sometimes called adaptive capacity) of a system to take over any disturbances without qualitatively altering the fundamental interactions that characterize it, and this ability can be defined as system resilience (Kaye and Quemada, 2017).

The role of intercropping on the rational use of land

Schröder and Köpke (2012) reiterated the positive value of the nitrogen land use rate in the case of intercropping broad bean and oilseeds (eg saffron and mustard) regardless of the type of soil they were grown in.

Intercropping barley and pea highlighted a number of real benefits, including higher land productivity (12-32% higher compared to the variant where barley was cultivated as a monoculture), an increased quality of biomass (high content of nitrogen and protein), a significant accumulation of carbon and nitrogen in the biomass of the soil surface, as well as a higher net exchange of CO2 and a gross rate of photosynthesis within ecosystem. However, the significance of using the intercropping system has varied greatly depending on the growing conditions and the proportion of species that have been used (Hauggaard-Nielsen et al., 2009; Jensen, 1996; Lauk and Lauk, 2008).

The agronomic parameters used to compare the yields of intercropping and monocropping systems are the land equivalent ratio (LER) (Mead & Willey, 1980) and the relative value total (RVT) (De Wit and van den Bergh, 1965; Schultz et al., 1982).

The land equivalent ratio represents the proportion of land needed to produce a certain amount of yield in the monoculture system as opposed to the intercropping system.
Overall, studies on the use of intercropping provide a conclusive reason to investigate the association between brassicas - pulses, given their potential to use less land in order to supply the same productive yield as monocultures (Shanmugam et al., 2021).

Relating intercropping with greenhouse gases (GHG)

In recent studies, both energy and carbon footprint analyzes have been used to determine crop production efficiency (Pratibha et al., 2015; Ozalp et al., 2018) and the sustainability of different soil fertilizer regimes (Pergola et al., 2018; Guardia et al., 2019).
Assessment of the carbon footprint is an important feature in rating the impact of a production system on global warming / climate change (Wiedmann and Minx, 2007). The higher the yield of crops, the lower the carbon footprint per kilogram (Pishgar-Komleh et al., 2017).
Worldwide, previous studies have demonstrated the impact of vegetable cultivation on global warming due to high emissions caused by energy consumption, agricultural works, use of fertilizers or irrigation (Torrellas et al., 2012; Khoshnevisan et al., 2014; Plawecki et al., 2014; Bartzas et al., 2015; Clavreul et al., 2017; Ntinas et al., 2017; Zarei et al., 2019).
The main greenhouse gases resulting from mismanagement of agricultural practices are carbon dioxide, methane and nitrogen oxide (IPCC, 2007).
The resulting carbon footprint of producing 1 kilogram of vegetables in the intercropping system is about one-fifth compared with monoculture, which highlights the importance of intercropping in terms of GHG mitigation and, consequently, environmental impact.
De Jesus Pereira et al. (2020) showed that greenhouse gas emissions were higher in the case of monoculture vegetable systems (25,273 kg CO_2 eq/ha), compared to the ones where intercropping has been chosen (16,368 kg CO_2 eq/ha).
In terms of soil carbon stock, the intercropping system emitted less CO_2 into the atmosphere (690 kg CO2 eq/ha) compared to the monocropping system (1,380 kg CO_2 eq/ha) over a twenty-year period, due to the fact that the area used in the case of the intercropping was smaller.
Several studies (Chirinda et al., 2010; Hwang et al., 2017) have shown that when nitrogen availability was increased, more N_2O was produced by nitrification and denitrification processes due to the proliferation of microorganisms. However, a number of researchers have reported that the use of agro-ecological crops can reduce N2O emissions compared to systems without cover crops by increasing the consumption of nitrogen of the so called “catch crop”, especially if non-pulses crops are used (Muhammad et al., 2019).

Effects of using the intercropping system in relieving salinization phenomena and nitrate accumulations of soil levels

Some previous studies have found that the stress caused by increased salinity could be alleviated by intercropping cash crops with some plant species capable of removing this excess (Aksoy et al., 2003).
Turfgrass represents a category of plant species with a higher tolerance to salinity because they had to adapt and survive into soils with a high degree of salinity during their phylogenetic development or to be irrigated with recycled / sewage water with a high salt content (Huang et al., 2014). Consequently, most turfgrass species are an excellent companion for the main horticultural crops, in order to alleviate the stress caused by the high salinity of the soils.
Turfgrasses constitutes the category of soil-covering plants, having a fibrous root system which are being distributed in the upper layer of soil, in the first 10 cm (Lyons et al., 2011). In contrast, most vegetables belong to the category of plants with a pivoting root system, with an overwhelming proportion of roots in the lower layer of the soil, up to a depth of 80 cm (Thorup-Kristensen and van den Boogaard, 1998; Vansteenkiste and et al., 2014).
Intercropping different species of turfgrasses with high-value vegetable crops is mainly based on the assumption that shallow rooting of turfgrass species does not lead to competition for nutrients *per se* but, on the contrary, could

absorb salt ions that have accumulated on the soil surface and eliminate their negative effects into the vegetable production system (Hu et. al., 2020).

The degree of salinity tolerance and also salt absorption may vary depending on the species and varieties of turfgrass (Chavarria et al., 2019; Soliman et al., 2018; Uddin et al., 2012). Some Bermuda grass varieties (*Cynodon* spp.) have been shown to be tolerant to a degree of salinity between 50-200 mM NaCl, without adversely affecting plant growth (Hu et al., 2012). Dong et al. (2019) showed that some species of turfgrass could excel in accumulating a higher amount of salt ions and heavy metals. Xia et al. (2019) highlighted the beneficial effect of intercropping with alfalfa *(Medicago sativa)* in inhibiting soil alkalization and salinization and improving its quality. Simpson et al. (2018) found that the association with purslane (*Portulaca oleracea*) could alleviate the salinity stress and could increase the productive yield and quality of watermelon fruits (*Cucumis melo*). Kilic et al. (2008) outlined a decrease in soil salt level and the elimination of the stress caused by it in an orchard where an intercropping system with purslane (*Portulaca oleracea*) was chosen.

About 80% of the amount of nitrates to which humans are exposed comes from vegetables (Rathod et al., 2016). Nitrates themselves are relatively harmless, but they have the ability to be reduced quite easily to nitrites, which can then be converted to nitrosamines, considered to have carcinogenic potential (Lundberg et al., 2008).

Therefore, the amount of nitrites and nitrates in vegetable products should be minimized in order to ensure a qualitatively safe vegetable production (Kalaycioglu and Erim, 2019). Nitrates can be absorbed directly by the roots of plants and can be transported to other organs through the nitrogen nutrition phenomenon (Wang et al., 2018). In plants, nitrates can be reduced to nitrites and further to ammonia by nitrogen reductase that occurs in plastids. Ammonia can be further assimilated in order to form amino acids through the synthesis of glutamine and glutamate (Coskun et al., 2017). Nitrate accumulation in vegetables is mainly correlated with nitrate soil level (Marousek et al., 2017).

It has been found that the use of green manure in intercropping systems reduces the risk of nitrate leaching both in the conventional system (Manevski et al., 2015; Mariotti et al., 2015) and also in the organic growing of cereals and vegetables (Whitmore and Schröder, 2007). Thorup-Kristensen et al., 2012).

Regarding the nitrate content, Hu et al. (2020) have shown that intercropping cauliflower with different species of turfgrass had a significant impact on it, both in the soil and in the rhizosphere area of cauliflower, compared to the control variant, as follows: 73.3% and 60.1% in the case of *Paspalum vaginatum,* 68.9% and 52.7%, at *Eremochloa ophiuroidesde*, succeeded by *Festuca arundinacea* (67.4% and 49%) and *Cynodon dactylon* (65% and 44%). The lowest impact was recorded in the cauliflower - Kentucky bluegrass (*Poa pratensis*) association, both in the rhizosphere and in soil, with 30.7% and, respectively, 35.7%. Intercropping cauliflower with *Paspalum vaginatum* and *Eremochloa ophiuroides* also significantly reduced the nitrate content of young cauliflower shoots by 46.4% and 29%, compared to the control variant.

Hu et al. (2020) presents the current methods of mitigating the problems related to soil salinization and nitrate accumulation of the vegetable growing systems: i) use of water (from rainfall or irrigation) to remove salts accumulated in the soil surface layer (Du et al., 2019; Zhang et al., 2020); (ii) rational fertilization management programs to reduce the accumulation of soil salts (Machado and Serralheiro, 2017); (iii) applying amendments in order to absorb soil salts and reduce stress on vegetable crops (Fan et al., 2016).

The influence of intercropping on crop yields

In organic vegetable growing, adopting the intercropping system can improve both the yield and the efficiency of nitrogen use, by associating complementary species regarding the use of resources (Shanmugam et al., 2021). When two vegetable species are intercropped, the dominant ones can increase both their productive yield and nutrient uptake (Zhang & Li, 2003), while the production of the other crop is reduced due to interspecific competition for nutrients.

The beneficial effect on the yield in the case of the intercropping system is shown in Table 1.

Table 1. List of some intercropping types and their benefits on vegetable and non vegetable crop yields

Intercropped species	The type of beneficial effect on yield	Author
Faba bean (*Vicia faba* L.) – White mustard (*Sinapis alba)* and cabbage *(Brassica oleracea* L. var. *capitata*)	- Higher yields for the Brassicaceae crops	(Schröder & Köpke, 2012; Lepse et al., 2017; Shanmugam et al., 2021)
Faba bean (*Vicia faba* L.) – Garlic (*Allium sativum* L.)	- Nitrogen transfer to the cash crop	(Tang et al., 2018; Thilakarathna et al., 2016)
Pea *(Pisum sativum L.)* – Wheat (*Triticum aestivum*) / Barley *(Hordeum vulgare)*	- Higher yields; - Improvement of grain and fodder quality	(Hauggaard-Nielsen et al., 2009; Carr et al., 2004; Lauk and Lauk, 2008)
Cucumbers (*Cucumis sativus* L.*)* – Lettuce (*Lactuca sativa* L.)	- Higher yields	(Rezende et al*.,* 2010)
Tomatoes (*Solanum lycopersicum L.*) – Lettuce (*Lactuca sativa* L.)	- Higher yields	(Cecílio Filho et al*.,* 2011)
Cauliflower (*Brassica oleracea* var. *Botrytis)* – *Paspalum vaginatum/Festuca arundinacea*	- Higher yields	(Hu et al., 2020)
Leek *(Allium porum L.)* – White clover (*Trifolium repens*)	- Higher yields when clover was sowed after leek planting	(Kolota and Adamczewski-Sowinska, 2004; den Hollander et al., 2007)
Leek *(Allium porum L.)* – Ryegrass (*Lolium spp.)*	- Higher yields when ryegrass was sowed six weeks after leek planting	(Müller-Schärer, 1996)
Maze (*Zea mays* L.) – Green manures spp.	- Increase of the dry matter content in corn grains	(Uchino et al*.,* 2009)
Tomates (*Solanum lycopersicum L.*) – Italian clover *(Trifolium incarnatum)*	- Higher yields for the cash crop	(Diacono et al*.*, 2021)
Cabbage (*Brassica oleracea* L. var. *capitata*) – Chilli peppers *(Capsicum annuum* L.*)*	- Higher yields	(Leong and Zaharah, 1991)
Pulses – Grains	- Higher yields for cereals when legumes are used as green manures	(Bedoussac and Justies, 2010; Bedoussac et al., 2015)
Broccoli (*Brassica oleracea* var. *italica)* – Beans (*Phaseolus vulgaris* L.) / Potatoes *(Solanum tuberosum* L.*)*	- Higher yields	(Santos et al., 2002)
Cabbage (*Brassica oleracea* L. var. *capitata*) – Romaine type lettuce (*Lactuca sativa* L. var. *longifoila*), / Leaf lettuce (*L. sativa* L. var. *crispa*) / Onion (*Allium cepa* L.) / dwarf bean (*Phaseolus vulgaris* L. var. *nanus*)	- Optimizes both yield and profitability	(Guvenc and Yildirim, 2006)
Faba bean (*Vicia faba* L.) - Wheat (*Triticum aestivum* L.)	- The growth and yield of cereal crop increased by 19% -28% and, respectively, 20% - 28%	(Xiao et al., 2018).

The impact of intercropping system on diseases and pests control

Over the past decades, studying the intercropping system effects on diseases and pests has favored the accumulation of a considerable number of bibliographic data. The general effects of using intercropping system in vegetable production are linked to the suppression of most pest and disease populations (Theunissen, 1994b).

Some of the intercropping scheme on which research has been conducted in terms of diseases and pests control are presented in Tables 2 and 3.

Table 2. List of some intercropping types and their benefits on vegetable pest control

Intercropped species	Insect population assessment	Author
Bean (*Phaseolus vulgaris* L.) – winter wheat (*Triticum aestivum* L.)	- *Empoasca fabae, Lygus lineolaris, Aphis fabae, Systena frontalis*	(Tingey and Lamont, 1988)
Brussels sprouts (*Brassica oleracea* var. *gemmifera*) – *Spergula arvensis*	- *Mamestra brassicae, Evergestis forficalis, Brevicoryne brassicae*	(Theunissen and Den Ouden, 1980)
Brussels sprouts (*Brassica oleracea* var. *gemmifera*) – Tomatoes *(Solanum lycopersicum* L.*)*	- *Phylotreta cruciferae, Plutella xylostella, Aleyrodes brassicae*	(Tahvanainen and Root, 1972; Philips, 1977)
Cabbage (*Brassica oleracea* L. var. *capitata*) – Tomatoes *(Solanum lycopersicum* L.*)*	- *Plutella xylostella*	(Burandy and Raros,1975)
Beans (*Phaseolus vulgaris* L.) – Grass weeds (*Eleusine* and / *Leptochloa*)	- *Empoasca kraemeri*	(Altieri et al.,1977)
Cabbage (*Brassica oleracea* L. var. *capitata*) – Living mulches (*Agrostis stolonifera, Festuca rubra, Poa pratensis, Trifolium repens)*	- *Phylotreta cruciferae Brevicoryne brassicae*	(Andow et al., 1986)
Cabbage (*Brassica oleracea* L. var. *capitata*) – Beans (*Phaseolus vulgaris* L.)	- *Delia radicum, Delia floralis*	(Hofsvang, 1991)
Cabbage (*Brassica oleracea* L. var. *capitata*) – *Trifolium* spp. (*T. repens, T. subterraneum*)	- *Mamestra brassicae, Brevicoryne brassicae Delia brassicae*	(Theunissen et al*.,* 1995)
Carrot (*Daucus carota*) – Onion *(Allium cepa* L.*)*	- *Psila rosae* (carrot), *Thrips tabaci* (Onion), *Cavariella aegopodii*	(Uvah and Coaker*,* 1984)
Leek *(Allium porum L.) – Trifolium subterraneum*	- *Thrips tabaci*	(Theunissen and Schelling, 1993)

Table 3. List of some intercropping types and their benefits on vegetable disease control

Intercropped species	Disease assessment	Author
Leek *(Allium porum* L.*) – Trifolium subterraneum*	- *Puccinia allii*	(Theunissen et al*.*, 1996)
Barley (*Hordeum vulgare*) – Pea (*Pisum sativum*), Lupin (*Lupinus* L.), Faba bean (*Vicia faba*)	- *Pyrenophora teres, Puccinia hordei*	(Hauggaard-Nielsen et al., 2008)
Wheat (*Triticum aestivum* L.) - Faba bean (*Vicia faba*)	- Wheat powderly mildeaw	(Chen et al., 2007)
Barley (*Hordeum vulgare*) – Wheat (*Triticum aestivum* L.)	- Seed head disease	(Naudin et al., 2009)
Barley (*Hordeum vulgare*) – Lupin (*Lupinus* L.)	- *Pleiochaeta setosa*	(Hauggaard-Nielsen et al., 2008)
Tomatoes (*Solanum lycopersicum* L.) – Kale (*Brassica oleracea* L. var. *acephala)*, Onion (*Allium cepa* L.)	- *Tomato spotted wilt virus (TSWV)*	(Ramkat et al., 2008)
Potato *(Solanum tuberosum* L.*)* - Grass-Clover	- *Phytophtora infestans*	(Bouws and Finckh, 2008)
Potato *(Solanum tuberosum* L.*)* - Faba bean (*Vicia faba*)	- *Phytophtora infestans*	(Garrett et al., 2001)
Cabbage (*Brassica oleracea* L. var. *capitata*) – Garlic *(Allium sativum* L.*)*	- *Sclerotium cepivorum*	(Zewde et al., 2007)
Tomatoes (*Solanum lycopersicum* L.) – Cucumbers (*Cucumis sativus* L.*)*	- Yellow leaf curl	(Al-Musa, 1982)
Tomatoes (*Solanum lycopersicum* L.) – Cowpea (*Vigna unguiculata* L.)	- *Pseudomonas solana-cearum*	(Michel et al., 1997)
Chilli peppers (*Capsicum annuum*) – Maize (*Zea mays* L.)	- *Phytophtora capsici*	(Sun et al., 2006; Zu et al., 2008)
Watermelon (*Citrullus lanatus)* – Rice (*Oryza sativa*)	- *Fusarium oxysporum*	(Su et al., 2008)

Some effects of the intercropping system on weed management

The intercropping system can represent a technological link in weed suppression, although the results obtained so far are variable (Vandermeer, 1989, Stefan et al., 2021). The positive effects on weed suppression have been shown in a wide range of crops, including maize, rye, soybeans, zucchini, summer cabbage, dwarf beans and tomatoes (Ilnicky & Enache, 1992). Furthermore, some clover species such as *Trifolium repens, T. pratense*, *T. fragiferum* and *T. dubium* turned out to be suitable for use in combination with a main crop / cash crop for the same purpose. If the crops are set up on rows, mowing the secondary associated crop can represent a suitable way to prevent tall weeds from flowering and seeding.

When the role of intercropping is to suppress weeds, its effects are expressed according to the savings made in terms of control measures. Stefan et al., (2021) show that even if intercropping does not necessarily reduce biomass or weed diversity, using cereals in association can play a pivotal role in reducing the pressure the weeds exert on cash crops. Therefore, it is preferable for intercropping systems to include cereals if weed control is one of the objectives.

CONCLUSIONS

The results obtained so far suggest that the intercropping systems could represent an approach of interest in all types of agriculture, but that it could be ideal for organic farming practice.

Embracing the intercropping system is more expensive, requires a high level of managerial skills, but, more importantly, a different philosophy on the part of the farmer, focusing on an ecosystem-oriented agriculture.

As long as cheap pesticides will not constitute a limiting factor for conventional farmers, by adopting a sets of environmental laws, they will be advantaged from production costs point of view.

There is an inverse relationship between the carbon footprint and crop yields.

Growing vegetables in an intercropping system increases productivity, maximizes the use of environmental resources and optimizes the use of inputs, balancing the system from an ecological point of view.

The restrictions on the use of pesticides and certain fertilizers that characterize organic farming make it suitable for enacting the intercropping system, as it corresponds exactly to its philosophy, patterns and methods. The small scale, as well as the biological and ecological diversity of farms in the unconventional growing systems makes them liable for intercropping as it does not require a completely different crop management.

Root system interactions can play an important role regarding the relationships between crops within intercropping (beneficial or competing) and nutrients. Thus, the complementary use of resources under the intercropping system improves the nitrogen content.

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CONSUMER PREFERENCES FOR KIWANO FRUIT, A NEWLY INTRODUCED CROP IN ROMANIA

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Abstract

One of the main tasks of the breeding programs is introduction of new vegetables crops in order to provide vegetable diversity to the growing population. At Vegetable Research Development Station Buzau, Romania, Cucumis metuliferus has been acclimatized and today seedlings and seeds of 'Tempus' variety can be sold. Kiwano, it has multiple health benefits and plays an important role in supporting diverse and nutritious diets. But the fruit is not well known and is underutilised, due to the lack of promotion. Hence, the goal of this study was to investigate the consumer preference and also to popularize the fruit to a large scale of people. In our survey we have investigated a number of 57 respondents, aged between 18 and over 45 years. From our results, 51 people are opened to include the kiwano fruit in their diets. Also, a percentage of 85.9% from our respondents are interested in growing the jelly melon. It can be concluded that kiwano has a great commercial potential and, aimed at certain markets, could be an excellent source of cash for small farmers.

***Key words**: Cucumis metuliferus, jelly melon, nutrition, questionnaire, survey.*

INTRODUCTION

In the present, the food industry is in search for healthy products since the worldwide health crisis redirected the consumer attitude, perception, and awareness to a diet rich in natural and high-quality products (Šeregelj et al., 2022; Amicarelli et al., 2021). In this context, one the main task in breeding programs is introduction of new vegetables crops in order to provide vegetable diversity to the growing population. At Vegetable Research Development Station Buzau, Romania, *Cucumis metuliferus* (kiwano, jelly-fruit, horned melon) has been acclimatized and today seedlings and seeds of 'Tempus' variety can be sold. Kiwano is a monoecious vegetable, that can be grown in open field or in greenhouses. From our research the best results were recorded in the greenhouse, because 'Tempus' is a late variety.

Horned melon is a climbing annual, with yellow-orange fruit and a juicy, jelly-like mesocarp. The plant origin goes back to Africa and nowadays is a speciality fruit in European markets. Several studies agree that the fruit is rich in various phytochemical components important in the daily diet. Due to the remarkable ease of expansion of this fruit, horned melon could represent an economically advantageous nutritional source to alleviate malnutrition or provide phytochemicals to the food and pharmaceutical industries (Vieira et al., 2021; Ferrara, 2018)

In the literature, the relevant data on the nutritional value and phytochemical profile of horned melon fruit/pulp is available. Different studies have been proven the antioxidant potential of edible parts (Bölek, 2020; Mastusaka and Kawabata, 2010; Motlhanka, 2008) or that horned melon seeds are reducing blood cholesterol levels (Usman et al., 2015). Jimman et al. (2010) and Gotep (2010) demonstrated that the glycosides extracted from kiwano pulp have antihyperglycemic activity and also are preventing both heart attacks and strokes. Omale et al. (2011) and Wannang et al. (2008) confirmed the potential benefits of horned melon for the treatment of ulcer disease. Other studies also support the anti-inflammatory properties of the kiwano fruit (Vieira et al., 2021). It was also identified antifungal activity (Nwadiaro et al. 2015; Aliero and Gumi, 2012) and antiviral activity (Nimzing, 2009; Amagon et al., 2012) of horned melon.

Even so is not so common in Europe, and particularly in Romania, kiwano fruit is a popular snack in different parts of the world, like Africa, Australia, New Zeeland and parts of US.

Over the past two decades, consumer demand for niche products (including exotic and unusual fruits) has grown substantially. People are interested in buying new, various type of fruits and vegetables in order to switch their menu. Even so this is a worldwide trend, in Romania people are still reluctant about changing their diet. In our study, we have investigated the consumer preference about the newly introduced fruit and it benefits. Understanding consumers and popularize the fruits is a key to improving our activity.

MATERIALS AND METHODS

The fruits of *Cucumis metuliferus* 'Tempus' variety were grown in the research greenhouse of Vegetable Research Development Station Buzau, Romania. The seeds were sown in the first decade of March in plastic pots with 70 cubes with a volume of 50 mL/cube in a mixture of peat and sand. The seedlings were planted 45 days after sprouting (3-4 leaf stage) in a sandy-loam soil, very well drain and the planting scheme used was 120 x 60 cm. Kiwano is an annual climbing vegetable and needs support in order to fully develop. Throughout the vegetation period, no serious pathogens were reported in order to damage the crop. The fruits were harvested at physiological maturity and were analysed by our colleagues from Department of Chemistry, Physics and Environment, "Dunarea de Jos" University of Galati. The biochemicals results (Busuioc et al., 2020) have shown that kiwano fruit has a high content in vitamin C (6.22 mg/10 g fruit), a good concentration of polyphenols and a beneficial antioxidant capacity.

Our survey included 17 questions, in the first part was described the demographic profile of the respondents, followed by the main characteristics of the fruit and in the last section we discussed about medicinal proprieties, price and consumer preferences. Information used in this study was obtained from a survey of 57 respondents from Buzau County, Romania, aged over 18 conducted at Vegetable Research Development Station Buzau, Romania. The resulted data were analysed with descriptive statistic methods.

RESULTS AND DISCUSSIONS

Cucumis metuliferus (Figure 1) is an accessible and easy crop to grow in the greenhouse or polytunnel. It has a long vegetation period and is a late-variety, with fruits reaching maturity at the beginning of September. Unharmed fruits can be kept for almost a year at room temperature. The crop has no serious pest, and can be grown successfully in organic systems. The yield potential of the crop is very high, and the crop maintenance cost are low due to genetic resistance of the species.

Figure 1. Mature and immature fruit of kiwano

The fruits were harvested at technological maturity and were share within our respondents. In Figure 2 is shown the fruit section of 'Tempus' variety.

Figure 2. Fruit section of 'Tempus' variety

The information in this study was obtained from a survey of Romanian consumers, aged over 18 and the demographic profile of the respondents are shown in Table 1.

Table 1. Demographic profile of respondents

Variable	Consumers	
	No.	%
Sex		
- Men	30	52.6
- Women	27	47.4
Area		
- Urban	29	50.8
- Rural	28	49.2
Age		
- 18-24	21	36.8
- 25-35	9	15.8
- 35-45	6	10.6
- Over 45	21	36.8
Studies		
- Elementary school	15	26.3
- High school	20	35.1
- College	22	38.6

Most of our respondents are young people with age under 24 (36.8%) or over 45 (36.8%). Mainly are people with college (38.6%) and high school (35.1%) studies.

In the second part of the survey, it was discussed about the main component of fruit: flavour - defined as being made up of taste (sour, sweet-sour, aromatic, neutral), aroma – texture (juicy, jelly-like, fleshy), fruit smell (citrusy, kiwi-like, cucumber-like, other) and shape (if consumers are tempted to buy it).

Despite the odd-looking fruit, horned melon was tempted to buy for a percentage of 31.6% respondents, while for 54.4% respondents were in between. At the opposite pole, 5.3% of people were not interested in buying the fruit and 8.8% where barely inclined to purchase the fruit.

In regard for the main attributes of fruit (Table 2), 45.7% consumers think that kiwano has a kiwi-like smell, while others (33.3%) assimilate the smell to fresh cucumbers or unripe bananas (12.2%) or else with a citrusy smell (8.8%). The fruit taste was sweet-sour for 56.1% consumers, while others found it sour (19.3%). The fruit texture varied from fleshy (12.3%) to juicy (40.3%) and to jelly-like for 47.4% of consumers. The fruit taste reminds of kiwi fruit to 61.4% of respondents while others (26.3%) assimilate with fresh cucumber.

Table 2. Fruit attributes

Variable	Consumers	
	No.	%
Fruit smell		
- Citrusy	5	8.8
- Fresh cucumber	19	33.3
- Kiwi	26	45.7
- Other (unripe bananas)	7	12.2
Fruit taste		
- Aromatic	7	12.3
- Neutral	7	12.3
- Sour	11	19.3
- Sweet-sour	32	56.1
Fruit texture		
- Fleshy	7	12.3
- Jelly-like	27	47.4
- Juicy	23	40.3
What does the fruit taste remind you of?		
- Banana	5	8.8
- Cucumber	15	26.3
- Kiwi	35	61.4
- Other (banana-kiwi)	2	3.5

In the next part of our survey, we discussed about the potential utilization of horned melon fruit for health promotion, diseases prevention, and also about many health benefits to consumers' daily diet. After a short presentation of the kiwano fruit benefits we had had questions about how easy is to consume horned melon fruit, if they are willing to introduce the new fruit to their diet and if they will recommend it to other people (Table 3). In general, consumers we're opened to introduce kiwano fruit in their diet and 91.2% respondents will recommend it to other people. Horned melon is an easy and cash worthy crop and 85.9% respondents would like to grow it.

In the last part of our survey, it was an open-ended question about how much money will spend on one fruit. The mean results of the respondents were about 1.5 euros/fruit. Nowadays, in the sales market, the fruit can be bought for 1 euro.

The last question of our survey is a Likert scale (1-strongly disagree and 10-strongly agree) and refers to consumer preference for kiwano fruit. The majority of respondents noted with 8 the kiwano fruit (a pleasantly fruit).

Table 3. Potential utilization of kiwano fruit

Variable	Consumers	
	No.	%
Do you find the fruit easy to consume?		
- No	11	19.3
- Yes	46	80.7
Kiwano has a long shelf life, for almost a year, will you consume it daily?		
- No	14	24.6
- Yes	43	75.4
If you knew that kiwano has a lot of vitamins, minerals and zero calories will you consume it daily?		
- No	12	21.1
- Yes	45	78.9
Will you recommend the fruit to other people?		
- No	5	8.8
- Yes	52	91.2
Kiwano has zero calories and a high protein content, will you use it in your diets?		
- No	6	10.5
- Yes	51	89.5
Horned melon is an easy crop to grow. Would you cultivate it?		
- No	8	14.1
- Yes	49	85.9

CONCLUSIONS

From our research it can be concluded that kiwano has a great commercial potential and, aimed at certain markets, could be an excellent source of cash for small farmers.

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THE ASSESSMENT OF SEEDLING QUALITY AIMED TO ENSURE SUSTAINABLE CABBAGE SEED PRODUCTION

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Abstract

Brassicaceae vegetables have been grown and consumed worldwide since ancient times and they are considered a functional food due to the presence of phytochemicals, mineral and vitamins necessary for human well-being. For this reason, species as cabbage are highly requested by consumers. At farm level, constrains related to seed production process highlight the importance of seed germination and seedling performance. The study is integrated in major research aimed to develop new modern scheme for cabbage seed production. The objective of this work was to assess seedling quality by measuring several morphological, phenological and physiological parameters with influence in performance of cabbage sustainable seedling production. The experiment was assessed on "Silviana" autumn cabbage, created at VRDS Bacau, and was conducted using a randomized complete block design with three replications. The work presents the results of germination, seedling growth dynamic, carotenes, xanthophylls and chlorophylls content as affected by different factors as variety, age and growing climate. This study reveals the importance of using qualitative seedlings during the process of seed production.

Key words: *Brassica, carotenes, xanthophylls, chlorophylls, growth.*

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is one of the most essential agricultural crops in the *Brassicaceae* family, which covers many important vegetable crops such as cabbage, kale, Brussels sprouts, cauliflower, broccoli, and kohlrabi and therefore has an ancient legacy of agriculture practices.

Cabbage is a leafy white biennial used as an annual vegetable because of its densely leafed (compact) heads. Cultivars come in a variety of forms, colours, and leaf textures.

Cabbage plants are classified as crinkled-leaf, loose-head or smooth-leaf, firm-head plants based on their leaf type.

Cultivated cabbage cultivars have a wide range of morphological features and are divided into two main groups: white (*Brassica oleracea* var. *capitata* f. *alba*), which is the common name for cabbage with green leaves, and red (*Brassica oleracea* var. *capitata* f. *rubra*), which is the common name for cabbage with red leaves (Singh et al., 2006).

Cabbage head is a large bud that contains the majority of the plant's leaves. It has rather low temperature needs; the biological threshold cannot be decreased any further than 5 and 6°C, and the ideal temperature for vegetation is set between 15 to 18°C. It can even withstand negative temperatures for a limited duration (Iosob et al., 2019).

Cabbage is frequently consumed because of its bioactive and antioxidant activities, along with anti-inflammatory and antibacterial characteristics (Cartea et al., 2011; Šamec et al., 2011).

Natural pigments give fruits and vegetables their distinctive colour, hence chlorophylls predominate in white cabbage cultivars (Fernández-León et al., 2010), and anthocyanins dominate in red cabbage cultivars, the colour of fruits and vegetables is primarily determined by their presence (Arapitsas et al., 2008).

Thus, according Lisiewska et al. (2004), the level and ratio of chlorophyll pigments have a significant impact on colour attraction. Colour is perhaps the most important attribute engaged in consumers' evaluations of products, according to Tijskens et al. (2001), and it plays a major contribution in the product's acceptability.

Chlorophylls are often accompanied along with carotenoid pigments, which range spectrum

colour from yellow to orange (Kidmose et al. 2001).
Chlorophylls are pigments that give plants and fruits their green colour and play an important part in photosynthesis. Vegetables' chlorophyll content is important for both their favourable development and their aesthetic attractiveness (Bulgari, 2017).
Its biodegradation is linked to cellular break-down and/or senescence, and it's frequently used to determine green vegetable quality loss (Hodges et al., 2000; Toivonen and Sweeney, 1998).
In fact, there has been evidence of a substantial link between chlorophyll concentration and overall aesthetic quality of vegetables (Aguero et al., 2008).
Furthermore, chlorophyll can be classified as a bioactive chemical since its naturally occurring dietary derivatives that have antioxidant and antimutagenic properties (Hsu et al., 2008; Ferruzzi et al., 2002; Ferruzzi and Blakeslee, 2007).
Carotenoids are important nutritional and functional elements. Green vegetables provide a reliable source of carotenoids, with cole crops being one of the most prevalent (Kalt 2005). β-carotene is among the most useful of the carotenes, evidencing pro-vitamin A activity (Nishino et al., 1999).
α-carotene, β-carotene, capsanthin, lutein, lycopene, and zeaxanthin are common carotenoids in plants. Carotene is a vitamin A precursor, and carotenoids with β rings have the highest provitamin A activity. In mammals, provitamin A carotenoids are converted to retinol and other linked retinols, which are involved in the visual cycle and gene control. (Rodríguez-Concepción, 2018).
Antioxidant compounds such as chlorophyll and carotenoids are found in cole crops (Kidmose et al. 2001).
With the exception of polyphenols, the major bioactive ingredient in *Brassicaceae* plants is vitamin C, which is among the most important micronutrients (Martín-Belloso and Fortuny, 2011). Pigment molecules as chlorophylls, carotenoids, and anthocyanins are also expressed when there is significant biological activity (Hsu et al., 2013).
Antioxidants are compounds that can cease or slow the lipid oxidation or other molecules by inhibiting or postponing the beginning or multiplication of oxidative chain reactions (Emmons et al., 1999; Velioglu et al., 1998).
Crop production, yield and quality are a critical concern in vegetable cultivation that is garnering more attention (Jablonska-Coglarek and Rosa 2002).
One of the most significant inputs in high-yield vegetable crop development and in seed production is the seedling quality. High-quality seedlings are required for proper stand establishment and homogenous plant growth. Traditionally, nursery production has centred on growing seedlings in a cost-effective and efficient manner. Presently, there is an increasing concern in decreasing seedling production's environmental impact.
Seeds encounter a variety of issues in terms of production, postharvest storage, and future quality. Furthermore, given the effects of global warming as a symptom of climate change, various stress factors may result in poor seed effectiveness in terms of decreased germination, imbalanced seedling emergence, poor seedling establishment, and counterproductive modifications of root cell architecture, resulting in a significant yield reduction (Rakshit and Singh, 2018).
Common germination evaluations can be used to determine seed performance in the field (Johnson and Wax, 1978), but subsequent establishment and growth projections are not always factual (Heydecker, 1972).
The success of producing healthy and well-growing seedlings is heavily influenced by the quality and germinability of seeds. Different production strategies can improve germinability and seedling health (Himanen and Nygren, 2015).
Seed quality, germination and early seedling vigour are critical factors in the success among many crops. Environmental variables such as temperature and humidity influence the germination of most seeds (Chachalis and Reddy, 2000; Taylorson, 1987).
The ability for germination and the rate of seed germination are affected by temperature, whereas soil moisture is required for germination (Bewley and Black, 1994). Germination is hampered by a lack of moisture. Early seedling survival is influenced by the moisture level of the growth medium. The

influence of relative humidity on seedling development in most plants is similarly as significant (Went, 1958). The aim of this study was to evaluate seedling quality by assessing a variety of morphological, phenological, and physiological characteristics that have an impact on the performance of cabbage seedling production.

MATERIALS AND METHODS

Plant material

In the experiment, the autumn white cabbage variety "Silviana" patented by the Vegetable Research and Development Station Bacau, was utilised in the trials required to fulfil the specified aim.

"Silviana" cultivar is an autumn cabbage type that is highly resistant to cracking and has a high yield potential. The leaves have a fine texture, the head form in longitudinal section is round to elliptical, and the colour of the leaves is raw green to medium intensity green.

It also contains a vitamin C concentration of 44.50 mg per 100 g, a cellulose level up to 1.15% and a production potential of 100 to 120 t ha^{-1}. The height of roughly 18-20 cm, the circumference of 18-21 cm, the average weight of 1.6-2.7 kg, the lack or very faint presence of anthocyanin colour of the covering leaves and the yellow tint of the inside leaves are further characteristics of the vegetative bud.

Site description

The studies were arranged and carried out in the VRDS experimental greenhouse which is situated in the north-eastern section of Bacau, on a river terrace with an altitude of 5-7 meters and a height of 165 meters above sea level, in the Bistrița - Siret interfluve, about 4 kilometres north of their confluence.

Experimental design

Three distinct dates were chosen for sowing. The first took place on 27 May 2021, followed by the second on June 28, 2021, and the third on 30 July 2021, in plastic pots filled with commercial peat-based potting material. The pots were arranged in a greenhouse compartment on rolling benches.

The experiment was carried out using a three-replication randomized complete block design. The plants were equally watered as needed with tap water (usually one time per day) by overhead misting after seedling emergence. A 40-day-old cabbage seedling was used for the trials, which was sowed in alveolar palettes according to the three epochs stated above.

After 40 days, when the plants had achieved proper planting age, the cabbage samples were removed from the greenhouse and transported to the laboratory for analysis. Plant samples were hand-picked at random followed by assessment of several morphological traits.

Germination

For a control test ISTA regulations were followed when conducting germination testing (International Seed Testing Association).

On Petri dishes with filter paper wet with tap water, three replicas of 100 seeds each lot were sowed. The seeds were germinated on a germinator for 16 hours in darkness and 8 hours in white fluorescent light, respectively, at alternating temperatures of 20 °C and 30 °C. The final germination percentage (FGP) was calculated. For each period the germination capacity was assessed in greenhouse conditions, by counting the days from sowing to emergence.

Pigments determination

The spectrophotometric method was applied, which is quick and readily available, with the BOECO S-20 Spectrophometer. 1g of fresh sample (leaves fresh tissue) was extracted with 80% acetone (v/v). Successive extractions were performed until the residue has become colourless and then was adjusted to 100 ml fixed volume. The absorbance of extracts was measured at 663, 646 and 470 nm against blank (acetone 80%). Formulas used for calculation of pigments content were those developed by Mackinney (1941) and the values were expressed in mg $100g^{-1}$:

$$\text{Chlorophyll a} = [(12{,}21 \times DO_{663}) - (2{,}81 \times DO_{646})] \times 5$$

$$\text{Chlorophyll b} = [(20{,}13 \times DO_{646}) - (5{,}03 \times DO_{663})] \times 5$$

$$\text{Carotenoids and xanthophylls} = [(1000 \times DO_{470}) - (3{,}27 \times \text{Chl. a}) - (1{,}04 \times \text{Chl. b})] / 229$$

Seedling growth dynamic

The seedlings morphological characters analysis was performed on all 3 sets of plants at the age of 40 days, each set being represented by a sowing period. Determinations have been made in this regard on the thickness of the seedling at the stem base (mm), the height of the seedling at planting maturity (cm), number of leaves, total seedling weight (g), weight of aerial part of the seedling (g), and on roots weight (g), using the ruler, scale (Radwag PS 600/C/2) and the caliper.

Statistical analysis

Statistical methods were used to evaluate the collected morphological data. The evaluation of the obtained analytical data was performed by statistical means. The IBM SPSS Statistics programme, version 26.0, was used for statistical analysis. The ANOVA test was used to examine the differences between the means, followed by the Tukey's posthoc test. At the threshold of $P < 0.05$, a significant difference was evaluated. The results were reported as means ± standard errors.

RESULTS AND DISCUSSIONS

In the recent period a significant information on how to produce high-quality seedlings was developed. The production of well-growing seedling is mandatory, because of its influence in crop competitiveness in ever-changing environment. Our focus was on investigation of seedling morphology and a few physiological parameters as influenced by different sowing periods. The general objective is to develop new modern scheme for cabbage seed production, seedling management being one important link of the process.

Table 1. The results obtained from the analysis of some environmental conditions

Sowing time	Light intensity (W / m^2)	Atmospheric humidity (%)
Period 1 (27.05.2021)	214.16±10.29ab	79.56±1.49a
Period 2 (28.06.2021)	238.21±7.02a	74.48±1.18b
Period 3 (30.07.2021)	206.82±8.61b	74.27±1.34b

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Table 1 shows the differences in terms of light intensity and atmospheric humidity for each sowing period. The first period was sown on the 27.05.2021 and on 31.05.2021 plants had emerged; thus 4 days were needed for emergence. For the second period the sowing took place on 28.06.2021 and 3 days later, on 01.07.2021 the seedlings started to emerge. In last period, the third one, the emergence took place in 3 days, with a sowing date on 30.07.2021 and an emergence date on 02.08.2021.

The outcomes of the analyses done on the morphological characters of white cabbage seedlings according to the sowing period is reported in the following section.

Table 2. The results obtained from the analysis of some morphological characters of seedlings

Sowing time	The diameter of the seedling at the stem base (mm)	The height of the seedling at planting maturity (cm)	Number of leaves
Period 1 (27.05.2021)	3.88±0.11a	18.51±0.75a	5.60±0.26a
Period 2 (28.06.2021)	3.43±0.12b	18.61±0.64a	4.90±0.18ab
Period 3 (30.07.2021)	3.21±0.10b	15.85±0.51b	4.70±0.15b

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Table 3. The results obtained from the analysis of some morphological characters of seedlings

Sowing time	Total seedling weight (g)	Weight of aerial part (g)	Roots weight (g)
Period 1 (27.05.2021)	5.30±0.27a	5.11±0.28a	0.188±0.011a
Period 2 (28.06.2021)	4.80±0.24a	4.62±0.23a	0.186±0.009a
Period 3 (30.07.2021)	3.53±0.17b	3.40±0.17b	0.137±0.009b

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Tables 2 and 3 shows the differences obtained from the analysis of morphological characters performed on seedlings at the age of 40 days, for each sowing period. Our interest was to detect the most suitable sowing period to ensure the development of high robust seedlings. The assessment of robustness included thickness, expressed by measuring the diameter of stem base, seedling height at planting maturity, number of developed leaves in a specific period, total fresh weight of seedlings, roots and aerial part.

Measurements of stem base diameter showed a small variation of the mean value of this parameter between three investigated periods. The average values identified ranged from 3.21 mm (period 3) to 3.88 mm (period 1).

When comparing the height of the seedling at planting maturity from all the three periods, we can observe that the average varied between 15.85 cm in period 3 (30.07.2021) and 18.61 cm in period 2 (28.06.2021), with the highest consistency reported in period 2. In terms of the number of leaves the average value fluctuated between 4.70 and 5.60, with the highest value recorded in period 1. For the first sowing period, it can be seen a significantly greater development of the plant's aerial components based on these preliminary data.

Regarding the total weight of seedlings, significant variations of the average per period can be observed, with a maximum of 5.30 g recorded in the first period and a minimum of 3.53 g in the third period.

In specific cases, fresh weight of the above ground section of seedlings taken under observation, average values ranged from 3.40 g to 5.11 g, similarly to total weight of seedlings, period 1 recorded the highest value, while period 3 recorded the lowest.

The root mass averages obtained fluctuated between 0.188 g and 0.137 g, with 0.188 g recorded in period 1 and followed closely by period 2 with a mean value of 0.186 g and lastly 0.137 g obtained by period 3.

All traits were investigated during the juvenile period. The seedling phase and the post-planting phase are included in this period which is characterized by intense vegetative growth, that is correlated with the value of temperature, soil moisture and the degree of supply of plants with mineral substances. At suboptimal temperatures and in conditions of inadequate supply of water and minerals, leaf growth is inhibited. During this period the ambient factors cannot determine the floral induction.

Our preliminary results confirm the fact that the first sowing period led to the best growing process of seedlings. In a period of 40 days, the seedlings reached the average of total weight of 5.30 g, the stem base diameter of 3.88 mm, the mean height at planting maturity of 18.51 cm, an average of total number of leaves of 5.6, the weight of aerial part of 5.11 g, and an average of 0.188 g for the weight of the roots.

Chlorophyll content (mg/100 g fresh tissue) of cabbage seedlings at different sowing stages are presented in Table 4.

Chlorophyll ratio, carotenoids and xanthophylls content and ratio (mg/100 g fresh tissue) of cabbage seedling at different sowing are presented in Table 5.

Table 4. Chlorophyll content (mg/100g fresh tissue)

Sowing time	Chlorophyll A	Chlorophyll B	Total chlorophyll A+B
Period 1 (27.05.2021)	7.08±0.79b	8.14±0.87b	15.22±1.29b
Period 2 (28.06.2021)	8.67±0.55ab	7.16±1.14b	15.83±1.01b
Period 3 (30.07.2021)	10.49±1.14a	25.71±1.33a	36.20±2.03a

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Table 5. Carotenoids and Xanthophylls content and chlorophyll ratio (mg/100g fresh tissue)

Sowing time	Carotenoids and Xanthophylls C X	Chlorophyll ratio A/B	Total chlorophyll / Carotenoids and Xanthophylls ratio
Period 1 (27.05.2021)	3.70±0.38c	0.93±0.09b	4.24±0.22b
Period 2 (28.06.2021)	2.05±0.28b	1.63±0.31a	10.73±2.94a
Period 3 (30.07.2021)	5.57±0.28a	0.41±0.04b	6.49±0.17ab

Values represent the average ± standard error. The lowercase letters represent the results of the Tukey test for $P < 0.05$.

Chlorophyll has a significant role in photosynthesis, and light regime is essential factor for chlorophyll synthesis.

Tables 4 and 5 show the differences obtained from the analysis of leaf pigments performed on 3 sets of seedlings aged 40 days, each set represented by a sowing period.

The average amount of chlorophyll A pigments ranged between 7.08 mg/100 g and 10.49 mg/100 g, with the highest value being recorded by seedlings from period 3 and the lowest value being reported by period 1.

It is observed that in the case of chlorophyll B, the average varied from 7.16 mg/100 g fresh tissue to 25.71 mg/100 g, with the highest value set by period 3 and the lowest value by period 2.

In terms of total chlorophyll pigments, the period with the sowing date of 30.07.2021, namely period 3, had the highest mean with a maximum value of 36.20 mg/100 g.

Therefore, in the case of seedlings from period 3, the average content of carotene and xanthophylls reached the maximum of 5.57 mg/100 g fresh tissue.

In the case of the chlorophyll A/chlorophyll B ratio, the greatest average of 1.63 mg/100 g was marked by period 2.

The average ratio of total chlorophyll/carotene and xanthophyll fluctuated between 4.24 mg/ 100 g and 10.73 mg/100 g, with the highest number being reported by period 2 with the date of sowing on 28.06.2021.

The preliminary data obtained from the cabbage seedling study revealed significant variance between sowing periods.

In view of the findings, we may conclude that the third period produced the highest levels of chlorophyll A and B pigments, as well as carotenoids pigments.

CONCLUSIONS

The research on seedling quality will support the development of modern seed production technology and will allow and facilitate the development of criteria for easy evaluation and identification of competitive planting material.

Based on these preliminary data, we observed that the first sowing period ensured significantly greater development of the plant's aerial components: number of leaves, the height of the seedling at planting maturity, the diameter of the seedling at the stem base and weight of the aerial part.

This can be explained by temperature, light and humidity regime in favour of vegetative growing of cabbage seedlings.

The preliminary data of cabbage seedling chlorophyll investigation revealed significant variance between sowing periods, third period produced the highest levels A and B chlorophyll pigments as well as carotenoids pigments.

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EVALUATION AND CONSERVATION OF GERMPLASM RESOURCES OF *SOLANUM MELONGENA* L. OWNED BY PLANT GENETIC RESOURCES BANK BUZĂU

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Abstract

Plant Genetic Resources Bank (PGRB) Buzau holds a valuable collection of eggplant (Solanum melongena L.) genotypes consisting of 286 genotypes. Of these, 62 genotypes are genetically stabilized, 86 genotypes are in an advanced stage of breeding, and 138 genotypes are in the segregating or recently introduced category, which are not sufficiently known in terms of character expression and stability in the lineage. In the present work, 62 genetically stabilized lines, mainly composed of local populations and old traditional varieties, were studied. During the growing period phenological, biometric observations and laboratory analyses were made using UPOV and IGPRI guidelines. Regarding productivity and resistance to the main pathogens, especially Verticillium wilt, it was found that the old local populations were the most vigorous and productive.
The research was completed with the recording of data regarding the genotypic and phenotypic expressivity specific to each cultivar, regeneration of the seed stock that will be directed to controlled atmosphere storage cells, and a part will be directed to research units, education, gene banks and farmers, for multiplication and technological transfer.

Key words: *eggplants, local population, variety, cultivar, genotype, phenotype.*

INTRODUCTION

The common or brinjal eggplant (*Solanum melongena* L.) belongs to the Leptostemonum Clade (the "spiny" solanums) of the species-rich genus *Solanum* (Solanaceae) (Knapp et al., 2013). Eggplants (*Solanum melongena* L.) are an important solanaceous crop, which is phenotypically very diverse but genotypically moderate. Eggplant (*Solanum melongena* L.), also known as brinjal in Southern Asia and aubergine in France and Britain, is the fifth economically most important vegetable in the Solanaceae family after tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), chili (*Capsicum annuum* L.), and tobacco (*Nicotiana tabacum* L.) (Oladosu et al., 2021).Worldwide, the largest producer of eggplants is China, with a production of 36,593,224 tons, followed by India with a production of 12,777,000 tons, Egypt 1,341,312 tons, Turkey 835,422 tons and Indonesia 618,202 tons. Eggplant (*Solanum melongena* L., Solanaceae; 2n = 2× = 24) ranks third in the genus *Solanum*, after potato and tomato, in total production and economic importance and is the most important Solanaceae crop native to the Old World (Daunay et al., 2001). At the European level, Italy and Spain are in first place for aubergine production with 304,690 tons and 282,200 tons, respectively (Figure 1).

Figure 1. Eggplant producing countries

Eggplant cultivation and consumption have spread to almost all parts of the world. It is ranked among the top 10 vegetables for high content of phenolic acids and antioxidant properties. Eggplants differ widely in size, shape, colour, and compositional traits. The purple-coloured eggplant is preferred by the consumers over the white and green ones (Mahanta & Dipankar 2020).

According to research carried out over the years, the cultivation of eggplant has been widespread on many continents, and is considered to have its origins in Africa, Asia and Europe.

Eggplant is the fifth economically most important vegetable in the Solanaceae family after tomato, potato, chili, and tobacco. Apart from the well-cultivated brinjal or aubergine eggplant (*Solanum melongena* L.), two other underutilized eggplant species, the African eggplant (*S. macrocarpon* L.) and the scarlet eggplant (*S. aethiopicum* L.), were also cultivated with local importance where the leaves and fruits are used for food and medicinal purposes (Oladosu et al., 2021). In the world ranking, Romania ranks 25th, with a production of 74,040 tons (FAO 2020).

Several traditional plant-breeding practices have been carried out for producing new varieties that can withstand with such changing climatic conditions besides increasing the productivity. These time-consuming practices could make considerable progress in crop improvement using selective germplasm, however, resulted in loss of biodiversity in the process (Chhapekar et al., 2016). The principal aim of the research on evaluation and conservation of genetic resources is to obtain genotypes with high fruit quality, to increase fruit/plant yield and to find genotypes with increased resistance to diseases and pests that are specific to vegetable growing regions of Romania. Eggplant is susceptible to numerous diseases viz., bacterial wilt; fusarium wilt; verticillium wilt, early blight, leaf spot, potato virus-Y (PVY), tobacco ring spot virus, tomato spotted wilt virus (TSWV), phytoplasma, and root-knot nematode. Due to these diseases, quality and quantity of eggplant production is adversely affected (Singh et al., 2019). Agronomic properties such as fruit uniformity, increased yield, and resistance to biotic and abiotic stress has been the primary objective of traditional plant breeders. An increase in the global population, degradation of soil nutrients, and climate change have contributed to the declining quality and quantity of cultivated arable land; hence, disease resistance and improved fruit yield have been the major breeding priorities (Oladosu et al., 2021) Therefore, the theme of the presented work focuses on the evaluation of the diversity of genetic resources available in the germplasm collection of BRGV Buzau, to allow the continuation of the breeding work on this species. Over the last 50 years, dependence upon commercial hybrids and advanced cultivars, as well as the neglect of traditional landraces, has led to a vast reduction in horticultural and agricultural biodiversity within the most popular species (Samuels, 2015). The need to evaluate eggplant genotypes for their inclusion in the bank's breeding and conservation programs arises in response to the action of various stressors that disrupt and require changes in agriculture. In this context we include abiotic stress caused by increased salinity levels in the soil, increasing periods of drought, seasonal lag, rising average annual temperatures, and flooding. Due to global climate changes and various anthropogenic activities, the occurrence of environmental stresses that limit yield is frequent in major eggplant producing areas (Alam & Salimullah, 2021). In the category of biotic stress, we are talking about increasingly aggressive attacks by specific diseases and pests. Together, these factors are forcing farmers to use increasing amounts of preventive and control substances, with a strong impact on both consumer health and the environment.

MATERIALS AND METHODS

In present, PGRB Buzau holds a valuable collection of eggplant (*Solanum melongena* L.) genotypes consisting of 286 genotypes. Of these, 62 genotypes are genetically stabilized, 86 genotypes are in an advanced stage of breeding and 138 genotypes are in the segregating or recently introduced category, which are not sufficiently known in terms of character expression and stability in the progeny. In the present work, 62 genetically stabilized lines were studied, mainly composed of local populations and old traditional varieties (Figure 2).

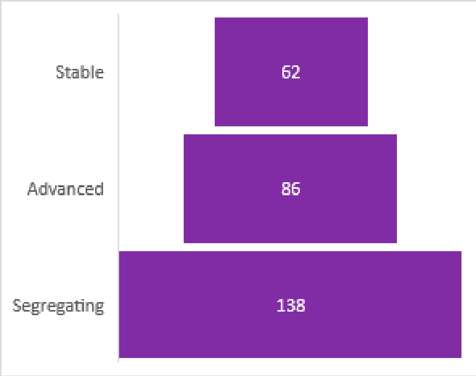

Figure 2. PGRB Buzau eggplant germplasm collection

The eggplant crop was established in the open field. The crop technology applied was the standard one for this species, i.e., the crop was established by seedling, obtained by direct seeding into 70-hole trays. The age of the seedlings at the time of planting was 60 days from sowing. The cropping pattern used was 70 cm between rows and 35-40 cm between plants per row (Figure 3).

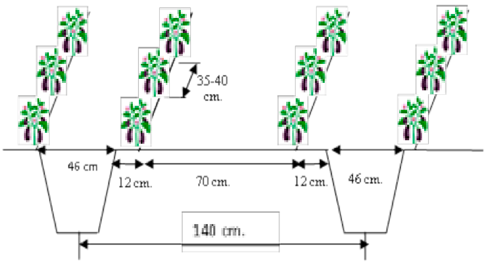

Figure 3. Eggplant crop establishment scheme

During the growing season, phenological observations and biometric determinations were carried out for each genotype under study using observation sheets conforming to the descriptors recommended by UPOV and IGPRI (Figure 4). Fruit measurements were taken at approximately 60-80 days from planting, meaning July-August, depending on the variety. Statistical analyses were performed using SPSS software.

1	Length(cm)	
2	Diameter (cm)	Bazal /Median /Apical
3	Shape	Globular ovoid obovate piriform club ellipsoid cylindric
4	Fruit curvature	Weak medium strong very strong
5	Pistil point shape	
6	Pistil point dimension (cm)	
7	Fruit apex	Indented flattened rounded sharp
8	Exocarp color at harvest maturity	
9	Fruit color intensity at harvest	Very light / light/ medium/ dark/ very dark
10	Patches	Absent / Present
11	Stripes	Absent / Present
12	The prominence of streaks/fruit	weak / medium / absent
13	Density of streaks/fruit	rare / medium / dens
14	Fruit glossiness	weak / medium / strong
15	Ribs	Absent or very weak / weak/median/strong/very strong
16	Anthocyanin. Col. under calyx	Absent / present
17	Calyx anthocyanin coloration	Absent / present
18	Anthocyanin color intensity	weak / medium / strong
19	Calyx dimension (cm)	
20	Sepals no.	
21	Sepals length (cm)	
22	Thorns on calyx	Absent or very weak/ weak/ medium/strong/very strong
23	Calyx blistering	Very weak/weak/medium/strong/very strong
24	Peduncle length (cm)	
25	Flesh color	white /yellow/green
26	Exocarp at phys. maturity.	yellow/orange/brown/red/________
27	Age of phis. mat.	early/ median/ tardy
28	Seed dispersion in fruit	
29	Seed number/fruit	
30	Seed weight/fruit	

Figure 4. Fruit description sheet

RESULTS AND DISCUSSIONS

The main purpose of the research was to evaluate the germplasm database in terms of genetic stability, expressiveness of traits and their maintenance in the lineage.
Genotypes that showed genetic stability and distinct phenotypic expressivity were promoted from the collection field to the working field and subjected to intensive breeding work (Figure 5).
The main plant characteristics are shown in Table 1, with emphasis on plant height, plant branching, plant breadth, growth habit, leaf blade color, peduncle length, intensity of purple color on flower and anthocyanin coloration on stem.

Figure 5. *Solanum melongena* L. genotypes held by BRGV Buzău

Table 1. Main plant characteristics for the genotypes under study at PGRB Buzău

Genotype	Plant height (cm)	Plant branching (cm)	Plant breadth (cm)	Growth habit	Leaf blade color (upper surface)	Peduncle length (cm)	Intensity of purple color on flower	Stem: anthocyanin coloration
1BG2021	52	2	37	Climbing	Green	5,32	Medium	Absent
2BG2021	58	3	45	Clumping	Green	4,69	Medium	Absent
3BG2021	49	2	41	Clumping	Green	3,9	Light	Present
4BG2021	45	3	43	Clumping	Green	6,35	Light	Absent
5BG2021	43	2	33	Climbing	Green	6,68	Medium	Absent
6BG2021	51	3	45	Clumping	Light green	1,5	Whitish	Absent
7BG2021	42	2	36	Clumping	Green	5,63	Medium	Present
8BG2021	46	3	42	Clumping	Greenish violet	4,75	Dark	Absent
9BG2021	39	3	36	Clumping	Light green	5,67	Light	Absent
10BG2021	67	3	41	Climbing	Green	4,43	Light	Absent
11BG2021	41	2	31	Climbing	Light green	3,63	Light	Absent
12BG2021	46	3	33	Climbing	Light green	2,67	Light	Present
13BG2021	69	3	45	Climbing	Green	6,34	Medium	Absent
14BG2021	50	2	39	Climbing	Green	4,91	Light	Absent
15BG2021	38	2	37	Clumping	Light green	6,09	Dark	Absent
16BG2021	44	2	41	Clumping	Green	6,38	Medium	Absent
17BG2021	75	3	53	Clumping	Light green	6,81	Light	Absent
18BG2021	43	3	39	Clumping	Green	5,37	Dark	Absent
19BG2021	47	2	33	Climbing	Green	11,8	Dark	Present
20BG2021	49	2	33	Climbing	Green	5,25	Dark	Absent
21BG2021	46	3	34	Climbing	Green	8,66	Dark	Absent
22BG2021	53	2	64	Climbing	Light green	5,95	Light	Absent
23BG2021	55	3	67	Clumping	Greenish violet	2,03	Medium	Present
24BG2021	62	3	37	Clumping	Greenish violet	5,01	Dark	Present
25BG2021	50	2	52	Climbing	Light green	3,72	Light	Absent
26BG2021	55	3	41	Climbing	Green	7,45	Light	Absent
27BG2021	51	3	39	Climbing	Light green	5,89	Medium	Absent
28BG2021	57	2	49	Clumping	Green	10,29	Medium	Present
29BG2021	55	2	49	Clumping	Green	4,21	Light	Absent
30BG2021	60	3	52	Clumping	Greenish violet	3,39	Light	Present
31BG2021	61	3	43	Climbing	Green	13,78	Medium	Absent
32BG2021	65	3	61	Clumping	Light green	4,72	Light	Absent
33BG2021	69	3	56	Clumping	Greenish violet	3,45	Medium	Present
34BG2021	71	3	42	Climbing	Dark green	7,66	Dark	Absent
35BG2021	48	2	36	Climbing	Green	6,06	Dark	Absent
36BG2021	52	2	45	Clumping	Greenish violet	8,63	Light	Present
37BG2021	50	2	44	Clumping	Greenish violet	6,09	Light	Present
38BG2021	60	3	39	Climbing	Dark green	5,04	Light	Absent
39BG2021	75	3	55	Clumping	Green	5,75	Light	Absent
40BG2021	62	3	58	Clumping	Greenish violet	7,27	Light	Present
41BG2021	72	3	66	Clumping	Green	7,36	Dark	Absent
42BG2021	69	3	62	Clumping	Dark green	4,95	Whitish	Absent
43BG2021	67	3	58	Clumping	Greenish violet	4,87	Dark	Present
44BG2021	49	2	37	Climbing	Greenish violet	3,1	Medium	Present
45BG2021	55	3	51	Clumping	Dark green	7,05	Medium	Absent
46BG2021	59	3	50	Clumping	Light green	4,5	Light	Absent
47BG2021	65	3	58	Clumping	Greenish violet	6,31	Medium	Present
48BG2021	71	3	59	Climbing	Green	6,95	Medium	Absent
49BG2021	52	2	48	Clumping	Greenish violet	7,57	Medium	Present
50BG2021	47	2	43	Clumping	Green	7,99	Medium	Absent
51BG2021	46	3	39	Clumping	Dark green	4,9	Whitish	Absent
52BG2021	41	2	35	Clumping	Green	8,34	Medium	Absent
53BG2021	55	3	49	Clumping	Green	5,76	Medium	Absent
54BG2021	63	3	47	Clumping	Dark green	8,3	Dark	Absent
55BG2021	59	2	50	Clumping	Green	8,23	Dark	Absent
56BG2021	48	3	41	Clumping	Greenish violet	7,56	Light	Present

57BG2021	45	3	37	Clumping	Green	5,73	Medium	Absent
58BG2021	46	2	39	Clumping	Green	5,61	Medium	Absent
59BG2021	47	2	40	Clumping	Green	10,77	Medium	Absent
60BG2021	55	3	44	Climbing	Greenish violet	3,81	Light	Present
61BG2021	82	3	74	Clumping	Dark green	7,4	Dark	Absent
62BG2021	79	3	67	Clumping	Dark green	7,3	Dark	Absent

As shown in Table 1, the genotypes studied show morphological diversity.
Most genotypes showed heights between 40 and 60 cm. Regarding plant branching, it was observed that genotypes with 3 branches were in the majority, with 38 genotypes having 3 branches and 24 genotypes have 2 branches.
Grouping of the studied genotypes was performed using a dendrogram made with the AHC method (Figure 6), obtaining two clusters with 39 and 23 genotypes, respectively.
The main fruit characteristics are recorded in Table 2.
In terms of fruit weight, values ranging from 22 grams to 1274 grams were recorded, with the mention that the largest fruits were obtained in genotypes from local populations. A great diversity of fruits was observed, due to the different characteristics of the genotypes studied, with both shapes and colors being varied. Eggplants differ widely in size, shape, colour, and compositional traits.
The purple-coloured eggplant is preferred by the consumers over the white and green ones (Charu & Dipanakar, 2020).
Genotypes with the main color of skin at harvest maturity of white, purple, indigo, green, khaki, burgundy, purple black were found. Many of these genotypes presented patches or stripes on their skin.

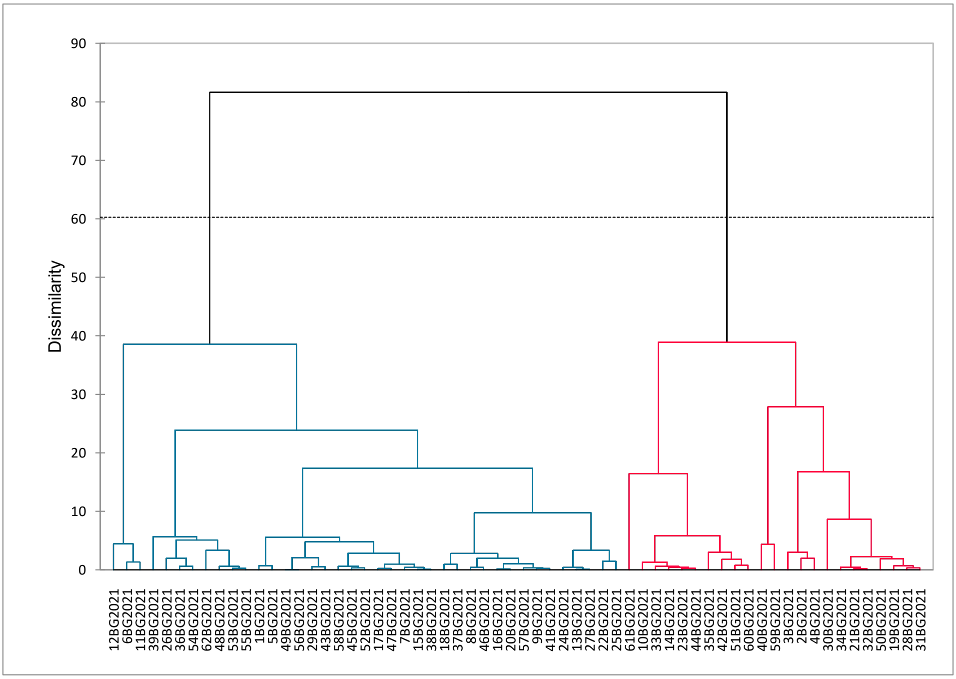

Figure 6. Dendrogram for studied genotypes

Table 2. Main fruit characteristics for the genotypes under study at PGRB Buzău

Genotype	Weight (g)	Length (cm)	Mid-diam (cm)	Pistil scar shape	Apex	Main color of skin at harvest maturity	Patches	Stripes	Ribs	Size of the calyx (cm)	Flesh color
1BG2021	315	21	5,68	circular	pointed	burgundy	present	present	very weak	6,66	greenish
2BG2021	779	17,5	11,02	linear	indented	lilac	present	present	mean	8,35	yellowish
3BG2021	323	10,08	8,89	linear	indented	lilac	present	present	weak	7,94	greenish
4BG2021	491	19	8,66	irregular	indented	white	absent	absent	very weak	8,41	whitish
5BG2021	447	21	7,03	irregular	rounded	indigo	present	present	very weak	6,81	greenish
6BG2021	27	2,41	4,6	irregular	indented	green	absent	absent	strong	1,6	green
7BG2021	483	20,5	7,46	irregular	indented	burgundy	present	present	absent	4,11	greenish
8BG2021	186	14,8	5,11	circular	indented	light purple	present	present	absent	3,16	greenish
9BG2021	316	19,4	5,91	linear	indented	white	absent	absent	very weak	3,86	whitish
10BG2021	582	12,7	11,33	linear	indented	white lilac	present	present	weak	4,31	whitish
11BG2021	115	6,88	6,03	circular	rounded	green	present	present	very weak	2,81	whitish
12BG2021	22	3,99	3,24	Puncti-form	rounded	lilac	absent	present	absent	1,42	whitish
13BG2021	198	15,11	4,64	irregular	flattened	indigo	absent	absent	absent	3,45	greenish
14BG2021	667	13,88	11,29	irregular	flattened	lilac	present	present	weak	4,32	whitish
15BG2021	383	19,5	6,49	circular	flattened	indigo	absent	absent	absent	4,1	greenish
16BG2021	295	20,3	5,26	circular	flattened	burgundy	absent	absent	absent	3,31	greenish
17BG2021	336	20,8	5,25	circular	rounded	lilac	present	present	very weak	3,22	whitish
18BG2021	349	19,4	6,57	circular	pointed	burgundy	present	absent	absent	3,54	greenish
19BG2021	631	24,5	7	irregular	flattened	purple black	absent	absent	absent	5,23	yellowish
20BG2021	336	19,6	6,21	irregular	rounded	purple black	present	absent	absent	3,64	yellowish
21BG2021	444	17,1	5,06	irregular	flattened	purple black	absent	absent	absent	5,2	yellowish
22BG2021	241	9,48	7,67	irregular	flattened	white	absent	absent	absent	3,84	yellowish
23BG2021	668	12,76	9,7	irregular	rounded	purple/white	present	present	very weak	4,79	whitish
24BG2021	195	19,4	4,2	circular	pointed	indigo	absent	present	weak	3,06	whitish
25BG2021	110	8,21	5,29	irregular	indented	white	absent	absent	absent	3,28	whitish
26BG2021	248	27,2	4,1	circular	rounded	white	absent	absent	very weak	1,97	whitish
27BG2021	234	15,6	5,35	circular	indented	purple/green	present	present	absent	3,15	greenish
28BG2021	510	19,7	8,18	irregular	flattened	indigo	absent	present	very weak	4,68	greenish
29BG2021	290	14,66	6,36	irregular	indented	white	absent	absent	very weak	3,93	whitish
30BG2021	575	12,84	11,64	irregular	indented	white	present	absent	very weak	5,04	whitish
31BG2021	565	19,9	7,87	irregular	flattened	indigo	absent	present	mean	4,77	yellowish
32BG2021	424	16,3	6,86	linear	indented	white	absent	absent	absent	5	whitish
33BG2021	492	12,1	9,74	linear	indented	white/purple	present	absent	absent	4,46	whitish
34BG2021	421	15,4	8,47	irregular	indented	indigo	absent	present	mean	4,6	yellowish
35BG2021	912	20,4	10,56	irregular	indented	indigo	absent	present	very weak	5,34	yellowish
36BG2021	289	32,3	4,16	circular	pointed	white/purple	present	absent	weak	2,88	whitish
37BG2021	165	21,9	4,41	circular	rounded	white/purple	present	absent	absent	2,84	whitish
38BG2021	377	17,5	6,79	irregular	indented	white	absent	absent	absent	4,36	greenish
39BG2021	232	26,6	4,22	puncti-form	rounded	light green	absent	absent	absent	3,36	greenish
40BG2021	337	27,6	5	circular	rounded	white/purple	present	absent	absent	3,54	whitish
41BG2021	410	20,4	6,37	irregular	flattened	purple	absent	absent	absent	4,47	yellowish
42BG2021	630	11,66	15,4	linear	indented	light green	present	present	strong	5,63	yellowish
43BG2021	249	10,33	8,14	irregular	indented	purple	absent	present	average	3,91	whitish
44BG2021	623	13,01	11,19	circular	flattened	white/purple	present	absent	weak	5,41	whitish
45BG2021	564	20,4	6,03	irregular	rounded	white/purple	absent	present	absent	4,57	yellowish
46BG2021	260	15,31	5,81	irregular	indented	white	absent	absent	absent	3,3	whitish
47BG2021	307	21,7	5,94	circular	pointed	purple/green	present	absent	absent	4,04	greenish
48BG2021	359	23,3	5,28	irregular	indented	indigo	absent	absent	absent	3,68	yellowish
49BG2021	467	15,6	7,57	irregular	flattened	green/purple	present	present	absent	5,19	greenish
50BG2021	560	16,7	9,19	linear	indented	purple	absent	absent	weak	5,48	yellowish
51BG2021	769	12,56	15,34	linear	indented	khaki green	present	present	strong	4,77	greenish
52BG2021	621	20,5	4,3	irregular	indented	indigo	absent	absent	absent	5,06	yellowish
53BG2021	327	19,3	5,71	circular	rounded	indigo	absent	absent	absent	3,76	greenish
54BG2021	470	27,4	5,3	linear	indented	indigo	absent	absent	absent	2,9	greenish
55BG2021	356	23,6	5,24	circular	rounded	indigo	absent	absent	absent	3,82	greenish
56BG2021	455	15,36	7,25	circular	flattened	indigo-purple	absent	absent	very weak	5,44	greenish
57BG2021	443	21,2	6,69	irregular	indented	indigo	absent	absent	absent	3,64	yellowish
58BG2021	456	25,8	6,13	irregular	rounded	indigo	absent	absent	absent	4,03	greenish
59BG2021	544	24,6	6,39	irregular	rounded	indigo	absent	absent	absent	4,43	yellowish
60BG2021	995	15,5	13,13	irregular	indented	white/purple	present	absent	mean	4,99	whitish
61BG2021	1274	29,1	10,6	irregular	rounded	purple	absent	present	absent	5,1	yellowish
62BG2021	668	22,4	8,1	puncti-form	rounded	purple	absent	present	absent	2,5	yellowish

Table 3. Mean values for the main characteristics of the studied genotypes

Genotype	Fruit length(cm)	Fruit weight (g)	Median diameter (cm)	Plant height (cm)	Plant breadth (cm)
1BG2021	21± 2 ^{ghij}	359 ±15,39 ^{ab}	5,68 ± 0,05 ^{tuvwxy}	52 ± 2,61 ^{lmno}	37 ± 3,41 ^{stuvwx}
2BG2021	17,5± 1,61 ^{klm}	844± 4,00 ^{d}	11,02 ± 0,57 ^{cd}	58 ± 3,85 ^{hijk}	45 ± 3,29 ^{klmnopwr}
3BG2021	10,08± 1,16 ^{wxy}	350± 3,29 ac	8,89 ± 0,64 ^{fg}	49 ± 3,63 ^{nopqr}	41 ± 3,40 ^{pqrstuv}
4BG2021	19± 1,41 ^{jkl}	548 ± 3,22 p	8,66 ± 0,46 ^{fgh}	45 ± 2,61^{qrstu}	43 ± 2,83 ^{nopqrst}
5BG2021	21± 1,41 ^{ghij}	506 ± 4,26 s	7,03 ± 0,22 ^{lmnop}	43 ± 1,63 ^{stuvw}	33 ± 4,24^{wx}
6BG2021	2,41± 0,89 ^{aa}	36 ± 4,43 an	4,6 ± 0,50 ^{zaaabac}	51 ± 3,41^{mnop}	45 ± 4,29 ^{lmnopqr}
7BG2021	20,5± 1,61 ^{ghij}	507±5,29 s	7,46 ± 0,40 ^{jklmn}	42 ± 4,43 ^{tuvw}	36 ± 4,00 ^{tuvwx}
8BG2021	14,8± 1,75 ^{pqr}	213 ± 2,83 ^{ak}	5,11 ± 0,25 ^{wxyzaa}	46 ± 4,60^{pqrstu}	42 ± 3,03 ^{pqrstuv}
9BG2021	19,2± 1,65 ^{jk}	362 ± 3,63 ^{ab}	5,91 ± 0,60 ^{rstuvwx}	39 ± 5,31^{vw}	36 ± 2,87 ^{x}
10BG2021	12,7± 1,18 ^{rstu}	654 ± 4,24 ^{k}	11,33 ± 0,51 ^{cd}	67 ± 4,24^{def}	41 ± 4,20 ^{wx}
11BG2021	6,88± 1,89 ^{z}	144 ± 3,41^{am}	6,03 ± 0,28 ^{qrstuv}	41 ± 2,83^{uvw}	31 ± 3,45 ^{mnopqr}
12BG2021	3,99± 1,43 ^{aa}	28 ± 2,00 ^{ao}	3,24 ± 0,24 ^{ad}	46 ± 3,03^{pqrstu}	33 ± 4,43 ^{rstuvw}
13BG2021	15,11± 1,58 ^{opq}	233 ± 2,45 ^{aj}	4,64 ± 0,51^{zaaabac}	69 ± 2,45^{de}	45 ± 4,82 ^{stuvwx}
14BG2021	13,88± 1,96 ^{qrst}	698 ± 4,43 ^{h}	11,29 ± 0,60^{cd}	50 ± 3,49^{mnopq}	39 ± 2,86 ^{pqrstuv}
15BG2021	19,5± 1,48 ^{ijk}	411 ±4,00 ^{y}	6,49 ± 0,34^{opqrst}	38 ± 3,41^{w}	37 ± 3,69 ^{fghij}
16BG2021	20,3± 1,38 ^{ghij}	331 ±3,03 ^{ad}	5,26 ± 0,26 ^{vwxyz}	44 ± 3,03^{rstuv}	41 ± 3,44 ^{rstuvwx}
17BG2021	20,8± 0,99 ^{ghij}	398 ± 3,95 ^{z}	5,25 ± 0,26 ^{vwxyz}	75 ± 3,13^{bc}	53 ± 4,84 ^{wx}
18BG2021	19,4± ,142 ^{jk}	422 ± 6,36 ^{x}	6,57 ± 0,36 ^{opqrs}	43 ± 3,24^{stuvw}	39 ± 5,40 ^{wx}
19BG2021	24,5± 1,49 ^{de}	687 ±3,03 ^{i}	7 ± 1,41^{lmnop}	47 ± 3,09^{opqrst}	33 ± 3,48 ^{vwx}
20BG2021	19,6± 1,64 ^{ijk}	399 ± 5,02 ^{z}	6,21 ± 0,76 ^{pqrst}	49 ± 3,41^{nopqr}	33 ± 2,86 ^{bc}
21BG2021	17,1± 1,20 ^{lmno}	494 ± 5,02 ^{t}	5,06 ± 0,73 ^{xyzaa}	46 ± 2,61^{pqrstu}	34 ± 4,49 ^{b}
22BG2021	9,48± 1,46 ^{xy}	301 ± 3,03 ^{aeaf}	7,67 ± 0,58 ^{ijkl}	53 ± 3,41^{klmn}	64 ± 4,24 ^{stuvwx}
23BG2021	12,76± 0,94 ^{rstu}	725 ± 3,35 ^{f}	9,7 ± 0,67 ^{e}	55 ± 3,63^{jklm}	67 ± 3,85 ^{ghijk}
24BG2021	19,4± 1,19 ^{jk}	243 ±3,85 ^{ai}	4,2 ± 0,69 ^{abac}	62 ± 3,03^{fghi}	37 ± 4,34 ^{pqrstuv}
25BG2021	8,21± 1,40 ^{yz}	163 ± 3,63 ^{al}	5,29 ± 0,39 ^{abac}	50 ± 4,00^{mnopq}	52 ± 3,69 ^{rstuvw}
26BG2021	27,2± 1,08 ^{bc}	305 ± 4,10 ^{ae}	4,1 ± 0,58 ^{ac}	55 ± 3,63^{jklm}	41 ± 4,53 ^{ijklmno}
27BG2021	15,6± 1,59 ^{mnopq}	292 ± 3,63 ^{ag}	5,35 ± 0,23 ^{uvwxyz}	51 ± 3,51^{mnop}	39 ± 4,63 ^{ijklmno}
28BG2021	19,7± 1,62 ^{hij}	558 ± 6,96 ^{o}	8,18 ± 0,64 ^{ghij}	57 ± 3,03^{ijkl}	49 ± 3,09^{ghijkl}
29BG2021	14,66± 1,22 ^{pqrs}	328 ± 4,43 ^{ad}	6,36 ± 0,18 ^{pqrst}	55 ± 2,63^{jklm}	49 ± 3,51 ^{nopqrst}
30BG2021	12,84± 0,75^{rstu}	621 ± 3,85 ^{l}	11,64 ± 0,55 ^{c}	60 ± 4,00 ^{ghij}	52 ± 3,46 ^{bcde}
31BG2021	19,9± 2,05 ^{hij}	614 ± 7,46 ^{l}	7,87 ± 0,78 ^{hijk}	61 ± 2,04 ^{ghij}	43 ± 4,24 ^{defgh}
32BG2021	16,3± 1,01^{mnop}	481 ± 5,66 ^{u}	6,86 ± 0,61 ^{lmnopq}	65 ± 3,22 ^{efg}	61 ± 4,45 ^{opqrstu}
33BG2021	12,1± 1,24 ^{tuv}	564 ± 3,41 ^{o}	9,74 ± 0,57 ^{e}	69 ± 3,41 ^{de}	56 ± 4,69 ^{opqrstu}
34BG2021	15,4± 1,17 ^{mnopq}	473 ± 3,03 ^{v}	8,47 ± 0,15 ^{fghi}	71 ± 2,61^{cd}	42 ± 5,40 opqrs^{tu}
35BG2021	20,4± 1,68 ^{ghij}	962 ± 5,40 ^{c}	10,56 ± 0,26 ^{d}	48 ± 3,42 ^{nopqrs}	36 ± 5,02 ^{opqrstu}
36BG2021	32,3± 1,14 ^{a}	349 ± 6,81 ^{ac}	4,16 ± 0,26 ^{ac}	52 ± 3,43^{lmnopq}	45 ± 5,40 ^{opqrstu}
37BG2021	21,9± 1,37 ^{fgh}	211 ± 5,22 ^{ak}	4,41 ± 0,21^{aaabac}	50 ± 2,83^{ghij}	44 ± 4,47^{opqrstu}
38BG2021	17,5± 1,47 ^{klmn}	422 ± 5,25 ^{x}	6,79 ± 0,33 ^{mnopq}	60 ± 3,47 ^{ghij}	39 ± 5,02 ^{opqrstu}
39BG2021	26,6± 0,77 ^{c}	272 ± 5,62 ^{ah}	4,22 ± 0,19 ^{abac}	75 ± 4,00 ^{bc}	55 ± 5,25 ^{opqrstu}
40BG2021	27,6± 1,27 ^{bc}	410 ± 6,00 ^{y}	5 ± 1,25 ^{yzaaab}	62 ± 2,61^{fghi}	58 ± 4,43 ^{opqrstu}
41BG2021	20,4± 0,60 ^{ghij}	458 ± 6,00 ^{w}	6,37 ± 0,41 ^{pqrst}	72 ± 2,51^{cd}	66 ± 4,82 ^{opqrstu}
42BG2021	11,66± 1,27 ^{uvw}	678 ± 5,62 ^{j}	15,4 ± 1,00 ^{a}	69 ± 2,67^{de}	62 ± 4,44 ^{opqrstu}
43BG2021	10,33± 0,75 ^{vwx}	296 ± 4,24 ^{afag}	8,14 ± 0,54 ^{ghij}	67 ± 3,03^{def}	58 ± 4,00 ^{opqrstu}
44BG2021	13,01± 1,19 ^{rstu}	674 ± 4,34 ^{j}	11,19 ± 0,57 ^{cd}	49 ± 4,82 ^{nopqr}	37 ± 3,53 ^{opqrstu}
45BG2021	20,4± 1 ^{ghij}	615 ± 4,43 ^{l}	6,03 ± 0,32 ^{qrstuv}	55 ± 3,63 ^{jklm}	51 ± 3,61 ^{opqrstu}
46BG2021	15,31± 1,53 ^{nopq}	308 ± 4,82 ^{ae}	5,81 ± 0,30 ^{stuvwxy}	59 ± 3,45^{hij}	50 ± 3,85 ^{opqrstu}
47BG2021	21,7± 0,65 ^{fghi}	373 ± 5,10 ^{aa}	5,94 ± 0,34 ^{rstuvw}	65 ±2,00 ^{efg}	58 ± 4,30 ^{opqrstu}
48BG2021	23,3± 0,94 ^{ef}	424 ± 5,40 ^{x}	5,28 ± 0,22 ^{vwxyz}	71 ± 2,66 ^{cd}	59 ± 4,46 ^{opqrstu}
49BG2021	15,6± 1,17 ^{mnopq}	532 ± 3,85^{q}	7,57 ± 0,31 ^{jklm}	52 ± 3,22 ^{lmno}	48 ± 2,93 ^{opqrstu}
50BG2021	16,7± 1,25 ^{mnop}	605 ± 4,86 ^{m}	9,19 ± 0,53 ^{ef}	47 ± 3,85 ^{opqrst}	43 ± 3,85 ^{opqrstu}
51BG2021	12,56± 1,39 ^{stu}	810 ± 6,26 ^{e}	15,34 ± 0,37 ^{a}	46 ± 3,03 ^{pqrstu}	39 ± 4,05 ^{opqrstu}
52BG2021	20,5± 1,47 ^{ghij}	651 ± 4,47 ^{k}	4,3 ± 0,34^{aaabac}	41 ±2,83 ^{uvw}	35 ± 4,63 ^{opqrstu}
53BG2021	19,3± 0,65^{jk}	379 ± 4,90 ^{aa}	5,71 ± 0,21 ^{tuvwxy}	55 ± 2,61 ^{jklm}	49 ± 4,51 ^{opqrstu}
54BG2021	27,4± 0,58 ^{bc}	521 ± 3,85 ^{r}	5,3 ± 0,26 ^{uvwxyz}	63 ± 3,27 ^{fgh}	47 ± 5,22 ^{opqrstu}
55BG2021	23,6± 1,35 ^{ef}	418 ± 4,90 ^{x}	5,24 ± 0,28 ^{vwxyz}	59 ±4,24^{hij}	50 ± 3,41 ^{opqrstu}
56BG2021	15,36± 1,02 ^{mnopq}	494 ± 5,02 ^{t}	7,25 ± 0,40 ^{klmno}	48 ±2,81 ^{nopqrs}	41 ± 4,35 ^{opqrstu}
57BG2021	21,2± 0,88 ^{ghij}	467 ± 5,83 ^{v}	6,69 ± 0,31 ^{nopqr}	45 ± 2,02^{qrstu}	37 ± 4,82 ^{opqrstu}
58BG2021	25,8± 1,07 ^{cd}	502 ± 5,02 ^{s}	6,13 ± 0,14 ^{qrstu}	46 ± 3,41^{pqrstu}	39 ± 4,82 ^{opqrstu}
59BG2021	24,6± 1, 09 ^{de}	593 ± 3,63 ^{n}	6,39 ±0,29 ^{pqrst}	47± 3,03 ^{opqrst}	40 ± 5,02 ^{opqrstu}
60BG2021	15,5± 1,47 ^{mnopq}	1053 ± 4,34^{b}	13,13 ± 0,23 ^{b}	55 ± 4,00 ^{jklm}	44 ± 4,90 ^{opqrstu}
61BG2021	29,1± 1,43^{b}	1308 ± 4,10^{a}	10,6 ± 0,62^{d}	82 ±3,02^{a}	74 ± 5,62 ^{opqrstu}
62BG2021	22,4± 1^{fg}	712 ± 3,63^{g}	8,1 ± 0,34^{ghij}	79 ±4,90 ^{ab}	67 ± 5,02 ^{opqrstu}

* values represent mean ± standard deviation; * letters represents Duncan test results with 95% confidence interval and p<0.05%

As we can see in Table 3, among the studied genotypes there is a great morphological diversity.
This morphological diversity indicated that the application of specific breeding and selection methods can achieve a considerable improvement in this crop.

CONCLUSIONS

The research led to the enrichment and evaluation of the PGRB Buzau germplasm collection with new genotypes, accumulating valuable information in the database of the Buzau Genebank.
Researches concluded with the organization of a valuable germplasm collection in order to be evaluated according to their genetic stability and the directions of use.
From the classification of the 286 genotypes, 62 of these have been identified as genetically stable, 86 genotypes are in an advanced stage of breeding, and 138 genotypes are in the segregating or recently introduced category, which are not sufficiently known in terms of character expression and stability in the lineage.
Regarding productivity and resistance to the main pathogens, especially Verticillium, it was found that the old local populations, especially those coming from Colibasi area, Giurgiu county, and the old Danubiana and Bucurestene varieties were the most vigorous and productive.
The research was completed with the recording of data regarding the genotypic and phenotypic expressivity specific to each cultivar, regeneration of the seed stock that will be directed to controlled atmosphere storage cells, and a part will be directed to research units, education, gene banks and farmers, for multiplication and technological transfer.

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EFFECTS OF ORGANIC INPUTS APPLICATION ON YIELD AND QUALITATIVE PARAMETERS OF TOMATOES AND PEPPERS

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Abstract

The new regulation of organic cultivation starting 2022, and The Action plan for organic production in the EU, have as main aim the extinction of organically cultivated surfaces and to ensure trackability of products in a manner that will allow the significant decrease of the negative agricultural impact on environment and in human health. Due to the restrictions on organic foods, not many inputs as fertilizers or growth promoters are available for organic vegetable application. Products of natural origin are used in horticultural crops as a stimulator of the growth and development of plants. The present study was conducted in frame of a project developed in ADER program. In frame of the project research aims to identify, evaluate, tase, develop and validate methods for the analysis of nutrients and contaminants from inputs usable in organic farming. The project strategy includes application of two organic inputs in condition of certified organic field at SCDL Bacau on three important vegetable species: tomatoes, peppers, and cucumbers. The work presents the developed protocols in terms of application to highlight the potential of products to enhance quality and yield parameters, for two of three species, namely solanaceous. Assessment of total dry matter, total soluble solids, carotenes, lycopene, chlorophylls, and xanthophylls content and yield potential were accomplished. Application of these inputs may contribute to enhanced growth, yield, and resistance against specific pathogens, as well as the positive impact of content and activity of certain bioactive compounds.

Key words: *solanaceous, vegetables, ecological inputs, quality.*

INTRODUCTION

According to the Food and Agriculture Organization (FAO), sustainable agriculture is the successful management of resources for agriculture to satisfy changing human needs while maintaining or enhancing the quality of environment and conserving natural resources.
Organic farming is the essential substitute to traditional cultivation for promoting or restoring biodiversity in agricultural landscapes (Tscharntke et al., 2021). To support the long-term cultivation of the plants, soil fertility must be maintained and improved. The use of chemical fertilizers is prohibited in organic farming. Thus, to maintain soil fertility, it is necessary to replace inputs with specific management practices by cultivating legumes, green manures or deep-rooted plants in a multiannual rotation program, incorporating natural fertilizers resulting from animal husbandry and other organic matter. from organic farming within the permissible limits specified by EU regulations. (Reganold et al., 2016; Meemken et al., 2018)

In organic farming the use of any substance obtained by chemical synthesis processes is prohibited and it is desired to reduce to a minimum any use of inputs from farms (these are allowed only exceptionally and under the control of inspection bodies). In order not to have any doubts about the inputs that can be used in organic farming in the European Union, the Commission has drawn up a list of fertilizers and amendments authorized under Regulations (EEC) No. 2092/91 and (EC) no. 834/2007, (CE) nr.889 / 2008, (CE) nr.848 / 2018 and (CEE) nr. 2164/2019. Organic fertilizers are an alternative to synthetic fertilizers and provide the nutritional requirements for healthy plant growth, micronutrients metals such as iron, zinc, manganese and copper. (Souri, 2016; Souri et al., 2018; Laily et al., 2021).
Organic farming will face a shortage of fertilizers due to climatic conditions that cause rapid mineralization of soil organic matter. Foliar fertilizers are used to support and supplement the needs of microelement plants. Thus, nutrients can be absorbed by plants through the leaves, which is influenced by

various factors as plant type, time and type of application, temperature, weather, etc., and the effects are visible shortly after application.

Sweet peppers (*Capsicum annuum*) are consumed for its high nutritional value (vitamin A, C, folate and macro elements: phosphorus, calcium, potassium) (Apostol et al., 2020; Guilherme et al., 2020; Silva et al., 2021). A two-year study at the Research Institute of Vegetable Crops at Skierniewice, Polonia showed that organic fruits of peppers had a higher average fruit weight, less wastes and more intensive redness as compared to conventional fruits. Meantime, organic pepper containned more ascorbic acid, β-carotene, soluble phenols and total flavonoids (Szafirowska et al., 2009).

The yield and quality of tomato are the subject of significant influence of fertilization method (Hasnain, 2020). In an open field experiment, (Bilalis et al. 2018) obtained the highest lycopene content through the application of seaweed compost compared with tomatoes managed with inorganic fertilizer. The same study shows a significantly higher total soluble solids and total solids to titratable acidity on tomatoes grown organically (Bilalis et al.,). Hasnain, 2020 stated that the screening of literature allows to classify the developed studies as follows: the most fertilization studies of tomato have generally only evaluated the effects on plants (Ravindran et al., 2019) or soil quality (He, 2016), solely. There are also studies focused on evaluation of both the plants and soil quality but only a limited number of parameters were the subject of investigations (Muchanga et al., 2020)

Exploring and understanding both the effects of fertilizer and application time, ration, as well as detecting the effects facilitate comprehensive evaluations of soil and plant and ensures significant support for production practice for the technologies of sustainable agriculture practice. More detailed work is mandatory necessary and should be explored to improve the development of sustainable agriculture.

MATERIALS AND METHODS

Legislation frame and place of experiment: the experimental device was developed in accordance with the provisions of EC Regulation 834/2007, Regulation (EC) no. 889/2008 and Regulation (EC) no. 848/2018 on organic farming. The experiment was performed in research field of the S.C.D.L. Bacău, in the polygon of ecological agriculture on an evolved medium alluvial soil, with a loam-sandy texture, the pH value was between 6.2-6.8, with a humus content of 2 -2.6%. The landfill is in interfluve of Bistriţa - Siret on an evolved alluvial land. Cultivation techniques are those adopted in the conditions of organic farming, in accordance with the regulations in force. The seedlings were produced in separate enclosures, in the greenhouse, under the conditions imposed by the regulations for organic farming. *Solanaceae* seedlings were produced in nutrient cubes and alveolar palettes. For weed control mulching with black polyethylene foil was applied and manually works if the case. The control of diseases and pests was done only with admitted products: against diseases: copper products - Boille Bordellaise - sulfur microns; for pests control Oleorgan, Lima Ko, Konflic, nettle macerate. The planting was fulfilled in the optimal period for the Bacău area on May 14, according to specific planting schemes devoted to the ecological culture of vegetables.

Applied inputs for impact evaluation: Treatments using Codamix and Ecoaminoalga plus (two fertilizers approved for organic farming) were applied to test the effectiveness on two vegetable species: peppers and tomatoes

Management of products application based on protocols developed by SCDL and presented at section of results.

Biological material selected for this study was represented tomatoes and peppers patented varieties of SCDL Bacau: DarianaBac - sweet pepper and Bacuni - tomato variety.

DarianaBac - sweet pepper is an early variety, with a vegetation period of maximum 120 days until the first harvest. The plant has an average vigor. The plants have 50% erect port and 50% of plants are horizontal. Color of fruits is yellow, with green nuances at technological maturity and bright red at physiological maturity. Shape is trapeze in longitudinal section. Length of fruits is 9,5-11,5 cm and diameter is 7,5-8 cm. Number of lobs 3-4. Pulp's thickness - 7,5-8,5 mm. Average weight of fruit - 80-120 g.

Unibac is a mid-early variety of tomatoes. The plants have determined growth and the robustly

is medium. The plant's height is 60-75 cm. The fruits are spherical-flattened shaped, red-brick colored at physiological maturity. The average weight of fruits is 70-90 g, and the number of seed lodge is four to five, and firmness is good. Over 65% from the entire production is included in I and extra quality. Yield destination is fresh consumption and canning.
Average yield potential of variety is 80-100 t ha^{-1}. Climatic data were registered during entire period of vegetation for each experimental crop. Starting one week before the first treatment and up to four weeks after the last treatment, the following data were daily recorded: average, minimum and maximum temperature (ºC); precipitation (quantity in mm). Any meteorological event that could influenced the quality and persistence of the treatments or their results (prolonged periods of drought, heavy rains, excessively low temperatures, etc.) were noted. For each species the values for are displayed in Table 1 and Table 2.

Table 1. Climatic data registered during experimental period of pepper

pepper	Air Temperature (◦C)		Solar Radiation (W/m2)		Air Relative Humidity (%)		Wind Speed (m/s)		Soil Temperature (◦C)		Daily evapo transpiration (ET0) (mm)	
	avg		avg		avg		avg		avg		avg	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Ist treatment	12,76	20,69	275	265	51,21	57,30	2,0	1,7	15,5	17,3	4,2	4,6
IInd treatment	22,87	25,77	258	272	73,84	78,10	0,4	0,8	23,9	24,8	4,3	4,0
IIIrd treatment	22,82	21,44	266	279	73,29	68,68	0,6	0,8	22,3	23,3	4,5	4,1

Table 2. Climatic data registered during experimental period of tomatoes

tomatoes	Air Temperature (◦C)		Solar Radiation (W/m2)		Air Relative Humidity (%)		Wind Speed (m/s)		Soil Temperature (◦C)		Daily evapo transpiration (ET0) (mm)	
	avg		avg		avg		avg		avg		avg	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Ist treatment	12,76	20,69	275	265	51,21	57,30	2,0	1,7	15,5	17,3	4,2	4,6
IInd treatment	17,48	18,22	280	238	61,99	65,95	0,9	0,7	15,7	17,3	4,3	4,2
IIIrd treatment	20,79	25,77	188	272	89,72	78,10	0,5	0,8	22,5	24,8	3,3	4,0

Table 3a. Soil characteristics of pepper experimental device

Assessed parameter	clayey cambic chernozem soil (values at 0,20 m deep)
ph	6,7
Humus content	2,3 (%)
Azot (%)	0,120
P mobil (ppm)	125
P mobil (ppm) corrected	115
K mobil (ppm)	200
Cu (mg/ kg)	31,6
Zn (mg/ kg)	68
Fe (%)	2,13
Mn (mg/ kg)	788
Ca (mg/ kg)	1105
Mg (mg/ kg)	4355
S-SO4	10

Table 3b. Soil characteristics of tomatoes experimental device

Assessed parameter	clayey cambic chernozem soil (values at deep of)	
	0,20 m	0,40 m
ph	6,62	6,19
Humus content	2 (%)	1,8
Azot (%)	0,110	0,110
P mobil (ppm)	138	82
P mobil (ppm) corrected	127	82
K mobil (ppm)	185	115
Cu (mg/ kg)	31,6	
Zn (mg/ kg)	68	
Fe (%)	2,13	
Mn (mg/ kg)	788	
Ca (mg/ kg)	1105	
Mg (mg/ kg)	4355	
S-SO4	10	

Soil investigations
The investigated parameters reflect their values at 20 cm deep, respectively 40 cm as presented in Table 3a and Table 3b
Plant investigation
Morphological investigations: size, number of inflorescences, foliage color, vigor, shape, appearance, color.
Biological and phenological observations: date of sowing, number of days till emergence, number of days till appearance of true leaves, number of days till appearance of the first flowers, number of days till first fruits development, date of first and last harvest. The quality of the tomato and peppers genotypes cultivated in organic conditions under application of organic inputs was assessed by determining the total soluble solids (TSS), dry matter (DM), carotene, lycopene carotene, lycopene. The fruits collected from each variant at physiological stage of maturity.
The total soluble solids content (TSS) was quantified using a handheld high precision portable refractometer. The samples were previously homogenized in a homogenizer. The results are expressed in ◦Brix, according to 932.12 methods (AOAC, 2005) Two measurements were performed for each homogenate sample and the results were expressed in ◦Brix.
The dry matter content (DM) was determined by drying fresh homogenized samples in a forced air drying oven (Biobase,) at 103 ± 2°C for 24 h until a constant mass was obtained (AOAC, 2000). The content was expressed in %. Differences from 100% was represented by water content. The mineral quantity was assessed my measurements and report to 100% from fresh weight of material, after calcination at 1000°C.
Antioxidants as β-carotene and lycopene from peppers and tomatoes fruits treated were extracted using petroleum ether and the quantitative dosing was performed spectrophotometric at different wavelengths respectively 452 nm for β-carotene and 472 nm for lycopene using spectrophotometer, against them blank represented by petroleum ether (Dobrin, 2019).
Statistical Analysis The results were reported as means ± standard errors. The ANOVA test was used to highlight the statistical significance among genotype characteristics and crop system differences. Where the differences were significant, Duncan's test ($p < 0.05$) multiple comparison tests was used.

RESULTS AND DISCUSSIONS

The results are presented for each investigated species, to highlight the best influence in terms of yield and quality following the application of two organic fertilizers at different moments during vegetation period.
The applied scheme for evaluation of inputs influence on pepper and tomatoes plants was based on a protocol special developed for these investigations. The protocol includes planting scheme, number and moments of treatments application, investigations to be followed. The protocol is the subject of a separate publication, here a synthesis is provided to follow up the experiment.
The experimental device for each crop was linear with three variants, displayed in three replicates: unfertilized control, and F1, F2 (two different foliar fertilization variants). Treatments were applied in three moments during vegetation cycle starting in third decade of May, continuing at interval of one month for the second application and another month for the third one. Method of organic fertilizer administration was leaf spray, concentration per application dose 2.5 liters/ ha/ treatment, concentration solution 0.25% Amount of solution applied/ ha: 1000 liters.
Capsicum annuum species under the influence of organic fertilizer application
The analyses of data registered in 2020 experimental year showed the influence of foliar application on earliness (Table 4).

Table 4. Phenological investigation of pepper, 2020-2021

Experimental variant 2020	Days to emergence	Seedling age (days)	Days till starting flowering	Days till fruit development	Days till first harvest 2020
A-VMt	5.67±0.33a	70.33±0.33a	98.00±0.57a	103.33±0.33a	174.00±1.00a
A-VF1	5.67±0.33a	70.33±0.33a	95.33±0.33a	100.33±0.88a	165.67±1.85a
A-VF2	6.00±0.0a	70.00±0.0a	95.33±0.88a	100.67±1.20a	167.00±3.46a

Tukey P < 0.05: Mean±Std. erro

Experimental variant 2021	Days to emergence	Seedling age (days)	Days till starting flowering	Days till fruit development	Days till first harvest
A-VMt	5.33±0.33a	69.67±0.33a	108.67±0.33a	115.00±0.57a	179.33±1.20a
A-VF1	5.00±0.0a	70.00±0.0a	104.67±0.88b	110.67±0.88b	173.33±0.66b
A-VF2	5.00±0.0a	70.00±0.0a	106.00±0.57ab	111.67±0.88ab	175.33±0.33b

Tukey P < 0.05: Mean±Std.error

The date of the first harvest varied depending on the fertilization variant: A-VF1, A-VF2 were early comparing the control variant. It is observed that variant A-VF1, A-VF2 the fruits reached the technological maturity faster compared to the control variant. Date of last harvest was 08.10.2020. The total number of harvested fruits per plant was 5 to 8.

The evolution of the sweet pepper Dariana Bac variety, for 2021 was slightly delayed compared to the average of recent years due to the low temperature conditions in the early stages of plant growth and development (Table 4). The date of the first harvest varied depending on the fertilization variant: The fruits in A-VF1 variant were harvested after 173.33±0.66 days from emergence and fruits from A-VF2 after 175.33±0.33. Same influence as in 2020 was confirmed in 2021, in all fruits of A-VF1 variant reached the technological maturity faster compared to the control variant. In A-VF2 variant the fruits reached the technological maturity faster compared to the control variant. Date of the last harvest 01.10.2021, was due to the first frost, total number of harvested fruits per plant being 3 to 6.

Related the influence of organic fertilizer application on yield we observed that both experimental years the values registered by control variant was lower compared to the fertilized variants. The best results were found in A-V1F2 which had a production of over 36.5 t/ha and in A-V2F2 - 32.9 t/ha.

Another sequence of our investigation was to analyse the influence of the application of two variants of foliar fertilization on some physiological parameters of fruits *Capsicum annum* L. var. *grossum* Sendt.

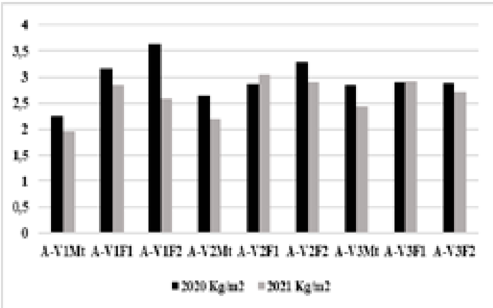

Figure 1. Influence of organic fertilizer application on peppers yield in 2020 and 2021

The total dry matter is composed of both organic and inorganic compounds, soluble or insoluble, and may be a quality indicator for peppers. The analysis of the peppers from the experimental variants was performed during the maturity period. The control variant had substantially equal values in three replicates, with an average of 9.54% Compared to the control, the application of the first fertilization variant led to higher accumulations. Compared to the control, the application of the first fertilization variant led to higher accumulations 9.60%, and the second fertilization variant, reported to the control, led to the highest accumulations of soluble dry matter 9,71%.

The soluble dry matter (TSS) is one of the most accessible and important indicators in estimation of quality, the soluble dry matter being composed mainly of soluble carbohydrates (glucose, fructose and sucrose), along with which are organic acids, amino acids and minerals.

The fertilization treatments lead to accumulation in peppers fruits of high TSS content (Table 5).

Table 5. Physiological investigations of peppers fruits

Peppers	DM	Water %	Minerals%	TSS Brix	Caroten mg 100g-1	Lycopene mg 100g-1
A-Mt	9.54±0.38a	90.45±0.38a	0.68±0.237a	6.59±0.11b	7.95±0.73ab	5.01±0.56ab
A-F1	9.60±0.51a	90.39±0.51a	0.46±0.012a	7.61±0.13a	7.14±0.24b	4.05±0.20b
A-F2	9.71±0.33a	90.28±0.33a	0.70±0.106a	7.48±0.34ab	11.15±1.05a	7.76±1.23a

Lycopene, the main red pigment in pepper fruits, shows a quantitative increase during the ripening period. Differences between lycopene levels can occur as results of different influences as variety, sampling method, preparation, method of determination, natural fruit variation, fertilization, agro-climatic conditions, soil properties, solar radiation, geographical origin, and post-harvest conditions. According to Igbokwe et al. (Igbokwe, 2013), the content of lycopene in red peppers can have values nine times more than green peppers. There are studies that report double the amount of lycopene in the red areas compared to the green ones. After integration of two experimental years, we can preliminarily conclude the positive influence of the two fertilizers in the case of yield, accumulations of total dry matter, soluble dry matter, partially carotene and lycopene.

Lycopersicum esculentum species under the influence of organic fertilizer application

Similar protocol including the linear experimental device, three replicates, application of two products in three phases during vegetation period was applied in case of tomatoes in purpose of evaluation the influence of organic fertilization on phenology and in the quality of tomatoes fruits.

Method of administration: leaf spray Concentration / application dose: application dose 2.5 liters / ha / treatment, concentration solution 0.25%.

Observations before applying the first treatment (plants with minimum 8 true leaves) and after applying the treatments. Efficacity was assessed by observations, measurements, and investigations before the first treatment and after each treatment. From the observations undertaken on the fertilized variants, in 2020, we conclude on the following aspects: there is difference related the number of inflorescences (8-11) in case of organic fertilizer application and (7-9) in control variant.

The height of the plant under organic fertilization reached (65-80 cm) compared to the control variant where the values of plants height ranged from 55-70 cm. During 2021, the number of developed inflorescences was (10-11) and the height of the plant (65-85 cm) in treated variants against control variant were number of inflorescences was 8-10 and plant height 60-75 cm. By visually assessment of foliage color was estimated as dark green, at all treated variants compared to the control which was green.

The beginning of maturation varied depending on the fertilization variant. The application of organic fertilizer conducted to an early fruit maturation (Table 6).

During 2021 experiments (Table 7) we observed that the beginning of maturation varied depending on the fertilization variant. In the control variant there were observed delays compared to the variants where organic fertilizer was applied.

It is observed that in variant T-VF1, in all replicates, and variant T-VF2 in two of three replicates, the beginning of maturation was achieved faster compared to the control variant.

Table 6. Phenological investigations of tomatoes, subject of treatments with two different organic fertilizers in 2020

Varianta	Days to emergence (days) 2020	Seedling age (days) 2020	Days till starting flowering 2020	Days till fruit development 2020	Days till harvest 2020
T-VMt	3.33±0.33a	47.67±0.33a	70.67±0.33a	78.67±0.33a	118.33±0.88a
T-VF1	4.33±0.66a	46.67±0.66a	69.33±0.33a	76.33±0.33a	103.33±9.68a
T-VF2	4.00±0.57a	47.00±0.57a	69.89±0.35a	76.67±0.88a	115.00±1.00a

Table 7. Phenological investigations of tomatoes, subject of treatments with two different organic fertilizers in 2021

Varianta	Days to emergence (days) 2021	Seedling age (days) 2021	Days till starting flowering 2021	Days till fruit development 2021	Days till harvest 2021
T-VMt	5.00±0.0a	45.00±0.0a	68.00±0.0a	75.67±0.33a	115.33±0.33a
T-VF1	4.67±0.33a	45.44±0.33a	66.67±0.33a	73.67±0.33b	100.67±9.83a
T-VF2	4.67±0.33a	45.33±0.33a	67.00±0.57a	74.33±0.33ab	111.67±0.33a

From the production point of view (Figure 2) in 2020 the T-VF1 fertilization variant stands out, which had an increase in production compared to the control variant of over 1.5 kg/m^2. T-V2F1 had a production of over 7.1 kg/m^2, and T-V3F1 - 6.91 kg/m^2.

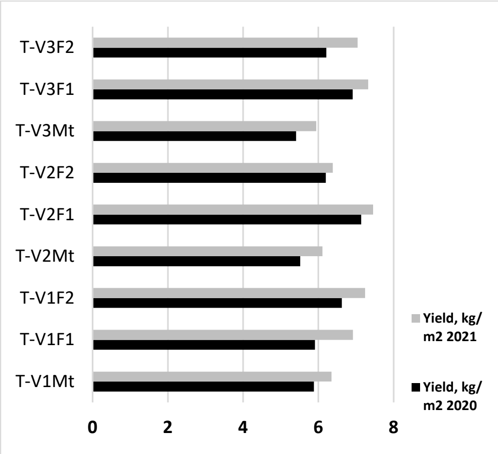

Figure 2 Influence of organic fertilizer application on tomatoes yield in 2020 and 2021

During experimental year of 2021, the T-VF1 fertilization had an increase in production compared to the control variant of over 1.34 kg/m^2. T-V2F1 had a production of over 7.45 kg/m^2. T-V3F1 obtained 7.32 kg/ m^2.

In both years, the average weight of the fruit was positively influenced by the application organic fertilizers against the control. According to our investigations in control variant the fruit weight varied from 56-70 g; T-VF1: 70-90 g/fruit; T-VF2: 65-85 g. during the first experimental year and a similar tendency was observed in the second experimental year, when fruits of control variant weighed 60-70 g; those from T-VF1: 70-85 g; and the fruits of T-VF2: 70-80 g.

Organic tomatoes were evaluated in terms of quality at harvest, in the ripening stage.

The fruits were harvested at the (6) stage of maturity, having an average diameter of 68.39 mm and an average height of 46.32 mm. The fruits were harvested by hand from each variant, the average samples on each repetition to correspond to the uniformity of size and maturity.

The analysis of tomatoes from the experimental variants was performed during the maturity period and highlighted a superior level of accumulation of total dry matter in variants where the fertilizer was applied, 5.35% and 5,72%, against control variant 5,19%. Same positive influence was observed in case of TSS accumulation, both treated variants, having superior accumulation comparing control (Table 8).

Table 8. Phisiological investigation of tomatoes

Varianta	DM %	Water %	Mineralse %	TSS Brix	Caroten mg 100g-1	Lycopene mg 100g-1
T-Mt	5.19±0.51a	94.81±0.51a	0.50±0.018b	4.33±0.15a	4.40±0.20a	3.50±0.17b
T-F1	5.35±0.47a	94.64±0.47a	0.57±0.006a	4.81±0.09a	4.62±0.26a	4.39±0.20ab
T-F2	5.72±0.25a	94.28±0.25a	0.55±0.010ab	4.82±0.10a	4.83±0.22a	4.69±0.37a

Regarding the influence of the fertilization variants, we found that both variants in the three repetitions allowed significantly higher accumulations of carotene and lycopene, compared to the control variants. Carotene and lycopene are the main pigments that give the specific color for tomato fruits, depending on variety, maturation phase, phytosanitary condition. They play an important role in determining the degree of ripeness and for assessing the commercial quality of tomatoes fruits. The synthesis of carotene and lycopene takes place simultaneously with the biodegradation of chlorophyll in tomato fruits.

The carotene and lycopene content represents an indicator for the degree of maturity and quality of the fruit. The color fruit playing a key role in consumer acceptance. Regarding the influence of the fertilization variants, we found that both variants in the three repetitions allowed significantly higher accumulations of carotene and lycopene, compared to the control variants.

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CONCLUSIONS

Related the influence of organic fertilizer application on pepper: after integration of two experimental years, we can preliminarily conclude the positive influence of the two fertilizers in the case of peppers yield, earliness, accumulations of total dry matter, soluble dry matter, partially carotene and lycopene.

Related the influence of organic fertilizer application on tomatoes - the application of organic fertilizer conducted to an early tomatoes fruit maturation. The positive influence of the two fertilizers is found even after the second season of experimentation, so we can conclude in the case of accumulations of total dry matter, soluble dry matter, carotene and lycopene that they were superior compared to the control. Related the influence of organic fertilizer application on yield we observed that both experimental years the values registered by control variant was lower compared to the fertilized variants.

The presented data are preliminary results of the major study, and more investigations will continue. The general objective of the research is to identify, evaluate, test, develop and validate the methods for the analysis of nutrients and contaminants from inputs usable in organic farming.

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THE EFFECT OF FERTILIZATION REGIME ON EGGPLANT CROPS UNDER GREENHOUSE

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Abstract

Eggplants (Solanum melongena L.*) have become in the last years one of the most appreciated vegetables worldwide due to their high nutritional value. Eggplant fruits contain significant amounts of carbohydrates, mineral salts, vitamins and polyphenols. In order to increase productive potential of eggplant, it is necessary to ensure the optimal level for all the environmental factors as well as the specific technological ones. Soil is the main source of mineral nutrients and water for plants, its ability to provide the needed nutrients varies depending on its level of fertility. Eggplant fruit quality is determined by nutrients quantity and quality. In addition, at the University of Life Sciences from Iasi, an experiment was carried out during 2020-2021 growth season with the purpose to investigate the effect of three different fertilization regimes on the fruit quality and fruit yield and its component of eggplant cultivar Mirval F1 and Bleach Pearl F1 under tunnels. The research indicated significant values in terms of quality and quantity for the microbiologically fertilized Mirval hybrid.*

Key words: *biometrical indicators, cultivars, differentiated fertilizers, eggplant, polytunnels.*

INTRODUCTION

Eggplants (*Solanum melongena* L.) have become increasingly popular in the last 3-4 decades, due to high consumer preferences, but also to the fact that this species finds favourable growing conditions, both in the field and in protected areas, which ensures good economic efficiency (Valerga et al., 2020).
Eggplants are a crop with a particular food and economic impact among vegetable crops because they have a high ecological plasticity, are appreciated by consumers and can be grown in diverse systems (Stoleru et al., 2014).
The growth and development of eggplant plants is influenced by the presence of nutrients.
In the last 30 years, new varieties and hybrids have been introduced in the Romanian market, which most of the time have not adapted to the new environmental conditions and to the high consumer preferences.
Recent research has highlighted that the main factors on which the quantity and quality of the harvest depend in organic and conventional systems are: cultivar, fertilization and irrigation (Caruso et al., 2019).
Nitrogen fertilization at 180 kg/ha increased eggplant yield in the field from 14.8 t/ha in the unfertilized variant to 25.43 t/ha (He et al., 2021).
Biological fertilization significantly influences fruit mass. In the Traviata F_1 hybrid grown in the solar, fruit mass, ranged from 67 g in the unfertilized up to 332.5 g. in the biologically fertilized version (Kostadinov et al., 2019).
Chemical potassium fertilization significantly influenced the total yield in a field eggplant cultivar, ranging from 20.6 t/ha in the unfertilized variant to 37.3 t/ha in the 135 kg/ha potassium fertilized variant (Hochmuth et al., 1993).
In an experiment on eggplant cultivar established in the Mediterranean area, obtained a total yield that ranged from 43.7 t/ha for the organically fertilized to 50.1 t/ha for the chemically fertilized (Leogrande et al, 2014).
Specific research by Smith (1979) highlighted that the N:K ratio in grafted eggplant crops should vary between 1:1 to 1:2 for optimal growth and development, otherwise vegetative growth is induced (Bletsos, 2003) at the expense of poor fruiting (Smith, 1979).

The height of eggplant plants varies within fairly wide limits depending on the number of irrigations, from 97.2 cm in the 12-day irrigated variant to 112.4 cm in the 6-day irrigated variant (He et al., 2021).
Total production in eggplant correlates positively with physiological indicators such as total chlorophyll content, photosynthesis rate, gas exchange, etc.
Total chlorophyll ranged from 0.7 mg/g fresh matter to 1.3 mg/kg fresh matter (Li et al., 2019).
Chlorophyll ranged from 34.56 mg/g fresh matter in the eggplant cultivars Kemer treated with 100 mM NaCl, up to 52.92 mg/g fresh substance in the control Kemer cultivar.
Total chlorophyll ranged from 0.70 mg/g fresh matter in the 80% field capacity irrigated variant to 1.17 mg/g fresh matter in the 100% field capacity irrigated variant.
Chlorophyll a ranged from 0.30 mg/g of FW in the variant irrigated at 40% field capacity to 0.80 mg/g of FW in the variant irrigated at 100% field capacity. Chlorophyll b ranged from 0.28 mg/g FW at the 60% soil capacity irrigated variant to 0.38 la mg/g fresh substance at the 100% soil capacity irrigated variant (Al-Muwayhi, 2019).
The height of eggplant plants ranged from 79.5 cm in the unfertilized variant to 112.7 cm in the variant fertilized with 500 g/m^2 biochar. Fruit weight per plant ranged from 371.4 g in the control variant to 584 g in the variant fertilised with 500 g/m^2 biochar. Chlorophyll a varied from 1.2 mg/g fresh matter in the non-fertilised variant to 8 mg/g fresh matter in the variant fertilised with 500 g/m^2 biochar. Chlorophyll b ranged from 1.1 mg/g fresh matter in the unfertilized variant to 3.4 mg/g fresh matter in the 500 g/m^2 biochar fertilized variant (Ebrahimi et al., 2021).
The influence of photosynthesis rate on the second eggplant cultivar ranged widely from 3.03 µmol m^{-2} s^{-1} for determinations made on the mid-plant leaves of the Black Pearl cultivar to 20.95 µmol m^{-2} s^{-1} for determinations made on the tip leaves of the Aragon cultivar (Acatrinei, 2019).
In the case of *Solanum aethiopicum* L., cv. Legon, temperature influences the rate of photosynthesis, the results varied in wide limits from 9.8 mg CO_2 dm^{-2}h^{-1}, in the fruiting phase at 40^{0}C temperature, to 23.5 mg CO_2 dm^{-2}h^{-1}, in the flowering phase at 30^{0}C temperature (Nkansah, 2001).
The main aim of the research was to investigate the possibility of using new cultivars and fertilisation regimes to increase the quantity and quality of eggplants.

MATERIALS AND METHODS

Experimental site

The research has been carried out during 2020-2021, in the didactic field of the "V. Adamachi" Farm of the University for Life Sciences of Iasi on an eggplant crop, established by seedling between 20-22 April, in a plot on an area of 200 square meters.
The technology of seedling production and crop maintenance was similar to organic crops (Stoleru et al., 2014).
Eggplant crop was preceded by cucumbers on the soil used in the present investigation. The soil was alluvial cambic chernozem soil with the following characteristics: pH 7.2; 3.2% organic matter; 31% clay; 28 mg/kg N, 1.83% K_2O; 1.13% CaO; 0.19% P_2O_5; 0.16% MgO; 0.46% Na_2O; 3.96% Fe_2O_3; 0.11% MnO; 49 ppm Cu; 103 ppm Zn; EC 416 µS/cm.
During the two years, the biological material used was three new hybrid cultivars of eggplant peas Mirval, Aragon and Black Pearl (Figures 1 and 2).

Figure 1. Mirval F1 cultivar

Figure 2. Black Pearl F1 cultivar

An experimental protocol was proposed to achieve optimal nutrition, using Nutrispore® fertilizers, Micoseeds® compared to a control, unfertilised. The combination of the two factors resulted in nine experimental variants. The experiment was a polyfactorial design, in divided plots with three replicates per variant, the area of a replicate being 7.4 m², corresponding to 18 plants per replicate.
Nutrispore® is a solid, water-soluble, chemical fertilizer based on N, P, K, Mg, and complexed with microorganisms of the genus *Glomus* sp.,

in order to provide dual benefits to the crop in terms of fertilization and vigorous plant growth, while also providing benefits to the cultivated soil.

At the first growth stage, 144 g of Nutrispore® - NPK Nutrispore® - NPK (MgO) 30-10-10 with Boron (B), Iron (Fe), Manganese (Mn), Zinc (Zn) were provided.

At the second application, Nutrispore® NPK (MgO) 15-10-30 with Bor (B), Iron (Fe), Manganese (Mn), Zinc (Zn) - 425 kg/ha and Nutrispore® NPK 12-48-8 with Bor (B), Iron (Fe), Manganese (Mn), Zinc (Zn) - 400 kg/ha were applied.

Micoseed® MB is a fertilizer based on *Glomus* sp., *Beauveria* sp., *Metarhizium* sp. and *Trichoderma* sp. (Stoleru et al., 2014). The organic fertilizer was complexed twice during the growing season with Nutryaction® which provides food for microorganisms, ensures growth of eggplant plants and provides enhanced activity of the required microflora. It provides better protection against stress caused by adverse biotic environment and stimulates the development of the root and foliar system of eggplants.

The fertilizers were applied to the soil at a dose of 800 kg/ha for the chemical treatment. Chemical fertilizer was applied three times as follows: 50% of the total amount in coincidence with the final soil preparation prior to planting; 25% when the first fruit reached a 2-3 cm; the last dose (25%) when the fruit of the third cluster reached a 2-3 cm. Biological fertilizer was applied two times as follows: 30 kg/ha at soil preparation and 30 kg/ha when the fruit reached a 2-3 cm from third cluster. The biological fertilizer was complexed with 1.5 L/ha for two time of Nutryaction® applied by fertirrigation system.

Biometrical analyses

In each plot and at each harvest, the number and weight of fruits as well as the mean fruit weight on random 5 fruit samples, were determined. The yield (kg/hg) was calculated by using the following formula: (plants/ha × fruits/plant × average fruit weight)/1000. The plant heights (cm) were measured after the last harvest in each experimental treatment.

Photosynthesis determination

Photosynthesis (μmol $CO_2 \cdot m^{2} \cdot s^{-1}$), was measured with a portable compact system LCi (ADC Bioscientific UK Ltd., Global House, Geddings Road, Hoddesdon, Herts, EN11 0NT, UK), with a Broad Leaf Chamber, with an area of 6.4 cm^2, between 9-10 am. The measurements were performed the day before harvesting, from 9 to 10 am.

Statistical analysis. The experimental results are expressed as means ± SD. The data were statistically processed by two-way ANOVA and Tukey's test was performed for mean separation at $p \leq 0.05$, using SPSS software version 21 (IBM Microsoft, New York, USA).

RESULTS AND DISCUSSIONS

The results on the influence of fertilization on the morphological characters of the analysed cultivars are presented in Table 1.

Fruit diameter for the three cultivars did not show significant differences from the experimental mean of 83.93 mm regardless of the variant. According to the experimental variant, the fruit diameter varied from 74.54 mm for the non-fertilized cultivar Mirval to 86.22 mm for the control cultivar Aragon.

Fruit height for the experimental variants ranged from 17.02 cm in the chemically fertilized Aragon cultivar to 18.68 in the organically fertilized Black Pearl cultivar. Compared to the experimental mean of 18.04 cm the differences were significant at $p \leq 0.05$.

The number of fruits per plant for the analysed cultivars varied according to the experimental variation from 5.77 fruits in the control Black Pearl to 7.93 fruits in the chemically fertilized cultivar Mirval. The values for number of fruits showed significant differences from the experimental mean of 6.56 for $p \leq 0.05$.

The mean mass of a fruit for the experimental variants ranged from 340 g for the Mirval control and Black Pearl control cultivar to 490 g for the organically fertilized Aragon cultivar. Differences from the experimental mean of 388 g were significant at $p \leq 0.5$.

Results on the individual influence of fertilization on leaf surface phosphoactive radiation, substomatal CO_2 concentration, transpiration, stomatal conductance, photosynthesis rate and water use efficiency are presented in Table 2.

Table 1. Results on the influence of fertilization on morphological characters in the analysed cultivars

Treatment	Diameter (mm)	Fruit height (cm)	Fruit mass (g)	No. of fruits
A x Ct	86.22±2.35ns	17.03±0.31bc	0.35±0.01b	6.77±0.23bcd
A x Mo	83.19±1.14ns	18.31±0.22abc	0.49±0.01a	6.87±0.18bc
A x Ch	82.63±0.94ns	17.02±0.29c	0.38±0.01b	7.57±0.19ab
M x Ct	74.51±22.58ns	18.58±0.19ab	0.34±0.01b	6.67±0.38bcd
M x Mo	85.79±1.85ns	18.19±0.37abc	0.39±0.01b	6.97±0.07ab
M x Ch	83.49±0.52ns	17.87±0.33abc	0.39±0.00b	7.93±0.09a
BP x Ct	84.09±2.25ns	18.50±0.46abc	0.34±0.02b	5.77±0.23d
BP x Mo	82.52±0.94ns	18.68±0.33a	0.47±0.02a	5.87±0.18cd
BP x Ch	82.50±1.41ns	18.22±0.23abc	0.36±0.02b	6.57±0.19bcd

*Values represent mean ± standard error. Lowercase letters represent Tukey's test results for $p \leq 0.05$ (a- represents lowest value and ns - not significant; A-Aragon; M-Mirval; BP-Black Pearl; Ch - chemical; Mo - microbiological; Ct - control).

The results presented in Table 2 show that the photosynthesis rate was significantly influenced by fertilization, with results ranging from 2.32 in the chemically fertilized cultivar Aragon to 7.31 in the unfertilized variety of the same cultivar.

The results from the statistical point of view on the influence of the cultivar on the transpiration process show that significant results were obtained, ranging from 2.61 in the case of the chemically fertilized variant, in the Aragon cultivar, to 4.66 in the chemically fertilized variant, in the Black Pearl cultivar.

Carbon dioxide, consisting of one carbon atom and two oxygen atoms, is a labile anhydride of carbonic acid ($CO_2.H_2O \sim H_2CO_3$), a chemical compound resulting from the oxidation of carbon, mostly of organic origin. On the other hand, it is a by-product in industrial processes.

Data on the influence of eggplant sorting on CO_2 concentration are presented in Table 2.

From the results obtained, it appears that significant values were obtained, ranging from 336.20 for the cultivar Mirval in the non-fertilized variety to 372.14 for the cultivar Aragon in the chemically fertilized variety.

Measurements recorded for stomatal conductance show that significant values were recorded, ranging from 0.21 in the case of the cultivar Black Pearl in the non-fertilized variety, to 0.46 in the cultivar Mirval in the non-fertilized variety.

Data on water use efficiency show that significant values ranging from 0.89 for the chemically fertilized cultivar Aragon to 2.51 for the non-fertilized variety of the same cultivar were recorded. The recorded data on leaf surface phosphoactive radiation show that significant values ranging from 186.39 were obtained for the organically fertilized cultivar Black Pearl to 316.94 for the chemically fertilized variety of the same cultivar.

Table 2. Results on the influence of fertilization on physiological traits in the analysed cultivars

Treatment	Phosphoactive radiation at the leaf surface (µmol $m^2 s^{-1}$)	CO_2 concentration (vpm) - Ci	Sweat (mol H_2O $m^{-2} s^{-1}$) - E	Stomatal conductance (mol CO_2 $m^{-2} s^{-1}$) - gs	Photosynthesis rate (µmolCO_2 $m^2 s^{-1}$) - A	Water use efficiency A/E
A x Ct	277.02±5.27ab	345.61±2.57bc	2.91±0.02 d	0.46±0.01b	7.31±0.42 a	2.51±0.12a
A x Mo	295.07±18.72ab	347.56±3.00bc	3.28±0.07 c	0.44±0.00a	6.45±0.31a	1.97±0.07b
A x Ch	233.76±12.73abc	372.14±1.84 a	2.61±0.03e	0.23±0.01bcd	2.32±0.18c	0.89±0.07c
M x Ct	274.51±22.58ab	336.20±3.44 c	3.19±0.05c	0.29±0.01	6.08±0.16a	1.91±0.08b
M x Mo	221.41±14.83bc	354.24±5.07 b	3.25±0.07c	0.26±0.00cd	3.27±0.48bc	1.01±0.17c
M x Ch	234.42±15.91abc	352.00±1.97bc	3.47±0.06c	0.22±0.01bc	3.19±0.10bc	0.92±0.05c
BP x Ct	231.19±5.58bc	350.14±1.73bc	3.31±0.08c	0.21±0.01cd	3.03±0.23bc	0.92±0.07c
BP x Mo	186.39±7.34c	343.29±2.54bc	3.94±0.05b	0.25±0.01d	4.24±0.20b	1.08±0.04c
BP x Ch	316.94±31.12a	347.67±5.95bc	4.66±0.05a	0.30±0.00a	4.20±0.67b	0.90±0.14c

*Values represent mean ± standard error. Lowercase letters represent Tukey's test results for $p \leq 0.05$ (a- represents highest value; A-Aragon; M-Mirval; BP-Black Pearl; Ch - chemical; Mo - microbiological; Ct - control).

In the experimental period 2020-2022, the influence of cultivar on eggplant production was investigated.
The results obtained for the yields in t/ha of Aragon, Mirval and Black Pearl cultivars are shown in Figure 3.
It can be seen that the highest fruit yield was obtained in organically fertilized cultivar Aragon 82.56 t/ha and the lowest yield was obtained in unfertilized cultivar Black Pearl 54.05 t/ha.
The cultivar Aragon obtained the highest fruit yield of 82.56 t/ha in the organically fertilized cultivar and the lowest of 59.03 in the non-fertilized cultivar. Differences from the experimental mean of 71.11 t/ha were significant for $p \leq 0.5$.
The Mirval cultivar obtained the highest fruit yield of 78.57 t/ha with chemical fertilization and the lowest of 63.36 with the non-fertilized variant. Compared to the experimental mean of 71.11 t/ha the results obtained were significant.
The Black Pearl cultivar obtained the highest fruit yield of 80.02 t/ha under chemical fertilization and the lowest of 54.05 under the non-fertilized variant. The differences from the experimental mean of 71.11 t/ha were significant for $p \leq 0.5$.
The yield results following fertilization indicated that the only cultivar that responded best to the biological fertilization was the cultivar Aragon while to chemical fertilization the cultivars Mirval and Black Pearl responded best.

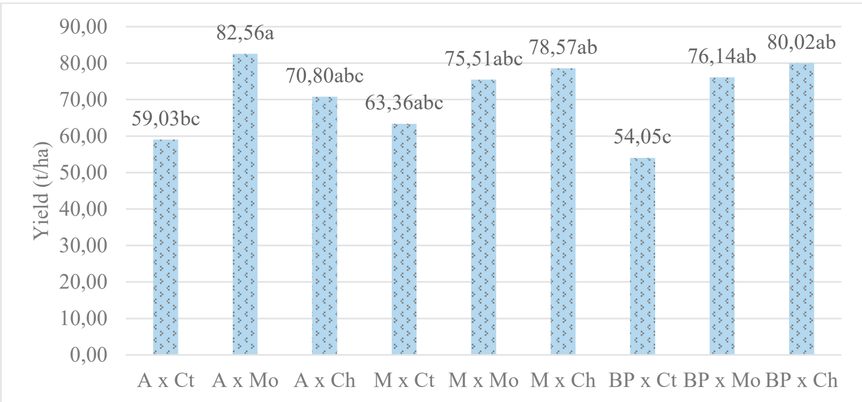

Figure 3. Eggplant production results 2020-2021
(Lowercase letters represent Tukey test results for $p \leq 0.05$ (a- represents highest value; A-Aragon; M-Mirval; BP-Black Pearl; Ch - chemical; Mo - microbiological; Ct - control).

CONCLUSIONS

The obtained results highlight the positive effect of chemical and biological fertilization regardless of cultivar, both in terms of physiological and yield indicators.
The new cultivars introduced into the crop provide high yields per unit area, the highest being achieved by Aragon F1.
Biologically fertilised varieties provide high yields compared to chemically fertilised varieties, in the case of Aragon even higher than with synthetic fertilisers.
The high yields of organically fertilised variants are also due to higher photosynthetic activity which correlates positively with the yield achieved.
Fruit diameter and height are in general genetically determined characteristics, therefore differences between varieties are not significantly influenced by fertilisation but by cultivar.
The number of fruits per plant and their mass are superior to the control variants, making significant differences.

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REVIEW ON IMPROVING TOMATO CULTURE TECHNOLOGY IN PROTECTED SYSTEM FOR ENVIRONMENTAL PROTECTION AND INCREASING PRODUCTIVITY USING PGPR

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Abstract

Tomato (Solanum lycopersicum L.)is one of the world's most prolific vegetables . Researchers are still looking for solutions to improve crop technology using plant protection and stimulation products that are neither harmful to the environment or the plants they treat. Many technologies for cultivating and treating plants with Bacillus spp. have been developed. For this reason, we analyzed the influence of PGPR on two tomato hybrids Cocete F1 and Nissos F1 in the greenhouse. The results showed that there weren't considerable differences in the quantitative characteristics of plant studied. Regarding stem thickness, the variants had values between 0.5 cm and 0.7 cm. The number of leaves was 16.8 = F1 Cocete 15.9 = F1 Cocete+PGPR, 16.6 = Nissos F1, 16.5 = Nissos F1 + PGPR). The plants height for Cocete F1 was 193.1 cm and 188.1 cm for Cocete F1 + PGPR, while for Nissos F1, the plants had 218.9 cm and 211.3 for Nissos F1 + PGPR. In terms of inflorescences number, Cocete F1 recorded 6.4 and 5.7 for Cocete F1 + PGPR whereas Nissos F1 had 5.1 and Nissos F1 + PGPR 4.9. Referring to the flowers number, the hybrid Cocete F1 in both variants registered similar values (30 flowers/plant) but Nissos F1, Nissos F1 + PGPR had 24.8 and 26.7, respectively. The number of fruits per plant was 14.8 for Cocete F1 while other variants produced 16 fruits/plant.

Key words: *biologic, tomatoes , bacteria, PGPR.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most productive vegetables in the world. It is consumed in a wide variety and has a beneficial impact on human health mainly due to the high content of lycopene, folic acid, ascorbic acid, flavonoids, α-tocopherol, potassium and phenolic compounds (Daniela Erba et al., 2013).

In 2019, world production of tomatoes was 180 million tons, and China was the main producing country with 62 million tons, contributing 35% of total world production (FAO, 2019, http://www.fao.org).

Tomatoes have been grown in our country for about two centuries, they have a special economic and food importance, being consumed in all seasons.

For the purpose to achieve higher quality production, researchers have sought and are looking for solutions to improve crop technology using various plant protection and stimulation products. A first step in this area was the use of products that do not affect the environment and thus the treated plants. Over the years, various technologies for cultivating and treating plants with Bacillus spp. have been developed in order to increase plant productivity but also to reduce plant residues, thus helping to protect the consumer.

Bacillus spp. is a bacterium that has been shown to be beneficial in various fields. Studies are still being conducted today to determine its usefulness.

Regarding the studied field, *Bacillus* spp. Was distinguished both by positive effects on the microorganisms existing in the soil but also by a better assimilation of nutrients, which led to increased productivity. It is also used to control certain diseases specific to both tomato cultivation and fruit growing. The rhizosphere provides essential habitat for microbial communities and facilitates a variety of plant-microorganism interactions. Members of the genus *Bacillus* are an important group of rhizobacteria that promote plant growth (PGPR), which improve crop growth and yield. PGPR improves plant growth and yield by facilitating the absorption of mineral nutrients

(Sadaf et al., 2020). *Bacillus* spp. also enhances the main transport functions of nutrients from soil to plant by solubilizing insoluble zinc compounds and increasing the bioavailability of zinc in soil (Muhammad et al., 2017).

Another important factor in maintaining the optimal balance in the soil of microorganisms is the way of irrigation. Drip irrigation has a significant role in the distribution of soil moisture, pH, porosity and growth of tomato roots, as well as their degree of branching.

Underground drip irrigation showed the highest values of the root index, the most intense positive root-soil-microorganism interactions and the highest fruit yield. The effects of surface drip irrigation on the development of tomato roots and soil microbial communities were lower, and the resulting fruit yield was also lower (Jingwei Wang et al., 2022).

In greenhouse plants, an obvious stimulant effect was observed on the quantity and quality of fruit when *Bacillus* spp. Was applied on the substrate in high concentration. The results showed the potential of *Bacillus* spp. to stimulate tomato production in the expected range for greenhouse cultivation. Due to the yields and the quality of the obtained fruits, it was estimated that the profitability of the treatment was 2.5 times higher than the control (Karina A et al., 2021). Bacillus spp. is also used in the biological control of various diseases that can occur in tomato cultivation. Bacillus spp. has a strong antibacterial activity in vitro against various pathogenic plant bacteria, being extremely effective in reducing the development of symptoms of three bacterial plant diseases, namely, bacterial wilt of tomatoes, bacterial staining of peach leaves and bacterial staining of red pepper leaves in pot experiments. The application of the bacterium Bacillus, effectively suppressed the development of bacterial wilting of tomatoes in the greenhouse experiment, with an effectiveness in controlling the disease, being comparable to that of dazomet, a commercial pesticide (Seong Mi Im et al., 2020).

From this research it is admitted that the use of Bacillus spp. Can have as effective results as chemicals, but without toxic residues for both the environment and the consumer.

Inoculation of *Bacillus* spp. improved the ability of tomatoes to defend against the yellow leaf curl virus, TYLCV by activating gene expression and enzymatic activity related to systemic resistance in tomatoes. Specifically, the rate of infection and the symptoms, disease level and symptoms present on the leaves decreased in tomato plants treated with Bacillus spp. Meanwhile, treatment with Bacillus spp. positively influenced the development of roots, green color of leaves and growth of tomato shoots. Secondly, culture-dependent microbiological analysis showed that *Bacillus* spp. increased the bacterial-fungal ratio and the number of beneficial microorganisms, while decreasing the number of pathogenic fungi in the rhizosphere (Qiao Guo et al., 2019).

In order to maintain plant health, research has evolved to the application of carbon-based nanomaterials that have significantly reduced the incidence and severity of *F. oxysporum* in inoculated tomato plants. Also, all carbon-based nanomaterials (CNM) treatments increased the yield of tomato fruit when the plants were inoculated with *F. oxysporum*. However, the application of carbon nanotubes (CNTs) has shown the best results. The application of CNMs can be a tool to increase the content of biocomposites and increase the nutritional quality of tomato fruits, because the production of this type of compound is stimulated. The decrease in the incidence and severity of *F. oxysporum* observed in this study is not fully explained by the modification of the antioxidant defense system of tomato, because the plants that received treatment with CNMs behaved similarly to the positive controls inoculated with the pathogen. However, NMCs may be a product that can be applied in the field as an alternative to traditional pesticides. Therefore, several studies are needed to consider biochemical, micromorphological, genetic or other relevant physiological processes of plants in order to properly understand how CNMs induce tolerance or resistance to pathogens. At present, this technology is not yet available for agricultural production, however, with the increase in research on this topic, it is possible that commercial products based on nanomaterials will be available in the near future (Yolanda González-García et al., 2022).

The application of treatments with *Bacillus* spp. proved to be useful during the storage period after harvest. In cherry tomatoes, they have been shown to play an important role in controlling foodborne pathogens in postharvest fruit. The treatments produced 2 complementary effects. On the one hand, at high concentrations, it showed strong antibacterial activity against *B. subtilis* by inhibiting cell division and oxidative phosphorylation, in addition, it reduced the motility of biofilm formation in *B. subtilis*. On the other hand, at low concentrations they increased the antioxidant capacity of cherry tomatoes and induced an ethylene-based defense response. It has been found that the application of treatments with *Bacillus* spp. Delays senescence in cherry tomatoes (Gui-Yang Zhu et al., 2021).

MATERIALS AND METHODS

The experiment took place in didactic greenhouses of the Agricultural University of Athens from September 2021 to February 2022. The main purpose of the experiment was to observe and monitor the main quantitative and qualitative characteristics of planted hybrids in the presence and/or absence of PGPR (Plant Growth-Promoting Rhizobacteria).
In order to perform the experiment, two hybrids Cocete F1 and Nissos F1 were used.
During the growing season, the crop was cared for according to known technology, using both chemical and biological products to control diseases and pests.
The fertilization recipe was calculated using the NUTRISENSE program (software for the nutrition and fertilization of hydroponic cultivated crops).
The quantitative observations that were made during the vegetation period were: the thickness of the package, the number of leaves, the number of inflorescences, the number of flowers and fruits and the total height of the plants.
The determinations were performed at 5 repetitions with 6 plants/repetition at an interval of 10 days. In order to establish the differences between the variants, the analysis of the variant, Test F and Test T was used.

RESULTS AND DISCUSSIONS

After 45 days from planting, the Cocete F1 hybrid did not register any significant difference between the plants treated with PGPR and the plants without treatment (P <0.76) with values of 0.7 cm for both variants studied (Figure 1).

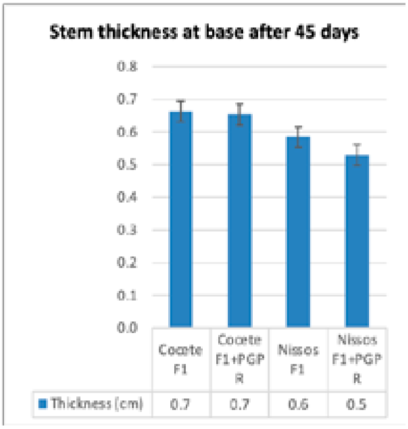

Figure 1. Stem thickness at base after 45 days

Regarding the hybrid Nissos F1 and Nisssos F1 + PGPR there was a significant difference (P <0.01) so that Nissos F1 recorded the value of 0.6 cm and Nisssos F1 + PGPR 0.5 cm.

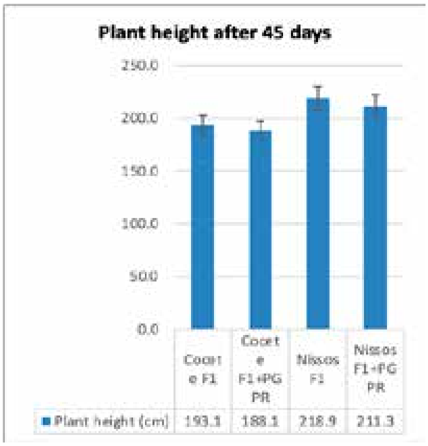

Figure 2. Plant height after 45 days

The height of the plant was determined after the removal of the main shoot. The results confirmed that there is a significant difference between PGPR-treated and untreated plants (P <0.03) for the Nissos F1 hybrid, respectively Nissos F1 recorded a plant height of 218.9 cm and Nisssos F1 + PGPR 211, 3 cm (Figure 2).
There was no significant difference for the Cocete F1 hybrid (P <0.15), with only 5 cm of

difference between the control plants and those treated with PGPR.

Regarding the number of leaves, the results confirmed that there is a significant difference between the plants treated with PGPR and the plants without treatment (P <0.0008) for the hybrid Cocete F1. However, for the Nissos hybrid, there was no significant difference (P <0.76) (Figure 3).

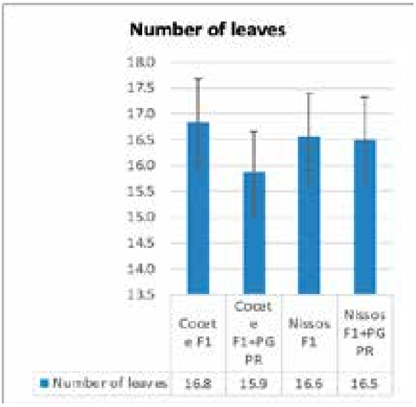

Figure 3. Number of leaves

Given the number of inflorescences (Figure 4). the analysis showed a significant difference between the plants treated with PGPR and the plants without treatment (P <0.0004) for the hybrid Cocete F1, so that the variant without PGPR had an average of 6.4 inflorescences/ plant and the variant with PGPR recorded 5.7 inflorescence/plant.

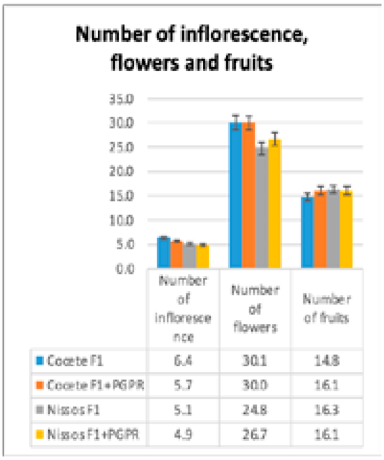

Figure 4. Number of inflorescence, flowers and fruits

There were no significant differences for the Nissos F1 hybrid (P <0.17), both variants having values close to 5.

Regarding the number of flowers, the analysis did not show a significant difference between the plants treated with PGPR and the plants without treatment for both hybrids (P <0.11 Nissos F1, P <0.96 Cocete)F1).

The values for Cocete F1 were similar of 30 flowers/plant, and for Nissos F1 it registered a number of 25 flowers/plant.

For the number of fruits, the results did not show a significant difference between the plants treated with PGPR and the plants without treatment for both hybrids (P <0.82 Nissos, P <0.19 Cocete).

The values for Cocete F1 were around 15 fruits/plant, and for Nissos F1 it was 16 fruits/ plant.

The distance between the inflorescences for the hybrid Cocete F1 and Cocete F1 + PGPR did not register significant values from the point of view of the statistical analysis (P <0.83), the two variants having similar and close values (Figure 5).

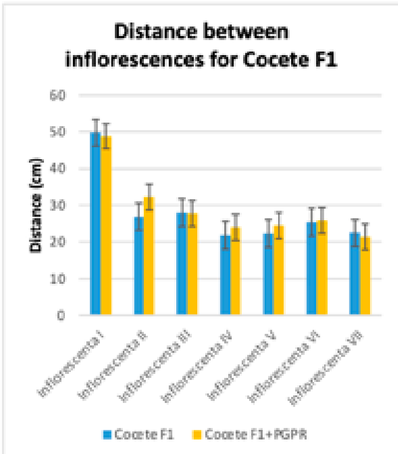

Figure 5. Distance between inflorescences for Cocete F1

In the case of the Nissos F1 hybrid, there were no significant differences in the presence/ absence of PGPR (P <0.83), as the varieties studied had similar values (Figure 6).

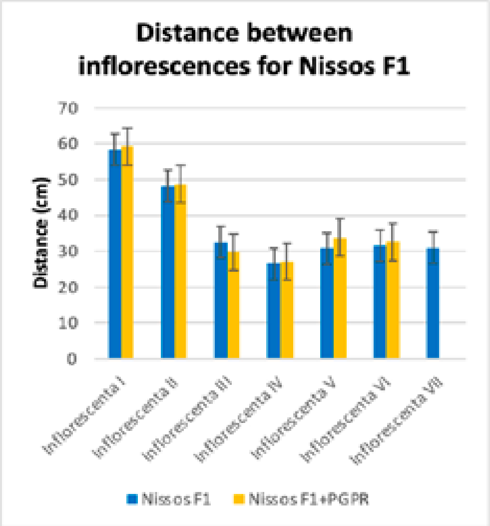

Figure 6. Distance between inflorescences for Nissos F1

CONCLUSIONS

The following results were obtained from this study:

In terms of stem thickness and plant height after removal of the main shoot, a significant difference was obtained between plants treated with PGPR and plants without treatment for the Nissos hybrid. No differences were observed for the Cocete hybrid.

For the number of flowers and fruits, there was no significant difference between PGPR-treated plants and untreated plants on both hybrids.

The number of leaves and the number of inflorescences were different, the difference being significant between the plants treated with PGPR and the plants without treatment for the hybrid Cocete. No difference was observed for Nissos.

The distance between the inflorescences for both studied variants was not influenced by the presence / absence of PGPR.

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ECOLOGICAL DIVERSITY OF THE EPIGEAL INVERTEBRATE FAUNA FROM AN EXPERIMENTAL BELL PEPPER CROP AT SCDL BUZĂU – ROMANIA

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Abstract

Ecological diversity is an important parameter in assessing the state of an ecological system. This paper presents the results of a study conducted in 2019-2020 on the abundance and ecological diversity of useful and harmful insect communities from an experimental crop of bell pepper organized at the Research-Development Station for Vegetable Growing Buzău. The experiment included five variants in four replications disposed in completed randomized block, untreated, diatomite in three doses (52.5 g, 105 g, 210 g) and Trichoderma asperellum Td85 strain. The diatomite is of autochthonous from the Pătârlagele deposit and the antagonistic fungus Trichoderma from the microorganism collection of the Research and Development Institute for Plant Protection Bucharest. Samples were collected every two weeks by the Barber soil trap method. Data collected in experimental crop of bell pepper under this study revealed a total of 1,455 individuals in 2019 and 1,634 in 2020, respectively, belonging to 124 species, 104 genera, 58 families, 17 orders, 8 classes and 3 phyla. The ecological diversity of present species was estimated using the diversity indexes, Margalef, Menhinick, Shannon-Wiener and Simpson.

***Key words**: diatomite, pepper, soil fauna, Trichoderma.*

INTRODUCTION

Bell pepper (*Capsicum annuum* L.), also known as sweet pepper, is one of the most important vegetable crops in Romania cultivated in the field and protected areas, as well. At the level of 2018 the total pepper cultivated area was 18 000 ha with a production of 229 mil kg, which makes to rank Romania 15th in the world (AgroStandard, 2020). Like all vegetables, bell pepper is a valorous natural source of vitamins and minerals good for human health. It is contains fibres, too.

Bell pepper crop is a suitable host for a range of pests and diseases with a severe negative impact on plants and fruits, the most common being aphids (*Aulacorthum solani*, *Macrosiphum euphorbiae*, *Myzus persicae*), tobacco trips (*Thrips tabaci* Lind), two-spotted spider mite (*Tetranychus urticae* Koch), corn earworm (*Helicoverpa armigera* Hbn.), greenhouse whitefly (*Trialeurodes vaporariorum* Westw) *Bemisia tabaci* and the southern green stink bugs (*Nezara viridula*) *Halyomorpha halysand, Pectobacterium* sp., *Alternaria* spp. (Boiu-Sicuia et al, 2017). In context of the current climatic change expressed by high temperatures and deficit of precipitation, the incidence of diseases and pest attack on plants is increasing. Although chemical products are a rapid and effective means, they cause many unwanted side effects such as pest resistance and toxic residues in fruit production. It is therefore highly necessary to find and implement new means of plant protection to replace chemicals as much as possible, being at the same time environmentaly friendly and to ensure stable, sustainable and helthy productions.

The diatomaceous earth and *Trichoderma* species can become good candidates to obtain nontoxic insecticides. As literature reveals the products based on diatomaceous earth and *Trichoderma* have become increasingly used in recent years in ecological and integrated pest management both in the field, protected areas and storehouses (Petrisor et al., 2019).

Diatomaceous earth is an inert dust rich in silicon dioxide present in fresh-water or marine environments as well as in terrestrial ecosystems (Zeni et al., 2021) that kills insects by penetrating their epicuticles. *Trichoderma* spp. is also present in soil and interact with plants resulting in beneficial effects such as stimulation of plant defence, development of roots, promotion of plant growth (Petrisor et al., 2019).

Epigeal fauna is an important component of the total fauna community and by its diversity plays an essential role in the functioning of the ecosystems. Some of the species have characteristics of bioindicators that offer important information about the quality of environment (Cunha, 2021).

In this study there are presented data on the ecological diversity of the epigeal invertebrate fauna collected in an experimental bell pepper crop conducted in a cropping system with medicinal plants and integrated pest management, that included treatments based on diatomaceous earth and *Trichoderma asperellum*. These data complement those previously published in Florescu et al., 2021.

MATERIALS AND METHODS

Location and area of the study. The experimental bell paper (*Capsicum annuum* L.), was set up in the field of the Vegetable Research and Development Station in Buzău County (S-E Romania). The varieties used were Buzau 10 in 2019 and Cantemir in 2020. Five variants of four replicates each were organized following randomized block method. Area of one replicate was 7mp. Variants consisted of V_1 (untreated), V_2 (52.5 g diatomaceous earth), V_3 (105 g diatomaceous earth), V_4 (210 g diatomaceous earth) and V_5 (bioinoculant *Trichoderma asperellum* Td85 strain). Diatomaceous earth was local product obtained from Pătârlagele deposit (Buzău County) and the antagonistic fungus was provided from the collection of microorganism of the Research and Development Institute for Plant Protection.

Technology and treatments applied. The diatomaceous earth was used as (i) powder applied after planting along the row of pepper plants in June and (ii) dispersion applied on soil and plants in vegetative season in July (Florescu et al., 2021). The pepper plants were accompanied by medicinal plants (marygolds and litchi tomato) produced at the VRDS Buzau.

Insect trapping. The mobile fauna on the soil surface was collected using the soil traps method. We used Barber type traps installed in the soil, four traps in each variant, from June to October each year. A trap consisted of a 400 ml glass jar introduced into the soil with the opening at the ground level. The recipients were filled in a proportion of 50-70% with 4% dish soap solution. The entomophaunistic material was collected every two weeks and conserved in 70% alcohol and analysed in the laboratory. Species identification was performed under stereomicroscop SZ 61 Olympus by the second author of this paper.

Statistical interpretation of data. To estimate the diversity of species we follow two types of diversity indices which are commonly used in ecological studies.

The Margalef and Menhinick species diversity indices include the richness species and total abundance. Margalef index (D_{Mg}) is given by the formula: $D_{Mg}=\frac{S-1}{\mathrm{Ln}\ N}$, where S is number of species and N is total number of individuals in the sample (Margalef, 1968). Menhinick index (Whittaker, 1977) was calculated using formula: $D_{Mn}=\frac{S}{\sqrt{N}}$.

The Shannon-Wiener and Simpson indexes consider both species richness and species evenness. The formula for Shannon-Wiener diversity index (Shannon & Weaver, 1949) was: $H' = \sum_{i=1}^{R} p_i \, ln \, p_i$, where: p_i is relative abundance of all species. Simpson index was defined as in the formula $D = 1 - \sum_{i=1}^{S} p_i^2$ (Preda, 2020).

RESULTS AND DISCUSSIONS

In this paper it is assessed the diversity of the invertebrates species mobile on the soil surface collected in the experimental pepper crop in which treatments with products based on diatomaceous earth and Trichoderma were applied to improve the status of plants and control pests. The fauna of invertebrates obtained in the present experiment was analysed in terms of taxonomic structure, numerical density and relative abundance as well as ecological parameters and the results were included in a previously reported study (Florescu et al., 2021).

In present paper there are presented the results regarding the diversity of epigeal invertebrate fauna collected in the same experimental pepper crop, following various species diversity indices. The total fauna (pest and useful) of epigeal invertebrates investigated area counted a number of 1,455 individuals in 2019 and 1,634 in 2020 (Figure 1). The invertebrates in 2019 belonged to 117 species distributed in 93 genera, 55 families, 17 orders, 7 classes and 3 phyla and those in 2020 belonged to 124 species distributed in 104 genera, 58 families, 17 orders, 8 classes and 3 phyla.

The annual relative abundances distributed by principal orders is presented in the graph in Figure 2.

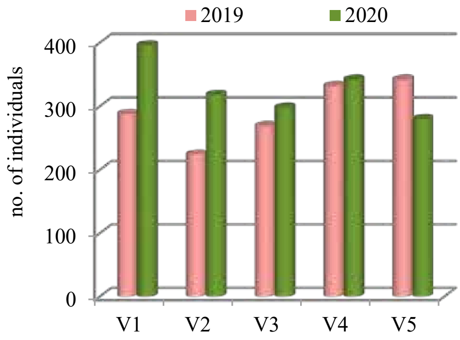

Figure 1. Abundance of total fauna in pepper crop

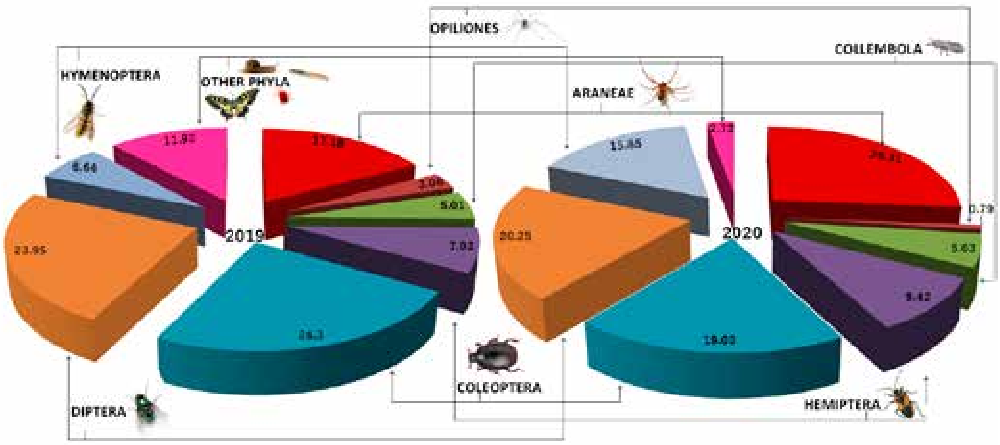

Figure 2. The most abundant order of species in useful and harmful fauna in the years 2019 and 2020

The highest values of relative abundance in 2019 were reached by the species of Coleoptera order (24.3%) followed by Diptera (23.95%) and Aranea (17.18%). In 2020, Aranea was found to be most abundant (26.31%) followed by Diptera (20.25%), Coleoptera (19.03%) and Hymenoptera (15.85%).

The species diversity. Four important indexes measuring ecological diversity of species communities were calculated in this study using excel calculation sheets.

The synthetic data, distinctly on functional groups and treatments, are presented in Tables 1 and 2.

Table 1. Species diversity indices of mobile invertebrate pest fauna in experimental pepper crop

Index values/Year	2019					2020				
	V_1	V_2	V_3	V_4	V_5	V_1	V_2	V_3	V_4	V_5
No of individuals (N)	119	68	60	134	124	84	81	74	94	71
No of species (S)	30	19	13	28	23	16	21	21	18	16
Margalef index (D_{Mg})	6.08	4.28	2.93	5.52	4.56	3.39	4.56	3.70	3.74	2.81
Menhinick index (D_{Mn})	2.75	2.30	1.68	2.42	2.07	1.75	2.33	2.44	1.86	1.90
Shannon-Wiener (H’)	2.60	2.28	1.69	2.75	2.49	2.41	2.40	2.34	2.35	2.07
Evenness (E_H)	0.76	0.77	0.66	0.81	0.79	0.87	0.79	0.76	0.81	0.74
Simpson index (D)	0.87	0.83	0.71	0.89	0.87	0.88	0.85	0.83	0.86	0.81
Evenness (E_{1-D})	0.13	0.18	0.31	0.11	0.14	0.13	0.16	0.18	0.15	0.20

Table 2. Species diversity indices of mobile invertebrate useful fauna in experimental pepper crop

Index values /Year	2019					2020				
	V_1	V_2	V_3	V_4	V_5	V_1	V_2	V_3	V_4	V_5
No of individuals (N)	169	156	209	198	218	312	237	224	248	209
No of species (S)	47	40	35	38	39	34	36	44	50	41
Margalef index (D_{Mg})	8.97	7.72	6.37	7.01	7.06	5.75	6.41	7.95	8.89	7.49
Menhinick index D_{Mn})	3.62	3.20	2.42	2.70	2.64	1.92	2.34	2.94	3.18	2.84
Shannon-Wiener (H')	3.04	2.97	2.64	2.73	2.19	2.60	2.52	2.78	2.81	2.36
Evenness (E_H)	0.78	0.80	0.74	0.75	2.59	0.73	0.70	0.73	0.71	0.63
Simpson index (D)	0.90	0.97	0.87	0.97	0.89	0.84	0.84	0.98	0.99	0.99
Evenness (E_{1-D})	0.10	0.03	0.13	0.03	0.11	0.16	0.16	0.02	0.01	0.01

The number of individuals and richness of species differed both between functional groups, pest and useful fauna, but also between treatments. The highest values were in pest fauna in both year of observation. The highest total number of species was found in untreated variant (77 species) in 2019 and in 210 g diatomaceous earth treatment (68 species) in 2020, while the lowest was found in 105 g diatomaceous earth variant (48 species) in 2019 and untreated variant (50 species) in 2020. No relation was observed between number of species and increased number of individuals among the treatments.

The Margalef richnness index (D_{Mg}) whose calculation was based on the number of species and abundance showed the highest values. At the experiment level, the values of this index were higher for useful fauna than for pest fauna in the 2-year study and higher in 2019 than in 2020 in both functional categories, between 5.75 and 8.98 and between 2.93 and 6.08 respectively. Among treatments, the values of D_{Mg} lower than 3 were found in 2019 in variants corresponding to 105 g diatomaceous earth and bioinoculant *Trichoderma asperellum* and lower than 6 were found in 105 g diatomaceous earth and untreated variants.

The Menhinick index which depend as the Margalef index on the species richness in connection to number of specimens, showed values with similar fluctuations.

The Shannon-Wiener diversity index is the most used to estimate the diversity of a community of species (plants or animals) at the level of ecological systems in a specific area or habitat. This index depends on both species richness and the uniformity of species distribution in the total number of individuals in the sample. The values of Shannon-Wiener index increases with the number of species and evenness. Therefore, the communities with a large number of species that are uniform distributed are the most diverse (Spellerberg, 2003). In present study, the Shannon-Wiener index expressed a good diversity of the invertebrate epigeal fauna in a stable state in crop variants in both years, ranging in 2019 from 1.69 to 2.75 for pest fauna and from 2.07 to 2.42 for useful fauna, and in 2020 from 2.19 to 3.04 for pest fauna and from 2.36 to 2.81 for useful fauna. The presented values of Shannon-Wiener diversity index may suggest that the interventions with diatomaceous earth and fungus inoculant in experimental pepper crop don't seem to affect the community of epigeal invertebrates.

The Simpson diversity index presented high values in the present study, from 0.71 to 0.97 in 2019 and from 0.81 to 0.99 in 2020 revealing a good quality of species diversity in the epigeal fauna present in experimented pepper crop.

CONCLUSIONS

The present study represent an important step in the examination and estimation of the ecological diversity of epigeal invertebrates in pepper crop.

The values of the species diversity indices based on the species richness relative to number of specimens as well as on the species richness and the uniformity of species distribution in the total number of individuals in the sample, suggest that diversity of epigeal invertebrates in pepper crop in not affected by treatments with diatomaceous earth and fungus inoculant used experimentally in pepper crop.

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STUDIES REGARDING YIELD POTENTIAL OF SOME GARDEN PEAS ACCESSIONS SOWN IN DIFFERENT DECADES

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Abstract

Pisum sativum also known as garden peas is one of the major food legumes that can grow in different regions, and it ranks the fourth in world food legume productions next to soybean, peanut, and dry bean. At Vegetable Research Development Station Buzau, a new breeding program of peas has started and 15 stable accessions were taken into study. The focus of the present study was to evaluated the yield potential and the best suited sown decade for 15 peas accessions. The crops were severely affected by excessive rainfall, (in May and June) which however, it was found that the accessions sown in the first decade had higher yields compared to the accessions in the second decade, both at green peas maturity and at physiological maturity. Thus, the lowest value for weight of 1000 grains was at A11 with 212.04 g, and the highest was obtained by A1 with 455.25g. In terms of yield potential, it was noted that A8 had the lowest value/sqm (102.01 g), and A10 was the accession with the highest weight/sqm (295.53 g), at physiological maturity. A9 was the earliest accession, followed by A1, and the latest accessions were A6 and A7.

Key words: *expressiveness, Pisum sativum, phenotype.*

INTRODUCTION

Soil, as a living environment of plants, is the main determinant factor of the flora in a certain area and affects both the condition of crop plants and weeds (Haliniarz et al., 2014). One of the most suitable legumes crop cold tolerant and frost resistance is garden peas (Nemecek et al., 2008).

The contribution of legumes, especially garden peas, is one of the key factors in ensuring soil fertility and in supporting the production of cereals in dry or developing areas (Jacobsen et al., 2012).

Peas are an annual plant specific to the cold season, with importance in human and animal nutrition (Mihailović and Mikić, 2010; Chețan et al., 2015). The root is pivoting, and on the young branches a large number of atmospheric nitrogen-fixing nodules grow following symbiosis with *Rhizobiu*m bacteria, thus contributing to the improvement of soil fertility (Bohlool et al., 1992; Cass et al., 1994; Singh et al., 2007; Tago et al., 2011; Matsumiya et al., 2013; Ferguson, 2013; Şimon et al., 2014). Also, the root system is characterized by a high capacity of absorption and solubilization of potassium and phosphorus (Muntean et al., 2008), from this reason it is a good precursor to other vegetable crops and is of particular importance in organic farming (Vînătoru et al., 2019).

In the current context characterized by important climate changes that may have adverse effects on agricultural crops, peas may become the most important legume for grains grown in Romania and in the European Union, due to its high adaptability, short vegetation period and high contents of proteins and energetic substances. In addition, grain peas can help ensure the biological balance of our planet, reduce pollution, improve soil fertility and reduce energy consumption caused by the production and use of chemical fertilizers (Chirilă, 1990).

For this reasons, Vegetable Research and Development Station (VRDS) Buzau has started an breeding program for this species (Barcanu et al., 2019; Gherase et al., 2021), in order to obtain competitive varieties on the market, with resistance to abiotic stress caused by the constantly changing environment.

The vegetation period is a hereditary controlled trait, influenced by the climatic factors characteristic of the landforms and represents the necessary period from sprouting to harvest maturation. This property is relatively stable only for the area where the biological material was created. Without a well-developed ecological plasticity, the vegetation period registers significant deviations if a genotype is cultivated in other pedoclimatic conditions.

In the Official Catalogue of Species and Varieties of Cultivated Crops from Romania only 11 cultivars are registered. One of this, ‘Getica’ variety was registered by VRDS Buzau in 2005. In 2021, 3 varieties of garden peas were approved under the name ’Banat’, ‘Crișana’ and ‘Muntenia’.

The aim of the study was to evaluate the production potential at physiological maturity of fifteen pea accessions and to identify the most suitable sowing period.

MATERIALS AND METHODS

The Breeding and Biodiversity Laboratory of VRDS Buzau has a valuable germplasm collection of *Pisum sativum.*

The study aimed the evaluation of fifteen accessions of garden bean noted A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14 and A15. For control variant is was used Getica cultivar, A15. The studied accessions were sown in two decades to identify the optimal sowing period in order to obtain a higher yield and quality production.

The studies were made for two consecutive years (2020-2021). The quantitative and qualitative observations were made using descriptors by the International Union for the Protection of New Varieties of Plants (UPOV 2006, 2015). The maximum temperature, minimum temperature, rainfalls in the studied years and average multiannual temperatures and rainfall are shown in Table 1.

The seeds were sown manually and the -sowing scheme used was 14 cm between plants and 70 cm between rows. The first decade was sown on March 1st, and the second decade was sown on April 1st, on both years of study.

Table 1. Mean of temperature and rainfall during studied years and multiannual

2020	March	April	May	June	July
Temp. max °C	14.35	19.93	22.77	28.3	30.77
Temp. min °C	3.32	4.8	10.7	16.16	18.09
Average °C	8.83	12.36	16.73	22.23	24.43
Rainfall mm	29.3	6.9	102.5	78.1	75
2021	**March**	**April**	**May**	**June**	**July**
Temp. max °C	10.25	14.86	22.67	25.73	31.32
Temp. min °C	0.38	4.23	11.45	15.73	19.25
Average °C	5.53	9.55	17.06	20.73	25.29
Rainfall mm	85.42	81.56	80.05	218.09	23
Multian	**March**	**April**	**May**	**June**	**July**
Average °C	5.2	11.4	17.1	21	22.9
Rainfall mm	26.09	40.6	68.8	80.8	67.7

The experiments were organized in randomized block design. In order to establish the quality of seeds, the total titratable acidity, the dry matter and total soluble solids content were measured at harvest maturity. Total titratable acidity was determined using 5 g of ground seeds diluted in 25 mL of double distilled water. The titratable acidity of seeds was determined by titration with 0.1 N NaOH until pH reach 8.1. The dry matter content (DM) was determined with KERN DBS60-3 thermobalance. The total soluble solids (TSS) were measured with digital refractometer KERN OPTICS ORF 1RS Methods described by us in details in other research articles (Agapie et al., 2020).

The yield was related to the quantity of dried seeds, reached physiological maturity because the aim of the researches was to obtain seeds and also to identify the most suitable sowing period. For statistical analysis, ANOVA was used followed by Duncan test.

RESULTS AND DISCUSSIONS

Throughout the vegetation period, phenological observations were made for both decades, on two years of study. In Table 2 and Table 3 are presented the phenological observation of year 2021. The phenological data of year 2020 were three to five days earlier than those in 2021 (data not shown).

The seed sown in the first decade sprouted faster than the those sown in the second decade This is due to higher temperatures in April which favoured better and faster germination.

But, from a qualitative point of view, the accessions have decreased production, as the plants have been more exposed to high temperatures in the summer months, knowing that peas are one of the plants that do not prefer high temperatures.

Therefore, we recommend sowing in February-March, as early as possible, when the weather allows. The vegetation period varied from semi-early varieties (A1, A5, A10), to semi-late varieties (A2, A3, A4, A8, L10, L11, L12, L15) and late varieties with a vegetation period of over 90 days (A6, A7, A13).

Table 2. Phenological observation of garden pea accessions - first decade

Accession	Sowing date	Germination date	Flowering period	Emergence of the first flat pod	Harvest fresh pods	Harvest dry pods
A1	01.03	29.03-31.03	07.05-13.05	19.05	09.06	02.07
A2	01.03	31.03-01.04	19.05-21.05	24.05	15.06	09.07
A3	01.03	29.03-04.04	14.05-16.05	21.05	12.06	05.07
A4	01.03	29.03-01.04	15.05-21.05	24.05	15.06	09.07
A5	01.03	29.03-30.03	13.05-17.05	15.05	09.06	02.07
A6	01.03	01.04-05.04	24.05-27.05	04.06	26.06	18.07
A7	01.03	01.04-05.04	24.05-26.05	31.05	24.06	13.07
A8	01.03	29.03-04.04	17.05-21.05	24.05	15.06	09.07
A9	01.03	30.03-01.04	07.05-10.05	12.05	10.06	03.07
A10	01.03	31.03-01.04	16.05-18.05	21.05	12.06	05.07
A11	01.03	29.03-30.03	15.05-21.05	25.05	16.06	10.07
A12	01.03	01.04-05.04	17.05-21.05	13.05	11.06	11.07
A13	01.03	31.03-02.04	17.05-23.05	28.05	25.06	23.07
A14	01.03	29.03-01.04	18.05-25.05	28.05	28.06	23.07
A15	01.03	29.03-03.04	14.05-17.05	16.05	09.06	02.07

Table 3. Phenological observation of garden pea accessions - second decade

Accession	Sowing date	Germination date	Flowering period	Emergence of the first flat pod	Harvest fresh pods	Harvest dry pods
A1	01.04	13.04-16.04	17.05-20.05	28.05	18.06	09.07
A2	01.04	13.04-16.04	20.05-26.05	31.05	21.06	12.07
A3	01.04	12.04-14.04	19.05-25.05	31.05	21.06	12.07
A4	01.04	13.04-15.04	24.05-29.05	03.06	24.06	15.07
A5	01.04	12.04-14.04	18.05-24.05	02.06	24.06	15.07
A6	01.04	18.04-21.04	27.05-03.06	14.06	26.06	18.07
A7	01.04	17.04-21.04	26.05-01.06	09.06	24.06	13.07
A8	01.04	13.03-15.04	24.05-29.05	03.06	24.06	15.07
A9	01.04	0.03-12.04	15.05-19.05	25.05	18.06	19.07
A10	01.04	13.04-16.04	21.05-26.05	30.05	21.06	12.07
A11	01.04	14.03-17.03	22.05-27.05	02.06	25.06	16.07
A12	01.04	13.04-16.04	22.05-31.05	04.06	28.06	16.07
A13	01.04	15.04-19.04	23.05-30.05	04.06	28.06	16.07
A14	01.04	13.03-16.04	20.05-26.05	02.06	24.06	15.07
A15	01.04	13.03-16.04	19.05-25.05	29.05	18.06	09.07

As we can see in Table 1, the year 2021 was atypical. Precipitation far exceeded the average of multiannual precipitation in previous years. In March the multiannual average was exceeded by 58.52 l/m^2, in April

the average was exceeded by 40.96 l/m^2, the average in May was exceeded by 11.25 l/m^2.

The highest amount of precipitation was recorded in June, 218.09 l/m^2, thus exceeding 137.29 l/m^2. Due to heavy rainfall, the production and quality of pea seeds have been significantly affected. Water stress is one of the most important factors that can affect the plant. Excess water causes root rot or can lead to mould on the foliar system.

At the same time, the appearance of *Erysiphe pisi* was reported and was present on all shoot system. The most prone to powdery mildew attack were A5 and A15 cultivars while A4, A7 and A13 cultivar were tolerant.

Tolerance means the ability of the plant to avoid crop losses, even if the symptoms of the disease are present. The accessions that showed resistance will be retained and studied in future improvement programs.
The TSS content varies between 8.78°Brix (A7) to 19.56°Brix (A10) (Figure 1).
For a pea variety to be considered a sweet pea, it must have at least 10°Brix. From our research, most all accessions studied had a high content in TSS.
The higher the acidity, the easier it is to control the microbial damage of processed products.

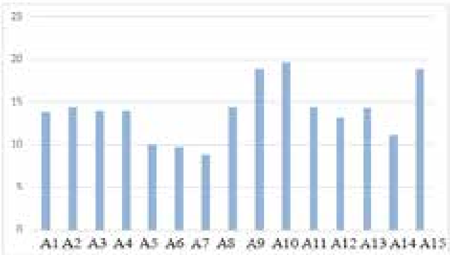

Figure 1. The mean soluble solids content (°Brix) of investigated *P. sativum* accessions

Pea crop is used mainly for industrialization. The percentage of titratable acidity varies between 0.79 g/l (A10) to 1.56 g/l (A9) (Figure 2). These values demonstrate the suitability of using the studied genotypes in the processed industry. The dry matter content varies from 21.18% (A11) to 36.84% (A3). Mostly accessions had the dry matter content exceeding 25% (Figure 3).

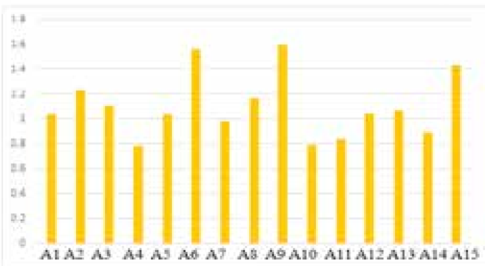

Figure 2. The mean titratable acidity content (g citric acid/100 mg vegetal product) of investigated *P. sativum* accessions

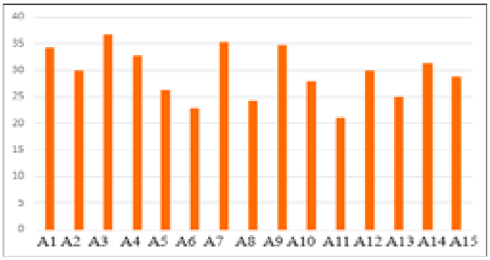

Figure 3. The mean dry matter content of investigated *P. sativum* accessions

The analysis of variance showed significant differences regarding the yield of seeds/plant (Figure 4), more accurate, in the first sowing decade A14 had the highest values (47.26g), while A6 had the smallest record with just 6.25 g/plant.
In the second sowing decade, the accessions behave differently, A4 had the highest values, with 29.19 g/plant, while A15 had 6.37 g/plant. A14 had a lower value with 26.85 g/plant less than in the second decade.
The accessions A7, A10, A13, A1, A3, A11 and A9 had similar values in both decades.
Concerning the yield of pods/plant (Figure 5),
A14 had the higher value in the first decade, with 126.58 g/plant, while A11 had just 16.23 g/plant. In the second decade, A4 had the higher value, 62.94 g, while A15 had just 14.78 g/plant.
A4 registered a decrease with 70.36 g in the second decade, while A14 recorded an increase with 40.65 g/plant in the second decade. Similar values between sowing decades were recorded by A 13, A10, A9, A6, A3, A11 and A1.

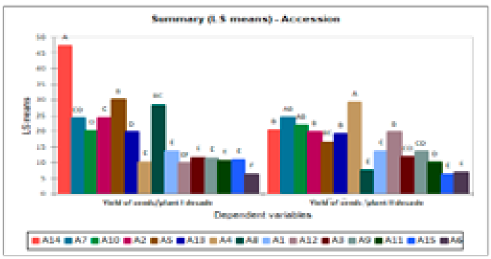

Figure 4. The mean Yield of seeds/plants (g) depending on the sowing decade

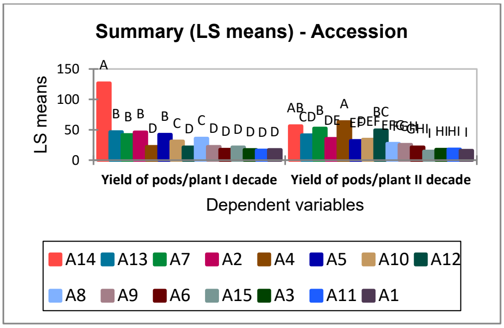

Figure 5. The mean yield of seeds/plants (g) depending on the sowing decade

Regarding the number of pods obtained per plant (Figure 6), it was observed that the number of pods per plant differed depending on the growing season, so it was found that accession A14 had 21 pods in the first epoch and in the second epoch the number was reduced to 7 pods/ plant.

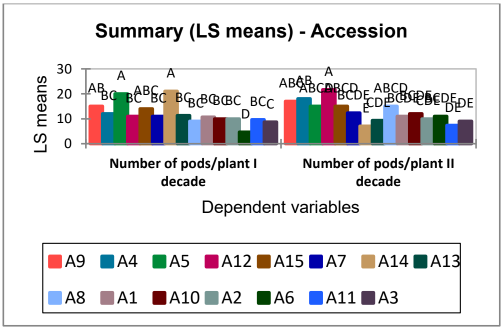

Figure 6. Number of pods/plants depending on the sowing decade

The number of pods per plant on accessions A1 and A2 did not differ according to the time of sowing, while accession A12, in the second epoch registered a higher number of pods per plant (25) compared to the first epoch (11).
These differences can be justified by the fact that each accession behaves differently depending on the growing conditions. Therefore, it is the breeder who decides the right accessions for his goals (Bos 2007).
The accessions A10, A13, A9, A1 and A3 have the highest yield of dry seeds per square meter with over 250 g/m^2, as shown in Table 4.
It should be noted that the same accessions had good results in the previous year regarding the seed production (Gherase et al., 2021). The accessions A4 and A11 had poor results compared to the previous year, as they were affected by excess rainfall. The accession A6 had lower production because it is a type of mange-tout pea, from which the young pods are harvested and consumed, while A8 had the lowest production, being attack more by pathogens. The yield was reduced because in May the accessions bloom, and the fruit setting rate was low.
Stages development of pea pods accession A1 can be found in (Figure 7).

Figure 7. Development stages of pea pods accession A1

Table 4. The Mean values of 1000 seeds mass and weight of dry seeds per square meter

Accessions	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15 (CV)
Mass of 1000 seeds (g)	445.25	423.30	311.15	390.56	276.24	370.52	247.61	435.57	223.41	244.89	212.04	335.68	236.60	439.22	341.32
Difference between control variant - CV (%)	**130.45**	124.02	91.16	114.43	80.93	108.56	72.54	127.61	65.45	71.75	**62.12**	98.34	69.31	128.68	100
Weight g/1 m^2	253.75	217.51	250.96	106.62	129.2	125.94	135.76	102.01	260.03	295.53	143.3	215.71	261.94	109.32	123.77
Difference between control variant - CV (%)	205.02	175.74	202.76	86.14	104.39	101.75	109.69	**82.42**	210.09	**238.77**	115.78	174.28	211.63	88.33	100

CONCLUSIONS

The purpose of our study was to identify the most suitable sowing period. It was noticed that the accessions behave differently. For example, A10 had the highest yield/m^2, with over 295 g/m^2. In terms of yield of pods/plant A14 had the highest values in the first decade and in the second decade the yield registered a noticeable decrease. At the opposite pole, the A4 had the highest pod yield/plant in the second decade.
It was noticed that A1, A3, A10 and A13 behave similar in both decades. We could

conclude that the sowing period did not affect the growth stage.

Some accessions were selected for desirable agronomic characteristics and will be useful in the breeding program to obtain new genotypes adapted to the pedo-climatic conditions of Romania.

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ANATOMICAL AND BIOCHEMICAL RESEARCH ON THE SPECIES *OCIMUM BASILICUM* L. (LAMIACEAE) CULTIVATED IN THE NUTRIENT FILM TECHNIQUE SYSTEM

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Abstract:

The article presents aspects regarding the anatomy and composition of volatile oil in the species Ocimum basilicum L. *(Lamiaceae), cultivar 'Aromat de Buzău'. Anatomical observations were made on cross-sections in the stems and lamina of the leaf. The epidermis of the leaf is unilayered, with the outer walls covered by a thin cuticle. Glandular and nonglandular hairs are present on both epidermis, especially on the lower epidermis. There are short glandular hairs, capitate or peltate and the non-glandular ones are unicellular or multicellular, sharp or with a curved tip. The stomata are present in both epidermis but more numerous in the lower epidermis, being of the diacytic type. The leaf mesophylle is bifacial with palisade tissue under the upper epidermis consisting of a single row of elongated cells, rich in chloroplasts and spongy tissue, below the lower epidermis, consisting of 3-4 rows of ovoid cells and with intercellular spaces. The oil has been extracted during the flowering period, by hydro-distillation and analyzed by gas chromatography – mass spectrometry (GC-MS). The main chemical compounds present in the volatile oil were: methyl chavicol (63.56 %), linalool (17.10 %), 1,8-cineole (4.01%) and α-epi-cadinol (2.64 %).*

Key words: *Ocimum, anatomy, secretory hairs, volatile oil, linalool.*

INTRODUCTION

The species *Ocimum basilicum* belongs to the Lamiaceae family, it is a plant with strong aromatic and medicinal properties being used for pharmaceutical, food and cosmetic purposes. (Nurzyńska-Wierdak et al., 2012). Due to its unique aroma, it is a plant often used in the fragrance industry, since ancient times. The interest in cultivating herbs and spices has increased recently, including for the species *Ocimum basilicum.* A number of new cultivars were introduced and gradually expanded into culture, differing in terms of morphology (colour, leaf shape, height, so on), production, and chemical composition.
All these features give the plant uses in various industries. Basil leaves and young shoots can be used in flavouring wines as well as for making teas and infusions (Vînătoru et al., 2019). The *Ocimum basilicum* 'Aromat de Buzău' is used as an aromatic, food, medicinal, ornamental, and melliferous plant. The flavours identified in this cultivar are varied depending on the presence of chemical compounds, such as: lemon flavour given by citral, anise and clove flavour provided by eugenol, camphor flavour imprinted by the high content of camphor and camphene, anise flavour given by the substance called anethole (Jailawi et al., 2021).
The production of volatile oil is carried out by secretory structures, in the species of the Lamiaceae family being frequently found pelted and capitate secretory hairs (Fahn, 1988; Berciu (Boz), 2009). The predominant chemical compounds in the volatile oil extracted from *Ocimum basilicum* plants were: methyl chavicol (estragole), 1,8-cineole, methyl eugenol, linalool (Elsherbiny et al., 2016), linalool, eugenol, methyl eugenol (Akgül, 1989), 1,8-cineole, linalool, geraniol, α-trans-bergamotene, epi-α-cadinol, methyl chavicol, nerol (Sajjadi, 2006), linalool, 1, 8-

cineole, eugenol, α-cubebene, methyl cinnamate, caryophyllene, β-ocimene, and α-farnesene (El-Soud et al., 2015), 1,8-cineole, linalool, camphor, α-terpineol, methyl chavicol and eugenol (Barcelos et al., 2013).

The composition of volatile oil can be influenced by environmental conditions, genetics, different chemotypes (Özcan & Chalchat, 2002; Nassar et al., 2014) as well as the application of treatments and fertilizers to plants.

The results obtained by Burducea et al. (2018) have shown that conventional fertilizers can increase fresh yield, while organic fertilizers positively change the composition of volatile oil, leading to increased crop quality.

Growing basil plants in the greenhouse with hydroponic system allows high yields and commercial quality both for the fresh market and for the essential oils industry (Guerrero-Lagunes et al., 2020). Previous studies have shown that the volatile oil of the *Ocimum basilicum* species is rich in substances that possess antibacterial, anti-insecticidal and antifungal activities.

The purpose of this study is to analyze anatomically and biochemically the basil plants grown in the hydroponic system.

MATERIALS AND METHODS

Material

The biological material used was represented by plants of *Ocimum basilicum* cultivar 'Aromat de Buzău', grown in hydroponic system in the greenhouse within the Research Center for Studies of the Food Quality and Agricultural Products, U.S.A.M.V. Bucharest. The analyses were performed during the flowering period, in May, 2021.

Anatomical and micromorphological characterization

Anatomical and micromorphological analyses were performed on the stems, leaves and petals of the *Ocimum basilicum* 'Aromat de Buzău' (Figures 1-3). In the stems, cross sections made at at the level of the internodes were made, and in the leaf in the middle part of the lamina of the leaf.

The sections were clarified with chloral hydrate and stained with carmine-alum and iodine green, according to the classical method (Morlova et al., 1966).

Observations and photographs were taken with the LEICA DM 1000 LED optical microscope, LEICA DFC 295 video camera and LEICA S 8 APO stereomicroscope, using 10x lenses, and micromorphological observations using by the Inspect S50 scanning electron microscope (SEM), existing in the endowment of the Research Center for Studies of Food Quality and Agricultural Products.

Volatile oil analysis

The extraction of volatile oil was made from the whole plant (stems, leaves, flowers).

The fresh harvested plants (300 g) were hydro-distilled for 3 h in a Clevenger-type apparatus. Analysis of the essential oils was performed on an Agilent 6890 GC coupled with a 5973 Network single quadruple mass spectrophoto-meter detector in Electron Ionization (EI) mode and 7673 injector on a HP-5MS capillary column (30 m × 0.25 mm id, 0.25 µm film thicknesses). The following column oven operating conditions were employed: 50°C for 8 min, then a 4°C/min ramp to 280°C. Helium was used as carrier gas with a constant flow of 1.0 mL/min, injection volume 3 µL with a split ratio 50: 1. The temperatures for inlet, MS transfer line and ion source was 250°C, 250°C and 230°C, respectively. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 50-550 amu at 2 scan/s (Ion et al., 2020).

RESULTS AND DISCUSSIONS

Stem anatomy

The anatomy of the stem is similarly to the species in the *Lamiaceae* family (Metcalfe & Chalk, 1983; Toma & Rugină, 1985; Garner & Catherine, 2017). The outline of the cross section is rectangular, with four rounded ribs. In the cross section, the epidermis, the cortex and the central cylinder can be seen (Figure 1a). The epidermis consists of a single row of cells, with the outer walls covered by a thin cuticle. In the epidermis, from place-to-place stomata and uniseriate nongladular hairs (with 3-5 cells) curved, very rarely capitate glandular hairs, with one or two cells were observed (Figure 1b).

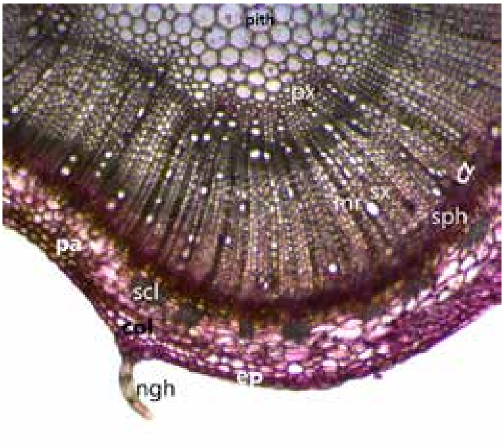

Figure 1a. Transversal section of the *Ocimum basilicum* 'Aromat de Buzău' stem: ep – epidermis; col – collenchyma; pa – parenchyma; scl – sclerenchyma; sph – secondary phloem; sx – secondary xylem; cz - cambium zone; mr – medullar race, ob. 10 x

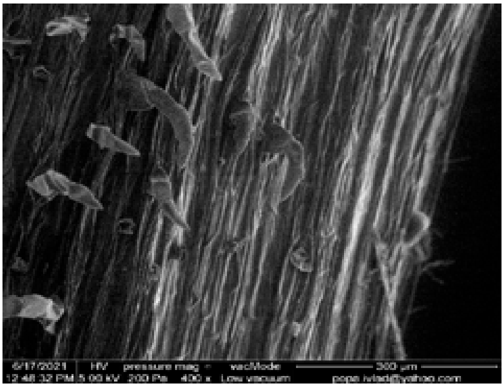

Figure 1b. Scanning electron microscopy (SEM) of the *Ocimum basilicum* 'Aromat de Buzău' stem,400 x

The bark consists of cords of angular colenchyma in the 4 ribs (3-4 rows of cells), and in the rest bands of parenchyma (chlorenchyma) 3-4 rows of cells. Along the ribs, at the level of the parenchymal tissue, there are also large air cavities. The last layer of the bark is primary type endoderm. In the central cylinder, the conducting bundles are collateral, of secondary origin, forming two concentric rings. On the outside is the thin secondary phloem ring, consisting of sieved vessels, attachment cells and cellulosic parenchyma. The phloem has thin segments of sclerenchymal fibers at the periphery. Inside there is the thick secondary xylem ring, made up of wooden vessels, wooden parenchyma and wood fibers. The xylem vessels are separated by sclerified medullary rays. The conducting bundles are larger at the corners. The wood of primary origin is reduced, and the wooden vessels are separated by parenchymal medullary rays. The marrow is parenchymal, cellulose in nature, multi-layered, 10-14 rows of cells.

Leaf anatomy

The epidermis of the leaf seen from the front is made up of cells with strongly wavy walls.

The stomata are present in both epidermis, but more numerous in the lower epidermis, the leaf being amphistomatic with diacytic stomata (Figure 2a, 2b).

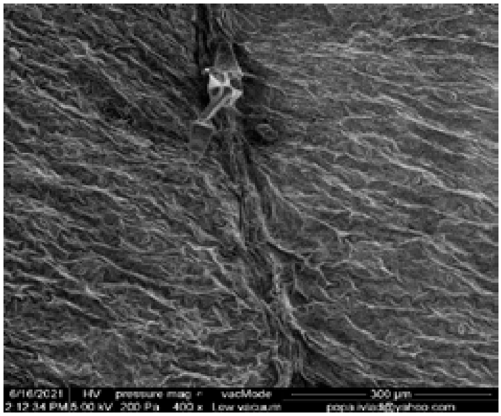

Figure 2a. Scanning electron microscopy (SEM) image of the upper leaf epidermis of the *Ocimum basilicum* 'Aromat de Buzău' 400x

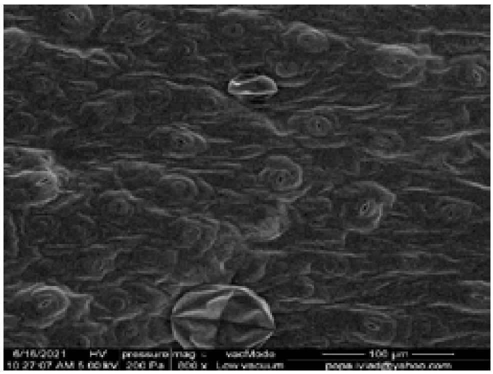

Figure 2b. Scanning electron microscopy (SEM) image of the lower leaf epidermis of the *Ocimum basilicum* 'Aromat de Buzău', 800 x

In some of the published articles it was noted that the stomatas are anomocytic type on the leaves of basil (Nassar et al., 2014). The median rib is strongly prominent on the lower face, with a hypodermic colenchyma and a free woody conducting bundle, located centrally, with xylem on the outside and phloem on the inside. On the adaxial side, numerous bent uniseriate non-glandular hairs are present, consisting of 3-5 cells, rarely hairs capitate or peltate secretory. In cross-section, both epidermis are unistratified, consisting of tabular cells tightly joined together, with the outer walls covered by a thin cuticle. Some regions of the upper and lower epidermis are made up of papillary cells, consisting of much larger oval cells.
In both epidermis there are nongladular and glandular hairs, peltate or capitate and scaly specific to the basil leaf (Zamfirache et al., 2008; Trettel et al., 2019; Poursaeid et al., 2020).

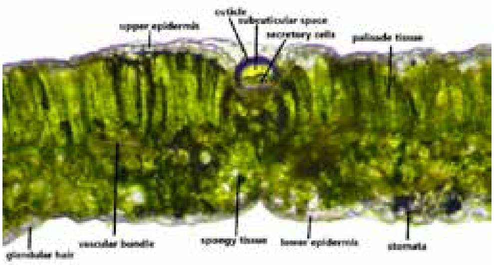

Figure 2c. Transversal section in the lamina leaf of the *Ocimum basilicum* de 'Aromat Buzău', ob. 10 x

The capitate hairs are composed of a base cell, a short stem cell, and a terminal cell consisting of either an elongated cell or two broad cells, seen in both epidermis. The peltate hairs consist of four terminal cells, seen in the lower epidermis. The scaly hairs were observed in the upper epidermis, being made up of a basal cell, on which secretory cells arranged in the rosette are present. The leaf mesophyll is bifacial, with palisade tissue under the upper epidermis, consisting of a single row of elongated cells, rich in chloroplasts and spongy tissue, below the lower epidermis, consisting of 3-4 rows of ovoid cells and with intercellular spaces.

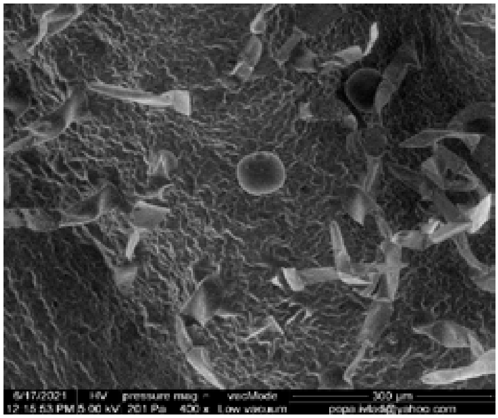

Figure 3. Scanning electron microscopy (SEM) image of the petal flower of the *Ocimum basilicum* 'Aromat de Buzău', 400 x

The composition of the volatile oil
Ocimum basilicum species has numerous local cultivars and populations, which have a very varied chemical composition of volatile oil (Burzo, 2015). Characteristic of the volatile oil are as follows: high content of methyl chavicol (63.56%), linalool (17.10%), followed smaller amounts of 1,8-cineole (4.01%) and α-epi cadinol (2.64%) were found, as showed in Figure 4.

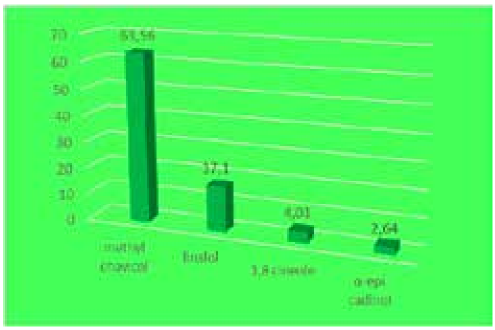

Figure 4. Major chemical compounds in the *Ocimum basilicum* 'Aromat de Buzău'

In addition, some compounds were detected in quantities of 1.06 % to 1.73% such as: δ-guaien (1.06%), β-ocimene (1.27%) and germacrene D (1.73%). Other constituents (the remainder which comprised 48 components) had a value below 1% (from 0.02% α-phellandrene to 0.95% bicyclogermacrene).

Other studies found that the chemical compound methyl chavicol (estragole) was also noted in the volatile oil of the species *Ocimum ciliatum* having the highest share (87.6%) followed by methyl eugenol (2.6%) and 1,8 - cineole (1.7%). Studies conducted by Moghaddam et al. (2014) showed that the oil extracted from this species had antimicrobial activity on all bacteria tested. The chemical compound linalool is a high weight monoterpenoid, commonly found in the composition of *Ocimum basilicum* volatile oil.

It has a role as a plant metabolite, a antimicrobial agent and, a fragrance (source:https://pubchem.ncbi.nlm.nih.gov/compound/Linalool).

The research conducted by Jailawi (2021) shows that the main chemical compounds present in the volatile oil extracted from the 'Aromat de Buzău' grown in hydroponic system during the flowering period were: linalool 27.52%, methyleugenol 17.48%, 1,8-cineole 6.69%, α-epi-cadinol 5.78% and α-bergamotene 4.69%. From the analyzes performed by Al Abbasy et al. (2015) it is showed that linalool has highest amount (69.87%) compared to other bioactive compounds considered major such as: geraniol (9.75%), p-allylanisole (6.02%), 1,8-cineole (4.90%), trans-α-bergamotene (2.36%) and neryl acetate (1.24%). Also, the chemical compound, linalool, predominated in the volatile oil analyzed by Guerrero-Lagunes et al. (2020) in plants grown in a greenhouse in a hydroponic system. *Ocimum basilicum* is characterized by a high linalool content, which makes it useful in the food, pharmaceutical and perfumery industries (Al Abbasy et al., 2015).

The species *Ocimum basilicum* is characterized by a high linalool content, which makes it useful in the food, pharmaceutical and perfumery industries (Al Abbasy et al., 2015).

The category of the main chemical compounds present in the volatile oil of *Ocimum basilicum* also includes 1.8 cineole (eucalyptol).

Following the studies performed by Omer et al., (2008) of three species of basil and four varieties grown in saline soil, it was showed that the main chemical compounds were: linalool (19.93%-40.41%), 1,8 - cineole (19.1%-10.52%), methyl chavicol (28.92%-46.48%), eugenol (4.08 %), γ-elemene (4.18%-3.22%) α-trans-bergamotene (2.52%- 4.57%), germacrene D (4.04%), t-cadinol (4.17%-10.9%). Eucalyptol is a terpenoid oxide with anti-inflammatory and antioxidant effects in various conditions such as pancreatitis, respiratory, cardiovascular and neurodegenerative diseases (Seol & Kim, 2016). The results obtained are in accordance with those of the literature.

CONCLUSIONS

The species *Ocimum basilicum* 'Aromat de Buzău' showed secretory hairs on the epidermis.

Curved uniseriate nonglandular hairs, are present in the epidermis of the basil stem.

The leaf blade is amphistomatic, with diacytic type stomata.

The nongladular and glandular hairs, peltate or capitate and scaly are present in both epidermis of the leaf.

The mesophile of the lamina is bifacial, with palisade tissue under the upper epidermis, consisting of a single row of elongated cells and spongy tissue, below the lower epidermis, consisting of 3-4 rows of ovoid cells and with intercellular spaces.

Regarding the composition of the volatile oil, the study shows that the *Ocimum basilicum* plants, the 'Aromat de Buzău', is an important source of chemical compounds that can be used in different industries.

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COMPARATIVE RESEARCH ON NEW TOMATO HYBRIDS FOR SPRING CULTURE IN SOLARIUM

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Abstract

The research was made with a tomato spring culture, in solarium, in 2016 and 2017, in Fierbintii de Jos village, Ialomita county, on an area of 2000 m^2. Four new tomato hybrids were used respectively Zadurella F1, Matissimo F1, Yigido F1 and SV 4224 TH F1. The tips of the main stems were removed at four inflorescences and the layout of the experiment was made in randomized blocks with three repetitions. The tomatoes were cultivated on a soil with neutral pH, with an optimal content of ammonium nitrate and average content of nitric nitrogen, phosphorus, organic matter, calcium, sodium and soluble salts, with very low content of potassium and very high of magnesium. The main fertilization was made through applying 30t/ha of organic fertilizers while preparing the soil. During the vegetation period the fertilization was phasial with products from the Tecamin brand, appropriate for each phenophase in part. The results obtained proved that all hybrids used were highly productive, the commercial production per each plant being between 2.48 kg and 3.62 kg. The commercial production per m2 varied between 9.42 kg and 13.76 kg. The average fruit weight was between 167 and 214 g. All hybrids produced large, firm fruits, with a content of dry soluble substance of roughly 5%, the titratable acidity between 0.22% for Yigido F1 and 0.43 % for Zadurella F1. The fruits had a C vitamin content between 14.42 mg/100 g at Yigido F1 and 23.00 mg/100 g at Zadurella F1.

Key words: *biochemical composition, vegetative growth, protected culture, production.*

INTRODUCTION

Tomatoes (*Solanum lycopersicon* L.) pertain to the *Solanaceae* family and are perennial plants in their original cultivation area, however in some regions they are cultivated as annual plants (Kalloo, 1993). They are cultivated all over the world on a surface of over 4.7 mil ha with a production of over 177 mil tones (FAO, 2016). Tomatoes have a very high capacity of adapting to various culture systems. It is the species with the highest expansion form all vegetable species because they are highly productive, the production can be echeloned throughout the whole year and is capitalized in various ways (Hoza et Hoza, 2013). Moreover the fruits are very rich in vitamins, mineral salts, lycopene and other elements highly beneficial for human health (Coyago-Cruz et al., 2017; Dinu et al., 2017). Currently this species is very much studied by researchers around the world in order to improve the culture technology. Although it is cultivated in culture systems, new solutions are researched to increase plant productiveness. The focus is to ameliorate the culture assortment by using hybrids with undetermined growth, especially for protected cultures or the ones conducted in greenhouses (Benalfew et al., 2016; Soarea et al., 2015). Grafting tomatoes on other botanically related species increases the resistance to various stressors, increases its production and quality (Bogoescu et al., 2008, Huang et al., 2015). The use of complex fertilizers with a very high degree of solubility and absorption by plants increases the yield of the crop (Apahidean et al., 2002; Sima et al., 2007; Becherescu et al., 2015). The management of tomato plants with several stems reduces the number of plants per sqm, and increases production (Hoza 2013) and cultivation on different crop substrates gives the possibility of monoculture (Apahidean et al., 2008). Due to the existence of a high number of tomato hybrids with superior features the necessity emerged to study the response of some hybrids in specific conditions of each country or each grower in order to recommend an optimal assortment structure for each hybrid destination The present paper aimed to investigate the above mentioned matter. Undoubtedly, more researches will be conducted in Romania in order to understand the hybrid responses and to choose the best ones for culture.

MATERIALS AND METHODS

The seedlings were transplanted in pots of 200 ml when the first pair of real leaves emerged, the substratum used was the same as the one for seeding. During the process of seedling production the following works have been conducted: airing the space in order to regulate temperature, humidity and oxygen, repeated irrigations to maintain a moist soil as to avoid hydric stress, three phasial fertilizations with Tecamin Raiz, one before transplanting with concentration of 0.2% and two after transplanting with concentration of 0.3% and phytosanitary treatments with Previcur Energy 0.1% and Dithane M45 0.2%. Ten days before planting the temperature dropped gradually to $12C^0$-$15C^0$ in order to harden plants and increase their resistance to the lower temperatures of the planting area.

The plantation of seedlings was conducted manually in the first decade of March, at 90cm/40cm/40 cm, resulting a culture density of 38461 plants/ha.

The culture was set up on a soil with neutral pH, an optimum content of ammonium nitrate and average content of nitric nitrogen, phosphorus, organic matter, calcium, sodium and soluble salts. The soil had a very low content of potassium and a very high one of magnesium. In order to ensure a balanced nutrition the organic fertilization, 30t/ha, was applied when preparing the soil and afterwards the phasial fertilization was applied during the vegetation period, for each phase in part. In culture the actions taken were the following: the airing of the space to regulate temperature was made gradually though the opening of the doors and lateral walls, the irrigation was conducted daily trough the drip system to maintain the moisture of the soil, plants were supported on strings, totally pruned, the removal of the main stem tip was made after the 4th inflorescence, phytosanitary treatments were done alternatively with Bravo 500 SC 0.4 %; Switch 0.1 % and Topsin 70 WDG 0.1 % and foliar fertilizations were managed as per the fertilization planning.

The foliar fertilization plan matched the vegetation phenophase and the products used were from the Tecamin brand as it follows: 2 weeks after planting in order to rebuilt the radicular system Tecamin Max 0.2 % and Tecknokel Amino mix 0.2 % were applied; During the vegetative growth was used Tecamin Max 0.2 %; before efflorescing Tecnokel Amino Mix 0.2%; fruit formation from first inflorescence Tecamin Max 0.2%; fruit growth Tecamin Brix 0.2% + Tecnokel CaB 0.2%; beginning of harvest Tecnokel CaB 0.2%; until culture end Tecnokel Amino Mix 0.2%, Tecamin Brix 0.2% and Tecnokel CaB 0.2%.

Measurements were made to note plant growth and the number of flowers and fruits in inflorescences, the fruit formation percentage, the production per plant, production and m^2 and biochemical tests for fruits. It was quantified: the content of C vitamin though titration with potassium iodate, the titratable acidity through titration with sodium hydroxide with phenophthalein as indicator, the firmness of fruits using the penetrometer and the dry soluble substance using the refractometer.

The statistical calculation was done through the variant analysis.

RESULTS AND DISCUSSIONS

Analysing the results obtained from the point of view of the vegetative growth of studied hybrids it was noticed that the hybrid had a slight impact on the growth process (Table 1). Thus, plant height up to the forth inflorescence – after which the tip of the main stem was removed – was under 1 m at Zadurella F1 and it was also correlated with the smaller distance between inflorescences. Hybrids Yigido F1 and SV 4224 TH F1 were more vigorous, the plant height being of 124 cm and the average distance between inflorescences of 31.0 cm.

Table 1. Biometrical features of hybrid tomato

Variant	Plants height (cm)	Average distance between:				Average (cm)
		Soil and first inflorescence (cm)	Inflorescence (cm)			
			1-2	2-3	3-4	
V1(Mt) –Zadurella F1	95.1	34.1	19.5	21.4	20.0	23.8
V2-Matissimo F1	110.6***	37.4	28.6	23.0	21.6	27.7***
V3-Yigido F1	124.2***	41.0	33.1	26.8	23.1	31.0***
V4-SV 4224 TH F1	124.6***	40.4	32.0	25.1	27.0	31.1***
Average	113.63					28.32
LSD 5%	0.43					0.71
LSD 1%	0.66					1.07
LSD 0.1%	1.05					1.71

The capacity of fructification of studied hybrids was quite uniform due to the genetic characteristics of the hybrids and the culture technology applied. The similarities between hybrids was highlighted by the number of flowers per plant which varied between 26.6 at Matissimo F1 and 27.9 la Zadurella F1, statistically assured, with negative values, which reached numbers lower that the control hybrid. The number of fruits formed was very significantly positive at Yigido and SV 4224 TH and insignificant at the hybrid Matissimo, the values being between 17.5 at Zadurella F1 and 20.3 la Yigido F1. Hybrids shown differences at fruit formation per plant. The top value was obtained by Yigido F1 (75.2 %) and the lowest by Zadurella F1 (62.7 %). The other hybrids had mediate values and the differences between hybrids in comparison to the control were highly significantly positive (Table 2).

Table 2. Number of flowers, fruits and fruit formation percentage for the hybrid tomato plants studied

Variant	Number of flowers/plant	Number of fruits/plant	% of fruit formation/ plant
V1-Zadurella (Mt)	27.9	17.5	62.7
V2-Matissimo	26.6^{ooo}	18.0 n	67.7***
V3-Yigido	27.0^{ooo}	20.3***	75.2***
V4-SV 4224 TH	27.5^{ooo}	19.3***	70.2***
Average	27.23	18.78	68.95
LSD 5%	0.25	0.68	0.47
LSD 1%	0.39	1.03	0.71
LSD 0.1%	0.62	1.64	1.14

The average weight of fruits was registered with differences between hybrids (Table 3). The smallest fruits were obtained at Zadurella F1 which also presented a lower vigour of plants in comparison to the other hybrids, respectively 167 g, while Matissimo F1 had the biggest fruits, respectively 214 g. By calculating the total production per plant it was observed that the highest production was obtained by Yigido F1, 4.02 kg/pl, followed by Matissimo F1 with 3.85 kg/pl. The control had 2.92 kg/pl. From the total production of fruits per plant roughly 10-15% from all fruits had quality parameters under the standard ones agreed for tomatoes. The commercial production was over 3 kg/pl for all hybrids except for Zadurella, which produced 2.48 kg/pl. The total production of tomatoes per m^2 showed the same tendency as the production per plant and it had values between 13.34 kg/m^2 and 14.63 kg/$m^{2,}$ in comparison to the control which had 11.01 kg/m^2. The commercial production was over 13 kg/m^2 at hybrids Yigido and Matissimo, 11.59 kg/m^2 at hybrid SV4224 TH and 9.42 kg/m^2 at hybrid Zadurella, all these results being assured statistically.

Table 3. Production of tomatoes

Variant	Average weight of fruit (g)	Total production		Commercial production	
		kg/pl	kg/m^2	kg/pl	kg/m^2
V1-Zadurella (Mt)	167	2.92	11.01	2.48	9.42
V2-Matissimo	214***	3.85***	14.63	3.47***	13.19
V3-Yigido	194***	4.02***	15.28	3.62***	13.76
V4-SV 4224 TH	182***	3.51***	13.34	3.05***	11.59
Average	189.25	3.57		3.15	
LSD 5%	4.93	0.12		0.13	
LSD 1%	7.46	0.19		0.19	
LSD 0.1%	11.88	0.30		0.31	

Taking into consideration the biochemical composition of fruits differences were registered between hybrids (Table 4). Thus, for hybrids with a higher production the content of C vitamin was lower, 14.42 mg/100 g at Yigido F1 and 17.83 mg/100 g at Matissimo F1. For hybrids with a lower production the content of C vitamin was higher 23.00 mg/100 g at Zadurella F1 and 20.11 mg/100 g at SV 424 TH F1. The results obtained were similar to the ones from professional literature (Chattopadhyay et al., 2013; Dinu et al., 2017).

Titratable acidity was quite different between hybrids and had values of 0.22 % at Yigido F1 and 0.43 % at Zadurella F1 matching the research of Caliman (Bogoescu et al., 2008). All hybrids presented a good firmness, however Yigido F1 had the lowest value. The total content of soluble dry substance did not have very different values between hybrids, all values were insignificant proving all hybrids are from the same category from the consumption point of view as shown by the professional literature as well (Kalloo, 1993).

Table 4. Some quality features of tomato fruits

Variant	Vitamin C (mg/100 g)	Acidity (%)	Pulp firmness (kg/cm²)	Dry soluble substance (%)
V1-Zadurella (Mt)	23.00	0.43	1.15	5.08
V2 – Matissimo	17.83^{ooo}	0.35	1.06	5.00 N
V3 – Yigido	14.42^{ooo}	0.22	0.98	5.20 N
V4 – SV 4224 TH	20.11^{ooo}	0.30	1.13	4.98 N
Average	18.84	0.32	1.08	5.06
LSD 5%	0.88			0.15
LSD 1%	1.33			0.23
LSD 0,1%	2.13			0.36

CONCLUSIONS

For the research conducted on some hybrid tomato in spring culture the following conclusions can be withdrawn:

The studied hybrids had a very good vegetative growth and manifested a high tolerance at diseases and pests. The total number of flowers per plant was uniform between hybrids, reaching values of 26.6 at Matissimo F1 and 27.9 la Zadurella F1. The number of fruits formed was smaller than the number of flowers and was influenced by the hybrid used, reaching values between 17.5 at Zadurella F1 and 20.3 la Yigido F1. The commercial production exceeded 3 kg/pl at 3 hybrids; only Zadurella had 2.48 kg/pl. The commercial production per m^2 had a similar tendency with the total one, highlighting Yigido F1 as the best hybrid with 13.76 kg/m^2. The control hybrid Zadurella F1 produced 9.42 kg/m^2. The content of C vitamin was in inverse proportion with the production obtained, higher for hybrids with smaller production and lower for hybrids with high production. Fruits had different acidity from one hybrid to the other which could contribute to the diversification of the tomato assortment and to satisfying all consumer taste. The firmness of fruits was registered between 0.98 kg/cm^2 at Yigido F1 and 1.15 kg/cm^2 at Zadurella F1.

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THE IMPACT OF FOLIAR FERTILIZERS ON THE PRODUCTION OF FIELD-GROWN ZUCCHINI

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Abstract

In Romania, zucchini crop is extending more and more as it is one of the vegetable ~~cultures~~ preferred especially by the population around larger cities, where a higher variety of produces is necessary. It is a vegetable species rich in minerals and vitamins, being prepared in different forms. For these reasons, there are concerns regarding the improvement of crop technology through the use of high quality seedling material, of foliar fertilizers with high impact on the production capacity of plants and environmentally friendly, this being also the purpose of the present research. For the research, the following were used: three hybrids F1, Eskenderany, Opal and Ismalia, and three organic fertilizers, Cropmax 0.1%, Aur verde Extra 0.1% and Bombardier 0.15%. After data processing and results interpretation, it was observed that all the hybrids that had been fertilized with Aur verde Extra 0.1% produced the best results in what concerns the number of flowers per plant. The fruit production per plant and the fruit production per square meter were higher for the F1 hybrids Eskenderany and Ismalia fertilized with Aur verde Extra 0.1%, while for Opal F1 the highest production per plant and per m^2 was obtained after applying Cropmax 0.1%. The highest increase of the production per plant was obtained for all hybrids fertilized with Cropmax 0.1%, while the highest profit per m^2 was obtained for Eskenderany F1 and Ismalia F1 fertilized with Aur verde Extra 0.1% and for Opal F1 fertilized with Cropmax 0.1%.

Key words: *foliar fertilizers, hybrids, yield, zucchini.*

INTRODUCTION

Zucchini *(Cucurbita pepo,* var. *oblonga*) is part of the *Cucurbitaceae* family and it originates from South and Central America (Peru, Mexico), Africa, where it was brought from during ancient times also to Europe (Ciofu et al., 2004), being amongst the oldest known plants for human diet. In Romania, zucchini is grown throughout the country, but in the neighborhood of larger cities it is cultivated more. This vegetable is appreciated by the consumers for its nutritional properties, important for the organism and the seeds' anthelmintic effect. Several varieties and hybrids are cultivated, and for identifying them DNA measurements are made, using the SSR method (Verdone et al., 2018). Vegetables from the Cucurbitacee family have a positive influence on human health, and various studies have clearly indicated that these vegetables have antioxidant, antidiabetic, anti-inflammatory and purgative properties (Becherescu et al., 2010; Becherescu et al., 2012; Mittler, 2002; Rolnik and Olas, 2020). After harvest, zucchini must be consumed as soon as possible, within 1-2 days when it is harvested with flower, and within 3-4 days if it is larger in size. It can be cultivated in field or in protected spaces. There are concerns regarding organic crops as it is a species of international interest. Research conducted on loamy soil, in ecological system, showed a higher accumulation of K in the fruit compared to other crops on sandy soil (Maggio et al., 2013), while using green fertilizers prior to the crop leads to good yields (Ciacciua et al., 2015). Using green fertilizers based on *Crotalaria juncea* L. *(Fabaceae)* to the prior crop ensures the quantity of N necessary for the following zucchini culture (De Oliveira et al., 2017). Using compost in the organic crop ensured a good tolerance of the zucchini plants against *Phytophtora* sp. through its favorable influence on the rhizosphere (Cucu et al., 2020). Using organic mulch ensure a better plant tolerance against diseases and pests, helping reduce the number of protective treatments (Robacer et al., 2016). Reduced soil works and using ameliorative plants (*Trifolium* sp. *and Lolium* sp.) within bio culture determined a net superior fruit quality, expressed through their antioxidant activity and phenol content, compared to the classical culture technology with deep works (Bucki et al., 2021). Using compost based on rice and sugarcane waste determined an increase in soil fertility, expressed in the C/N ratio, and also a good plant growth (Farid et al., 2022). The comparative study of several varieties in bio and classical systems showed that for the bio crop, the content in active elements was higher than for the classical culture. The content in essential amino acids was higher by 1.3%, K by 9%, Mg by 67%, Na by 29%, tocopherol 4 times higher etc. Also, differences were recorded amongst the varieties within the same crop system, which indicates that the system and variety are responsible for the quality of the edible part (Armesto et al., 2020).

Zucchini is a species tolerant to the quality of irrigation water. Using water with NaCl concentration of up to 80 mM (Villor et al., 2000), respectively with content of Ca up to 3mM (Neocleous et al., 2018) did not affect the production or its quality. Research conducted by Savvas et al. (2009) showed that zucchini tolerates saline water for irrigation if 1 mM Si is added, and water salinity favorably influenced zucchini plants' tolerance to mildew.

The comparative study of applying irrigation water through dripping and underground showed higher efficiency of the dripping irrigation regarding production, but in what concerns the quality underground irrigation proved more effective (Rouphael et al., 2006). Concerning the idea to efficiently user irrigation water within greenhouse, several watering moments were tested, and irrigating at a water deficit with pressure of -25 kPa and applying 2 l/m^2 water ensured the best result, a production of 15 kg/m^2 (Contreras et al., 2017). Zucchini crop in hydroponics with nutritional solutions of EC 2.2 dS/m^{-1} and 4.4 dS/m^{-1} concentrations showed a good plant behavior. For the 4.4 dS/m^{-1} concentration it was recorded a slight increase in the number of male flowers, but the content in dry substance was higher compared to the 2.2 dS/m^{-1} concentration (Liopa-Tsakalidi et al., 2015).

For classical crop in the field, correct application of pesticides does not determine the accumulation of certain residues above the accepted limits (Oliva et al., 2017). A lot of fields are infested with nematodes. Research regarding nematodes tolerance for the zucchini plants showed that varieties and hybrids manifest a different tolerance, genetically controlled, but in order for the culture to succeed, the nematodes population must be kept under control, either through chemical treatments, either through prior crops with repellent effect (Soledad et al., 2019).

Vegetable waste resulted after clearing the zucchini crop represent a source for obtaining biogas, while research showed a good yield, either directly, either through treatments with different substances that accelerate anaerobic fermentation (Gu et al., 2020; Gu et al., 2021).

In order to stimulate the growth capacity of zucchini plants, stem cutting and stimulating the rooting of the shoots from the nods that are in contact with the soil were tried, but these interventions did not determine the growth of biomass compared to the control (Low et al., 2011).

MATERIALS AND METHODS

Research was carried out within the Vidra village, Ilfov county, in 2020. The area is favorable for vegetable crop being close to Bucharest, which represents the main sales market. The experiment was organized with two variable factors, one the hybrid and the other the foliar fertilizer used during the vegetation period. The main objective was to evaluate the influence of hybrids and fertilizing products on the growth and fructification of zucchini plants in the climatic conditions of the Vidra vegetable area. The biological material used was represented by 3 cultivars as it follows:

Eskenderany F1, early hybrid (40-50 days), has light-green colored fruit and is productive. Plants are vigorous, semi-right, compact and resistant to lower temperatures, being recommended for early crop. Fruit is uniform, cylindric, with thin, bright epidermis, with whitish small spots.

Opal F1, early hybrid (55 days), is productive, has a high capacity to adapt for different environmental conditions. Plants are semi-compact, which allows an easier harvest. Fruit is cream colored and shaped as elongated cylinder.

Ismalia F1**,** is an early hybrid, cold tolerant, recommended for open field and within protected crop. Plants are vigorous, compact. Fruit is whitish with a slight greenish tinge, cylindric and uniform. It is resistant to disease, transport and mechanical damages.

Three stimulation and foliar fertilization products were used, applied at a 10-day interval. Cropmax, the organic stimulator for plant metabolism, which contains amino acids, microelements, polysaccharides, vitamins and ensures a very good growth for roots and foliar mass, increases the plants' capacity to easily adapt to stressful conditions.

Aur verde Extra, complex foliar fertilizer with NPK and microelements (120 g/l N, 80 g/l P205, 105 g/l K20, 0.3 g/l MgO, 0.6 g/l B, 0.2 g/l Cu, 0.5 g/l Fe, 0.3 g/l Mn, 0.2 g/l Zn), is applied to vegetables, decorative plants, fruit trees and vines, at a 7-14 day interval. It has effects on the fast and vigorous plant growth, increases productive yield.

Bombardier, biostimulator and fertilizer resulted from the controlled bacterial fermentation of several plants, contains micronutrients, organic matter, vitamins, coenzymes, proteins, auxins and antioxidants necessary for plants (free amino acids 16.51%, total nitrogen 10.60%, P 0.64%, K 0.25%, polysaccharides 7.87%, total humic extract 29.34, total organic matter 76.70%, total organic carbon 38.10%). It has effects on plant growth and development, adaptation to stress and on the increase in fruit quality.

From combining the two factors, 12 variants resulted, each variant organized 3 repetitions and 5 plants per repetition, as it follows:

V1 - Eskenderany F1+Cropmax 0.1%,
V2 - Eskenderany F1+Aur verde extra 0.1%,
V3 - Eskenderany F1+Bombardier 0.15%,
V4 - Eskenderani F1 nonfertilized,
V5 - Opal F1+ Cropmax 0.1%,
V6 - Opal F1+Aur verde extra 0.1%,
V7 - Opal F1+Bombardier 0.15%,
V8 - Opal F1 nonfertilized,
V9 - Ismalia F1+Cropmax 0.1%,
V10 - Ismalia F1+Aur verde extra 0.1%,
V11 - Ismalia F1+Bombardier 0.15%,
V12 - Ismalia F1 nonfertilized.

Zucchini crop, as a thermophilic species, was set up through direct sowing on April 24th, at a distance of 0.9/0.9 m, resulting in 1.23 pl/m^2. Plant emergence was over 70% after 7 days, while 9 days after it was fully emerged, temperature during the day being 20-25ºC, while during the night only 6-7ºC. During the vegetation period, specific maintenance works were applied, except for phasal fertilization, which was performed according to the experimental variants. When plants began to grow, weekly measurements were made regarding plant height and diameter, number of flowers and fruit formed, determination of fruit size through their length and diameter, average fruit weight and calculating yield, according on the cultivated hybrid and fertilizer used. The interpretation of the results was made using the variant analysis and calculating the correlation coefficient.

RESULTS AND DISCUSSIONS

The comparative study of 3 zucchini varieties, fertilized with 3 products, showed a different reaction. Thus, what concerns plant diameter, fertilization determined a higher growth for all hybrids, the differences in diameter being influenced by the fertilization and less by the hybrid used (Table 1). The more vigorous hybrid was Eskenderany F1, with an average plant diameter of 84.73 cm. the combination between Opal hybrid and Bombardier fertilizer gave the highest plant vigor, 87 cm. Generally, Ismalia T1 hybrid had plants with a smaller diameter.

Plant height was influenced more by the fertilizer. Bombardier 0.15%, determined a different hybrid reaction, the highest plant growth was recorded for Eskenderany F1 hybrid, reaching values of 99.9 cm, while Cropmax and Aur verde Extra had similar influences on plant growth (Figure 1).

Table 1. Plant diameter for several zucchini varieties in different fertilization conditions (cm)

Variety/ Fertilizer	Cropmax 0.1%	Aur Verde Extra 0.1%	Bombardier 0.15%	Nonfertilized	Average (cm)	Significance
Eskenderany	86.65	85.55	86.25	80.45	84.73	N
Opal	84.30	85.45	87.00	78.50	83.81	N
Ismalia	84.95	83.70	82.55	78.70	82.47	N
Media	85.30	84.90	85.26	79.22	83.67	Mt
LSD 5%					2.03	
LSD 1%					3.08	
LSD 0.1%					4.90	

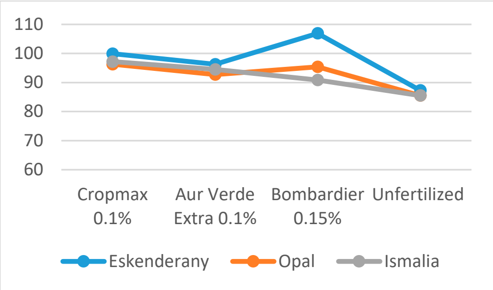

Figure 1. Plant height for studied zucchini hybrids (cm)

Fruit size was influenced more by the hybrid, and for the same hybrid by the fertilizer. The longest fruit was recorded for Opal F1 hybrid, with different values depending on the fertilizer, while the smallest fruit was recorded for Ismalia (Figure 2a.).

Fruit diameter was slightly higher for Eskenerany F1 (Figure 2b.)

The fructification process was influenced more by the hybrid and its biological particularities, differences between hybrids being larger than between fertilizers.

In what concern the number of formed flowers, the fertilizers did not influence at the same level all 3 hybrids, from which the conclusion is drawn that the influence was determined by the combination between the hybrid and fertilizer (Table 2).

The highest blooming capacity was recorded for Eskenderany F1, with more than 16.7 flowers per plant, having a maximum value of 17.4 for Cropmax fertilization and a minimum value of 15.97 for nonfertilized plants.

The Opal F1 hybrid the lowest blooming capacity, with approximately 11.65 flowers per plant, while Ismalia F1 had intermediary values between the other two hybrids, with an average value of 14.60 flowers per plant. For the hybrids Opal and Ismalia, the Aur Verde Extra 0.1% fertilizer slightly influenced the blooming, followed by Cropmax. Differences between hybrids, but also between fertilizers for the same hybrid, were statistically ensured.

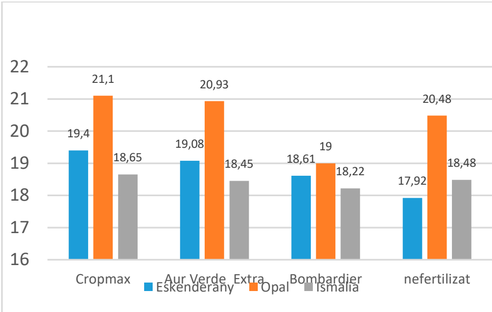

Figure 2a. Fruit length (cm)

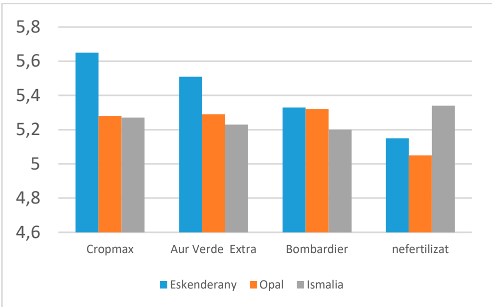

Figure 2b. Fruit diameter (cm)

The fructification capacity, expressed through the number of harvested fruit followed, in general, the same tendency as the blooming, but in this case it was recorded a better influence of the fertilizer Aur verde Extra 0.1% for all hybrids, closely followed by Cropmax 0.1% (Table 3).

Table 2. Influence of fertilization on the number of flowers for several hybrids (piece)

Variant/ Repetition	R1	R2	R3	Average (Pieces)	Difference %	The meaning
V1	18.8	17.2	16.2	17.40	121.14	***
V2	18.2	16.6	17.0	17.27	120.21	***
V3	17.0	15.6	17.4	16.67	116.03	**
V4	15.2	16.0	16.7	15.97	111.16	*
V5	12.8	11.6	11.8	12.07	84.01	oo
V6	13.0	11.0	13.2	12.40	86.33	oo
V7	12.0	10.8	10.6	11.13	77.51	ooo
V8	10.3	11.7	11.1	11.03	76.81	ooo
V9	14.6	15.2	15.4	15.07	104.89	N
V10	16.8	13.8	15.4	15.33	106.75	N
V11	16.8	13.2	14.4	14.80	103.04	N
V12	13.8	12.5	13.4	13.23	92.13	N
Average	14.94	13.77	14.38	14.36	100	Control
LSD 5%				1.36	9.81	
LSD 1%				2.45	13.36	
LSD 0.1%				2.82	17.96	

The highest number of fruit was harvested from the combination between Eskenderany and Aur Verde Extra 0.2%, de 17.15 fruit/plant, while the lowest number was harvested for Opal nonfertilized or fertilized with Bombardier 0.15%. The statistical analysis of data showed that compared to the experiment average, the hybrid Opal had values that were negatively statistically ensured, while Eskenderany had positive differences. In case of Ismalia, differences were not statistically ensured.

Table 3. Influence of fertilizer on fruit formation for several zucchini hybrids (piece)

Variants	R1	R2	R3	Average (Pieces)	Differences %	The meaning
V1	18.55	15.65	16.25	16.82	118.76	**
V2	18.50	16.65	16.30	17.15	121.53	**
V3	18.80	14.80	15.60	16.40	115.82	*
V4	15.05	15.90	14.90	15.28	107.93	N
V5	13.85	10.75	11.80	12.03	85.69	o
V6	12.95	11.20	12.80	12.32	86.98	o
V7	11.55	11.10	10.35	11.00	77.68	ooo
V8	10.90	11.10	11.00	11.00	77.68	ooo
V9	13.95	14.15	15.10	14.40	101.69	N
V10	16.20	14.42	14.45	15.02	106.09	N
V11	15.85	13.85	13.75	14.48	102.28	N
V12	13.50	13.55	12.64	13.23	93.00	N
Average	14.97	13.59	13.91	14.16	100	Control
DL5%				1.72	12.16	
DL 1%				2.35	16.59	
DL 0.1%				3.16	22.31	

In what regards the average fruit weight, differences were not as large as for the previously analyzed parameters; fertilizers had a rather low influence, not statistically ensured, except for one variant: Eskenderany+ Cropmax 0.1% (Table 4). The average value for fruit weight was between 376.5 g for Eskenderany and 361.58 g for Ismalia.

Table 4. Fertilizer influence on average fruit weight for several zucchini hybrids (g)

Variant	R1	R2	R3	Average (g)	Differences %	The meaning
V1	377	380	417	391.33	106.62	*
V2	372	377	394	381.00	103.81	N
V3	377	385	375	379.00	103.26	N
V4	375	339	350	354.67	96.63	N
V5	380	362	372	371.33	101.17	N
V6	369	351	377	365.67	99.63	N
V7	370	345	369	361.33	98.45	N
V8	361	357	361	359.67	97.99	N
V9	369	367	385	359.67	101.81	N
V10	377	356	349	373.67	98.27	N
V11	349	360	348	360.67	96.00	N
V12	367	345	349	352.33	96.36	N
Average	370.25	360.33	370.5	352.67	100	Control
DL5%				19.90	5.42	
DL 1%				27.08	7.37	
DL 0.1%				36.47	9.93	

Fruit production was influenced more by the hybrid and less by the fertilizer. The highest production per plant was obtained for the hybrid Eskenderany F1 of 6.18 kg, followed by Ismalia with 5.19 kg, while Opal F1 produced only 4.24 kg (Table 5a). For the same hybrid, the fertilizer Aur Verde Extra 0.1% had a better influence on Eskenderany F1 and Ismalia F1, while Cropmax 0,1% better influenced hibridul Opal F1. The production per unit of area (m^{-1}) followed the same distribution and it was slightly different for the same hybrid depending on the fertilizer, having values of 6.6-8.12 kg m^{-1} for Eskenderany F1, 6.27- 6.70 kg m^{-1} for Ismalia F1 and 4.85-5.59kg m^{-1} for Opal F1 (Table 5b.).

Correlations

The used fertilizers determined a good fruit formation, most flowers forming fruit, as it can be observed from the correlation coefficient between the two parameters r^2 = 0.99, even though it was a hot summer (Figure 3).

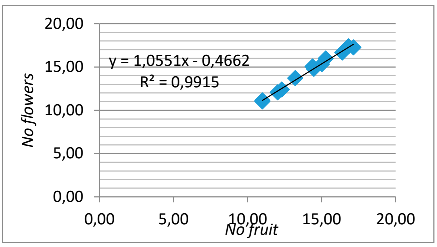

Figure 3. Correlation between number of flowers and number of fruits

In order to determine the influence of fruit size on the number of fruits formed per plant, the correlation coefficient was calculated (Figure 4). The value of the coefficient (r^2 = 0/004) did not show an independence between the two parameters neither positive nor negative, which indicates that fructification is determined by the genetically characteristics and applied culture technology.

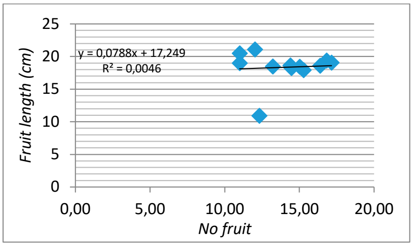

Figure 4. Correlation between fruit size and number of fruits per plant

Harvesting zucchinis for consumption, even at a larger size, did not determine a decrease in the capacity to form new fruit, the correlation between these two parameters showed a weak positive dependency, with a coefficient value of r^2 = 0.36 (Figure 5).

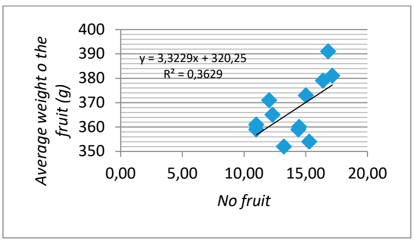

Figure 5. Correlation between fruit weight and number of fruits per plant

Table 5a. Fertilizer influence on the production capacity for several zucchini hybrids (kg/pl)

Variety/ Fertilizer	R1	R2	R3	Average (kg/pl)	Difference %	The meaning
V1	7.00	5.94	6.77	6.57	126.47	***
V2	6.89	6.28	6.64	6.60	127.11	****
V3	7.10	5.62	5.86	6.19	119.22	**
V4	5.65	5.39	5.22	5.36	99.84	N
V5	5.26	3.89	4.52	4.56	87.71	O
V6	4.78	3.93	4.83	4.51	86.88	O
V7	4.28	3.83	3.82	3.98	76.55	OOO
V8	3.93	3.93	3.97	3.94	75.91	OOO
V9	5.16	5.19	5.83	5.39	103.82	N
V10	6.11	5.13	5.12	5.45	104.97	N
V11	5.53	4.99	4.78	5.10	98.17	N
V12	4.95	4.68	4.92	4.85	93.36	N
Average	5.55	4.90	5.19	5.37	100	Mt
DL 5%				1.72	12.16	
DL 1%				2.35	16.59	
DL 0.1%				3.16	22.31	

Table 5b. Fertilizer influence on the production capacity for several zucchini hybrids (kg m^{-1})

Variety/ Fertilizer	R1	R2	R3	Average (kg m^{-1})	Difference %	The meaning
V1	8.61	7.30	8.32	8.08	125.12	***
V2	8.47	7.72	8.16	8.12	125.74	***
V3	8.73	6.91	7.20	7.61	117.00	**
V4	6.94	6.62	6.42	6.66	94.00	N
V5	6.46	4.78	5.52	5.59	103.17	o
V6	5.87	4.83	5.94	5.55	86.54	o
V7	5.26	4.71	4.69	4.89	85.92	ooo
V8	4.83	4.83	4.88	4.85	75.70	ooo
V9	6.34	6.38	7.17	6.63	102.71	N
V10	7.51	6.30	6.29	6.70	103.79	N
V11	6.80	6.13	5.87	6.27	97.08	N
V12	6.08	5.75	6.05	6.53	101.21	N
Average	6.83	6.46	6.29	6.46	100	Mt
LSD 5%				0.72	11.15	
LSD 1%				0.99	15.33	
LSD 0.1%				1.33	20.60	

CONCLUSIONS

The present research showed that the genotype has a great influence and manifests specifically for all characteristics related to productivity: number of flowers, number of fruits, average fruit weight and production. The used fertilizers influenced the production capacity for all hybrids, except for the fertilizer Bombardier in case of the hybrid Ismalia F1, compared to which the results were higher for the control variant. The combination between hybrid and fertilizer is important and influences the obtained production. Fruit size was more a characteristic of the hybrid and less an influence of the fertilizer.

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EFFECT OF USING ORGANIC FERTILIZERS ON THE PRODUCTIVE PARAMETERS OF TOMATOES UNDER FIELD METEOROLOGICAL CONDITIONS

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Abstract

In times of climate changes and increased demand for food purity a significant part of scientific research is directly addressed organic agriculture. The producers are increasingly orienting themselves to bio-production due to its long-run positive consequences both for human and environmental health. The role of organic production is clearly outlined in a number of papers and documents, related to the fight against climatic changes and soil and water contamination. This paper aims at answering the question of whether the biologically grown tomato variety Rugby presents a good alternative to the conventionally produced varieties with respect to their yield. The meteorological conditions during the vegetation period in 2021 have been analyzed in detail and basic productive parameters of the experimentally grown plants (number of standards and non-standard fruits; total weight in kg/da) have been compared for the two methods and three planting dates. At the first harvest in early summer, plants fertilized with mineral fertilizers NPK give better yields than unfertilized and fertilized with Arkobaleno. However, with the intensification of the unfavourable hydrothermal conditions by the end of the summer, the final total yield from the bio-fertilized plants for all the three planting dates already has better parameters not only compared to the unfertilized plants but also compared to the these grown after mineral fertilization. Biologically fertilized tomato plants show better productivity under conditions of over-wetting. Mineral fertilizers have a slight advantage in case of heat stress but the yields are comparable with those, from the plants, fed with the organic fertilizer Arkobaleno.

Key words: *yield, tomatoes, organic and mineral fertilizers, meteorological conditions.*

INTRODUCTION

In recent years, climate change has put serious pressure on all economic sectors, including agriculture. Global models predict temperature increase in Southeast Europe and Bulgaria by more than 2.0 degrees Celsius and an increase in the frequency of meteorological extremes (Alexandrov, 2010; 2011; Svetozarevic et al. 2021). Faced with this real threat, people are getting united by the idea of reducing the anthropogenic causes of the global warming. Soils and their health are identified as a key element in the fight against climate change. Their balanced management is one of the mechanisms for climate regulation and a path for biodiversity protection and conservation (Revised World Soil Charter, 2015). Adopted in Rio de Janeiro, Brazil, the 2012 document "The future we want" recognizes the economic and social importance of good governance for the land, including soil, and in particular its contribution to climate change reduction and water conservation. Plant organisms, in particular vegetable crops, extract trace elements and nutrients necessary for their growth and development, which are most often replenished by applying chemical fertilizers. In recent years, the quality of life and health status of people and nature requires more widespread use of organic nutrition and reduced utilization of chemical ones. The researches indicated that organic fertilization did not cause deficiencies in the nutrient content and yield of vegetables when compared with conventional fertilization, showing that ecological management can be used effectively (Herencia & Maqueda, 2016). Important advantages of organic fertilizers are the gradual release of micronutrients and maintaining a good soil balance. With their help optimal conditions are maintained for soil microorganisms, responsible for fertility and soil structure. Tomato (*Solanum lycopersicum*) is an important horticultural crop that also functions as a research model for the plant family of Solanaceae (Xu et al., 2017). Tomatoes are a major product for Bulgaria (Todorovska et al, 2013) and in particular for the dry region of the Upper Thracian lowlands and the Plovdiv field. Bulgarian tomato varieties are a symbol of our country and are recognizable around the world due to their excellent taste. Tomatoes are very sensitive to extreme phenomena of meteorological origin such as drought and overhumidification - a common phenomenon in the conditions of climate change in our country. These abiotic factors affect almost every stage of their life cycle. Depending on the phase of the plant and the duration of the stress period, abiotic stress can cause up to 70% loss of yield (Krishna et al, 2019; Adams et al, 2001). Extreme variations during the hot summer can damage the intermolecular interactions, necessary for proper growth, thus disrupting plant development and fruit set (Bita & Gerats, 2013). Are organic fertilizers a good alternative to the mineral ones in terms of final production amounts? Do they show good resistance to various weather conditions and are they able to reduce the abiotic pressure on tomatoes? This paper aims to analyze the impact of *mineral* and organic nutrition on early and total yield of Rugby tomatoes during a three-year period of outdoor cultivation for three planting dates.

MATERIALS AND METHODS

Meteorological measurements. The experimental work was carried out in the period 2019-2021 in UEEB (training and experimental base) of the Department of Horticulture at the Faculty of Viticulture and Horticulture at the Agricultural University - Plovdiv. The meteorological parameters were reported for each day of the vegetation period and for this purpose a meteorological station was installed with sensors for: air temperature (average, maximum, minimum in °C; relative humidity in %; precipitation in mm; solar radiation w/m^2). The measurements were compared with the norms proposed by the NIMH for the respective period.

Methodoloical formulation of the experiment. For the purposes of the study, the Bulgarian determinant variety Rugby, selectively adapted to the climatic conditions of our country, was used. For the needs of the experimental work, 40-45 day seedlings were produced, which were then planted in the field in phase 5-6 leaves on three dates, as follows: April 27; May 7; May 17. Three variants of fertilization were set for each planting date: 1st - unfertilized = control; 2nd - mineral fertilization; 3rd - organic fertilization. The experiment was based on three repetitions with 20 plants per repetition. The variant with mineral fertilization was set in a separate experimental plot. The mineral fertilization was carried out with the recommended norms for early field production with superphosphate (50% P_2O_5), potassium sulfate (35% K_2O), ammonium nitrate (33.5% N). For the organic fertilization Arkobaleno fertilizer in a dose of 100 kg ha-1was used. Prior to planting, the beds were mulched with silver black foil. Drip irrigation was applied by placing one drip hose per bed with drippers at a distance of 30 cm and a flow rate of 1.6 mm/h. The irrigation norm was keyed to the meteorological conditions and in particular to the amount of precipitation in the region in mm. Planting was done according to the scheme 160/50 cm with one row in the middle of the bed. Biometric readings were made periodically and at the same time for the batches, planted on the three different planting dates, starting from the moment of initial ripening of the batch, planted on the third one. Early and total standard yields were determined. The early yield was calculated in total in kg/ha from the first three harvests. Anova regression statistical method and two-factor analysis with significance levels $p \leq 0.05$ were used for evaluation of the two methods – biological fertilization and conventional fertilization – at the measured weather conditions in the open air.

RESULTS AND DISCUSSIONS

The planting, growth and development of tomatoes during the three experimental years took place under different weather conditions. The monthly averages and extremes of the main meteorological elements of the period are visualized in Table 1.

Table 1. Hydrothermal conditions during the tomatoes growing seasons 2019-2021

	Temperature in ° C						Precipitation, mm				Number of days	
Month	average T	δ T (°C)	max (°C)	date	min (°C)	date	Σ	Q/Qn	max	date	≥1	≥10
						2019						
May	18.4	1.3	32.4	28	2	9	22	34	6	31	6	0
June	23.4	2.5	34.2	8	10.6	4	197	364	79	3	11	5
July	23.5	0.6	36.4	3	13.2	19	68	135	27	11	8	2
August	24.7	2.7	36.4	22	12.4	7	31	81	20	3	5	1
						2020						
May	17.6	0.5	32	8	5.4	8	71	108	13	1	11	3
June	21.5	0.6	34.7	30	11.3	4	54	101	14	16	6	3
July	24.4	1.5	38	31	12	15	20	40	9	8	4	0
August	24.7	2.7	37	31	13.3	18	18	47	7	9	4	0
						2021						
May	18.5	1.4	31	24	4.6	10	35	54	14	31	6	2
June	21.9	1	38	25	10.1	5	58	108	23	11	11	1
July	26.3	3.4	39.4	30	14	26	36	73	10	9	4	1
August	25.7	3.7	41.6	2	12.9	31	25	66	13	19	3	1

The deviation from the climatic norm of the average air temperature by months varied widely - between 0.5°C in May 2020 and 3.7°C in August 2021. In the first summer month, during which the rooting and initial growth and development of the tomatoes took place, the deviation was 1.3°C in 2019 and 1.4°C in 2021, respectively. July was the hottest month with lowest temperature in 2019 - 23.5°C, and highest - in 2021 - 26.3°C, and deviation of 3.4°C from the climatic norm. In August both in 2019 and 2020 the average temperature was 24.7°C, while in the warmest 2021 year it was 25.7°C with a deviation of 3.7°C from the climatic norm for this month. The precipitation measurements showed conditions of over-wetting in 2019, when in July 79 mm for 24 hours were reported. All minimum temperature values were positive and frost damage conditions after planting the seedlings were not observed. Studies, related to heat stress in tomatoes, report decreased PSII electron transport under the influence of 43.0°C for 6 hours (Heckathorn et al., 1998).

The maximum temperature in July and August had its lowest value in 2019 - 36.4°C, while in 2021 it exceeded 39°C, reaching up to 41.6°C in August.

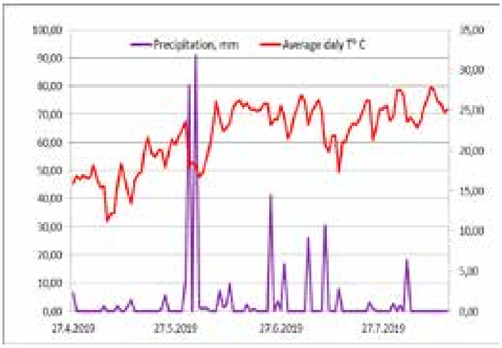


Figure 1. Average daily temperature and ∑ mm precipitation 27.04-27.08 2019

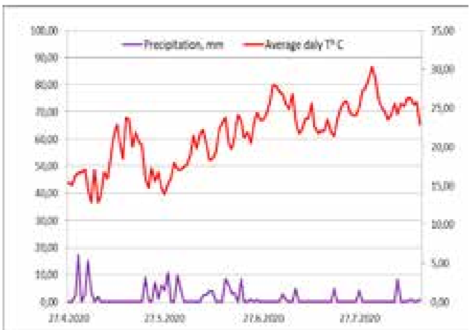


Figure 2. Average daily temperature and ∑ mm precipitation 27.04-27.07.2020

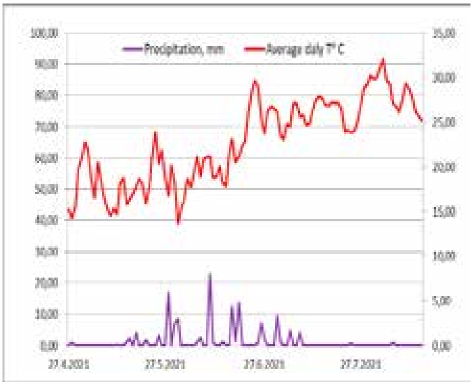


Figure 3. Average daily temperature and ∑ mm precipitation 27.04-27.07.2021

Solar radiation, daily rainfall and temperature values affect the development, productivity and taste of tomatoes. It is known that high maximum temperatures adversely affect the period of flowering and fertilization. Average temperatures of above 21.0°C and below 25.0°C are optimal for tomatoes (Geisenberg & Stewart 1986), with values above the optimum leading to lower seed and fruit yields (Peet et al. 1997). The most solar radiation was measured during the growing season of the plants, planted on 27.04, and the least - for the tomatoes with a period of development 17.05 - 29.08. During the period 27.04 - 15.08 (the earliest planting date) the most sunshine was registered in 2021 (16560.5 w/m^2).

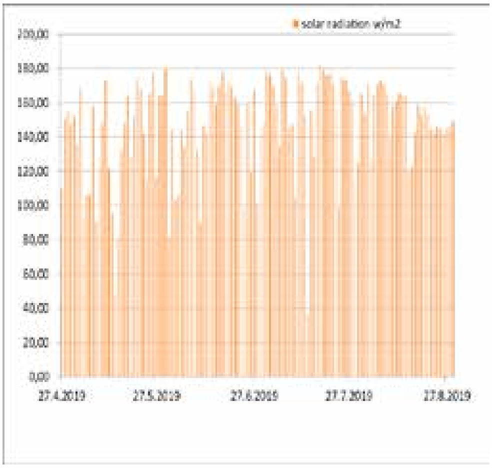
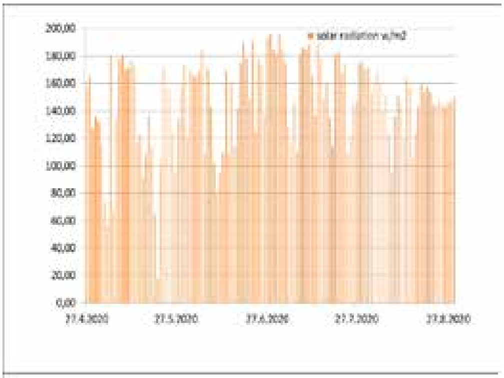
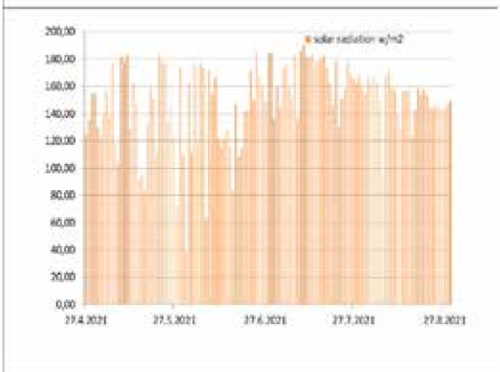


Figure 4. ∑ Solar radiation w/m^2 27.04-27.08 2019

In the second period 7.05-22.08 the solar energy is highest in 2020 (16188.5 w/m2), and in the third (17.05 - 29.08) - it is highest in 2019 (15843.95 w/m^2) (Table 2).





Figures 5 and 6. ∑ Solar radiation w/m^2 27.04-27.08. 2020; 27.04-27.08. 2021

The amounts of precipitation for the three-year period by days showed: over-wetting at the end of May 2019; need for irrigation in 2020 and 2021; and a well-defined drought in July 2021. According to the established irrigation norms (Zahariev et al, 1986), the water used for irrigation, required for optimal development of tomatoes in early and late cultivation, was 141.7 mm in 2020 and 178.2 mm in 2021. Since the the tomatoes, planted on the second date, grew at higher temperatures, the amount of water, required for irrigation increased to 303.1 mm in 2020 and 285.8 mm in 2021.

Air relative humidity (RH) between 55-60% is important for effective pollen production and pollination (OECD, 2017). In all three years, the average RH W % was within the favorable limits for the tomato plants. In 2019, the average relative humidity in % for the three periods batches of plants was between 64.7 and 66.9%. The values are lower in 2020 and 2021 due to the higher temperatures and the dry periods. In all three years, the average humidity in% was best for the development of the earliest planted tomatoes.

During the growing season of the tomatoes, planted on the second and third dates, the active temperature sums were higher than the ones, for the earliest planted pants, except for the year 2021, when the first period was the warmest with an active temperature sum of 2524°C.

Table 2. Agrometeorological conditions during the growing seasons 2019-2021

planting periods	Σ°C	Σp, mm	humidity %	w/m2
27.04.2019- 15.8.2019	2431,2	387,4	66,9	16292,9
7.05.2019- 22.8.2019	2438,5	384,8	66,3	16001,5
17.05.2019- 29.8.2019	2474,4	377,4	64,7	15843,9
27.04.2020- 15.8.2020	2398,4	168,3	65,7	16227,2
7.05.2020- 22.8.2020	2442,5	126,9	64,8	16188,5
17.05.2020- 29.8.2020	2395,2	125,7	64,2	15403,7
27.04.2021- 15.8.2021	2524,0	131,8	63,3	16560,5
7.05.2021 22.8.2021	2510,2	144,2	62,5	16164,1
17.05.2021- 29.8.2021	2506,0	152,4	63,0	15597,7

It can be noticed that the temperature sums for the three planting dates were very close within a year and with more than 100°C difference between the individual years. The average air temperatures were below the optimum in the initial stage of development in 2019 and 2020 and around the optimum in 2021. In all three years there was a decrease in temperature values at the end of May. The average values for the whole vegetation periods were lowest in 2019 - between 21.9°C and 22.7°C. They were above the upper optimal limit after July 27, except for 2019. In 2021, the average temperatures for the earliest and latest planting dates were higher than for the second one, with values of 23.6°C and 23.9°C, respectively. The most favorable in terms of temperature conditions with values around the optimal for tomatoes was 2019, and the most extreme – 2021, with an average temperature for July exceeding the optimal for growing the species (tomatoes). However, the combination of temperature and rainfall was most unfavorable in 2019, when the intense excessive rainfall created conditions for increase in diseases and pests and reduced quality and yields. Under conditions of irrigation, the most favorable for growing tomatoes were the thermal parameters of 2020.

Table 3. Early yield kg/da 2019-2021

2019				
factors	**1 date**	**2 date**	**3 date**	**means**
control	962.7	700.1	419.5	694.1
NPK	1427.9	947.8	566.9	980.9
Arkobaleno	1581.0	863.4	479.2	974.6
means	1323.9	837.1	488.5	
LSD	**p 0.05= 340.5**			
2020				
factors	**1 date**	**2 date**	**3 date**	**means**
control	1091.7	721.2	386.6	733.2
NPK	2046.2	834.5	557.2	1145.9
Arkobaleno	2176.8	963.8	569.2	1236.6
means	1771.6	839.8	504.3	
LSD	**p 0.05=541.3**			
2021				
factors	**1 date**	**2 date**	**3 date**	**means**
control	1703.6	838.5	379.9	974.0
NPK	2484.7	1598.3	823.8	1635.6
Arkobaleno	1916.9	1083.7	539.9	1180.1
means	2035.0	1173.5	581.2	
LSD	**p 0.05=227.3**			

As a result of the phenological stages, the best early yield in all three years was obtained from the first planted tomatoes, for which the yields after fertilizing with NPK and Arkobaleno were statistically significant compared to the control in 2021 - Table 3. Significant differences were found for the second and third batches of plants after using both mineral and organic fertilizers compared to the control plants. This shows that feeding at later dates and in extreme conditions affects the productivity of tomatoes in a positive direction.

In the total yield, the fed variants (NPK and Arkobaleno) are statistically significantly better, compared to the control, in the unfavorable in terms of humidity 2019 as well as in the warmer 2021 (Table 4) Later planted tomatoes had significantly higher yields in 2019 and 2020 compared to the control. In 2021, the best yield was observed from the tomatoes, planted on the second date.

Abiotic and biotic stress contributes 50% and 30% respectively to losses in agricultural productivity worldwide (Kumar & Verma, 2018). During the most unfavorable 2019 the yields from the control for the three growing periods were lower than the average as it follows: control - first date 26.9%; second date 24.9%; third date 29.9%. In 2020, the values were above the average for the period, namely: first date - 16.8%; second date 16.0% and third date 8.5%. In the extremely hot 2021 the deviation is positive, but less than in 2020: first date 10.5%; second date 8.9%; third date 21.4%.

Table 4. Yield kg/da 2019-2021

		2019		
factors	**1 date**	**2 date**	**3 date**	**means**
control	3070.8	2116.1	2515.8	2567.5
NPK	3514.9	2757.1	4063.6	3445.2
Arkobaleno	3321.7	2429.6	3279.9	3010.4
means	3302.4	2434.2	3286.4	
LSD	**p 0.05= 410.0**			
		2020		
factors	**1 date**	**2 date**	**3 date**	**means**
control	4123.0	3103.2	3436.5	**3554.2**
NPK	5428.0	4421.6	5763.9	**5204.5**
Arkobaleno	4262.9	3617.8	4366.9	**4082.5**
means	**4604.6**	**3714.2**	**4522.4**	
LSD	**p 0.05=855.9**			
		2021		
factors	**1 date**	**2 date**	**3 date**	**means**
control	4632.2	4830.8	4099.0	**4520.6**
NPK	5104.1	5738.6	5743.9	**5528.8**
Arkobaleno	5754.6	5929.2	4605.5	**5429.8**
means	**5163.6**	**5499.5**	**4816.1**	
LSD	**p 0.05=756.5**			

In general, the productivity is highest for the third planting date in 2021, as well as for the first and second dates in 2020. When feeding with mineral fertilizers, the negative deviation in the unfavorable 2019 was the largest of all variants for all three dates: first date 43.2%; second date 37.4%; third date 45.4%. In 2020, the productivity was above average with values: first date 13.4%; second date 7.2% and third date 12.1%. Mineral fertilization gave the best yields in 2021: first date 29%; second date 30.2% and 33.3%. Regarding Arkobaleno feeding, the deviation below the average yield for 2019 was first date 32.7%; second date 21.7%; third date 24.9%. Better yields were found on all three dates in 2020: first date - 23%; second date 11% and third date 19.6%. In the unfavorable year 2021, the yields were above the average, but with a lower % than in 2020: first date 9.7%, second date 10.7% and third date 5.3%.

CONCLUSIONS

The main meteorological parameters during the vegetation period of development of Rugby tomatoes, planted on three different dates and grown in a conventional and organic way in three consecutive years from 2019 to 2021, were taken into account. Despite the trends of warming and increasing thermal potential, extreme phenomena resulting from climate change have a rather negative impact on tomato productivity. At the second and third planting date at higher air temperature values and insufficient rainfall, tomatoes need doubling water for irrigation. An analysis of the early and total yields during the experimental period was made and the best harvest in 2021 was established, except for the third planting date, which showed good productivity in 2020. For the three dates of planting a significant positive effect of feeding with mineral NPK fertilizer and with organic fertilizers was observed. The Rugby variety gave good yields in conditions of irrigation in the extremely warm 2021, which focuses interest in its use in the warmer climate of the Upper Thracian lowlands. In case of over-wetting, biological fertilization gives better yields than the conventional one. During vegetation in periods with excessive temperatures, the plants, fed with a mineral NPK fertilizer, show better productivity. Feeding with Arkobaleno can be used to mitigate adverse events of meteorological origin and, given the increased requirements for food and soil purity, this organic fertilizer can be more widely used in the Upper Thracian lowlands.

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EVALUATION OF THE COMBINING ABILITY OF TOMATO GENITORS OBTAINED AT PGRB BUZAU

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Abstract

Hybrid tomatoes are grown in more than 95% of protected areas, rather than varieties. Romania has very few indigenous hybrids of this species, which led PGRB to launch a new tomato breeding program in order to obtain hybrids in accordance with the requirements of growers and consumers, involving in this program the native germplasm collection. PGRB owns a valuable germplasm collection for this species comprising 3084 lines. Of these, 1050 have been identified as genetically stabilized genotypes, 692 as genetically advanced genotypes and 1342 segregants. A number of 33 genitors have been selected after they successfully passed the general combining ability test and showed distinct phenotypic expressiveness. These genitors were involved in specific crosses and 19 of them manifested F1 reproductive heterosis. A number of 9 hybrids outperformed both genitors and genitors mean, from which H 14 recorded the highest percentage of heterobeltiosis of 84.1%. Three hybrids recorded mean values of estimated heterosis with an average of 27.7% (H4) and a number of 7 hybrids were below both the best parent and genitors mean.

Key words*: breeding, germplasm, heterosis, hybrid, selection.*

INTRODUCTION

Tomato is one of the most popular vegetable crops grown across the world. Its genetics most frequently studied among vegetable crops, resulted in the reorganization of its commercial exploitation of hybrid vigour since last hundred years.

Tomato has tremendous potential of heterosis for earliness, total yield, resistance attributes and uniformity. Hybrid tomato varieties will continue to predominate in high input agricultural systems and may also expand under some lower input systems where benefits can be demonstrated (Cheema, D. S., & Dhaliwal, 2005).

Therefore, the available germplasm must be replaced with newly evolved hybrids with attractive quality traits to attain high yield potential. Considering the present scenario, creation of hybrids is inevitable to enhance the crop yield. For this purpose, choice of parents is an important step that promotes a well-planned hybridization programme (Saeed, A., 2014). In tomato, heterosis has been exploited in F1 hybrids to a great extent for more than 50 years in many developed countries like USA, Europe, and Japan (Islam, M. et al., 2012).

Although tomatoes have recently arrived in Romania, after 1984, it has become the most used vegetable. Tomatoes have been introduced in numerous research studies. However, Romania is dependent on imports of hybrid tomato seeds for the establishment of crops in protected areas. More than 90% of the hybrid seeds for protected areas are of foreign origin and do not always meet the soil and climate requirements and consumer demands.

In Romania, the cultivation of hybrid tomatoes in protected areas shows an upward trend. In this regard, it was necessary to achieve a germplasm collection composed of genotypes with distinct phenotypic expressiveness, suitable for hybrid combinations, which have a genetic heritage of interest that can be transmitted and exploited.

It is well known that maintenance or preservation of germplasm involves two principal considerations: avoiding loss of genetic diversity and avoiding costs. Active collections are geared to meet the needs of the users of germplasm (Evgenidis, G. et al., 2011). The germplasm collection offers multiple possibilities both for obtaining hybrids with high production potential and for the safe preservation of genes of interest. Plants obtained from hybrid combinations must have superior qualities demanded by both consumers and processors.
High yield coupled with good processing qualities are the pre-requisites for the general acceptance of the hybrid by the farmers (Chattopadhyay A. et al., 2013). Breeding for high yield and other desirable traits requires information on the nature and magnitude of variations in the available materials, relationship of yield with other agronomic characters (Akinfasoye, J., 2011).
The fact that progeny derived from commercial parents exhibited improved traits, suggests that this parent-type is a profitable source for the generation of elite material. Little information exists on breeding potential of commercial tomato varieties for obtaining new lines via pedigree selection. Given the importance of knowing the breeding value of parents and other important genetic parameters in tomato improvement, it is necessary to investigate the breeding potential of commercial tomato varieties (Hernández-Leal, E., 2019).
The genetic material used to obtain high performance hybrids can come from local populations, established parents, commercial lines, varieties.
The evaluation of combining ability aims to identify potentially valuable and suitable parents to participate in the hybridisation process. Estimation of general combining ability (GCA) provides basic and important information for exploiting genetic potential of parents for development of superior and elite lines (Saeed, A., 2014). Hybrid combinations must exhibit the phenomenon of reproductive heterosis calculated as both estimated and manifested as percentage heterobeltiosis. At the same time a large source of variability within the species studied is obtained.

MATERIALS AND METHODS

The genetic material used in the present experiment comes from the germplasm base held by PGRB Buzau for this species consisting of a total of 3084 genotypes. They were classified according to the degree of genetic stability as follows: 1050 stable, 692 advanced, 1342 segregants (Figure 1).

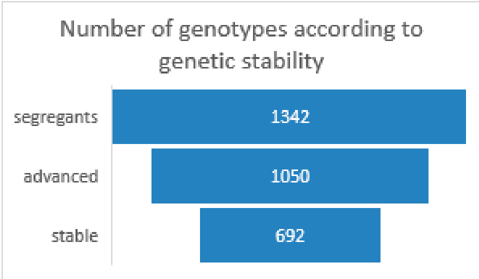

Figure 1. Germplasm collection

Of these, 31 parents have passed the test of general combining ability, being subjected in the present work to the test of specific combining ability. As a hybridization method, simple hybridization was used, obtaining the following crosses:

L19 ♀ x L10 ♂= **H1**
L22 ♀ x L12 ♂= **H2**
L23 ♀ x L15 ♂= **H3**
L26 ♀ x L10 ♂= **H4**
L311 ♀ x L312 ♂= **H5**
L312 ♀ x L508 A ♂= **H6**
L508 A ♀ x L517 A ♂= **H7**
L517 A ♀ x L522 ♂= **H8**
L12 ♀ x L312 ♂= **H9**
L15 ♀ x L508 A ♂= **H10**
L19 ♀ x L311 ♂= **H11**
L22 ♀ x L312 ♂= **H12**
L709 ♀ x L508 A ♂= **H13**
L724 ♀ x L517 A ♂= **H14**
L2000 ♀ x L522 ♂= **H15**
L709 ♀ x L22 ♂= **H16**
L10 ♀ x L23 ♂= **H17**
L12 ♀ x L26 C ♂= **H18**
L15 ♀ x L311 ♂= **H19**

The hybridization consisted in the castration of the flowers on the maternal parent, which is carried out when the petals begin to open, forming an angle of 25-30° to the vertical axis of the flower, and can continue until the mentioned angle reaches a maximum of 45° and the colour of the stamens is still greenish.
Hand pollination was carried out by inserting the style into the pollen tube in such a way that the pollen grains covered the entire surface of the stigma.

The stamens were harvested and after removing the petals, dried for 15-20 hours at 24°C, after which the pollen was extracted by shaking the stamens vigorously.
The pollen obtained was placed in glass tubes of 3-5 cm length and 2-3 mm inner diameter, fitted at one end with a cotton plug. The pollen was used immediately after extraction. It is important to have plenty of pollen available for making hybrid crosses. Since tomato vines bloom abundantly a ratio of one male for every four female plants is recommended, as proposed by Opena R.T et al., 2001.
The cultivation technology applied was the classical tomato cultivation in protected areas according to Vinatoru C. et al., 2019.
The experiment was laid out in a randomized complete block design with three replications, similar to the study conducted by Amaefula, C. et al., 2014. The crop establishment took place by producing seedlings in protected spaces, the planting scheme consisted of strips spaced at 120 cm, with 70 cm between rows and 35 cm between plants/row, in a palisade system.
The estimated heterosis was calculated based on the mean of the parents as follows:

$$[(F1 - MG) / MG] \times 100$$

where MG is the average of the parents and F1 is the resulting hybrid. Heterosis was estimated as better parent heterosis (BPH) as put forth by as follows:

$$BPH = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

Where $\overline{F_1}$ is the mean of hybrid, $\overline{BP}$ is the mean of the better parent (Amaefula, C. et al., 2014).
Measurements were recorded on days to fruiting, fruit weight, fruit length, fruit width, number of fruit per plant and yield per plant according to "descriptors for tomato" proposed by IPGRI, Italy (Saleem, M. Y. et al., 2009) and UPOV Guidelines for the conduct of tests for Distinctness, Uniformity and Stability.
Test of significance was done as described by Kumar et al., 2011. Components of the generation means were evaluated using Hayman model as explained by Singh & Chaudhary, 1985 as follows:

$$a = \overline{B_1} - \overline{B_2}$$

$$d = \overline{F_1} - 4\overline{F_2} - \left(\frac{1}{2}\right)\overline{P_1} - \left(\frac{1}{2}\right)\overline{P_2} + 2\overline{B_1} + 2\overline{B_2}$$

$$aa = 2\overline{B_1} + 2\overline{B_2} - 4\overline{F_2}$$

$$ad = \overline{B_1} - \left(\frac{1}{2}\right)\overline{P_1} - \overline{B_2} + \left(\frac{1}{2}\right)\overline{P_2}$$

$$dd = \overline{P_1} + \overline{P_2} + 2\overline{F_1} + 4\overline{F_2} - 4\overline{B_1} - 4\overline{B_2}$$

$$t \text{ value of effect} = \frac{\text{effect}}{\text{SE of effect}}$$

a = additive mean; d = dominance effect; aa = additive × additive; ad = additive by dominance; dd = dominance × dominance; B1 = mean of backcross to parent 1; B2 = mean of backcross to parent 2; P1 = mean of parent 1; P2 = mean of parent 2; F1 = mean of First filial generation; F2 = of mean second filial generation; SE = standard error.

Statistical calculations were performed using SPSS software, Pearson correlation coefficients were determined as well as variance analysis by ANOVA test followed by DUNCAN test with 95% confidence interval and p-value < 0.05%.

RESULTS AND DISCUSSIONS

Following the descriptors according to IGPRI and UPOV, the morphological and phenotypical characterization of parents selected from the germplasm collection, parents that have successfully passed the test of general combinatorial ability, was carried out.
Thus, the morphological characters observed and quantified in their case demonstrate a rich phenotypic variability. The availability to crossbreeding as well as the gene pool carried by these parents is the strengths of this parent collection.
The majority of parents showed indeterminate breeding but 2 parents showed semi-determinate breeding. The height of the plant varies between 180-290 cm, the number of leaves under the first inflorescence is on average 6, the type of inflorescence is compound for most parents and the number of inflorescences/plant is 6.4 on average, registering a maximum value of 10 inflorescences/plant in lines L 19, L 80 and L 311 with the specification that these lines present medium size fruits.
Concerning the phenotypic characters of the fruits of the parents selected to participate in the hybridization process and to obtain F1 hybrids, a wide range of variability of the main characters was observed. Different fruit shapes

were identified as follows: classic round, flattened, slightly flattened, ovoid, pruniform, cherry type, pomegranate type, banana type, etc. Fruit colour ranged from shades of yellow, orange yellow, red, burgundy, to indigo black. Fruit weight was recorded as average value at around 157 g, with a minimum at L 10 of 10 g and a maximum of 560 g at L 2000, expressing a very wide variability of this trait (Table 1 and Table 2) (Figure 2).

Table 1. Morphological characters of genitors

Crt.no.	Genitor	Type of growth	Plant height (cm)	Suckers no.	Fruits no/inflorescence	Total leaves no.	Inflorescence type	Inflorescence no./plant
1.	L10	**SP+**	200	18	4	24	biparous	9
2.	L 12	**SP+**	200	14	4	28	biparous	5
3.	L 15	**SP+**	180	10	6	26	biparous	6
4.	L19	**SP+**	200	12	6	24	uniparous	10
5.	L 22	**SP+**	180	6	4	25	multiparous	9
6.	L 23	**SP+**	200	10	6	24	biparous	6
7.	L 26 C	**SP+**	180	12	6	26	multiparous	5
8.	L 27 B	**SP+**	180	8	6	20	uniparous	5
9.	L 28	**SP+**	180	18	6	22	biparous	5
10.	L 64	SP	60	6	6	50	multiparous	6
11.	L 66	SP	60	6	5	25	biparous	6
12.	L 80	SP+	291	14	5	26	multiparous	10
13.	L 101	SP+	261	17	9	23	multiparous	5
14.	L 150	SP+	215	21	8	27	multiparous	5
15.	L 165	SP+	216	18	7	28	biparous	5
16.	L 306	SP+	268	11	7	26	uniparous	7
17.	L 307	SP+	185	16	7	25	multiparous	6
18.	L 308	SP+	288	10	6	31	multiparous	7
19.	L 309 A	SP+	251	11	7	28	multiparous	8
20.	L 311	SP+	232	9	6	32	uniparous	10
21.	L 312	SP+	281	13	7	30	uniparous	5
22.	L 508 A	SP+	253	11	7	41	multiparous	6
23.	L 517 A	SP+	251	10	7	38	multiparous	5
24.	L 522	SP+	272	13	5	21	multiparous	6
25.	L 524	SP+	268	11	5	27	multiparous	5
26.	L 532	SP+	165	9	6	33	multiparous	5
27.	L 548	SP+	189	10	5	31	multiparous	5
28.	L 631	SP+	271	12	6	36	multiparous	8
29.	L 709	SP+	291	12	5	32	multiparous	5
30.	L 724	SP+	263	13	7	28	biparous	9
31.	L 2000	SP+	250	18	12	35	multiparous	5

Table 2. External fruit characters of parents (mean values)

Crt.no.	Genitors	Fruit shape	Plant height (cm)	Fruit diameter (cm)	Unripe fruit colour	Ripe fruit colour	No. of locule s	Fruit weight (g)
1.	L10	rounded	2.0	2.0	Light green shoulder	Red	2	10
2.	L 12	rounded	2.0	2.0	Light green shoulder	Yellow	7	190
3.	L 15	rounded	5.0	5.5	Light green shoulder	Red	4	40
4.	L19	flattened	4.0	5.2	Light green	Pink	4	300
5.	L 22	flattened	6.3	8.0	Light green	Red	3	80
6.	L 23	rounded	5.0	5.5	Light green	Red	6	230
7.	L 26 C	rounded	6.0	6.0	Light green shoulder	red	7	150
8.	L 27 B	Slightly flattened	6.0	7.5	Light green shoulder	red	5	180
9.	L 28	Slightly flattened	5.5	6.3	Light green shoulder	red	5	335
10.	L 64	rounded	6.0	6.0	Light green	Dark red	8	165
11.	L 66	Slightly flattened	4.5	5.5	Light green	red	6	190
12.	L 80	ovate	3.6	2.1	Medium green	red	2	12,1
13.	L 101	rounded	5.2	6.443	Small shoulder	orange	5	128.2
14.	L 150	Bell pepper type	7.365	7.899	Medium green	red	4	82.82
15.	L 165	obovate	6.299	6.152	Medium green	red	4	142.01
16.	L 306	Cherry type	2.5	2.5	Green shoulder	red with orange stripes	3	160.95
17.	L 307	rounded	4.863	5.423	Medium green	yellow with green stripes	4	86.75
18.	L 308	Bell pepper type	5.5	8.5	Uniform green	red with orange stripes	5	178.9
19.	L 309 A	Rounded Slightly flattened	6.996	9.805	Very light green	red	11	351.6

20.	L 311	rounded	3.563	4.338	Green shoulders	yellow	4	41.94
21.	L 312	Cherry type	3.607	3.599	Uniform green	red and tan green	2	27.88
22.	L 508 A	rounded	4.638	4.992	Green shoulders	red with brown shoulders	3	65.43
23.	L 517 A	rounded	6.036	9.263	Light green shoulder	red	11	272.97
24.	L 522	Rounded Slightly flattened	5.5	9	Uniform green	yellow-orange	16	252.3
25.	L 524	Bell pepper type	8.9	7.4	Medium green	red	4	233.6
26.	L 532	Banana type	7.2	3.9	Striped green	yellow	3	75.6
27.	L 548	rounded	4.278	5.253	Very light green	orange	3	82.66
28.	L 631	obovate	6.971	4.569	Medium green shoulders	yellow	3	78.24
29.	L 709	obovate	4.709	2.387	Light green	red	2	17.19
30.	L 724	rounded	3.020	3.459	Purple green	Indigo black with red pistillate spot	2	23.43
31.	L 2000	cordate	9.5	10.3	Medium green	red	25	560

Figure 2. Fruit details-genitors and hybrids

The aim of the study was to obtain F1 hybrids that visibly show reproductive heterosis. Calculating the estimated heterosis, in relation to the mean of the parents, 7 hybrid combinations were lower than both parents and both parents' mean, 9 combinations exceeded both parents and parents' mean, with a maximum value for estimated heterosis of 55.05% in the hybrid combination L724 ♀ x L517 A ♂= H14. Relative to the best parent, the heterosis, called heterobeltiosis, expresses the percentage by which the hybrid combination is superior to the best parent. Thus, a maximum heterobeltiosis value of 84.1% was recorded for the combination L724 ♀ x L517 A ♂= H14. There is a definite correlation between the two calculated percentages of heterozygosity, with combinations showing both a high percentage of estimated heterozygosity and heterobeltiosis, hybrids H 17 and H 19 with values of 79.4% and 78.3% heterobeltiosis, respectively. The negative values recorded for BPH (heterobeltiosis) show that there may be large distances between the characters recorded by the parents. In terms of yield per plant, high values were recorded by hybrid combinations that had parents with wild characters that more easily transmit these traits (Table 3).

Table 3. Parents mean yields, crosses mean yields, estimation of heterosis and heterobeltiosis

Crt.no.	G1 ♀	Yield (kg/plant)	G2 ♂	Yield (kg/plant)	MG	Crosses	Hybrid yield (kg/plant)	Estimated heterosis (%)	BPH- (Heterobeltiosis) (%)
1.	L19	2,4	L10	3,0	2,7	L19 ♀ x L10 ♂= **H1**	2,200	-22.72	-26,6
2.	L 22	3,8	L 12	2,0	2,9	L22 ♀ x L12 ♂= **H2**	3,100	6.45	-18,42
3.	L 23	3,4	L 15	3,7	3,55	L23 ♀ x L15 ♂= **H3**	3,300	-7.57	-10,8
4.	L 26 C	4,5	L10	2,0	3,25	L26 ♀ x L10 ♂= **H4**	4,500	27.77	0
5.	L 311	3,1	L 312	2,7	2,9	L311 ♀ x L312 ♂= **H5**	4,560	36.40	45
6.	L 312	2,7	L 508 A	3,2	2,95	L312 ♀ x L508 A ♂= **H6**	3,396	13.13	6,44
7.	L 508 A	3,2	L 517 A	2,9	3,05	L508 A ♀ x L517 A ♂= **H7**	5,800	47.41	81,25
8.	L 517 A	2,9	L 522	3,2	3,05	L517 A ♀ x L522 ♂= **H8**	3,000	-1.66	-6,25
9.	L 12	2,0	L 312	2,7	2,35	L12 ♀ x L312 ♂= **H9**	4,752	50.54	75,92
10.	L 15	3,7	L 508 A	3,2	3,45	L15 ♀ x L508 A ♂= **H10**	6,150	43.90	66,2
11.	L19	2,4	L 311	3,1	2,75	L19 ♀ x L311 ♂= **H11**	4,200	34.52	35,4
12.	L 22	3,8	L 312	2,7	3,25	L22 ♀ x L312 ♂= **H12**	3,795	14.36	-0.26
13.	L 709	3,2	L 508 A	3,2	3,2	L709 ♀ x L508 A ♂= **H13**	5,270	39.27	64,6
14.	L 724	1,9	L 517 A	2,9	2,4	L724 ♀ x L517 A ♂= **H14**	5,340	55.05	84,1
15.	L 2000	2,5	L 522	3,2	2,85	L2000 ♀ x L522 ♂= **H15**	3,140	9.23	-1,8
16.	L 709	2,7	L 22	3,8	3,25	L709 ♀ x L22 ♂= **H16**	3,500	7.14	-7,8
17.	L10	3,0	L 23	3,4	3,2	L10 ♀ x L23 ♂= **H17**	6,100	47.54	79,41
18.	L 12	2,0	L 26 C	4,5	3,25	L12 ♀ x L26 C ♂= **H18**	6,200	47.58	37,7
19.	L 15	3,7	L 311	3,1	3,4	L15 ♀ x L311 ♂= **H19**	6,600	48.48	78,3

MG - genitors mean; G1 - genitor 1, G2 - genitor 2

An estimation of the effect of the additive genes according to the desired trait and breeding objective was made by calculation formulas (Table 4). The following quantitative traits were considered: fruit yield/plant, number of fruits/plant and average fruit weight.

The effect of the additive genes was quantified in the average additive value, with a maximum of 2.6% for the yield/plant trait in the case of hybrid H4, for the number of fruits/plant trait, high values were recorded for the hybrid combinations H 16 and H 17 with 159% and 172% respectively and for the average fruit/plant weight trait, H1 showed an additive effect of 278%.

The dominance effect denoted by **d** recorded a maximum of 1246% for the hybrid combination L2000 ♀ x L522 ♂= H15 for the fruit/plant yield trait. Another high value of the dominant gene effect was observed for fruit weight with 871% at H8. As for the **aa** effect (additive x additive), also in the case of the hybrid combination H15 it was observed to be the most pronounced, registering 1482% in the case of average fruit weight. The compound effect of additive and dominant (**ad**) genes recorded a maximum value in the case of the mean fruit weight trait in the H 15 hybrid with 153%. The **dd** effect (dominance x dominance) recorded a maximum for the hybrid combination H 16 in the case of mean fruit weight. Fruit yield/plant recorded a maximum value of the **dd** dominance effect in the case of H19 of 20.4%. The recorded percentages show the effect of reproductive heterosis, especially in terms of fruit weight, with a marked increase in this trait in the case of hybrids that showed reproductive dominance.

Heterozygosity values depend on the favourable accumulation of dominant allele genes in the F1 population.

Table 4. Effect of additive genes on the quantitative parameters of the resulting hybrid combinations

Characteristics	Crosses	m (%)	a (%)	d (%)	aa (%)	ad (%)	dd (%)
Fruit yield	L19 ♀ x L10 ♂= **H1**	2 ^{i}	-0,5 ^{abcgh}	1,30 ^{a}	1,8 ^{a}	-0,20 ^{abhi}	-1,8 ^{j}
	L22 ♀ x L12 ♂= **H2**	2,9 ^{h}	1,9 ^{ab}	-0,80 ^{ab}	-1 ^{cd}	1,00 ^{ab}	2,4 ^{gh}
	L23 ♀ x L15 ♂= **H3**	3,1 ^{gh}	-0,2 ^{abce}	0,55 ^{ab}	0,8 ^{ab}	-0,05 ^{abef}	-0,3 ^{ij}
	L26 ♀ x L10 ♂= **H4**	4,3 ^{e}	2,6 ^{a}	-3,95 ^{cd}	-5,2 ^{e}	1,35 ^{a}	8,7 ^{f}
	L311 ♀ x L312 ♂= **H5**	4,36 ^{e}	0,5 ^{abc}	-5,18 ^{def}	-6,84 ^{f}	0,30 ^{abd}	11,2 ^{e}
	L312 ♀ x L508 A ♂= **H6**	3,19 ^{gh}	-0,4 ^{abcfg}	-1,52 ^{b}	-1,96 ^{d}	-0,15 ^{abgh}	3,8 ^{g}
	L508 A ♀ x L517 A ♂= **H7**	5,6-0.5- ^{bc}	0,4 ^{abc}	-8,45 ^{gh}	-11,2 ^{hi}	0,25 ^{abd}	17,7 ^{bc}
	L517 A ♀ x L522 ♂= **H8**	2,8 ^{h}	-0,2 ^{abce}	-0,05 ^{ab}	0 ^{bc}	-0,05 ^{abf}	0,9 ^{hi}
	L12 ♀ x L312 ♂= **H9**	4,55 ^{de}	-0,6 ^{di}	-7,40 ^{fgh}	-9,8 ^{gh}	-0,25 ^{abi}	15,6 ^{cd}
	L15 ♀ x L508 A ♂= **H10**	5,95 ^{ab}	0,6 ^{abc}	-8,30 ^{gh}	-11 ^{hi}	0,35 ^{abcd}	17,4 ^{bc}
	L19 ♀ x L311 ♂= **H11**	4 ^{ef}	-0,6 ^{dh}	-4,55 ^{de}	-6 ^{ef}	-0,25 ^{abhi}	9,9 ^{ef}
	L22 ♀ x L312 ♂= **H12**	3,59 ^{fg}	1,2 ^{ab}	-1,82 ^{bc}	-2,36 ^{d}	0,65 ^{abc}	4,4 ^{g}
	L709 ♀ x L508 A ♂= **H13**	5,07 ^{cd}	0,1 ^{abce}	-6,41 ^{efg}	-8,48 ^{g}	0,10 ^{abe}	13,6 ^{d}
	L724 ♀ x L517 A ♂= **H14**	5,14 ^{cd}	-0,9 ^{bcgh}	-9,02 ^{h}	-11,96 ^{i}	-0,40 ^{abi}	18,8 ^{ab}
	L2000 ♀ x L522 ♂= **H15**	2,94 ^{h}	-0,6 ^{abcgh}	-1,07 ^{ab}	-1,36 ^{cd}	-0,25 ^{abi}	2,9 ^{gh}
	L709 ♀ x L22 ♂= **H16**	3,3 ^{gh}	-1 ^{bch}	-0,95 ^{ab}	-1,2 ^{cd}	-0,45 ^{abcd}	2,7 ^{gh}
	L10 ♀ x L23 ♂= **H17**	5,9 ^{ab}	-0,3 ^{abcef}	-8,90 ^{h}	-11,8 ^{i}	-0,10 ^{abg}	18,6 ^{ab}
	L12 ♀ x L26 C ♂= **H18**	6 ^{ab}	-2,4 ^{cdh}	-9,05 ^{h}	-12 ^{i}	-1,15 ^{bi}	18,9 ^{ab}
	L15 ♀ x L311 ♂= **H19**	6,4 ^{a}	0,7 ^{abc}	-9,80 ^{h}	-13 ^{i}	0,40 ^{abcd}	20,4 ^{a}
Number of fruits/plant	L19 ♀ x L10 ♂= **H1**	24 ^{e}	-170,000 ^{n}	265,000 ^{a}	356,000 ^{a}	-85,000 ^{m}	-530,000 ^{s}
	L22 ♀ x L12 ♂= **H2**	26 ^{de}	-1,000 ^{g}	-4,520 ^{m}	-10,000 ^{l}	-0,504 ^{h}	25,000 ^{d}
	L23 ♀ x L15 ♂= **H3**	29 ^{c}	-5,000 ^{h}	-7,540 ^{n}	-10,000 ^{l}	-2,504 ^{h}	19,000 ^{e}
	L26 ♀ x L10 ♂= **H4**	38 ^{a}	-174,000 ^{o}	219,540 ^{c}	292,000 ^{c}	-86,500 ^{m}	-431,000 ^{q}
	L311 ♀ x L312 ♂= **H5**	26 ^{de}	-23,000 ^{j}	178,000 ^{f}	238,000 ^{f}	-10,000 ^{i}	-348,000 ^{n}
	L312 ♀ x L508 A ♂= **H6**	25 ^{e}	53,000 ^{e}	136,000 ^{g}	187,000 ^{g}	27,000 ^{f}	-272,000 ^{m}
	L508 A ♀ x L517 A ♂= **H7**	25 ^{de}	36,000 ^{f}	2,000 ^{l}	8,000 ^{k}	17,000 ^{g}	-8,000 ^{h}
	L517 A ♀ x L522 ♂= **H8**	24 ^{e}	-2,000 ^{g}	-47,500 ^{p}	-60,000 ^{n}	-1,500 ^{h}	95,000 ^{b}
	L12 ♀ x L312 ♂= **H9**	18 ^{g}	-66,000 ^{l}	107,000 ^{i}	152,000 ^{h}	-28,000 ^{k}	-214,000 ^{l}
	L15 ♀ x L508 A ♂= **H10**	32 ^{b}	-12,000 ^{i}	3,502 ^{l}	8,000 ^{k}	-2,500 ^{h}	5,000 ^{f}
	L19 ♀ x L311 ♂= **H11**	21 ^{f}	-40,000 ^{k}	78,500 ^{j}	108,000 ^{i}	-17,500 ^{j}	-149,000 ^{j}
	L22 ♀ x L312 ♂= **H12**	33 ^{b}	-76,000 ^{m}	79,500 ^{j}	108,000 ^{i}	-37,500 ^{l}	-155,000 ^{k}
	L709 ♀ x L508 A ♂= **H13**	28 ^{cd}	133,000 ^{c}	248,500 ^{b}	333,600 ^{b}	66,500 ^{d}	-489,000 ^{r}
	L724 ♀ x L517 A ♂= **H14**	41 ^{a}	69,000 ^{d}	8,500 ^{k}	10,000 ^{k}	33,500 ^{e}	-1,040 ^{g}
	L2000 ♀ x L522 ♂= **H15**	34 ^{b}	-7,000 ^{h}	-83,000 ^{q}	-110,000 ^{o}	-3,000 ^{h}	170,000 ^{a}
	L709 ♀ x L22 ♂= **H16**	38 ^{a}	158,600 ^{b}	191,000 ^{e}	254,000 ^{e}	80,000 ^{c}	-374,000 ^{o}
	L10 ♀ x L23 ♂= **H17**	39 ^{a}	172,000 ^{a}	207,500 ^{d}	280,000 ^{d}	84,500 ^{b}	-411,000 ^{p}
	L12 ♀ x L26 C ♂= **H18**	38 ^{a}	-1,000 ^{g}	-39,000 ^{o}	-54,000 ^{m}	-1,000 ^{h}	86,000 ^{c}
	L15 ♀ x L311 ♂= **H19**	12 ^{h}	-40,000 ^{k}	132,000 ^{h}	15,000 ^{j}	150 ^{a}	-95,000 ^{i}
Average fruit weight	L19 ♀ x L10 ♂= **H1**	24,2 ^{e}	278,000 ^{b}	462,000 ^{f}	492,000 ^{f}	133,000 ^{b}	-520,000 ^{n}
	L22 ♀ x L12 ♂= **H2**	25,6 ^{de}	-104,000 ^{p}	375,000 ^{k}	400,000 ^{h}	-48,600 ^{l}	-414,000 ^{l}
	L23 ♀ x L15 ♂= **H3**	29,4 ^{c}	179,000 ^{d}	397,000 ^{i}	382,000 ^{i}	84,000 ^{d}	-310,000 ^{j}
	L26 ♀ x L10 ♂= **H4**	38,2 ^{a}	139,000 ^{f}	192,000 ^{n}	158,000 ^{l}	69,000 ^{e}	-80,000 ^{g}
	L311 ♀ x L312 ♂= **H5**	26,4 ^{de}	14,000 ^{j}	154,026 ^{q}	32,000 ^{q}	7,026 ^{g}	215,888 ^{d}
	L312 ♀ x L508 A ♂= **H6**	24,6 ^{e}	-38,000 ^{m}	257,562 ^{l}	72,000 ^{n}	-19,442 ^{i}	312,916 ^{b}
	L508 A ♀ x L517 A ♂= **H7**	25,4 ^{de}	-207,000 ^{q}	517,793 ^{e}	562,000 ^{d}	-103,223 ^{m}	-635,634 ^{p}
	L517 A ♀ x L522 ♂= **H8**	24,400 ^{e}	20,000 ^{i}	871,373 ^{b}	936,000 ^{b}	9,663 ^{g}	-1046,686 ^{r}
	L12 ♀ x L312 ♂= **H9**	17,600 ^{g}	164,000 ^{e}	442,000 ^{g}	348,000 ^{j}	82,800 ^{d}	-143,600 ^{i}
	L15 ♀ x L508 A ♂= **H10**	32,000 ^{b}	-25,000 ^{l}	133,500 ^{r}	66,000 ^{o}	-12,500 ^{h}	85,000 ^{f}
	L19 ♀ x L311 ♂= **H11**	21,400 ^{f}	248,000 ^{c}	562,000 ^{c}	568,000 ^{c}	119,000 ^{c}	-548,000 ^{o}
	L22 ♀ x L312 ♂= **H12**	32,600 ^{b}	51,000 ^{g}	179,000 ^{p}	78,000 ^{m}	25,400 ^{f}	130,000 ^{e}
	L709 ♀ x L508 A ♂= **H13**	27,600 ^{cd}	-50,000 ^{n}	184,905 ^{o}	48,000 ^{p}	-26,099 ^{j}	230,238 ^{c}
	L724 ♀ x L517 A ♂= **H14**	40,600 ^{a}	-247,000 ^{s}	389,787 ^{j}	418,000 ^{g}	-122,223 ^{o}	-463,634 ^{m}
	L2000 ♀ x L522 ♂= **H15**	34,400 ^{b}	307,000 ^{a}	1246,000 ^{a}	1482,000 ^{a}	153,000 ^{a}	-1948,000 ^{s}
	L709 ♀ x L22 ♂= **H16**	38,000 ^{a}	-65,000 ^{o}	219,411 ^{m}	34,000 ^{q}	-33,599 ^{k}	345,238 ^{a}
	L10 ♀ x L23 ♂= **H17**	38,600 ^{a}	-220,000 ^{r}	414,000 ^{h}	316,000 ^{k}	-110,000 ^{n}	-112,000 ^{h}
	L12 ♀ x L26 C ♂= **H18**	38,400 ^{a}	43,000 ^{h}	544,000 ^{d}	502,000 ^{e}	23,000 ^{f}	-392,000 ^{k}
	L15 ♀ x L311 ♂= **H19**	12,400 ^{h}	-3,000 ^{k}	-682,000 ^{s}	20,000 ^{r}	84,000 ^{d}	-743,000 ^{q}

a = additive mean; d = dominance effect; aa = additive × additive; ad = additive by dominance; dd = dominance × dominance.

*letters represent Duncan test results with 95% confidence interval and p<0.05%; CV-coefficient of variation

Taking into account the correlations between the effects of genes calculated according to the formulas mentioned above, it was identified that between the effect of additive genes and the effect of additive x dominance genes there is a positive trend dependence relationship, when one of the effects increases, automatically the other will increase. The R-squared coefficient of determination, calculated by Pearson's correlation of coefficients, was 1 (Figure 3).

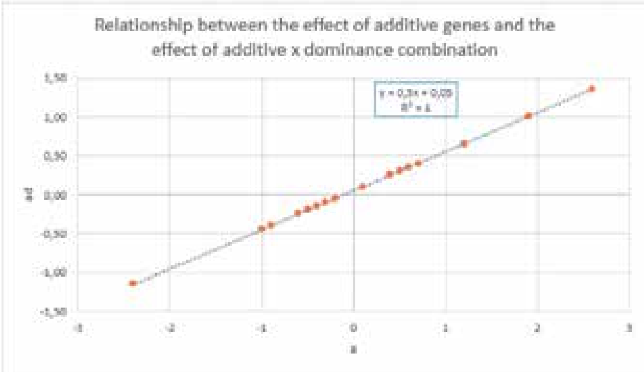

Figure 3. Relationship between the effect of additive genes and the effect of additive x dominance combination

CONCLUSIONS

The germplasm collection of tomato has been evaluated for genetic stability. From this germplasm base, 31 parents were selected by the general combining ability test and successfully passed this test. They were then subjected to the specific combining ability test, resulting in 19 hybrid combinations showing reproductive heterosis. Of these, hybrid H 14 recorded the highest percentage of estimated heterosis as well as heterobeltiosis, outperforming both the parent average and the best parent. Calculating the effect of additive genes, it was found that hybrids whose parents possessed wild genes significantly manifested the additive gene effect by recording high values of dominance and additive effect in the F1 population.

The hybrid combinations that demonstrated strong heterosis will be proposed for approval and patenting.

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THE INFLUENCE OF *FUSARIUM OXYSPORUM* AND *ALTERNARIA ALTERNATA* FUNGI ON VARIABILITY AND HERITABILITY OF THE TOMATO GROWTH CHARACTERISTICS

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Abstract

As a result of the analysis of the sensitivity of the tomato perspective lines to the culture filtrates (CF) Alternaria alternata and Fusarium oxysporum, it was found that in the most of the cases they did not significantly influence seeds germination, but in all the cases, inhibition of the embryonic root length and stem occurred. By bifactorial analysis of the variance it was found that for the seeds germination, the genotypic factor was the most important in the reaction to F. oxysporum and A. alternata isolates, and for the growth of embryonic root and stem in both variants a major influence belongs to the fungus isolate. Genotypic and phenotypic variations varied to a large extent depending on the isolate and the analyzed character. High coefficient of heritability in the broad sense (h^2 = 0.60-0.95) indicates a good heredity of the studied characters in the interaction with the isolates of F. oxysporum and A. alternata fungi. We mention that the coefficient of genotypic variation varied widely – 17.8-73.1% for the studied characters, which proves the genetic and environmental nature of their variability.

***Key words**: tomatoes, variability, growth organs, F. oxysporum, A. alternata.*

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.), an important widespread crop, is some of the most widely consumed and popular vegetables, widely grown worldwide and particularly profitable for growers (Roiola et al., 2014; Nasir et al., 2015).

Globally, tomatoes are an important part of a diverse and balanced diet (Willcox et al., 2003), providing a wide variety of nutrients (Ilahy et al., 2016), vitamins, carotenoids and phenolic compounds (Raiola et al., 2014; Martí et al., 2016; Li et al., 2018).

These bioactive compounds have a wide range of physiological properties, including antiinflammatory, antiallergenic, antimicrobial, vasodilating, antithrombotic, cardio-protective and antioxidant effects (Raiola et al, 2014). Also, fresh and processed tomatoes are the richest sources of antioxidant lycopene in the human diet (Viuda-Martos et al., 2014; Nasir et al., 2015), which protects the human body from free radicals and reduces the risk of cancer (Giovannucci, 1999).

Carotenoids and polyphenolic compounds contribute to the nutritional value of tomatoes and improve their functional attributes and sensory qualities, including taste, aroma and texture (Raiola et al., 2014; Tohge & Fernie, 2015; Martí et al., 2016).

Cultivated tomatoes have a limited genetic diversity due to their long selection and oriented towards certain traits during evolution and domestication (Bai & Lindhout, 2007; Blanca et al., 2015).

Tomato resistance to disease is often associated with a number of undesirable traits, which is why resistance donors are not acceptable in practice (Chaerani & Voorrips, 2006).

For these reasons, tomatoes are prone to a high incidence of disease, during the cultivation and post-harvest period being affected by over 200 diseases caused by various pathogens (King & Lively, 2012; Singh et al., 2017).

Recently, tomatoes have a high frequency of fungal pathogens of *Fusarium* spp. causing root rot at various stages of development, yellowing of lower leaves, vascular necrosis, wilting of plants, defoliation, and eventually plant death (Szczechura et al., 2013; McGovern, 2015; Bodah, 2017; Srinivas et al., 2019) and *Alternaria* spp. which is manifested by brown spots on leaves, shoots, fruits (Mamgain et al., 2013; Lupascu et al., 2015; Mihnea et al., 2018).

As alternariosis develops, defoliation occurs, starting with the older leaves and moving toward the younger ones, and necrotic lesions can be seen on flowers and stems (Strandberg, 1992). The disease can lead to complete defoliation, strongly influencing the photosynthetic efficiency of the plant, productivity and fruit quality (Rotem, 1994; Lawrence et al., 2000).

For the successful management of these diseases, it is necessary to use varieties with high genetic performance, a factor that is a decisive link of the innovative progress in agriculture and ensures the achievement of high quantities of high quality production, with required organoleptic properties (Carli et al., 2011; Ercolano et al., 2008; Mihnea et al., 2016; Seymour et al., 2002). Plantation rotation, fungicide diversification (Olaya et al., 2017; Malandrakis et al., 2018), the use of different agronomic methods, starting from the use of healthy seeds or seedlings, soil moisture control, removal of infected plants, plant debris and weeds, increasing plant vigor through proper fertilization management are decisive factors for achieving the desired goals (Foolad et al., 2008; Small et al., 2015; McGovern, 2015; Gamliel et al., 2009). Obtaining stable crops for tomatoes, reducing losses due to diseases and unfavorable environmental factors can be achieved by creating resistant varieties with ecological stability and high plasticity (Mihnea et al. 2016; Mihnea, 2017).

The aim of our research was to determine the influence of *F. oxysporum* and *A. alternata* fungi on the organs of growth and development of tomato plants in the early stage of ontogenesis, as well as on their variability and heredity.

MATERIALS AND METHODS

The experiments were carried out in laboratory conditions at the Laboratory of Applied Genetics, Institute of Genetics, Physiology and Plant Protection.

Mary Gratefully and the lines L 302, L 303, L 304, L 305, L 306, L 307, with agronomically valuable characteristics, obtained from the following hybrid combinations: F_{14} Potoc x F_1 (*Gruntovschi gribovschi x L. chilense*), F_7 (Maestro x Irisca), F_{12} (Novicioc x Iuliana), F_7 (Maestro x Irişca) F_8 (Mihaela x Dwarf Moneymaker), F_7 (Maestro x Dicaia roza), correspondingly, were used as material for the assessment of resistance to fungal diseases. Mary Gratefully approved variety was used as a control.

To establish pathogenic agents that cause root rot and brown leaf spot in tomatoes, they were initially isolated in aseptic conditions on must-agar and PDA (Potatoes Dextrosis Agar) media according to classical methods (Bilay, 1982). These media are best optimized for the isolation, cultivation and study of the morphologic-cultural characteristics of pathogens. Small fragments of tissue from the base of the stem, leaf, petiole of tomato plants were used. The fragments were sterilized in a 2% solution of calcium hypochloride for 1-2 min, then washed 2-3 times in bidistilled water, squeezed between 2 sheets of filter paper, and placed on a medium near the gas flame.

The species of causative agents were identified based on macro- and microscopic characteristics, according to mycological identification guides (Bilay, 1977; Barnett & Hunter, 1998; Ellis & Ellis, 1985).

Culture filtrates (CF) of 3 isolates of *F. oxysporum* and *A. alternata* fungi (CF1, CF2, CF3), isolated from tomato plants with signs of disease, were used.

CF were prepared by inoculating the mycelium in Czapek-Dox (Tuite, 1969) liquid medium and subsequently culturing at 22-24°C for 21 days.

Tomato seeds were treated with fungal CF for 16 hours. The seeds kept in distilled water served as control. The seedlings were grown out in three replications in Petri dishes on filter paper moistened with distilled water at a temperature of 24-25°C for 6 days. As a test index of plant reaction, served important growth and development characteristics of tomatoes in the early stage of ontogenesis – germination, root length and stem length.

The share of phytopathosystem components was determined by bifactorial analysis of the variance.

For the analysis of genetic variability, heritability and genetic progress, the following formulas were applied:

$\sigma^2_g = (MSS - MSE)/r;$

$\sigma^2_{ph} = \sigma^2_e + \sigma^2_G;$

$h^2 = \sigma^2_g / \sigma^2_{ph} \times 100\%$;
$PCV = 100 \times \sqrt{\sigma^2_{ph}} / X$;
$GCV = 100 \times \sqrt{\sigma^2_g} / X$;
$GA = K \times (\sigma P) \times h^2$;
$GA,\% = 100 \times K \times h^2 \times \sigma_{ph} / X$, in which:

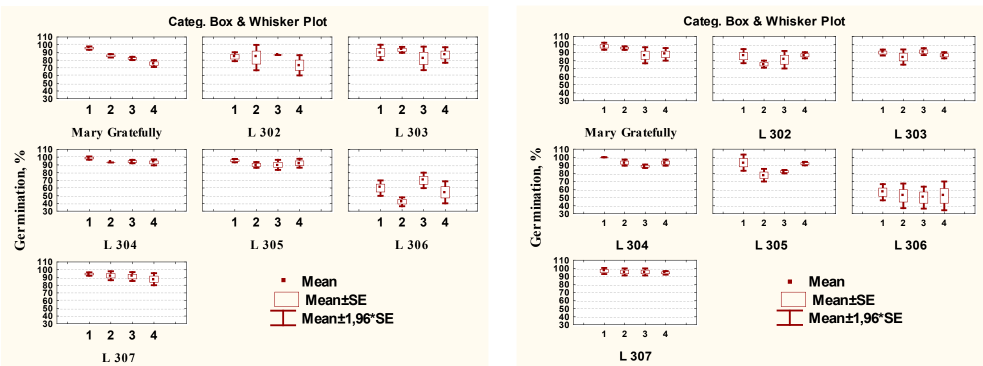
σ^2_g – genotypic variance; σ^2_{ph} – phenotypic variance; σ^2_e (error variance, or VE) = MSE; h^2 – coefficient of heritability in the broad sense; PCV – phenotypic coefficient of variation; GCV – coefficient of genotypic variation; X – general average of the character; GA – genetic advantage; K – selection differential = 2.06 at the selection pressure of 5%; σ_{ph} – general standard deviation of character (Rameeh, 2014; Adeniji, 2018; Balkan, 2018).

Cluster analysis was performed by the *k*-means method (Savary et al., 2010), programming 3 clusters according to the possible values of the characters: small, medium and high.
The data obtained were statistically processed in the software package STATISTICA 7.

RESULTS AND DISCUSSIONS

Testing of the reaction of tomato plants to the treatment of CF seeds of 3 isolates of the fungus *F. oxysporum* showed that under the action of pathogen metabolites in most lines there was an inhibition of germination, embryonic root length and stem. In this case, the reaction of the plants depended on the genotype, the factor analyzed and isolated.

Seed germination analysis showed that CF in 14 cases out of 28 produced inhibition by 2.3 - 9.3%, in 3 – 11.0-14.0% and in 2 cases more than 20%. A differentiated reaction of tomato genotypes was noted. In the Mary Gratefully variety under the influence of *F. oxysporum* CF 2, germination was inhibited by 14.0%, and in the lines L 302 and L 306 – stimulated by 2.7% and 16.7%, respectively. An inhibition was also observed with 21.0% in the Mary Gratefully variety under the influence of CF3 and with 29.7% in L 306 – under the influence of CF1 (Figure 1 A). The genotypes L 303, L 304 and L 307 showed resistance to all three isolates.
Regarding the *A. alternata* fungus, it was found that when treating the seeds with CF, under the action of the pathogen metabolites in most lines there was an insignificant repression of seed germination. *A. alternata* CF inhibited seed germination by 1.0… 11.8%. Insignificant stimulation was recorded at L 302 (1.3%) and L 303 (1.2%) (Figure 1 B). The L 303 and L 307 lines showed an increased resistance to all 3 isolates, which can be used in the improvement process as the most resistant.
Genotypes L 303 and L 307 have shown complex resistance and are of interest in breeding tomatoes as a source of resistance to *F. oxysporum* and *A. alternata*.

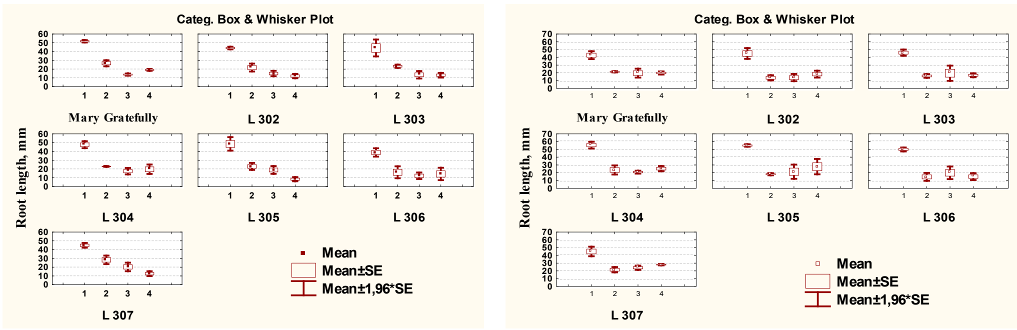


A B

Figure 1. Influence of *F. oxysporum* (A) and *A. alternata* (B) culture filtrates on seeds germination (%) on the Mary Gratefully and some tomato lines
Horizontal: 1 – H_2O (control), 2 – CF 1, 3 – CF 2, 4 – CF 3

Regarding root growth, it was found that *F. oxysporum* and *A. alternata* CF had a different effect (Figure 2). Thus, *F. oxysporum* CF1 inhibited root length by 36.4-53.0% in the genotypes under study. In the case of CF2 and CF3, there was a much stronger inhibition: 56.6-78.3% and 61.1-82.9% of the control, respectively.

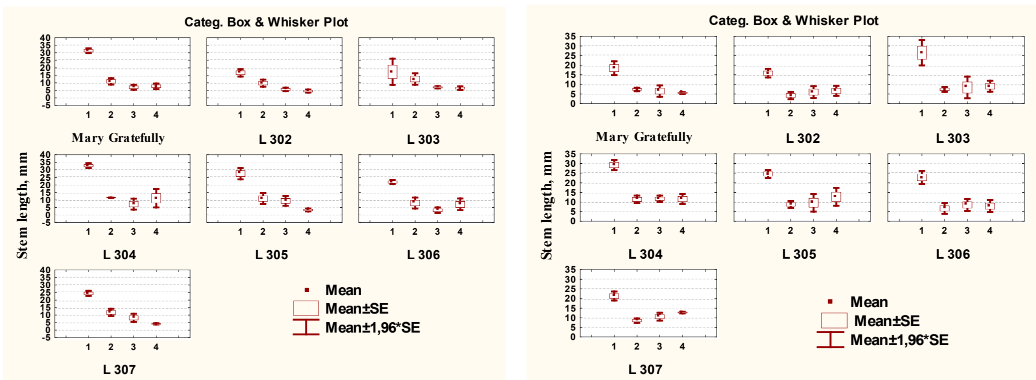
Genotypes were found to be quite sensitive to *A. alternata* CF. Thus, *A. alternata* CF inhibited root growth within 38.2-69.6%. The evaluated genotypes were the most strongly influenced by CF1 and CF2, the average values in relation to the control varying in the limits of 50.8-69.6% and 46.4-67.3%, respectively. There were strong inhibition at L 302, L 305, L 306. The lowest sensitivity of the embryonic root in the studied CF was recorded at L 307.



A B

Figure 2. Influence of *F. oxysporum* (A) and *A. alternata* (B) culture filtrates on root growth in tomato seedlings

In the case of the reaction of the strain to *F. oxysporum* CF, the inhibition in relation to the control was 34.6-88.3: CF1 – 34.6-66.0%, CF2 – 63.9-84.9%, CF3 – 64.9-88.3%, and for *A. alternata* CF – 40.7-72.6%: 40.7-72.6% for CF1, 49.5-70.7% – CF2 and 40.7-70.1% – CF3 (Figure 3). So, as in the case of the root, the stem was the most strongly affected by CF2 and CF3.



A B

Figure 3. Influence of *F. oxysporum* (A) and *A. alternata* (B) culture filtrates on stem growth in tomato seedlings

Cluster analysis (*k*-means method) showed that for all 3 studied characters, in the control variants and with *F. oxysporum* and *A. alternata* CF's, the intercluster variant was much higher than the intracluster one, which indicates that the 7 genotypes taken into account showed distinct pronounced differences. The only exception was *F. oxysporum* CF1 for root and stem length, in which the intercluster variance was lower than the intracluster variance, which indicates the poor specificity of the genotype reaction to this isolate (Tables 1, 2).

Table 1. Analysis of inter- and intraclusterian variance in the interaction of tomato genotypes with *F. oxysporum*

Variant	Interclusterian variance	df	Intraclusterian variance	df	F	p
Germination						
Control (H_2O)	967.924	2	102.890	4	18.81	0.01
FC1	2029.844	2	13.625	4	297.96	0.00
FC2	303.562	2	90.213	4	6.73	0.05
FC3	1116.617	2	33.880	4	65.92	0.00
Length of the root						
Control (H_2O)	67.902	2	28.940	4	4.69	0.09
FC1	36.204	2	44.485	4	1.63	0.30
FC2	36.640	2	12.497	4	5.86	0.06
FC3	73.239	2	12.250	4	11.96	0.02
Length of the stem						
Control (H_2O)	203.641	2	18.597	4	21.90	0.01
FC1	1.841	2	9.457	4	0.39	0.70
FC2	10.113	2	7.527	4	2.69	0.18
FC3	27.041	2	6.457	4	8.38	0.04

df= Freedom degree; F= Fisher's criterion; p= error

Table 2. Analysis of inter- and intraclusterian variance in the interaction of tomato genotypes with *A. alternata*

Variant	Interclusterian variance	df	Intraclusterian variance	df	F	p
Germination						
Control (H_2O)	12297.661	2	28.093	4	92.38	0.000
FC1	1402.510	2	46.0667	4	60.89	0.001
FC2	1257.202	2	102.087	4	24.63	0.006
FC3	1252.964	2	45.173	4	55.47	0.001
Length of the root						
Control (H_2O)	137.843	2	19.072	4	14.46	0.016
FC1	62.877	2	20.072	4	6.27	0.059
FC2	33.626	2	21.732	4	3.09	0.154
FC3	118.277	2	39.840	4	5.94	0.063
Length of the stem						
Control (H_2O)	76.240	2	26.617	4	5.73	0.067
FC1	15.528	2	9.547	4	3.25	0.145
FC2	23.808	2	2.032	4	23.44	0.006
FC3	45.364	2	2.125	4	42,70	0,002

df= Freedom degree; F= Fisher's criterion; p= error

By classification, based on the three characters and descriptive analysis of the clusters, have been identified genotypes of tomatoes that were located in cluster 3, with complex resistance to both pathogens – L 304, L 307; resistance to *F. oxysporum* – L 303, L 305. Germination, compared to other 2 characters, was a factor with higher discriminant capacity (Table 3).

Table 3. Descriptive analysis of clusters

Cluster	Character	*F. oxysporum*		*A.alternata*	
		x	Genotype	x	Genotype
1	Germination, %	55.5	L 306	51.5	L 306
	Root length, mm	14.2		16.3	
	Stem length, mm	6.0		7.7	
2	Germination, %	81.1	Mary Gratefully, L 302	85.7	Mary Gratefully, L 302, L 303, L 305
	Root length, mm	18.1		19.6	
	Stem length, mm	7.8		7.7	
3	Germination, %	90.5	L 303, L 304, L 305, L 307	93.5	L 304, L307
	Root length, mm	18.4		23.9	
	Stem length, mm	9.1		11.1	

The processing of experimental data by bifactorial analysis of the variance allowed to assess the variability and degree of influence of the isolate, genotype and their interaction in the share of the phenotypic manifestation of growth and development of tomato genotypes investigated. Thus, the contribution of the genotype, isolate and genotype x isolate interaction for seeds germination was found to be 83.9; 10.0 and 4.2% for *F. oxysporum* CF, and 90.1; 6.8; 1.5% – for *A. alternata* CF, which indicates that for seeds germination, the genotypic factor had the greatest importance in the reaction to the mentioned pathogens.

In the variant with CF *F. oxysporum*, the contribution of their genotype, isolation and interaction in the source of variability of root and stem length was 1.8; 97.2; 0.7% and 4.0; 93.6; 2.0%, respectively, and for treatment with CF *A. alternata* – 3.1; 95.7; 0.8% and 7.5; 91.0; 0.9%, respectively.

Therefore, for the growth of the embryonic root and the stem in both variants, a major influence belongs to the isolate (Table 4).

Table 4. Bifactorial analysis of *tomato genotype x fungal pathogen* relationships

Source of variation	Freedom degree	Length of the root		Length of the stem		Germination	
		The average sum of the squares	Contribution in the source of variation, %	The average sum of the squares	Contribution in the source of variation, %	The average sum of the squares	Contribution in the source of variation, %
F. oxysporum							
Genotype	6	80.33*	1.8	65.17*	4.0	2015.2*	83.9
Isolate	3	4428.55*	97.2	1534.67*	93.6	241.5*	10.0
Genotype x isolate	18	30.35*	0.7	32.24*	2.0	101.0*	4.2
Random effects	56	14.80	0.3	7.27	0.4	45.8	1.9
A. alternata							
Genotype	6	137.85*	3.1	86.03*	7.5	2585.3*	90.1
Isolate	3	4318.84*	95.7	1044.31*	91.0	194.4*	6.8
Genotype x isolate	18	33.87	0.8	10.33	0.9	44.3	1.5
Random effects	56	20.01	0.4	7.12	0.6	45.9	1.6

*- $p<0.05$ (significance liver).

In connection with the above, the genotypic and phenotypic variations of the analyzed characters varied considerably depending on the species of the fungus, which was reflected in the fairly wide amplitude of the heritability coefficient – 0.60-0.95 (Table 5).

Table 5. Genetic variability and heredity of tomato growth organs in early ontogenesis at interaction with some fungal pathogens

Parameter	Length of the root		Length of the stem		Germination	
	F. oxysporum	*A. alternata*	*F. oxysporum*	*A. alternata*	*F. oxysporum*	*A. alternata*
σ^2_g	21.84	39.3	19.3	78.9	656.5	846.5
σ^2_{ph}	36.64	59.3	26.57	86	702.3	892.4
h^2	0.60	0.66	0.73	0.92	0.93	0.95
GCV, %	17.75	23.2	36.22	73.1	30.39	34.5
PCV, %	24.46	28.4	42.49	76.3	31.46	35.4
PCV – GCV, %	6.71	5.2	6.27	3.2	1.07	0.9
GA	16.7	18.6	12.77	13.5	27.57	29.9
GA, %	67.5	68.7	105.3	111.4	32.73	35.5

σ^2_g = genotypic variance; σ^2_{ph} = phenotypic variance; h^2 = coefficient of heritability in the broad sense; PCV = phenotypic coefficient of variation; GCV = coefficient of genotypic variation; GA = genetic advantage.

At the same time, it was observed that the h^2 values of seeds germination under the action of isolates of both fungi and stem length in interaction with *F. oxysporum* CF were much higher compared to other variants, which proves the genetic determinism of these characters.
The coefficient of genotypic variation was also medium or high - 17.75-73.3% for the studied characters, which proves the genetic nature of their variability. The difference between PCV and GCV was 0.9-6.71% and reflects the differentiated response of growth organs to the action of fungal isolates.

CONCLUSIONS

As a result of the analysis of the tomatoes perspective lines on *F. oxysporum* and *A. alternata* isolates extracted from sicked plants, it was found that germination, compared to 2 other characters - embryonic root length and stem length, was a factor with higher discriminant capacity.
Under the influence of fungal pathogens culture filtrates, in all of the cases there was inhibition of growth of the embryonic root and the stem, but the degree of reaction of the plants depended on the genotype, the analyzed character and the isolate of the fungus.
Bifactorial analysis of the variance found that for seeds germination, the genotypic factor was most the important in the reaction to *F. oxysporum* and *A. alternata*, and for the growth of embryonic root and stem in both variants a major influence belonged to the fungus isolate.
The high coefficient of heritability in the broad sense (h^2 = 0.60-0.95) indicates a good heredity of the studied characters in the interaction with the isolates of *F. oxysporum* and *A. alternata* fungi. We mention that the coefficient of genotypic variation was also medium or high - 17.8-73.1% for the studied characters, which proves the genetic nature of their variability.

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THE CONTENT OF MICROELEMENTS IN BLOOM OF BROCCOFLOWER AFTER FOLIAR FERTILIZATION

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Abstract

World market of vegetables is becoming less conservative and has been opening itself to new cuisines, as well as to new products. Broccoflower, high quality, nice looking plant crossbred from broccoli and cauliflower, has been making a slow but steady breakthrough to the market. Meanwhile, fast life accompanied by fast food had led to epidemic of nutritional disorders. Lack of iron, zinc and manganese are amongst them. That, alongside other reasons had induced the field experiment with broccoflower, aiming to determine the effect of foliar fertilization on the content of microelements iron, manganese and zinc in the bloom of broccoflower. The experiment was set according to the method of random bloc design with three repetitions and four foliar treatments (control, zinc (0.125 g 0.1 L^{-1}),drin (0.1 mL 0.1 L^{-1}) and boron (0.175 g 0.1 L^{-1})). The highest determined results show 0.87 mg of iron, 0.61 mg of zinc and 0.27 mg of manganese in 100 g of fresh matter broccoflower bloom without statistically significant differences between treatments. Those results show just how well stocked broccoflower is with these minerals, which should be useful in this plant's breakthrough on domestic vegetable market, which is still quite conservative.

Key words: *broccoflower, iron, manganese, micronutrients, zinc.*

INTRODUCTION

A hybrid of broccoli (*Brassica oleracea* var. *italica*; broccoli) and cauliflower (*B. oleracea* var. *botrytis*; cauliflower) was bred in Dutch laboratories in the 1980s and named broccoflower in 1989. The goal of growing this plant is to produce an inflorescence that develops on a shortened stem, as in cauliflower, but the flower buds within the Shannon inflorescence are more pronounced in size. In broccoli, the bloom and apical portion of the bloom stem are used, as well as the lateral bloom that develop from the leaf axils after the apical bloom is harvested. In broccoflowerand cauliflower, top dominance is not pronounced and no side shoots with bloom are formed (Fabek, 2010).

As with other cabbage varieties, the optimum temperatures for vegetative growth of Shannon are 15 to 20°C. A combination of different climatic conditions and thus the growing area, appropriate choice of varieties and growing dates can ensure a continuous supply of broccoflower. Planting should be done in moisture-saturated soil (Lešić et al., 2002).

According to research by Csizinszky (1995), the determined minerals inbroccoflower was about 480 g P kg^{-1} DM (dry matter), 400 g Ca kg^{-1} DM, 180 g Mg kg^{-1} DM, and 550 g S kg^{-1} DM. For micronutrients, the copper content in all applied nitrogen treatments, which were 98, 196 and 294 kg N ha^{-1}, was determined about 12 mg Cu kg^{-1} DM, 22 mg Fe kg^{-1} DM and 41 mg Zn kg^{-1} DM For boron, with an increase in the amount of nitrogen consumed from 98 kg N to 294 kg N ha^{-1}, an increase in concentration from 29 to 39 mg B kg^{-1} DM was observed, while the manganese content increased from 35 to 43 mg Mn kg^{-1} DM.

The availability of iron depends primarily on the characteristics of the soil, especially the predominant clay minerals, the chemical and organic composition, and the pH reaction of the soil (Vukadinović and Vukadinović, 2011). Easily soluble Fe oxides are formed on very aerial soils, which have a slightly acidic to neutral pH, and this also complicates plant nutrition. Iron deficiency often occurs on alkaline soils that contain a lot of lime, especially if they are poor in organic matter. In addition to soil pH, the availability of iron is also influenced by the presence of antagonistic elements such as Mn, Cu, Zn, Ni, Co, Cr, which cause Fe chlorosis (Bergmann, 1992).

In plant tissue, about 80% of the iron is in the chloroplast stroma. Iron is involved in the structure of two types of proteins, the heme proteins and the Fe-S proteins. The heme protein group includes cytochromes, peroxidases, catalases, and leghemoglobin; the Fe-S protein group includes feredoxin, which is important in oxidation-reduction processes, especially FS I (Kirkby and Mengel, 2001).

The manganese content in plants depends strongly on the plant species, but also on the plant part or organ. Manganese plays an extremely important role in oxido-reduction processes. It is a component of a number of enzymes and activator of enolases, carboxylases, superoxide dismutases and other enzymes, but it is not a building block because it is only a component of manganese protein. It is also important for the reduction of nitrates, so in the absence of manganese, nitrates accumulate because they are reduced slowly. Good manganese availability reduces the need for N, P, K, and Ca without reducing yields, so manganese is important for more economical use of other nutrients in the soil (Vukadinović and Vukadinović, 2011).

The physiological role of Zn is very extensive and significant, especially in protein metabolism. It is an integral component of many enzymes, where it forms tetrahedral chelates as a divalent cation, i.e., binds the enzyme to the substrate. It is involved in the structure of the enzymes: carbonic anhydrase, dehydrogenase (malate, glutamate, etc.), alcohol dehydrogenase, superoxide dismutase, etc., and is also their activator. The importance of Zn is extremely important in the biosynthesis of DNA and RNA (RNA polymerase), protein synthesis (through RNA transport and influence on ribosome structure), auxin synthesis, i.e. it influences plant growth (through the influence on tryptophan synthesis) and stabilization of biomembranes. Zinc is transferred from roots to shoots through the xylem tissue. However, high concentrations of zinc have also been detected in the phloem, suggesting that zinc flows through both conducting tissues. Nitrogen-deficient plants do not show zinc retranslocation from older leaves, suggesting that zinc deficiency symptoms are more pronounced in nitrogen-deficient plants (Marschner, 1995).

To gain insight into the significance of the results obtained, we decided to compare the contents of the three microelements studied with the contents of the same microelements in broccoli and cauliflower. According to Parađiković (2011), the contents of dry matter varies in both crops. In broccoli, the range is larger and ranges from 9 to 13%, while cauliflower varies less with 11 to 13%.

According to USDA (2013), broccoli contains 0.52 mg Fe 100 g^{-1}FM (fresh matter), 0.35 mg Zn 100 g^{-1}FM, and 0.151 mg Mn 100 g^{-1}FM. Cauliflower contains 0.2 mg Fe 100 g^{-1}FM, 0.11 mg Zn 100 g^{-1}FM, and 0.082 mg Mn 100 g^{-1}FM.

The objective of this study was to determine the effect of foliar fertilization on the content of the microelements iron, manganese, and zinc in broccoflower bloom.

MATERIALS AND METHODS

For this study, a field fertilization trial with broccoflower (Amfora variety, Bejo company) was conducted in 2009 at the Maksimir Experimental Station of the Department of Vegetable Crops of the Faculty of Agriculture in Zagreb. The trial was carried out according to the complete randomized block design in three replicates and with four foliar treatments: Control (water application), Zinc (0.125 g 0.1 L^{-1}), Drin-organic liquid biostimulant (0.1 mL 0.1 L^{-1}), Boron (0.175 g 0.1 L^{-1}). Foliar treatmentswere applied twice: 1st Oct 2009 and 14th Oct 2009. Sowing was performed on 30th June 2009 and planting in the field on 31st July 2009 on the soil mulched with foil. The row spacing was 0.5 m and in the row 0.4 m, which corresponds to 5 plants per 1 m^2. The basic experimental plot consisted of two rows with 7 plants per row (14 plants per 1 plot). The harvest was carried out once.

The standard agricultural measures were used. Soil fertilization was applied before foil spreading on the soil surface based on the planned yield of 30 t ha^{-1} (667 kg NPK 7-20-30 ha^{-1}, 100 kg K_2SO_4 ha^{-1}, and 400 kg KAN ha^{-1}. Foliar fertilization with urea was also applied twice during the growing season.
During harvest, broccoflower bloom were randomly selected from each plot for the average sample for chemical analysis. Samples were dried at 105° C and then grinded and homogenized. Iron, zinc and manganese were determined after digestion with conc. HNO_3 and $HClO_4$ in a microwave oven by atomic absorption spectrometer (AOAC, 1995) in the laboratory of the Department of Plant Nutrition at the Faculty of Agriculture in Zagreb.
Statistical data processing was performed using the analysis of variance model (ANOVA). The SAS System for Win. ver 9.1 program (SAS Institute Inc.) was used. Tukey's test for significant thresholds (SAS, 2002-2003) was used to test the results. Data were processed using the statistical software package SAS System for Win Ver. 9.1 (SAS Institute Inc., 2002-2003).

RESULTS AND DISCUSSIONS

Table 1 shows significant differences in the dry matter content. The lowest percentage of dry matter was determined in the control treatment (9.1%), and the highest in the treatment with zinc (10.3%). The obtained results are significantly lower than those of cauliflower (11-13%) and are at the lower range of the results that can be obtained with broccoli (9-13%) (Parađiković, 2011).

Table 1. Dry matter content in broccoflower bloom, depending on treatment

Treatments	% dry matter (DM)
Control	9.1 b
Zinc	10.3 a
Drin	9.4 ab
Boron	9.7 ab

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

Iron content in dry matter showed significant variations, from 80.6 mg Fe kg^{-1} DM in the zinc treatment to 95.8 mg Fe kg^{-1} DM in the control treatment (Table 2). The nutrients content in 100 g of fresh matter, which is considered the norm in the diet, is usually used as a reference content when calculating the ability to meet the needs of certain minerals, vitamins, carbohydrates and other food components. The iron content in broccoflowerfresh matter ranges from 0.82 to 0.87 mg Fe 100 g^{-1}FM (Table 2) and no statistically significant difference was found between fertilizer treatments. These results are significantly higher than the iron content of broccoli (0.52 mg Fe 100 g^{-1} FM) and cauliflower (0.2 mg Fe 100 g^{-1} FM), according to USDA (2013).

Table 2. Broccoflowerbloom iron content in dry and fresh matter, depending on treatment

Treatments	mg Fe kg^{-1}DM	mg Fe 100 g^{-1} FM
Control	95.8 a	0.87
Zinc	90.6 c	0.82
Drin	90.8 ab	0.85
Boron	86.8 bc	0.84

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

In dry matter, again, the blooms of the control treatment are the richest with 28.5 mg Mn kg^{-1}DM and those of the zinc treatment the poorest with 25.9 mg Mn kg^{-1}DM. For fresh matter, the results are almost identical, ranging minimally from 0.26 to 0.27 mg Mn 100 g^{-1}FM. Compared to broccoli with 0.151 mg Mn 100 g^{-1}FM and cauliflower with 0.082 mg Mn 100 g^{-1}FM according to USDA (2013), we have once again results that prove to be significantly better (Table 3).

Table 3. Broccoflower bloom manganese content in dry and fresh matter, depending on treatment

Treatments	mg Mn kg^{-1}DM	mg Mn 100 g^{-1}FM
Control	28.5 a	0.26
Zinc	25.9 b	0.27
Drin	27.4 ab	0.26
Boron	26.4 b	0.26

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

Similar to iron, Table 4 shows that, again, the highest content of the observed element is found in the control treatment (68.1 mg Zn kg^{-1} DM) and the lowest in the zinc treatment (58.1 mg Zn kg^{-1} DM). As with iron, there are no significant differences in fresh matter, and zinc content ranges from 0.58 to 0.62 mg Zn 100 g^{-1} FM. Compared to broccoli (0.35 mg Zn 100 g^{-1} FM, according to USDA (2013) and cauliflower (0.11 mg Zn 100 g^{-1} FM, according to USDA (2013), broccoflower stands out as a much richer source of this mineral, even more so than for iron.

Tablica4. Broccoflower bloom zinc content in dry and fresh matter, depending on treatment

Treatments	mg Zn kg^{-1}DM	mg Zn 100 g^{-1}FM
Control	68.1 a	0.62
Zinc	58.1 b	0.59
Drin	64.2 ab	0.60
Boron	59.9 b	0.58

Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

Considering these results, it can be concluded that broccoflower is a very worthy agricultural culture. The first reason for this, is its excellent ability to uptake nutrients, which can be concluded from the very small variations in the results obtained in different treatments. One season may not be a reliable indicator, but the results obtained showed the cost-effectiveness of foliar fertilization in this crop under the given conditions, as the control treatment did not lag behind the others.

Regardless of the different treatments, the recorded contents of all nutrients showed that broccoflowercan be a significant source of these nutrients in the diet. Since these are nutrients, that a large part of today's population is deficient in, these results can be considered as an additional reason to increase the acreage of this crop and, consequently, the market for the studied product. The quality of the product was very high compared to its main competitors in terms of the parameters observed.

CONCLUSIONS

The obtained results showed the exceptional ability of broccoflower to accumulate micronutrients such as iron, manganese and zinc regardless the treatment. In 100 g of fresh broccoflower bloom, the highest levels of 0.87 mg Fe, 0.62 mg Zn, and 0.27 mg Mn were obtained, without statistically significant variations between treatments.

In addition to the other benefits, broccoflower offers the consumer, as well as the possibility of long-term storage with minimal loss of weight and quality, and there is little to counterbalance the reasons for growing this crop. So, it should suggest to increase acreage and market share so that the only real obstacle, the high price, can be removed. The data obtained gain particular significance in comparison with cauliflower and broccoli, the competitors, but also the "parents" of the observed crop.

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INCREASING THE NUTRITIONAL QUALITY OF *PLEUROTUS ERYNGII* BY GAMMA IRRADIATION OF LIVING MYCELIUM

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Abstract

Pleurotus eryngii, known as oyster mushroom, is a commercially cultivated edible mushroom, widely appreciated for its good taste and texture. In this study, we investigated the influence of low doses of gamma irradiation (between 100 and 300 Gy) applied to the living mycelium of two strains of P. eryngii (PeM39 and PeM41), in order to enhance the synthesis of bioactive metabolites with nutritional values. For this purpose, we determined the content of polyphenols, flavonoids, proteins and carbohydrates in ethanol extracts (70%) of P. eryngii fruiting bodies obtained from irradiated mycelium. The results showed that, in the fruiting bodies of both strains, the 300 Gy dose was optimal for stimulating an increase in phenols, flavonoid and carbohydrate content, and the 200 Gy dose was optimal for increasing protein content. Thus, gamma-irradiation treatment of mycelial inoculum may be an effective tool for stimulating the synthesis of secondary metabolites with antioxidant and nutritional properties in P. eryngii.

Key words: *Fruiting bodies, gamma irradiation, mycelium, metabolites synthesis, Pleurotus eryngii.*

INTRODUCTION

Pleurotus eryngii (also known as king oyster mushroom) is an edible mushroom cultivated widely in many regions of the world. *P. eryngii* is reportedly rich in protein, carbohydrates, unsaturated fatty acids, vitamins and other nutrients and is low in fat, making it a high-quality, low-calorie food. In addition, *P. eryngii* is also a rich source of the disaccharide, trehalose (Reis et al., 2012). Polysaccharides from *P. eryngii* have a variety of biological activities, including anti-oxidant, anti-hyperlipidaemia, antitumor, bacteriostatic and immune-regulatory (Zhang et al., 2020). Phenolic compounds are mushroom antioxidants which are strong radical scavengers and free radical inhibitors and phytonutrients (Michalak, 2006). Gamma irradiations are physically induced stresses that has been considered as the rapid and fast method to enhance the quality and quantity of plant characteristics (Patil et al., 2018). Bioactive compounds such as phenols and flavonoids can be induced under proper dose of radiation. Both polyphenols and flavonoids are chemical compounds, synthesized by the body, involved in antioxidant defence (Pelcaru et al., 2021). Plants exposed to gamma radiation produce various defence and antioxidant enzymes, many of which produce secondary metabolites that activate the induction of oxidative stress conditions. Gamma irradiation exerts its effects by inducing oxidative stress in the cells which increases the total phenol due to the release of phenolic compounds from glycosides components (Patil et al., 2018). Gamma irradiation is considered to be a physically induced stress on living organisms or cells. Radiation treatment can be a much faster way to quantitatively improve the chemical synthesis of antioxidant compounds that may play a role in defending irradiated tissue (Charbaji and Nabulsi, 1999). Most research uses gamma radiation as a conditioning agent for harvested mushrooms or their extracts to increase nutrient or antioxidant levels by breaking down existing compounds (Kim et al., 2009; Fernandes et al., 2012). In this study, we used gamma irradiation on the living mycelium of two isolates of *P. eryngii* (PeM39 and PeM41) as a tool to stimulate the production of biologically active metabolites in the fruiting bodies.

MATERIALS AND METHODS

Mushroom material. *Pleurotus eryngii* used in this study were purchased from the Research and Development Institute for Vegetable and Floriculture Vidra and consisted of two isolates named PeM39 and PeM41. Fruiting bodies of the two isolates were obtained from living mycelium irradiated at different irradiation doses.

Irradiation of the mycelium of *P. eryngii*. The irradiation was performed by using a ^{60}Co research irradiator GC-5000 (B.R.I.T. Mumbai, India) located in IRASM Radiation Processing Department of "Horia Hulubei" National Institute of Physics and Nuclear Engineering (Romania). Mycelial samples were acutely exposed to gamma rays at following average doses: 0 (control), 100, 200, and 300 Gy (Gray) respectively, at a dose rate of 0.8 Gy/s. An alanine dosimetry system was used for dose evaluation. The reference material for the doses is water. Irradiation temperature, as measured inside the irradiation chamber, was in the range of 27–28^{0}C.

Mycelium cultivation and mushroom production. The irradiated mycelium of the two *P. eryngii* isolates was grown at the Vidra Institute on a substrate composed of a mixture of sawdust, straw and corn stalks enriched with nitrogen-rich additives. The first carpophores appeared after about 10-15 days and after another few more days the mushrooms were harvested.

Preparation of *P. eryngii* extracts. The dried mushrooms were shredded using a blender. 50 ml of ethanol (95^0) was added to 5 g of powder of the two *P. eryngii* isolates. Rotary evaporator (Büchi, Model R-205V, Merck USA) was used to remove the solvents of the filtrate. The residues obtained were weighed and dissolved in 70% ethanol. The final concentration of the extracts was 100 mg / ml.

Total phenols content. The concentration of phenolic compounds in ethanol extracts of mushrooms was determined by using the Folin-Ciocâlteau (Sigma-Aldrich) method (Singleton, 1999). This is based on the reduction of the Folin-Ciocalteu reagent by phenolic compounds, which will form chromogens that can be detected spectrophotometrically at OD= 765 nm. For the determination of phenols, a calibration curve was carried out starting from standard solutions of Gallic acid. The total phenol content in the ethanol extracts was calculated based on the regression equation ($y = 0.001x + 0.0041$; $R = 0.9999$) obtained for different Gallic acid concentrations (50 - 500 mg/l) and expressed in milligrams of Gallic acid equivalents per liter extract (mg GAE/l). Both the samples and the reference substance were worked in triplicate, and their average absorbance value was calculated.

Total flavonoid content. The flavonoid content in ethanol extracts of *P. eryngii* isolates obtained from irradiated mycelium was quantified according to the colorimetric method of aluminum chloride, described by Piyanete et al., 2009. To achieve the reaction, 150 µl AlCl3 10% solution was added over the sample/ standard quercetin solution, and the mixtures was incubated at room temperature for 5 min., after which 200 µl of 1M sodium hydroxide solution was added sequentially. The standard curve was made of quercetin and comprised 5 dilutions (0.1, 0.5, 1.0, 1.5 and 2.5 mg/mL), starting from a stock solution of 1 mg/mL in 80% methanol. The absorbance was read at 510 nm, and the flavonoid content was calculated based on the regression equation: $y = 0.3604x + 0.0269$; $R = 0.9534$ and expressed in mg of quercetin equivalents (QE) per mL of extract.

Total proteins content. Total proteins content was quantified according to Bradford method (1976). The method is based on the use of Coomasie Blue G dye as reaction substrate. For determination of total proteins content a standard curve was made starting from standard BSA (bovine serum albumin) solutions (5 – 50 µg/ml). The reaction mixtures (Bradford reagent with samples or standard solutions of BSA) were measured spectrophotometrically at a wavelength of 595 nm. The total protein content was calculated based on the regression equation: $y = 0.0025x + 0.2233$; $R = 0.8099$. The results obtained were expressed in µg BSA/ml.

Total carbohydrates content. The total amount of carbohydrates was estimated using phenol-sulfuric acid method (Dubois et al., 1956) with a few modifications and proceeded with a curve where glucose concentrations ranging from 10 to 80 µg. After sample preparation, aliquots of 0.2 to 1.5 mL were pipetted into test tubes and the volume was filled until 2.0 mL with 0.8 mL of 5% (w/w) phenol and 5 mL of sulfuric acid. The tubes were then shaken and left to stand for 30 minutes for further reading in a spectrophotometer (Eppendorf UV-VIS) at 490 nm. The total sugar content in the tested samples was calculated based on the regression equation: $y = 0.0061x + 0.1014$; $R = 0.9987$. Results were expressed in µg glucose /ml.

Statistical Analysis. All experiments were performed in three replicates. Results were expressed as mean values (SEM) and standard deviation (±SD). The graphics were plotted by using Microsoft Office Excel 2010.

RESULTS AND DISCUSSIONS

Two *Pleurotus eryngii* isolates, PeM 31 and PeM 41, grown from mycelium irradiated at 100, 200 and 300 Gy, were harvested and analysed for total phenols, flavonoids, proteins and total sugars (carbohydrates). Analyses were performed on 70% ethanol extracts from the fruiting bodies of the two isolates.

Quantitative estimation of total phenol content. Phenols are important constituents of plants which are responsible for scavenging the free radical due to the presence of hydroxyl groups and it directly responsible for the anti-oxidative potential (Sharififar et al., 2009). The estimation of total phenolic content was expressed in terms of Gallic acid equivalent in milligram (mg) per litre. The calibration curve obtained from y equation ($y = 0.001x + 0.0041$; $R = 0.9999$) was used to determine the total phenol content (Figure 1). High phenol content values were found in both isolates of *P. eryngii* grown from mycelium irradiated at 200 and 300 Gy. Compared to the controls (PeM39 = 217.9±1.53 mg GAE/l and PeM41= 216.9±0.58 mg GAE/l, respectively), isolates from mycelia irradiated at 300 Gy showed the highest phenol concentrations: 312.23±2.91 mg GAE/l in *P. eryngii* PeM39 extracts and 237.96±1.53 mg GAE/l in *P. eryngii* PeM41 extracts. (Figures 1 and 2, Table 1).

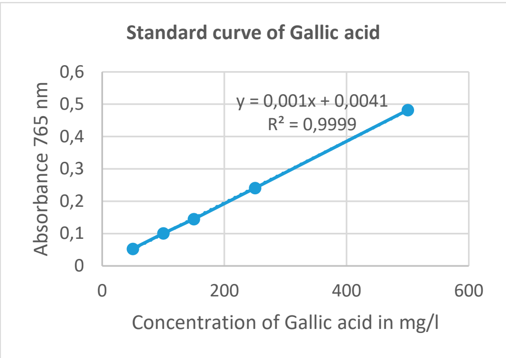

Figure 1. Gallic acid standard curve and the regression equation

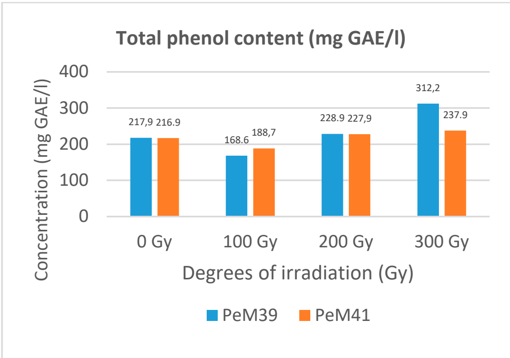

Figure 2. Total phenol content (mg GAE/l) in *P. eryngii* PeM39 and PeM41 from irradiated mycelium. Data represent the mean ± SD, $n = 3$

Estimation of total flavonoid content

Flavonoids are secondary metabolite having antioxidant properties (Wong and Chye, 2009). The total flavonoid content was expressed in terms of quercetin equivalent in mg/ ml. The calibration curve obtained from y equation ($y = 0.3604x + 0.0269$; $R = 0.9534$) was used to determine flavonoid content as shown in Figure 3. The total flavonoid content of the various extract of *P. eryngii* from gamma irradiated mycelium are represented in Figure 4 and Table 1. In this case, both isolates of *P. eryngii* grown from mycelium irradiated at 200 and 300 Gy showed the highest flavonoids concentrations in comparison to the controls (PeM39 = 1.53±0.01 mg QE/ml and PeM41 = 1.63±0.06 mg QE/ml). Irradiation dose of 300 Gy showed the highest amounts of content 2.15±0.09 mg QE/ml in PeM39 samples and 2.51±0.09 mg QE/ml in PeM41 samples.

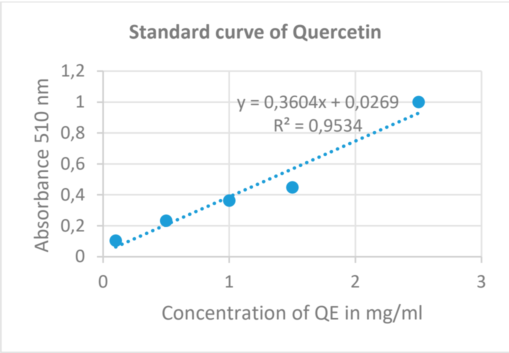

Figure 3. Standard curve of Quercetin and the regression equation

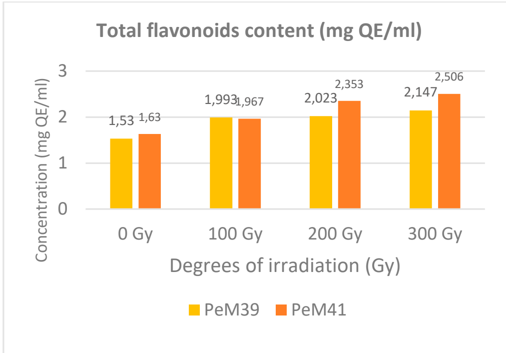

Figure 4. Total flavonoid content (mg QE / ml) in *P. eryngii* isolates from irradiated mycelium. Data represent the mean ± SD, n = 3

Estimation of total proteins content

The total protein content was calculated based on the regression equation: y = 0.0025x + 0.2233; R = 0.8099. The results obtained were expressed in µg BSA/ml (Figure 5). High protein content values were found in both isolates of *P. eryngii* grown from mycelium irradiated at 100 and 200 Gy. Compared to the controls, 14.08±3.44 mg µg BSA /ml in PeM39 extracts and 17.28±2.2 µg BSA /ml in PeM41 extracts, isolates from mycelia irradiated at 200 Gy showed the highest proteins values, 33.68±4.61 µg BSA /ml in *P. eryngii* PeM39 extracts and 21.68±0.2 µg BSA /ml in *P. eryngii* PeM41 extracts. (Figure 6, Table 1).

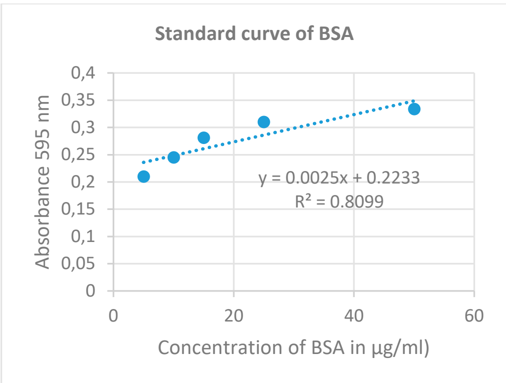

Figure 5. Standard curve of BSA and the regression equation

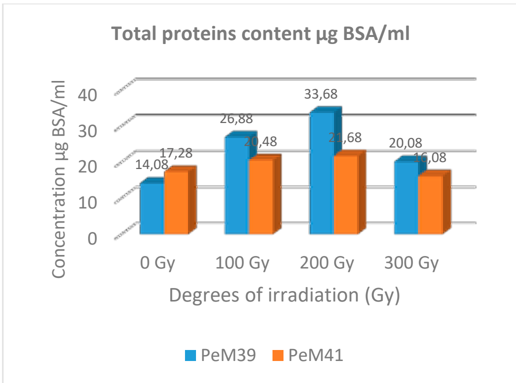

Figure 6. Total protein content (µg BSA / ml) in samples of *P. eryngii* isolates grown from irradiated mycelium. Data represent the mean ± SD, n = 3

Estimation of total carbohydrates

The concentration of total carbohydrates content in the tested samples was calculated based on the regression equation: y = 0.0061x + 0.1014; R = 0.9987 (Figure 7). Results were expressed in µg glucose /ml. In this study, the 92±6.53 µg glucose /ml and in PeM41 with 66.27±5.17 µg glucose /ml as compared to controls which are 51.41±5.79 µg glucose /ml highest total carbohydrates values were found in both isolates of *P. eryngii* grown from mycelium irradiated at 300 Gy. At this dose, highest values of total carbohydrates were found in PeM39 with 63.

for PeM39 and 56.16±1.25 µg glucose /ml for PeM41 (Figure 8 and Table 1). It was found that both isolates from mycelium irradiated at

100 Gy, the carbohydrate concentration decreased compared to the controls, and increased with increasing irradiation at 200 Gy and 300 Gy respectively.

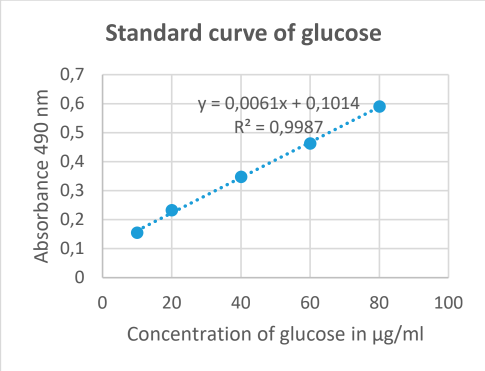

Figure 7. Standard curve of Glucose and the regression equation

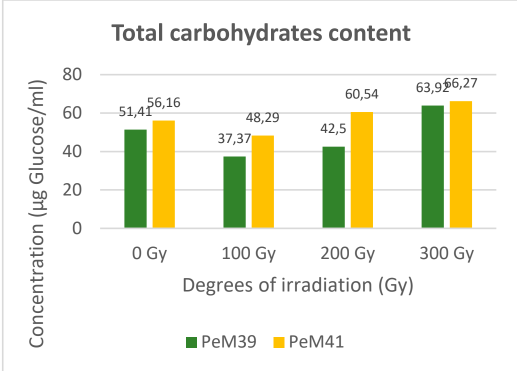

Figure 8. Total carbohydrate content (µg Glucose / ml) in *P. eryngii* isolates grown from irradiated mycelium. Data represent the mean ± SD, *n* = 3

Table 1 shows the data obtained on the total content of phenols, flavonoids, proteins and carbohydrates in ethanol extracts of *P. eryngii* PeM 39 and *P. eryngii* PeM 41 obtained from fruiting bodies results from mycelia subjected to different degrees of irradiation.

These data reveal that the total phenol content decreased in both isolates originating from fungal mycelium irradiated at 100 Gy and then increased from 200 Gy to 300 Gy.

This indicates that there is an accumulation of phenolic compounds in the body of the fungus with increasing irradiation doses. The same situation was found for the carbohydrate content. Initially, in both isolates grown from the fungal mycelium irradiated at 100 Gy the carbohydrate content decreased significantly, and then there was an increase in carbohydrate content with increasing irradiation doses.

Estimation of total flavonoid content showed that flavonoids accumulate in the fungus with increasing irradiation levels, which has been observed in both isolates.

It was also observed that the irradiation doses of 100 Gy and 200 Gy, to which the mycelium of the two isolates were subjected, were optimal for the accumulation of higher amounts of protein in the body of the fungus.

Table 1. Total phenols, flavonoids, proteins and carbohydrates content in fruiting bodies of *P. eryngii* isolates from gamma irradiated mycelium

Sample	Irradiation dose (Gray)	Total phenols (mg GAE /l)	Total flavonoid (mg QE /ml)	Proteins (µg BSA /ml)	Carbohydrate (µg Glucose /ml)
***P. eryngii* PeM 39**	**0**	217.90±1.53	1.53±0.01	14.08±3.44	51.41±5.79
	100	168.57±1.33	1.99±0.05	26.88±1.00	37.37±4.47
	200	228.90± 5.03	2.02±0.03	**33.68±4.61**	42.50±0.92
	300	**312.23±2.91**	**2.15±0.09**	20.08±0.64	**63.92±6.53**
Sample	**Irradiation dose (Gray)**	**Total phenols (mg GAE /l)**	**Total flavonoid (mg QE /ml)**	**Proteins (µg BSA /ml)**	**Carbohydrate (µg Glucose /ml)**
***P. eryngii* PeM 41**	**0**	216.90±0.58	1.63±0.06	17.28±2.20	56.16±1.25
	100	188.92±4.51	1.97±0.03	20.48±0.60	48.29±1.77
	200	227.91±8.39	2.35±0.22	**21.68±0.20**	60.54±4.52
	300	**237.96±1.53**	**2.51±0.09**	16.08±1.43	**66.27±5.17**

Data represent the mean ± SD, *n* = 3

CONCLUSIONS

As a result of these studies, it can be concluded that the increase in irradiation at doses ranging from 200 to 300 Gy, which was subjected to the mycelium from which the fruiting bodies resulted, leads to an accumulation of phenolic compounds, flavonoids and carbohydrates. Protein accumulation occurred only in isolates grown from mycelium irradiated at doses lower than 300 Gy. To our knowledge, this is the first work showing the use of sub lethal doses of gamma irradiation as a treatment of *P. eryngii* living mycelium for increasing synthesis of biologically active metabolites with antioxidant and nutritional properties in fruiting bodies of the fungus.

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MORPHOLOGICAL CHARACTERIZATION AND GENETIC VARIABILITY ASSESSMENT WITH SSR MARKERS IN SEVERAL TOMATO GENOTYPES

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Abstract

A complete morphological description of the tomato genotypes is necessary either for the new cultivars under approval, or for the recommendation of proper tomato cultivars in certain cultural conditions. In this study, the morphological and molecular diversity of 13 tomato genotypes were analyzed to identify distinctness among them. The genetic diversity was evaluated with 8 SSR markers. The efficiency of these markers to reveal the genetic differences with tomato genotypes was proven by: a mean number of scorable bands per marker of 6.62, of which 81.5% were polymorphic bands and the polymorphic information content of 0.764. The cluster analysis grouped the 13 tomato genotypes into two distinct clusters, depending on their type of growth, and inside each group in correlation with parental origin. The evaluation of the relevant characteristics with specific descriptors demonstrated the differences between the genotypes analyzed in terms of their type of growth and the different aspects of the leaves and fruits. Combining the morphological description with molecular methods proved to be efficient for the assessment of distinctiveness among analyzed tomatoes and necessary for documented recommendations for tomato growers.

Key words*: dendrogram, polymorphism, Solanum lycopersicum, standard descriptors, variability.*

INTRODUCTION

Global population growth and climate change are the main reasons for researchers to accelerate their investigations and promote new plant varieties to meet increasing demand for high-quality foods, with good nutritional value.
Tomato (*Solanum lycopersicum* L.) is one of the most popular and economically advantageous crops in the world because of its widely cultivated area all over the world, under a wide range of agro-climatic conditions, its nutritional value (Todorovska et al., 2014; Renna et al. 2018), and its extensive use for culinary purposes. Therefore, the tomato is intensively studied not only as a model plant for studies of classical and molecular genetics (Titeli et al. 2021; Xia et al. 2021) but also as a crop plant for which the quantity and quality of tomato production in different climatic conditions are of global interest (Gerszberg & Hnatuszko-Konka, 2017).
The morphological characterization and the assessment of genetic diversity with different molecular markers are considered important tools in exploring tomato germplasm aiming for efficient use of the accessions in breeding programs (Sun et al., 2012; Wang et al., 2016; Ronga et al., 2018; Al-Shammari & Hamdi, 2021).
In Romania, the outstanding achievements of the last 10 years in the development of new tomato varieties and their promotion in the consumer market, or in the processing industry, require an estimate of the genetic diversity of the cultivated accessions (Felföldi et al., 2021). Therefore, aiming to have efficient utilization, conservation and management of tomato genetic resources, the present study was conducted for the followings: (i) to evaluate and register the diversity of morphological traits for 13 tomato genotypes necessary for documentation of each accession; (ii) to assess the genetic diversity using SSR markers; (iii) to apply the cluster analysis using markers (SSR) to prove the discrimination capacity of tested SSR markers for genotypes identification and also to show the degree of variability among the analyzed genotypes.

MATERIALS AND METHODS

Plant material. The tomato genotypes presented in Table 1 were studied at NR&DIB Ştefăneşti-Argeş, Romania. The seedlings were obtained by germination of selected seeds in plastic trays on peat substrate. All the quality seedlings, with normal development and a rich root system, healthy and hardened, were planted in unheated greenhouse conditions (the varieties with undetermined growth), or in field conditions (the varieties with determined growth).

Table 1. Plant materials used in this study, their origin and main features

Tomato genotype	Important agronomic characters
Indeterminate growth	
Costate 21 Selection from the Pablo variety	-fruit weight (about 300 g per fruit) -special aspect of the fruit (sweet pepper appearance) -rich foliage density
Ștefănești 22 Rila variety selection	-high productivity per plant (over 3 kg). -tolerance to tomato-specific pests
Ștefănești 24 Rila variety selection	-could be grown in the greenhouse and in the field -good tolerance to disease and pest
HA1 Hybrid	-high tolerance to tomato-specific diseases -ability to adapt to different environmental conditions (type of soil and temperature) -sweet fruits with special taste
HA2 Hybrid	-produces many fruits in the bunches -special taste, very sweet
HA3 Hybrid	-the elongated shape of the fruit (capsicum appearance) -could be grown in protected areas and in the field
Determinate growth	
Argeș 20 Selection from the Argeş 11 variety	-fruit weight (about 200 g) -commercial appearance -good tolerance to tomato specific pests and different environmental conditions
Argeș 11 NotoriusxHeinz2274	-few seeds in the fruit -good productivity per plant
HB4 Hybrid	-long storage time, over 30 days - tolerance to the main diseases -recommended for growing in the field
HB5 Hybrid	-firmness of the fruits -resistance to cracking and sunburn -needs reduced numbers of practical works
HB6 Hybrid	-good production potential -tolerance to specific diseases and pests -recommended for broth production
HB7 Hybrid	-stability and uniformity of the main features -high content of carotenoid and vitamin C in the fruit
HB8 Hybrid	-good resistance to transport and storage -suitable for organic crop and greenhouse condition

Morphological description. Throughout the growth and development of the plants, observations were made and the morphological characteristics of the plants of each variety were registered according to the Descriptors for Tomato (*Lycopersicon* spp.) published by the International Plant Genetic Resources Institute (1996). This characterization includes measurements of whole plant height, size of floral components, fruit morphology and weight. All observations and measurements were carried out as indicated by the IPGRI descriptors, at certain plant organs, and at a certain phase of development.

DNA isolation. Young leaves were collected from adult tomato plants for total DNA extraction. This was performed according to the method recommended by Ahmed et al. (2009) with few modifications (Bădulescu et al., 2020). The total DNA extracted samples were verified for their quantity and quality by using a spectrophotometer BioPhotometer plus (Eppendorf).

DNA amplification. From each DNA sample were used 3 μl for amplification with 8 different SSR primers: SSR T-7, SSR47, SSR T-62, SSR63, SSR110, SSR111, SLM6-7, SLM6-12. The reaction mix contained: 5 μl of 5x FirePol Master Mix Ready to load (Solis BioDyne, Estonia), 2 μl of each forward and reverse primer, 3 μl DNA template, and water to a total volume of 25 μl. The amplification process was done with Techne TC-512 Thermal Cycler as follows: initial denaturation of 4 min at 94 °C; 35 cycles, each with 1 min denaturation at 94 °C, 1 min for primers annealing at 55 °C, and 2 min for elongation at 72°C; the final extension of 7 min at 72°C, and amplified products maintenance at 4°C.

Electrophoresis and DNA products visualization. After amplification were taken 5 μl for each sample to migrate on agarose gel (3.0% agarose with TAE buffer, and stained with ethidium bromide), at 75 volts for 1 hour. The migrated bands were visualized and photographed with Gene Flash Syngene Bio Imaging system under UV light. To estimate the size of amplified DNA bands was used the ladder Quick-Load Purple 50 bp DNA Ladder.

Data collection and analysis. For each SSR marker were recorded all scorable bands (the amplified DNA products), and were calculated the percentages of polymorphic bands: (number of polymorph bands/total number of bands) x100. The Polymorphism Information Content (PIC value) was calculated with the formula recommended by Botstein et al. (1980):

PIC = $1-\sum p^2$, where p represents each allele frequency for an SSR in all analyzed genotypes.
The amplified products were scored as present (1), or absent (0), and the similarity Dice coefficients were calculated for each genotype in comparison with all the others and for each marker (Dice, 1945). The similarity analyses and the dendrogram were done and produced using NTSYS-pc version 2.2. (Rohlf, 2000).

RESULTS AND DISCUSSIONS

Morphological characterization. All collected data with the main and distinct descriptors are presented in Table 2. In the first stage of evaluation, the seedling phase, we found that out of the total of 5 descriptors (7.1.1.), only for 2 descriptors (measurements for the length and width of the primary leaves) there were differences, the other characteristics regarding the green color of the hypocotyl, the intermediate coloration intensity and the presence of pubescence were the same in all genotypes.
In the case of genotypes with indeterminate growth, in greenhouse conditions, the plants were directed to a maximum height between 167 cm (Ştefăneşti 24) and 250 cm (Costate 21), and in the case of genotypes with determinate growth, the plant heights varied from 49 cm (Argeş 11 and Argeş 20) to 77 cm (HB7).
The morphological descriptors for stem and leaf (7.1.2.) highlighted several differences between genotypes: (i) the density of pubescence on the stem was slightly different between the genotypes with indeterminate growth (rare in HA2 and intermediate in the other 5 genotypes), and genotypes with determinate growth that expressed variations from rare to very dense (HB7); (ii) specific for the genotypes with determinate growth is the short length of the internodes, denoted by 3; (iii) dense foliage was noticed for the genotypes Costate 21, HA2 and HB7; (iv) the shape of the mature leaf is very different: potato-type (HB8), standard (Argeş 20, Ştefăneşti 22, HA1, HA2 and HB6), peruvian (Costate 21, HB4 and HB7), pimpinellifolium (Argeş 11, Ştefăneşti 24, HA3 and HB5).
A larger number of descriptors were used to characterize the inflorescences (7.2.1.), but obvious differences between genotypes were noticed only for the position of the style compared to that of the stamens. The Costate 21 variety stood out with its slightly exserted position at the moment of anthesis.
For a consumer, very important is the aspect of the fruit, its color, and shape.
The descriptors for the shape (7.2.2.5.) and size (7.2.2.6.) of the fruits revealed particularities of each tomato genotype: flattened (1) for Costate 21; slightly flattened (2) for Ştefăneşti 22, HA1, HB4, and HB7; round (3) for Argeş 20 and HB8; round elongated (4) for Ştefăneşti 24, Argeş 11 and HB6; cylindrical/long oblong (6) for HA2, HA3, and HB5. In correlation with this characteristic is the latitudinal cross-section shape of the fruit (7.2.2.29), which varied from irregular in Costate 21 variety, angular in Ştefăneşti 24 and HA3, to round in the other studied genotypes. Specific for each one is the color of the fruit at maturity (7.2.2.11), which varied from yellow for HA2, slightly orange for Ştefăneşti 24 and HB7, to red for the rest of described tomatoes. In correlation with this descriptor, the color of the pericarp (7.2.2.26) was noted, which varied from yellow (2) for HA2 and HB7 to orange (3) for Ştefăneşti 24, pink (4) for Ştefăneşti 22, HB4, and HB5, red (5) for all the other genotypes showing the same red external color of the fruit.
The differences among the harvested fruits of the same genotype in terms of fruit size (length /width), the average weight/fruit, and external color, were the basic criteria for assessing the homogeneity of fruit size, as follows: low (3) for Argeş 20, Ştefăneşti 24 and Costate 21; intermediate (5) for Argeş 11, HA1, HB4, HB5, and HB7; high (7) for Ştefăneşti 22, HA2, HA3, HB6, and HB8.
The descriptors for the average quantifiable evaluations regarding the weight and size of the fruit (7.2.2.8, 7.2.2.9, and 7.2.2.10), as well as the ones referring to the number of places, aspect of pistil scar, and fruit blossom end shape, are the specific ones of each genotype.
The presented results show the differences in terms of morphological descriptors among the analyzed genotypes, and the repetition of the records 3 years in a row proved the stability and genetic uniformity of the seed material.

Table 2. Evaluation of the morphological traits in tomato analyzed genotypes with standard descriptors

Genotype	Descriptors for tomato									
	7.1.2.1	7.1.2.2	7.1.2.3. (cm)	7.1.2.4.	7.1.2.5.	7.1.2.6.	7.1.2.9.	7.2.1.7.	7.2.2.5.	7.2.2.6.
Stefanesti 22	4	7	212	5	3	5	3	2	2	3
Stefanesti 24	4	5	167	5	5	5	5	2	4	3
Costate 21	4	7	250	5	5	7	4	3	1	3
HA1	4	7	198	5	5	5	3	2	2	3
HA2	4	5	175	3	3	7	3	2	6	2
HA3	4	7	210	5	5	5	5	1	6	4
Arges 11	2	3	50	3	3	3	5	2	4	3
Arges 20	2	3	50	3	3	3	3	1	3	5
HB4	2	3	69	3	3	5	4	1	2	5
HB5	2	3	65	5	3	5	5	1	6	3
HB6	2	3	65	5	3	5	3	1	4	3
HB7	2	3	77	7	3	7	4	1	2	5
HB8	2	3	68	5	3	5	2	2	3	3

Genotype	Descriptors for tomato								
	7.2.2.8. (g)	7.2.2.9. (mm)	7.2.2.10. (mm)	7.2.2.11	7.2.2.26	7.2.2.29.	7.2.2.31.	7.2.2.32	7.2.2.33
Stefanesti 22	199	6.6	7.1	5	4	1	5	2	2
Stefanesti 24	194	6.6	6.7	3	3	2	4	4	2
Costate 21	233	6.5	7.7	5	5	3	7	4	2
HA1	125	5.3	6.3	5	5	1	5	2	2
HA2	52	7.7	3.4	2	2	1	3	3	3
HA3	190	9.9	6	5	5	2	5	2	3
Arges 11	140	6.9	6	5	5	1	3	2	2
Arges 20	183	6.6	6.4	5	5	1	5	2	2
HB4	196	6.7	7.3	5	4	1	5	2	2
HB5	91	7.1	4.7	5	4	1	3	1	2
HB6	103	6.1	5.5	5	5	1	3	2	2
HB7	208	6.3	7.7	3	2	1	6	4	2
HB8	94	5.2	5.4	5	5	1	3	2	2

Genetic diversity using SSR markers. The reproducible amplification products obtained after PCR amplification were analyzed to evaluate the efficiency of the selected primers in confirming the genetic diversity of the analyzed tomato genotypes.

From the beginning, we chose to use only SSR primers that produce multiple bands with tomato varieties (Rajput et al., 2006; Saravanan et al., 2014). Also, for allele scoring, we considered the minor and shadow bands for proving the differences among genotypes, similar to Rodriguez et al. (2001), and Diklesh et al. (2016). After migration on agarose gel were noticed a total number of 315 bands, migrated at a distance corresponding to a base-pair number between 100 and 425. All the 8 tested SSRs had with the 13 tomato genotypes a mean number of 6.6 bands/primer and produced polymorphic bands in a proportion of 81.5%. Our results (Table 3) showed the efficiency of these SSRs for revealing the differences among analyzed genotypes. Moreover, the mean value for the polymorphic information content (PIC) for all SSRs of 0.764, is another reason to consider all tested SSRs as efficient markers in detecting the genetic polymorphism. The highest PIC values calculated for SSR 110, SSR 47, SSR 63, and SLM 6-7 are directly correlated with their highest values for the number of alleles and also, for the lowest value of the sum of the square for the allele frequencies of all genotypes. Thus, according to Serrote et al (2020), these markers could be considered the most informative ones for the efficient discrimination of the tomato genotypes.

The matrix with similarity coefficients (data not shown) was obtained by using UPGMA method, and the constructed dendrogram is presented in Figure 1. This dendrogram is based on the genetic similarity calculated for the tested SSRs and clearly revealed the clustering of tomato genotypes depending on their type of growth. The first cluster includes only genotypes with indeterminate growth (Costate 21, Ştefăneşti 22, Ştefăneşti 24, HA1, HA2 and HA3) and a genetic similarity ranging from 0.50 to 0.87. Distinct could be considered HA3, which has very low similarity coefficients with all the other tomato genotypes (0 with Ştefăneşti 24, or 0.25 with Ştefăneşti 22 and HA1, or 0.37 with HA2).

The second cluster grouped the tomato genotypes with determinate growth (Argeş 20, Argeş 11, HB4, HB5, HB6, HB7, and HB8), for which the similarity was ranging from 0.12 to 0.62. Within this group of genotypes, the HB4 hybrid is distinguished, with the lowest recorded values of similarity coefficients, from 0 to only 0.25 with the two varieties of Argeş.

Our evaluations with 8 polymorphic SSR markers, the data recorded in the matrix with similarity coefficients, and the obtained dendrogram, brought forward the following: (i) the two varieties of Ştefăneşti have a similarity coefficient of 0.5, both varieties coming from selections of the same variety, Rila; (ii) the similarity coefficient between the two Argeş varieties is only 0.25, due to the origin of the variety Argeş 20 from the variety Argeş 11, which is a complex hybrid.

Table 3. Results with relevant parameters proving the efficiency of tested SSR markers

Marker identifier	Band size intervals	Number of scorable alleles	Polymorphic alleles %	Polymorphic information content (PIC)
SSR 110	100-375	8	87.5	0.866
SSR 111	100-300	5	80.0	0.695
SSR T-7	100-275	4	75.0	0.635
SSR 47	100-350	8	87.5	0.806
SSR 62	100-425	6	83.3	0.734
SSR 63	100-350	9	85.7	0.847
SLM 6-7	100-400	9	77.8	0.837
SLM 6-12	100-350	4	75.0	0.697
Average±St.dev		6.62±2.1	81.5±5.2	0.764±0.08

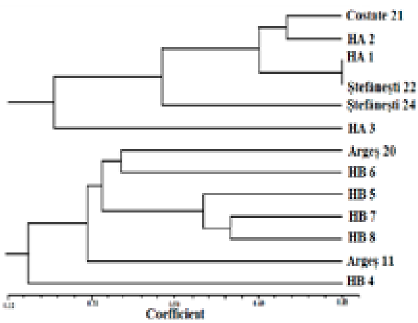

Figure 1. Dendrogram showing the genetic similarity among the 13 tomato genotypes derived by SSR markers

General considerations resulting from the research. The standard descriptors are the most used tool for the characterization and identification of tomato varieties (Vînătoru et al. 2016; Muşat et al. 2019; Salim et al. 2020). During the growing season different cultural conditions (soil nutrition, water supply, diurnal temperature variation, and different light spectral composition) are essential to optimize plant growth (Mihnea et al. 2019; Uleanu & Bădulescu, 2017; Vînătoru et al., 2015). Depending on these, the same genotype may have different morphological features. Thus, it is recommended to repeat at least 2 years consecutively the same procedure of analysis and evaluation of the plant organs from germination to fruit harvest, following a certain methodology. Similar to other economically important species, the assessment of genetic diversity among tomato varieties is performed based on morphological descriptors, and molecular characterization (Garcia et al., 2004). Starting from the generally accepted ideas that a high similarity value indicates a low degree of genetic variability (Arrufitasari et al., 2022), and that SSR markers producing multiple alleles per locus are considered suitable to characterize genetic diversity within or between populations (Kosman & Leonard, 2005), we consider the applied procedure to be appropriate for highlighting the genetic polymorphism among the analyzed tomato genotypes. The genetic similarity with the 13 tomato genotypes, based on 8 SSRs, ranging between 0.13 and 0.88, shows the great genetic variability among studied tomato genotypes and indicates a very useful genetic resource for breeding purposes.

Obviously, we agree with Benor et al. (2008), Kim et al. (2017), Castellana et al. (2020), Aziz et al. (2021) considering that a higher number of SSR markers would contribute to an even better characterization of the germplasm in tomatoes and to successful exploitation of the agronomically important traits.

CONCLUSIONS

The assessment of genetic diversity among 13 tomato genotypes with standardized morphological descriptors and molecular markers highlighted the high diversity of the main characteristics of the genotypes as a result of interaction between genetic information and the environment. Phenotypic diversity is valuable for a breeder, mainly for the selection of new genitors to improve the fruit quality traits and yield potential, but correlations with the genetic base of these traits increase its usefulness in breeding programmes.

The chosen SSRs for genetic evaluation and the results obtained after amplification confirmed that these markers are efficient and adequate for highlighting the distinctiveness among tomato genotypes. Interpretation of the results obtained with the eight SSR markers, as well as the similarity coefficients results, proved a very high degree of genetic diversity (between 13% and 88%) among the analyzed genotypes.

If the diversity of morphological characteristics is important for the curator (to identify accessions) and the breeder (to choose the appropriate material for certain breeding purposes), the assessment at the molecular level is that which brings certainty to the correct identification and characterization of tomato genotypes.

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EXPRESSIVENESS OF THE MAIN QUANTITATIVE CHARACTERS OF *RAPHANUS SATIVUS* VAR. *CAUDATUS* ACHIEVED AT PLANT GENETIC RESOURCES BANK BUZAU

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Abstract

PGRB Buzau has a valuable germplasm collection of Raphanus sativus. The bank's specialists have been involved in the conservation and improvement of this species' resources, obtaining two varieties registered in the Official Catalogue of Romanian Cultivated Plants. A large number of genotypes of this species was obtained due to the high degree of entomophily. Within the Bank, a considerable number of genotypes have been obtained, among which one valuable genotype has been biologically isolated from the others, showing genetic stability of the main characters, successfully passing the DUS test. The research undertaken in the present study reflects the expressiveness of the main quantitative characters of the newly obtained genotype. The genotype was evaluated in two growing environments, protected and field, and it was found that both quantitative traits in terms of plant height, height, number of shoots, pod production, pod size and yield per plant were clearly superior (1174 g pods/plant) in protected environments. In the field, production was significantly lower (8.7 g pods/plant) but with much lower cost prices.

Key words: *pod, genotype, expressivity, edible, entomophilous.*

INTRODUCTION

Radish green (*Raphanus sativus* var. *caudatus*) is a small-sized Brassicaceae family that grows as a tuber root and is a vegetable with a short growth period (Hidayat E., 2021). Research on wild vegetable plants and their possible introduction into the human diet for diversification of nutrient intake is important for the genetic conservation, propagation, improvement, and valorisation of these wild genotypes. The Food and Agriculture Organization recognizes the importance of consumption of vegetables (and fruits) as the inadequate consumption of these crops was the cause of 14% gastrointestinal cancer deaths, 11% of ischemic heart disease deaths and 9% of stroke deaths (FAO 2016).

Medicinal plants continue to play an important role in the lives of a significant proportion of the global population (Patel et all., 2021).

Recently, wild vegetable species have started to be prioritised in research and breeding programmes. Many are quite unaware that underutilized vegetables and wild food plants (that can be used as vegetables) can be tapped alongside the more popular vegetables in the market to increase vegetable consumption (Zhang et all., 2021). Very little is known about their use, genetic description, breeding possibilities and conservative selection.

Plants having India inception are *Oryza sativa*, rice; *Eleusine coracana*, African millet; *Cicer arietinum*, chickpea; *Phaseolus aconitifolius*, Mothbean; *Phaseolus calcaratus*, ricebean; *Dolichos biflorus*, Horsegram; *Vigna sinensis*, Asparagus bean; *Solanum melongena*, eggplant; *Raphanus caudatus*, Rats tail radish (Rao D.N., 2017).

Further research on the various wild relatives of cultivated vegetable plants is necessary to ensure food security. These have a superior capacity to adapt to changing climatic conditions and to attacks by various pathogens.

Raphanus sativus contains several domesticated varieties, such as cherry belle radish *(R. sativus* var. *radicula*), black radish (*R. sativus* var. *niger*), rat’s tail radish (*R. sativus* var.

caudatus), oilseed radish (*R. sativus* var. *oleiformis*), and the most widely planted cultivar of radish, *R. sativus* var. l*ongipinnatus* (Zhang et al., 2021).

In the West, it was introduced from Java (Indonesia) to England around 1815. It probably began to conquer new territories of the world through trade, at that time reaching America and Europe, where it was cultivated sporadically. In Romania, this species arrived quite late (Vînătoru et al., 2019).

PGRB Buzau has a valuable germplasm collection of *Raphanus sativus*. The bank's specialists have been involved in the conservation and improvement of this species' resources, obtaining two varieties registered in the Official Catalogue of Romanian Cultivated Plants.

As a result of the increasing interest in biodiversity conservation and gastronomic interest for new species, since 2000 a new variety of *Raphanus* has been studied which has not been cultivated in Romania so far, namely *Raphanus sativus* var. *caudatus*.

Edible parts of the plant are represented by the leaves which should be eaten when young, either raw or cooked, presenting a slightly spicy taste. The flowers can also be eaten raw. The most desirable part from the nutritional point of view is the seed-bearing silica.

It is recommended to eat them at an immature stage, before the seeds form, because as the seeds form, the silica seeds turn bitter and spicy.

They can be eaten raw, like common radishes. They can be stored in the refrigerator for about three weeks, frozen or pickled. In addition to other uses, the species can be successfully grown and used in microgreens.

Radish has been reported for the occurrence of flavones, flavonoids, isoflavone, catechins, isocatechins, coumarin lignans and anthocyanins that may contribute to its pharmacological potential (Agil et al., 2006).

MATERIALS AND METHODS

A large number of genotypes were obtained for this species due to the high degree of entomophily. At Bank level, a considerable number of genotypes in various stages of improvement have been obtained, of which 3 genotypes are genetically stabilised. Of these, the G2 genotype showed superiority in terms of genetic stability and yield capacity and was included in this study. In order to ensure the purity of the genotype, it was biologically isolated from the other genotypes in culture, demonstrating genetic stability of the main characters, successfully passing the DUS test.

The research undertaken in the present study shows the expressivity of the main quantitative characters of the newly obtained genotype.

Research on the establishment of the crop by both direct sowing and seedling has been developed.

Sowing in the field was carried out around 15th of March.

Sowing was carried out in 70-hole pallets in cold protected areas around 1st-5th March.

The field crop establishment scheme was as follows: the land was shaped with the L 445 tractor in aggregate with MMS 2.8 with 140 mm spacing, 94 cm at the crown and 70 cm between rows, and 12 cm from the edge of the crown, 46 cm ruler (Figure 1).

Sowing was carried out respecting distances of 70 cm between rows and 30 cm between plants/row.

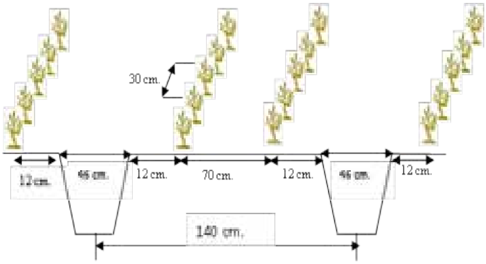

Figure 1. Field crop system

For the cultivation in protected areas, a planting pattern of 70 cm between strips and 35-40 cm between plants/row was used. In cold protected areas the crop is established after 15th of February (Figure 2).

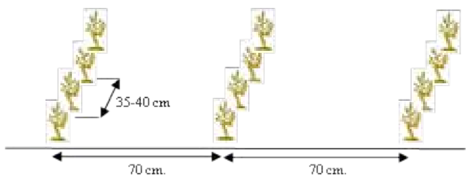

Figure 2. Protected areas crop system

Phenological observations, biometric and laboratory determinations were carried out during the growing season.

RESULTS AND DISCUSSIONS

Harvesting is recommended from the basis of the inflorescence to the apex.

In the open field, harvesting is carried out in stages, from the basis of the plant to the apex, when the pods are juicy and have a size between 3-12 cm, until the milk-wax phenophase is exhausted.

In protected areas, harvesting can be done in stages from 15th of April until 30 May (Figures 3, 4).

Figure 3. Flower and seed pod detail

Figure 4. Seed pods in different ripening stages

For seed production, it is recommended to establish the crop by seedling, with the mention that the seed pods reach maturity after 15th of June (Figure 5).

Figure 5. Fully ripened seed pods

Research has shown that in the soil and climatic conditions of our country, both in protected areas and in the open field, it is not possible to obtain plants with a high production of pods in very cold periods, because the species is entomophilous.

Aside from the fact that the flowers do not tolerate low temperatures below 6 degrees, it should be mentioned that at these temperatures, natural pollinators are absent.

Special attention should be paid to the biological isolation distances from other varieties and also to the seed lots.

After the seeds have reached the wax stage, they must be protected against pests, especially sparrows, as these seed pods are an important food source for them.

High temperatures of over 25 degrees cause the crop to ripen too rapidly.

The quantity of pods/plant decreases, their taste is significantly impaired, they become very bitter and especially the consistency of the pods changes, they lignify prematurely.

The main characteristics of the studied plants are presented in Table 1.

Table 1. Mean values of the main key features studied in field and protected areas

Characteristics	V1 C	CV%	V1 S	CV%
Plant height (cm)	132,8±15,4 ^{a}	11,6	188±18,7 ^{b}	9,93
Plant diameter (cm)	43,4±5,9 ^{a}	13,7	60±8,8 ^{b}	14,7
Main shoots no.	6±1,41 ^{a}	23,6	11±1,4 ^{b}	12,8
No. of pods/shoot	16±2 ^{a}	12,5	29±4,8 ^{b}	16,6
Shoot length (cm)	39,2±3,5 ^{a}	9,04	57±8,2 ^{b}	14,4
No. of silicva/ plant	102±9,6 ^{a}	9,4	170±33,4 ^{b}	19,6
Peduncle length (cm)	29±8 ^{a}	27,6	47,6±9,3 ^{b}	19,5

Pod length (mm)	110±17,6 [a]	16	146±34,1 [a]	23,4
Pod diam. (mm)	8,8±1,72 [a]	19,6	14±1,4 [b]	10,1
Pod seed no.	9±1,4 [a]	15,7	14±1,5 [b]	10,1
Pod weight (g)	3,17±1,05 [a]	33,25	5,8±2 [b]	34,2
Pod production/plant (gr)	666±262,2 [a]	39,4	986±257 [a]	26

*letters represent Duncan test results with a 95% confidence interval and p<0.05%

As seen in Table 1, the quantitative characters determined recorded significantly lower values for the crop established in the field in comparison to the one in the protected areas. This may demonstrate that the species prefers the conditions in protected areas, with the observation that these do not significantly influence seedpod length and seedpod/plant production (Figure 6).

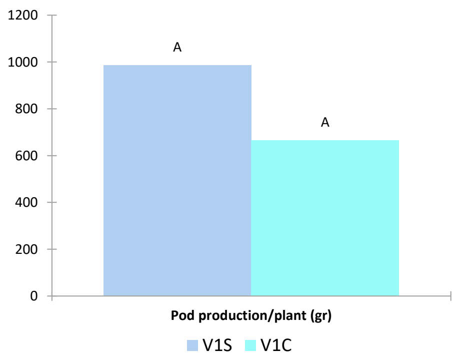

Figure 6. Similarity of pod production/plant for V1S and V1C

Significant differences influenced by the growing environment were observed in terms of plant height, number of shoots, number of seedpods/shoots, and number of seedpod/plant. An increase of more than 60% was observed in the protected area crop in terms of seedpod diameter and number of seeds in seedpods. (Figure 7).

Figure 7. Diferences of pod seed number for V1S and V1C

Determining correlations were made using Matrix of Pearson, as shown in Tables 2 and 3.

Table 2. Matrix of Pearson correlation coefficients for the main quantitative plant traits in V1C

Variables	H	PL	Pl	PD	S	pD	P/s	PS	SL	Pw	P/p
Plant height (cm) H	**1**										
Peduncle length (cm) PL	0,104	**1**									
Pod length (mm) Pl	0,140	**0,991**	**1**								
Plant diameter PD	-0,483	0,749	0,706	**1**							
Main shoots no. S	0,587	0,634	0,643	0,000	**1**						
Pod diam. (mm) pD	0,127	**1,000**	**0,990**	0,730	0,658	**1**					
Pods/shoot P/s	0,234	**0,984**	**0,966**	0,672	0,707	**0,988**	**1**				
Pod seed no. PS	0,257	-0,758	-0,723	**-0,950**	0,000	-0,740	-0,707	**1**			
Shoot.length (cm) SL	0,056	**0,992**	**0,994**	0,736	0,638	**0,991**	**0,959**	-0,718	**1**		
Pod weight (g) Pw	-0,636	0,632	0,641	0,821	0,040	0,614	0,493	-0,657	0,695	**1**	
Pods/plant P/p	0,183	**0,988**	**0,967**	0,725	0,646	**0,990**	**0,997**	-0,763	**0,961**	0,528	**1**

To determine the correlations between the main quantitative characteristics of *Raphanus sativus* var. *caudatus* plants studied under field culture conditions, it can be observed a maximum of 1 obtained by pod diameter and peduncle length, resulting in a close correlation between these two characters. A strong correlation of 0.997 was also observed between pods/plant and pods/shoot.

Table 3. Matrix of Pearson correlation coefficients for the main quantitative plant traits in V1S

Variables	PL	pL	pD	PS	Pw	P/p	H	PD	S	P/s	SL	p/p
Peduncle length (cm) PL	**1**											
Pod length (mm) pL	**0,955**	**1**										
Pod diam. (mm) pD	0,321	0,505	**1**									
Pod seed no. PS	**0,931**	**0,977**	0,600	**1**								
Pod weight (g) Pw	**0,943**	**0,989**	0,584	**0,997**	**1**							
Pods/plant (g) P/p	**0,930**	**0,976**	0,601	**1,000**	**0,997**	**1**						
Plant height (cm) H	**0,925**	**0,971**	0,606	**1,000**	**0,995**	**1,000**	**1**					
Plant diameter (cm) PD	**0,946**	**0,992**	0,578	**0,995**	**1,000**	**0,995**	**0,992**	**1**				
Main shoots no. S	0,595	0,572	-0,300	0,400	0,449	0,395	0,379	0,466	**1**			
Pods/shoot P/s	**0,941**	**0,987**	0,587	**0,998**	**1,000**	**0,998**	**0,996**	**0,999**	0,440	**1**		
Shoot length (cm) SL	**0,943**	**0,989**	0,585	**0,998**	**1,000**	**0,997**	**0,995**	**1,000**	0,447	**1,000**	**1**	
Pods/plant p/p	**0,937**	**0,984**	0,593	**0,999**	**0,999**	**0,999**	**0,998**	**0,998**	0,423	**1,000**	**0,999**	**1**

For protected area cultivation, numerous interdependencies were observed with a maximum value of 1 for the following traits: pods/plant and pod seed number, plant height and pod seed number, plant diameter and pod weight, pods/shoot and pod weight, shoot length and pod weight, shoot length and plant diameter, shoot length and pods/shoot, pods/plant and pods/shoot. These premises lead us to the conclusion that the species thrives very well in the growing conditions offered by cold protected areas.

Figure 8. *Raphanus sativus* var. *caudatus* crop detail

CONCLUSIONS

The research was completed with the successful acclimatization of the species in our country, a new genotype was obtained with expressive distinct phenotype to be registered at ISTIS for patenting and registration.

Its production potential has been evaluated both in protected areas and in the field, concluding that it can be grown with success early spring and late autumn, excluding the hot summer periods.

The cultivation technology of the species has been developed for both protected areas and the field.

The research led to the enrichment and evaluation of the PGRB Buzau germplasm collection with new genotypes, accumulating valuable information in the database of the Buzau Genebank.

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BIOCHEMICAL CHANGES DURING THE STORAGE OF SWEET POTATO ROOTS

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Abstract

The biochemical changes during storage targeted the total dry matter, total soluble solids content, the total and reducing sugar content, the glucose content as well as the starch content, from the roots of six sweet potato genotype (Ipomoea batatas L.*).*
Determinations were performed at harvest and then during storage after 30 days and 90 days. The results showed that the investigated biochemical indices vary during storage depending on genotype and period. Thus, after 30 days of storage, the DM content varied from 21.56% to 29.85% and then increased from 23.75% to 32.42% at 90 days of storage. For the TSS content, values between 9.20% (cultivar 2) and 12.80% (cultivar 3) were initially recorded, so that after 90 days of storage, the values increased from 11.3% (cultivar 6) to 16.26% (cultivar 3). The content of glucose and reducing sugars increased during the storage period of the sweet potato. Storage had little influence on the starch content. There was considerable genotypic variation of this constituent with a reduction of up to 11.30% after 90 days of storage.

Key words*: Ipomoea batatas; genotype, carbohydrate; storage.*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) belongs to *Convolvulaceae* family and originates in Central America, species from which the tuberous roots are consumed. The largest cultivated areas are in China, India, Japan, USA, but also in some European countries. Sweet potato is one of the crops with good adaptability to ecological conditions, the global production in 2020 being about 89.5 million t, and 55 million t in the EU (Eurostat, 2021).
Vegetables are essential foods for a well-balanced diet because they contain high concentrations of vitamins, minerals, fibers and phytochemicals that act as antioxidants. Sweet potatoes can be considered a staple food due to their abundant nutrients, antioxidant capacity and ability to grow in different climates. For a sustainable agriculture, it is recommended to promote those plant species that are more adaptable to the conditions of thermohydric stress and ensure a good stability of production (Matei et al., 2015).
Sweet potato is appreciated for its very high nutritional value, both for the tubers and for the young aerial parts. Thickened roots rich in carbohydrates, starch, minerals (Ca, Mn, Cu Fe, P, K) and vitamins (ßcaroten, vitamin C, B6) can be used in various food forms: bread, puree, soups, juices, French fries, desserts (Dinu et al., 2015).
Sweet potato leaves are important sources of nutrients and antioxidants and should be eaten as leafy vegetables in an attempt to reduce malnutrition, especially in developing countries (Dinu et al., 2021a; Dinu et al., 2018). Due to its high starch content, sweet potatoes are a good source of bioethanol (Lareo et al., 2013).
Root production is influenced by climate. It influences important metabolites in tubers, playing an essential role in the organoleptic properties of the species and in the level of resistance to stress and disease and implicitly to a low number of chemical treatments that are essential elements of sustainable agriculture (Dinu et al., 2021b). Climatic conditions, culture system and characteristics of the genotype have an impact on the quality parameters of grapes (Costea et al., 2015), tomatoes (Draghici & Pele, 2012) or *Luffa* spp. (Vinatoru et al., 2014).
Lebot (2009) states that sweet potato roots contain large amounts of carbohydrates, 80–

90% of the dry matter, most of which is starch (50-80%). Numerous studies have shown that during the storage of sweet potato roots there are a number of changes in carbohydrate components, such as a decrease in starch content and an increase in sugar content, especially reducing sugars (Takahata et al., 1995). Changes in the starch and sugar content of sweet potato roots during the storage process are attributed to the activities of endogenous amylolytic enzymes.

Sweet potato is a staple food in many parts of the world, especially in the tropics. In Romania, sweet potato is a less cultivated species, but the biochemical composition of tuberous roots makes it very important both in terms of food and industry, and therefore the culture should be expanded. The tuberous roots of the sweet potato genotype are differentiated by the different colour of the rhizoderm and the pulp, as well as by the nutritive composition. The sugar content of sweet potato roots varies depending on the genotype, the culture system or certain technological sequences (Dinu et al., 2021b). The content of free sugars and starch in sweet potato roots has a considerable impact on the processing.

Numerous studies have been carried out in large sweet potato growing countries with reference to the stages of handling the harvest and the methods of storage. Moyo et al. (2004) mentioned the storage of roots in pits in the ground or in environmental conditions, in bags, in some countries in South Africa. What is certain is the fact that this species has very thin root rhizoderm and if lesions occur during harvesting, they will reduce the storage period and implicitly loss of nutrients. Resistance or susceptibility to disease is largely due to genotype and environmental conditions (Paraschivu et al., 2020).

The storage of tuberous sweet potato roots is essential to ensure a constant supply both for the population consumption and especially for the food industry in juices production or flours obtaining used in pastry.

Therefore, keeping the sweet potato roots quality during storage is necessary both for the processing industry and to avoid large economic losses. During storage, the roots are very perishable because they have a high moisture content (60-75%), therefore mechanical strength is low, as well as high susceptibility to microbial degradation (Sugri et al., 2017). It is preferable to store them in cool spaces in order to preserve their quality as well as possible (Krochmal-Marczak et al., 2020). However, long-term storage at low temperatures results in cold-induced sweetening (CIS) (Amjad et al., 2020).

Sweet potatoes nutritional quality depends largely on the quantity and quality of carbohydrates. The aim of this study was to present the metabolic changes of the carbohydrate components of six sweet potato genotypes, which occurred during storage, in environmental conditions, over a period of 90 days after harvest.

MATERIALS AND METHODS

Six sweet potato genotypes from South Korea were used in this study, which differ in terms of the skin shape and colour and in flesh colour (Table 1). The experiment took place in the experimental field of the Faculties of Horticulture and Agronomy from Craiova, Romania, in 2019. After 160 days from the planting moment, the harvest was carried out and the roots were sorted and the healthy ones were stored at a temperature of 18-20^{0}C and 75% RH (relative humidity). The biochemical determinations concerned the DM, TSS content, total and reducing sugar content, glucose content, and starch content. The determinations were performed when harvested, then after 30 and 60 storage days. For the analysis, roots of 150-200 g were used; 6 of each genotype and then washed, chopped and well homogenized.

Table 1. Basic characteristics evaluated of sweet potato tubers

Genotype	The tuber shape	The colour of the skin/flesh
Genotype 1	Ovate-elongated	Yellow skin with white flesh
Genotype 2	Ovate-elongated	Yellow skin with white flesh
Genotype 3	Ovate	Purple skin with white flesh
Genotype 4	Ovate	Purple skin with white flesh
Genotype 5	Ovate	Red skin with orange flesh
Genotype 6	Ovate	Purple skin with purple flesh

The dry matter content (DM %) was determined gravimetrically by sample drying to a constant weight at 105°C.

Total soluble solids (TSS %) were determined using a digital refractometer (Kruss Optronic DR 301-95) at 20°C and expressed as %.
Reducing sugars (%) were extracted in distilled water (1:20 g:mL), 60 minutes at 60°C and assayed colorimetric at 540 nm with 3,5 dinitrosalicylic acid reagent using glucose as standard.
Glucose (%) content was assayed at 500 nm by glucose oxidase/peroxidase method (GOD/POD). Glucose oxidase (GOD) is used to oxidize glucose by the oxygen in the air to gluconolactone and hydrogen peroxide. Under the influence of peroxidase (POD) the hydrogen peroxide reacts with colour indicator forming a pink compound. The glucose content was calculated from calibration curve using glucose (5 mg/mL) as standard.
Total sugar content (%) and non-reducing sugars were converting by hydrochloric acid hydrolysis, 15 min at 100°C to reducing sugars. After neutralization, total sugar content (%) was assayed colorimetric with 3,5 dinitrosalicylic acid reagent at 540 nm. Non-reducing sugars (%) is the difference of total soluble sugars and reducing sugars.
The starch content (%) was determined by using Ewers polarimetric method. Starch from the ethanol-insoluble material is extracted into hot dilute hydrochloric acid (Soare et al., 2019). After having cooled, phosphotungstic acid is added to precipitate the proteins and the solution is filtered. The optical rotation of the filtrate is measured using a Carl Zeiss JENA polarimeter and the results were calculated with a specific optical rotation of the potato starch [α] D20°=185.4°. All the spectrophotometric measurements were performed with a Thermo Scientific Evolution 600 UV-Vis spectrophotometer with VISION PRO software.
Statistical calculations were performed in Microsoft Office Excel 2007. For the significance analysis, the data were analyzed by one-way ANOVA. Means were compared using LSD at 5% level of significance.

RESULTS AND DISCUSSIONS

DM and TSS content vary within the studied genotype and storage time (Table 2). When harvested, DM recorded values between 21.39% (genotype 4) and 27.53% (genotype 5) with an average of 24.88%. After 30 storage days, the dry matter content increased to values between 21.56% (genotype 4) and 29.85% (genotype 5) with an average value of 26.19% and after 90 storage days it increased to values between 23.75% (genotype 4) and 32.42% (genotype 5) with an average value of 28.56%. Both when harvested and after storage, genotype 4 has the lowest dry matter content and genotype 5 has the highest value. At 90 storage days, the values increased as a result of increased evaporation.
The change in dry matter content may be a result of physiological processes such as transpiration and respiration that occur during storage and which are influenced by the temperature and air relative humidity. These variations in dry matter content have been observed by several authors. Thus, after eight weeks of storage in normal environment temperature, the highest dry matter content (35.13%) of sweet potatoes was in a pot with moist soil and the lowest in dry sand (31.13%) (Bhattarai et al., 2021).
Krochmal-Marczak et al. (2020) in their study for 5 sweet potato cultivars stored for 6 months reported an increase in dry matter content from 26.61% when harvested to 29.19% (at 5°C) and up to 33.59% (at a temperature of 15°C).
Dandago & Gungula (2011) reported that dry matter content decreased after 5 months of storage for samples stored in pit with alternate layer of river sand and increased in samples stored in moist sawdust in wooden box. Also, Agbemafle at al. (2014), show that dry matter content was 40.68 % in freshly harvested sweet potato and increase to 43.85 % after six weeks storage in ambient condition. For storage in moist sawdust the dry matter content decreased to 37.20 % in week six and for sweet potatoes stored in wood ash the dry matter content increased to 49.09 % in week six.
The comparison of total soluble substance between sweet potato genotypes, at harvest, indicates values from 7.13% for genotype 2 to 8.78% for genotype 1. After 30 storage days, the recorded values show slight increases, from 9.2% for genotype 2 to 11.8% for genotype 3.

Table 2. DM and TSS content in sweet potato roots during storage

Genotype	DM %			TSS %		
	When harvested	30 storage days	90 storage days	When harvested	30 storage days	90 Storage days
Genotype 1	24.82 ab	25.11 c	27.34 c	8.78 a	9.80 ab	13.70 b
Genotype 2	25.19 ab	26.70 b	28.06 c	7.13 b	9.20 b	13.12 b
Genotype 3	26.04 a	27.18 b	29.92 b	7.91 ab	11.80 a	16.26 a
Genotype 4	21.39 b	21.56 d	23.75 d	7.90 ab	10.12 ab	12.40 bc
Genotype 5	27.53 a	29.85 a	32.42 a	8.48 ab	9.30 b	12.30 bc
Genotype 6	25.30 ab	26.76 b	29.85 b	8.36 ab	9.84 ab	11.30 c
$P \leq 0.05$	4.18	1.20	1.77	1.59	2.23	1.68

The differences between the averages indicated by different letters are significant ($p \leq 0.05$).

After 90 storage days the total soluble matter values increased from 11.3% for genotype 6 to 16.26% for genotype 3. The results show significant differences between genotypes. Our results are in agreement with reported data in other studies. In Ghana, during the 3 weeks of storage, the values of some varieties ranged from 6.2 to 9.55 g / 100g TSS (Adu-Kwarteng et al., 2014).

The increase in TSS may also be a result to the conversion of starch into monosaccharides.

The structure of carbohydrates in sweet potato roots influences the nutritional quality and processing characteristics. Generally speaking, longer storage periods of raw roots, prior to processing, result in low-strength raw material.

Regarding the quantitative and qualitative spectrum of sugars, it varies according to the genotype and the storage period (Table 3). Thus, when harvested the glucose content varied from 0.68% (genotype 4) to 1.75% (genotype 1), in order genotype 4 < genotype 6 < genotype 3 < genotype 5 < genotype 2 to < genotype 1. The glucose content increased after 30 storage days with values between 0.89% (genotype 4) and 2.07% (genotype 1), and after 90 storage days, the values also increased between 2.05% (genotype 3) and 3.87% (genotype 6). The results indicated that the highest glucose contents were recorded after a longer storage period for genotypes 5 and 6.

Total soluble content is another useful parameter for selecting genotypes that can accumulate higher levels of total sugar (Babeanu et al., 2017).

Reducing sugars present in sweet potatoes are glucose and fructose. In the present study, the content of reducing sugars varied from 2.23% (genotype 6) to 4.12% (genotype 5).

This indicator increased after 30 storage days to values between 3.35% (genotype 3) and 6.8% (genotype 1), and after 90 storage days; the values are much higher compared to harvest period from 4.87% (genotype 3) to 8.2% (genotype 1).

It can be observed that after 30 and 90 storage days, all genotypes get an increase in glucose and reducing sugars. During storage periods, the variation in reducing sugars was significantly different.

Table 3. Carbohydrates variation during sweet potato roots storage

Genotype	Glucose			Reducing sugars (%)				Total Sugars (%)	
	When harvested	30 storage days	90 storage days	When harvested	30 storage days	90 storage days	When harvested	30 storage days	90 storage days
Genotype 1	1.75 a	2,07 a	3.25 a	2.45 e	6.8 a	8.2 a	5.82 b	8.74 b	11.45 b
Genotype 2	1.18 b	1.64 ab	3.18 ab	3.17 c	5.06 b	6.32 c	5.67 b	7.42 d	10.8 cd
Genotype 3	0.97 bc	1.12 bc	2.05 c	2.84 d	3.35 d	4.87 d	7.62 a	9.72 a	12.60 a
Genotype 4	0.68 c	0.89 c	2.52 bc	3.35 b	4.13 c	5.44 d	7.34 a	8.48 b	9.54 e
Genotype 5	1.11 bc	1.76 a	3.78 a	4.12 a	5.07 b	6.8 bc	7.26 a	7.86 c	10.98 c
Genotype 6	0.83 bc	1.04 bc	3.87 a	2.23 f	4.12 c	7.36 b	6.72 ab	7.75 cd	10.41 d
$P \leq 0.05$	0.45	0.60	0.71	0.04	0.30	0.80	0.18	0.34	0.46

The differences between the averages indicated by different letters are significant ($p \leq 0.05$).

Obtained results are in agreement with reported data in other studies. Agbemafle et al. (2014) reported that during the six weeks storage period, the reducing sugar content of sweet potato stored under ambient conditions increased from 2.41 % to 3.24%, under moist saw dust increased to 3.74% and under wood ash the reducing sugar content increased to 2.84 % at the end of the storage period at the end of the storage period. In another study, for 3 cultures 12 weeks inside dry sand the content in reducing sugars increased from 3.16% and 4.66% to 4.72%-9.79% (Bhattarai et al., 2021).

Total soluble sugars present in sweet potato tubers are glucose, fructose and sucrose (Adu-Kwarteng et al., 2014). When harvested, total soluble sugars content varied from 5.67% (genotype 2) to 7.62% (genotype 3) ranging from genotype 2 < genotype 1 < genotype 6 < genotype 5 < genotype 4 < genotype 3. In all genotypes, total soluble sugars content increased after 30 storage days from 7.42% (genotype 2) to 9.72% (genotype 3) and after 90 storage days from 9.54% (genotype 4) to 11.45% (genotype 1). The results obtained pointed out the fact that the content in total soluble sugars varied significantly ($p < 0.05\%$) within the analysed genotype.

Total sugar content increase is also presented in other studies. After a 6 month storage in a climatic chamber with temperature control of 5 sweet potato cultivars, an increase from an average value of 5.27% when harvested to an average value of 5.97% at 5°C and an average value of 7.39% at 15°C was reported (Krochmal-Marczak et al., 2020).

Yamdeu et al. (2015), proved that low-temperature storage negligibly influenced starch and maltose contents of the tubers but induced a significant increase of reducing sugars and total soluble sugars.

Percentages of glucose, reducing sugars without glucose and non-reducing sugars in total sugar soluble in the 6 genotypes are presented in Figures 1, 2 and 3. The content of each indicator varies by genotype and storage period. The results obtained show that sucrose is the major component (except genotype 1, 2, and 5 after 30 days of storage).

Starch content in sweet potato tubers varies in terms of the the cultivar analyzed and storage time (Table 4). When harvested starch content has values ranging from 15.94% (Genotype 1) to 21.77% (genotype 5) in order, genotype 1 < genotype 6 < genotype 2 < genotype 3 < genotype 4 < genotype 5. After storage all genotypes record a decrease in starch content.

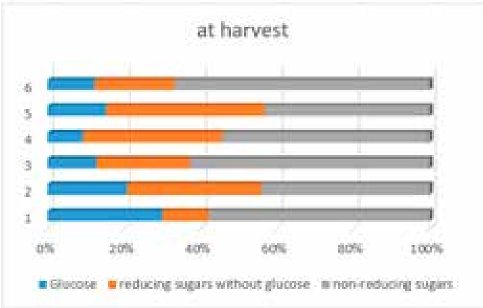

Figure 1. Content of glucose, reducing sugars and non-reducing sugars (%) in the 6 genotypes (%) at harvest

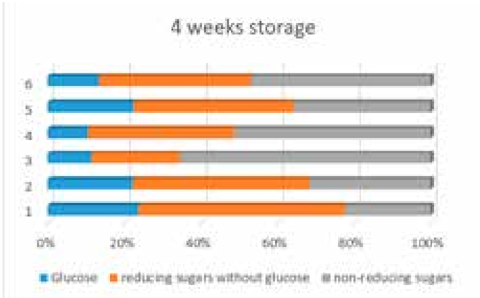

Figure 2. Content of glucose, reducing sugars and non-reducing sugars (%) in the 6 genotypes at 4 weeks storage

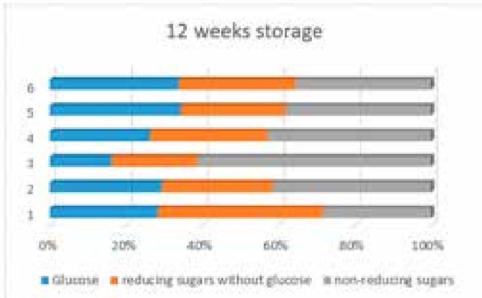

Figure 3. Content of glucose, reducing sugars and non-reducing sugars (%) in the 6 genotypes (%) at 12 weeks storage

Table 4. Evolution of starch content during the storage period of sweet potato roots

Genotype	Starch (%)		
	When harvested	30 days storage	90 days storage
Genotype 1	15.94 e	15.37 c	11.20 b
Genotype 2	17.06 c	17.06 b	13.73 ab
Genotype 3	20.41 b	18.11 b	12.06 b
Genotype 4	20.68 b	20.12 a	14.65 a
Genotype 5	21.77 a	20.29 a	15.12 a
Genotype 6	16.97 d	14.05 d	11.07 b
P≤0.05	0.57	1.06	1.82

The differences between the averages indicated by different letters are significant ($p \leq 0.05$)

The starch content after 30 storage days recorded values between 14.05% (genotype 6) and 20.29% (genotype 5), and after 90 storage days, the values were between 11.07% (genotype 6) and 15.12% (genotype 5).

Decreased starch content is illustrated by other studies, also. After 5 months of storage of 5 sweet potato cultivars, a decrease is reported from an average value of 19.16% when harvested to an average value of 17.16% at 5°C and an average value of 14.68% at 15°C (Krochmal-Marczak et al., 2020).

Recently, a decrease in the starch content was reported from values between 22.73% and 25.51% when harvested to values in the range of 15.12 -19.53 for 3 crops stored for 12 weeks inside dry sand (Bhattarai et al., 2021). In a research paper studying the effects of five different storage methods on the quality and nutritional composition of sweet potatoes is recorded that the amount of starch decreased from the initial value of 16.95 to various values as storage period progressed. The lowest value (10.03%) was obtained in sample stored in pits with layers of river sand under shade (Dandago & Gungula, 2011)

Zhang et al. (2002) and Niu et al. (2019) also observed a decrease in starch content during tuber storage depending on genotype. This variation in starch can be explained by the biochemical and physiological processes occurring during storage. The decrease in starch content in sweet potato roots and the increase in sugar content during storage is caused by enzymatic hydrolysis catalysed by α and β amylases. Their activity increases during storage (Nabubuya et al., 2012). After harvest, starch biosynthesis can also take place, using sucrose as a carbon source (Preiss, 1982).

CONCLUSIONS

The obtained results show that the quantitative and qualitative spectrum of sugars varies from one genotype to another and also in terms of storage period. When stored for 30 days, there is a slight increase in the content of soluble substances, glucose, reducing sugars and total soluble sugars and a decrease in the starch content. After 90 storage days, all genotypes have a higher carbohydrate content, which ensures a proper quality of consumption, but a more pronounced decrease in starch, the differences is given by the genotype. The highest total sugar content was for genotype 3 with 12.60%.

By carefully sorting the roots, managing the temperature and storage period, it can be recommended to keep the roots for a period of 90 days in environmental conditions, for small growers. Knowing these changes in sugars during storage can be useful in planning the seasons of establishment and harvesting at the optimal time, in choosing the genotype with valuable properties and shelf life.

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THE NUTRIENT SOLUTION OXYGENATION INFLUENCE ON THE GROWTH OF THE SPECIES *LACTUCA SATIVA* L. ROOT SYSTEM CULTIVATED IN THE NUTRIENT FILM TECHNIQUE (NFT) SYSTEM

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Abstract

Lettuce is a highly appreciated vegetable species as evidenced by the very high market demand, regardless of the season. Thus, it is cultivated in all cropping systems, both conventional and unconventional. Moreover, in order to meet the high demand, large-scale farmers are constantly working to find solutions to increase production, reduce the period of formation of the edible part, and reduce the number of fertilizers. Lettuce cultivation in the Nutrient Film Technique (NFT) system is most commonly used in all climatic zones. In the experiment performed at University of Agronomic Sciences and Veterinary Medicine of Bucharest, Hortinvest greenhouses, on lettuce culture in the NFT system we monitored the debit and oxygen content of the nutrient solution at the roots of lettuce plants and found that depending on its flow rate, the configuration the root system was different. We also found that there were correlations between the size of the root system and the mass of lettuce plants obtained in this system.

***Key words**: Lettuce, flow, roots, oxygenation.*

INTRODUCTION

The Nutrient Film Technique (NFT) cultivation system was developed in the late 1960s by Dr. Allan Cooper at the Glasshouse Crops Research Institute in Littlehampton, England (Graves, 1983). Over time, this cultivation system has proven to be the fastest type of liquid hydroponic system.

The main feature of the NFT system is that the nutrient solution flows like a thin film through a plastic channel that encloses the roots of the plants. Therefore, in the hydroponic system, the nutrient solution plays an essential role being the supplier of water, oxygen and all the essential mineral elements necessary to obtain high quality crops (Drew, 1997; Trejo-Téllez, 2012).

The importance of the oxygen availability in the plants root zone for their growth and development it is indicated by Moreno Roblero et al. (2020). In the same time, specific studies are required for different plant species in order to identify the necessary quantity of oxygen to maximize the benefits.

Concurrently, plant cultures in hydroponic system are not supplied at the maximum uptake water, nutrients, and oxygen (Blok et al, 2017; Gherghina et al., 2020; Al-Kinani et al. 2021; Jailawi et al., 2021).

In these conditions, in order to improve the lettuce crops, it is necessary to identify various methods and procedures for improving the oxygen uptake.

Moreno Roblero et al. (2020) outlined the necessity of the oxygen presence in the plant's roots zone in all their development stages and pointed out oxifertigation as an effects accelerator of the natural resources. Chun & Takakura (1994) revealed that in hydroponic cultures, it is necessary to have a high dissolved oxygen (DO) level in order to support a healthy roots respiration.

On the other hand, Urrestarazu & Mazuela (2005) identified that chemical oxygenation of the nutrient solution has positive effects on plants. Kurashina et al. (2019) studied the effects of the underwater ultrasound and DO supersaturation in the hydroponic cultures and demonstrated their direct effects on plants growth.

Another important aspect is the hydroponic technical structure. It was proved that the container gradient plays a big role in the nutrient solution oxygenation (López-Pozos et al., 2011). Colunje et al. (2021) studied the effects of nutrient solution oxygenation with ozone in pepper hydroponic cultures and identified the impact on plants growth.

The positive effect of oxygen on lettuce plant growth in a floating hydroponic system is identified by Goto et al. (1996). Suyantohadi et al. (2010) reveal that the lettuce plant hydroponic culture irrigated with low temperature water and with high concentrated dissolved oxygen (20-30 mg/L supply) gives a better harvest than in normal conditions.

Carrasco et al. (2011) identifies the necessary oxygen uptake using the peroxides or peracetic acid in nutrient solutions as a source in order to increase the fresh weight in the *rocket* (*Eruca sativa* Mill.) crop grown in floating system.

Kurashina (2019) study on lettuce cultivated in hydroponic system revealed that oxygen supersaturation is enhancing the plant growth, without degradating the chlorophyll from the leaves. According to Baiyin et al. (2021), in plants harvesting, the flow rate adjustment can improve the crop results.

Edwards & Asher (1974) have proven the importance of the nutrient solution flow rate on the hydroponic cultures. They have also outlined that lower nutrient solution flow rate reduces among others the plants nitrogen uptake.

Van Rooyen & Nicol (2022) identified that online plants pH measurement supports the control on plants nitrogen concentration in nitrification-hydroponic systems.

Sago & Shigemura (2018), have studied the nitrate content of the plants cultivated in controlled cultures. Their study conclusions were that including the necessary nitrate content in the nutrient solution at the beginning of the cultivation can lead to lower nitrate content butterhead lettuces crops.

Our objectives were to monitor the debit and oxygen content of the nutrient solution at the roots of lettuce plants cultivated in the NFT(Nutrient Film Technology) system and to analyse the results depending on its flow rate, the configuration the root system. Another objective was to analyse the correlations between the size of the root system and the mass of lettuce plants obtained in this system. In the same time, the nitrate content was calculated for each variant in order to analyse impact of the nutrient solution flow rate on the nitrate content.

MATERIALS AND METHODS

The experiment took place at University of Agronomic Sciences and Veterinary Medicine of Bucharest, Hortinvest greenhouses, on lettuce culture in the NFT system (Nutrient Film Technology), during the 10th March- 08th April 2020.For the experiment have been used the following lettuce assortments: Alanis, Aleppo and Kiribati, from seeds made available by Holland Farming Agro S.R.L. (Figure 1).

V1- Alanis V2 - Aleppo V3- Kiribati

Figure 1. The biological material used in the experiment

The oxygenation of the nutrient solution was achieved by increasing its flow rate. No oxygen from other sources was used for the experiment. The nutrient solution composition is presented in Table 1.

Table 1. Composition of nutrient solution used for fertilizing of lettuce seedlings in NFT crops and on perlite substrate

Components	Kg/1000 L H_2O
Fertilizer A	
Calcium nitrate	92.50
Potassium nitrate	25.00
Chelated iron (13%)	0.85
Ammonium nitrate	1.20
Fertilizer B	
Potassium nitrate	50.00
Potassium sulphate	4.00
Monopotassium phosphate	10.00
Ammonium monophosphate	6.00
Magnesium sulphate	35.00
Microelements	
Manganese	0.2
Boron	0.29
Zinc	0.09
Copper	0.02
Molybdenum	0.01

The flow rate variants (conventionally named F1, F2, F3 and F4) of the nutrient solution for each assortment were 1.0 L/min, 2.0 L/min, 2.5 L/min and 3.0 L/min (Table 2).

Table 2. Experimental variants

Cultivars	Nutrient solution flow (L/min)			
	Flow rate (F1)	Flow rate (F2)	Flow rate (F3)	Flow rate (F4)
V1 - Alanis	1.0	2.0	2.5	3.0
V2 - Aleppo	1.0	2.0	2.5	3.0
V3 - Kiribati	1.0	2.0	2.5	3.0

The seedlings were produced in a greenhouse in Jiffy seven pots and were 25 days old at planting. Planting in the NFT system took place in the greenhouse during the 10th March- 08th April 2020. All climatic factors, light, atmospheric humidity and temperature as well as the temperature of the nutrient solution were monitored. The observations and determinations made were aimed, in dynamics, at the growth of plants in diameter, the formation of the number of leaves. After 25 days from planting in the NFT system, the plants were harvested, on variants, their mass was determined by weighing, the length of the roots was measured. For each variant the dry matter content and the nitrate content were performed. Data were analysed statistically according to analysis of variance technique using analytical software and treatment means were compared using Tukey's test (Steel et al., 1997).

RESULTS AND DISCUSSIONS

The highest average edible mass of lettuce plants was recorded in the flow rate variant in which we distributed the nutrient solution at the value of 2.5 L/min, of 378.33 g/plant in the Alanis variety, head lettuce, 190.67 g Aleppo blond Lollo and 237.33 g of Kiribati oak leaf. The control flow rate variant, at a flow rate of 1 L/min, showed the lowest average edible mass of 228 g for F1 Alanis variety, 141 g/plant for F2 Aleppo variety and 201.67 g/plant for F3 Kiribati variety (Figure 2).

In the case of Alanis assortment, we have obtained average masses of lettuce plants between 228 g in F1 and 378.33 g in F3. We found that the differences in mass increased with the increase of the flow of the nutrient solution, being between 47.67 g at F2, by 20.91% over the flow rate control variant and by 150.33 g, respectively by 65.94% at F3. In the case of F4 the increase was by 123 g respectively by 54.24% over the flow rate control variant. From a statistical point of view, there were significant positive differences at F2 and distinctly very positive differences at very significant at F3 and F4 (Table 3).

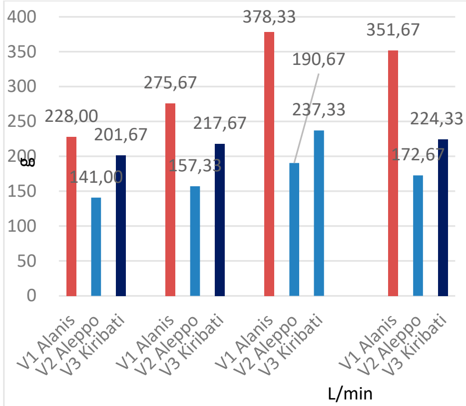


Figure 2. Average edible lettuce mass calculated for each assortment and for all four (4) flow rate variants (L/min)

Table 3. Average mass of lettuce plants in the Alanis assortment

Variants Alanis	Mass (g)	Difference (g)	(%)	Significance
F(0) average	308.42	80.42	135.27	**
F(1)	228.00	0.00	100.00	Ct
F(2)	275.67	47.67	120.91	*
F(3)	378.33	150.33	165.94	***
F(4)	351.67	123.67	154.24	***

DL5% = 45.310 DL5% in % = 19.8728
DL1% = 68.550 DL1% in % = 30.0658
DL01% = 109.210 DL01% in %= 47.8991

In the case of the Aleppo variety (Lollo bionda type) we found that the highest average mass of the plants was recorded in flow rate variant 3 where the flow of the nutrient solution was 2.5 L/min. In this flow rate variant, the difference in mass was 49.67 g and 35%, respectively, over the flow rate control variant aspect found by the statistical significance, very significant. In the case of F2, at a nutrient solution flow rate of 2.0 L/min the plant mass increases were insignificant (Table 4).

Also, in the case of the Kiribati variety, we found the same aspect regarding the average mass of the plants. At F3, at a flow rate of 2.5 L/min, the highest average mass of lettuce plants of 237.33 g was obtained compared to F1 of 201.67 g. Statistically, the differences were positively distinctly significant at F3 (Table 5).

Table 4. Average mass of lettuce plants in the Aleppo variety

Variants Aleppo	Mass (g)	Difference (g)	Difference (%)	Significance
F(0) average	165.42	24.42	117.32	*
F(1)	141.00	0.00	100.00	Ct
F(2)	157.33	16.33	111.58	N
F(3)	190.67	49.67	135.22	**
F(4)	172.67	31.67	122.46	*

DL5% =	21.240	DL5% in % =	15.0638
DL1% =	32.140	DL1% in % =	22.7943
DL0.1% =	51.200	DL0.1% in % =	36.3121

Table 5. Average mass of lettuce plants in the Kiribati variety

Variants Kiribati	Mass (g)	Difference (g)	Difference (%)	Significance
F(0) average	220.25	18.58	109.21	**
F(1)	201.67	0.00	100.00	Ct
F(2)	217.67	16.00	107.93	*
F(3)	237.33	35.67	117.69	***
F(4)	224.33	22.67	111.24	**

DL5% =	11.360	DL5% in % =	5.6331
DL1% =	17.190	DL1% in % =	8.5240
DL0.1% =	27.390	DL 0.1% in %=	13.5818

Figure 3 shows the aspect of the assortments Alanis and Kiribati at the best flow rate variant (F3) 2.5 L/min.

Figure 3. Aspect of lettuce culture for Alanis and Kiribati assortments

Figure 4 shows schematically the differences in the average mass of the plants compared to the flow rate control variant.

Analysing the length of lettuce roots after harvest in the Alanis variety, we found differences between flow rate variants. Thus, the longest root length (29.00 cm) was obtained in the flow rate variant where the flow was 1 L/min. At F4 at a nutrient solution flow rate of 3.0 L/min. the root length showed the lowest value (16.00 cm).

From a statistical point of view, flow rate variants 3 and 4 show the biggest differences and the difference from the flow rate control variant was negatively distinctly significant (Table 6).

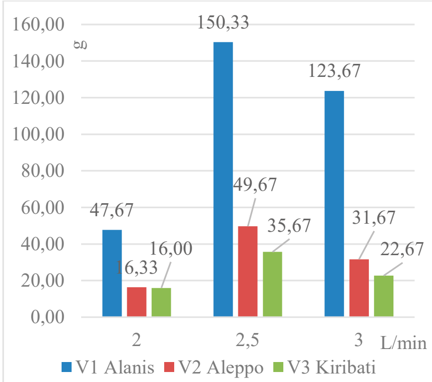


Figure 4. The difference in mass of the plants from the flow rate is 1 L/min (the flow rate control variant)

Table 6. The length of the lettuce roots in the Alanis variety at the flow rate of 1 L/minute

Variants Alanis	Roots (cm)	Difference (cm)	Difference (%)	Significance
F(0) average	23.25	-5.75	80.17	000
F(1)	29.00	0.00	100.00	Ct
F(2)	26.00	-3.00	89.66	00
F(3)	22.00	-7.00	75.86	000
F(4)	16.00	-13.00	55.17	000

DL5% =	1.880	DL5% in % =	6.4828
DL1% =	2.850	DL1% in % =	9.8276
DL0.1% =	4.550	DL0.1% in %=	15.6897

In the case of the Aleppo variety, we noticed a situation similar to the Alanis variety. In this case the longest roots were recorded at F1 (27.00 cm) and the shortest at F4 (18.50 cm). From a statistical point of view, we have noticed distinctly significant negative differences (Table 7).

Table 7. The length of the lettuce roots of the Aleppo variety

Variants Aleppo	Roots (cm)	Difference (cm)	Difference (%)	Significance
F(0) average	22.86	-4.14	84.68	000
F(1)	27.00	0.00	100.00	Ct
F(2)	23.70	-3.30	87.78	000
F(3)	22.25	-4.75	82.41	000
F(4)	18.50	-8.50	68.52	000

DL5% =	0.880	DL5% in % =	3.2593
DL1% =	1.330	DL1% in % =	4.9259
DL0.1% =	2.120	DL0.1% in %=	7.8519

In the Kiribati variety, the plants have developed a higher root system if the nutrient solution flow rate was 1 L/min. (28.75 cm) and lower with the increase of the flow rate through the culture trough of 24.50 cm at F2 with 2 L/min, 22.50 cm, at F3 with 2.5 L/min and 20.33 cm at F4 with 3 L/min. All data are also statistically confirmed and are significantly different from the flow rate control variant.

Table 8. Length of lettuce roots in the Kiribati variety

Variants Kiribati	Roots (cm)	Difference (cm)	(%)	Significance
F(0) average	24.02	-4.73	83.55	000
F(1)	28.75	0.00	100.00	Ct
F(2)	24.50	-4.25	85.22	000
F(3)	22.50	-6.25	78.26	000
F(4)	20.33	-8.42	70.71	000

DL5% =	1.230	DL5% in % =	4.2783
DL1% =	1.860	DL1% in % =	6.4696
DL0.1% =	2.960	DL0.1% in %=	10.2957

Figure 5 shows schematically the values regarding the length of the roots for the experimental flow rate variants. The highest values are observed at F1 with a flow rate of 1 L/min and the lowest at F4. In the F1 flow rate of 1 L/min, the length of the roots is 29.0 cm for Alanis, 27.0 cm for Aleppo, 28.75 cm for Kiribati assortment. On the other hand, in the case of a flow rate of 3 L/min, the length of the roots is 16 cm for Alanis, 18.50 cm for Aleppo, 20.33 cm for Kiribati assortment. At the flow rates of 2 L/min and 2.5 L/min, the length of the roots varies between the abovementioned values, as it is presented in the Figure 5.

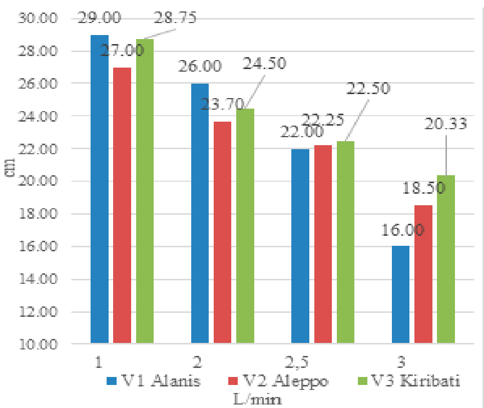


Figure 5. Root length in experimental flow rate variants

The correlation between plant mass and nutrient solution flow rate indicated a significantly different influence with the assortment. Thus, the Aleppo assortment showed a higher influence ($R^2 = 0.7846$) compared to the other varieties where the ratio was $R^2 = 0.5818$ for the Kiribati variety and $R^2 = 0.6092$ for the Aleppo assortment (Figure 6).

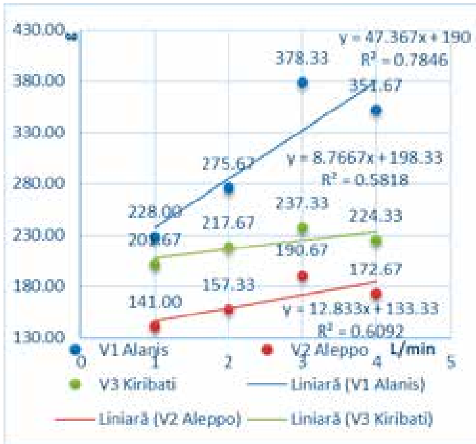


Figure 6. Influence of nutrient solution runoff velocity on average lettuce mass

The correlations between the length of the roots and the flow rate of the nutrient solution for the analysed varieties indicated very significant negative relations, which means that at low flow rates of 1 L/min the root length is higher and decreases with increasing nutrient flow rate. (Figure 7).

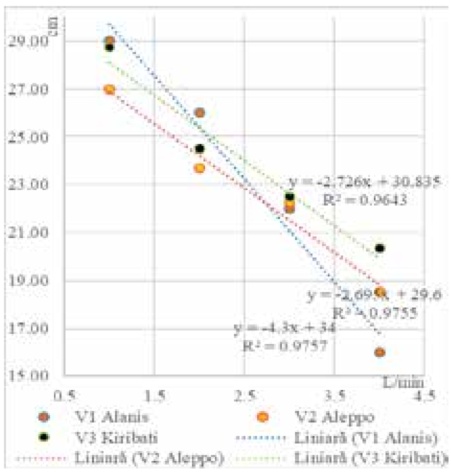
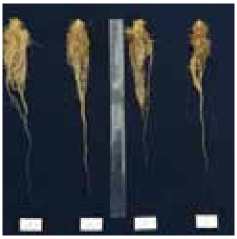


Figure 7. Influence of nutrient solution flow rate on lettuce root length

Figure 8 shows the roots of lettuce plants by varieties and flow rate variants:

a. Alanis

b. Aleppo



c. Kiribati

Figure 8. Appearance of roots in experimental flow rate variants a. Alanis; b. Aleppo; c Kiribati

The root volume varied between 14.18 cm^3 for the Alanis variety at the flow rate of the nutrient solution of 1.0 L/min and of 10.00 cm^3 for the flow rate variant with the flow of 3.0 L/min. In the case of the Aleppo variety, the root volume varied between 10.00 cm^3, the flow rate of 2.0 and 2.5 cm^3 and 12.00 cm^3, respectively, in flow rate variants 1 and 4. The Kiribati variety showed the largest root volume between 16.3 cm^3, a flow of 1.0 L/min and 14.00 cm^3 at a flow rate of 3.0 L/min (Figure 9).

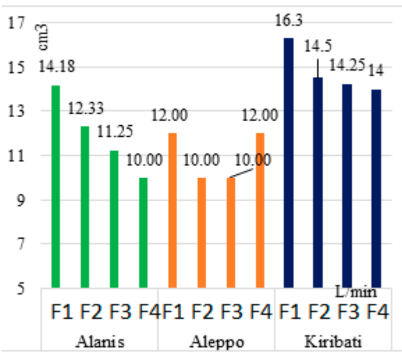


Figure 9. Root volume in experimental flow rate variants (F1, F2, F3, F4)

Regarding the average mass of the roots, it was found that the highest weight was achieved in all varieties at the variant with a flow rate of 1 L/min (Figure 10).

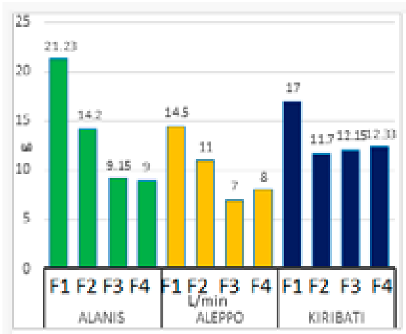


Figure 10. Average mass of lettuce roots in experimental flow rate variants (F1, F2, F3, F4)

The dissolved oxygen level (DO) was determined with a portable oxygen meter. Oxygen levels were determined upon entering and leaving the culture trough. We found that at the entrance the oxygen level was 7.0 mg/L. As the nutrient solution flowed through the culture trough, the roots of the plants consumed the initial oxygen and at the exit of the trough its level was different depending on its flow rate. In the case of the flow rate variant where the flow rate was 1 L/min at the outlet, the oxygen content of the nutrient solution was 4.1 mg /L and at 3 L/min 5.8 mg/L (Table 9).
Thus, the water temperature increases, the solubility of oxygen decreases.

Table 9. Oxygen consumption of plants in the nutrient solution

Flow rate	The amount of oxygen in the nutrient solution in the drain	Inlet solution temperature	Outlet solution temperature
L/min	mg/L	ºC	ºC
1.0	4.10	20.8	21.2
2.0	5.08	20.8	21.0
2.5	5.20	20.8	21.6
3.0	5.80	20.8	21.5

Concerning the nitrate content, it is to be specified that it was calculated in order to identify the nitrate content on plants cultivated in the system presented above. In the same time, the impact of the nutrient solution flow rate was the sole parameter taken into consideration for this analyse.

The nitrate content of lettuce plants was determined in the lowest amount for all lettuce varieties at a flow rate of 1 L/min. The highest nitrate content was identified in lettuces where the solution flow rate was 2.5 L/min. In the case of all flow rate variants, the nitrate content was below the limit allowed by the quality standard of 2,000 mg/kg for lettuce crops in the greenhouse during the winter.

According to the REGULATION (EU) NO. 1258/2011 OF THE COMMISSION of December 2/ 2011 amending Regulation (EC) No.1881/2006 as regards the maximum levels for nitrates in food, for fresh lettuce (*Lactuca sativa* L.), Lettuce grown in the greenhouse and lettuce grown in the air free, the maximum accepted nitrate level during the chosen culture period (during the 10th March- 08th April 2020) is 4,000 mg NO_3/kg except for the head lettuce (2,500 mg NO_3/kg) (Figure 11).

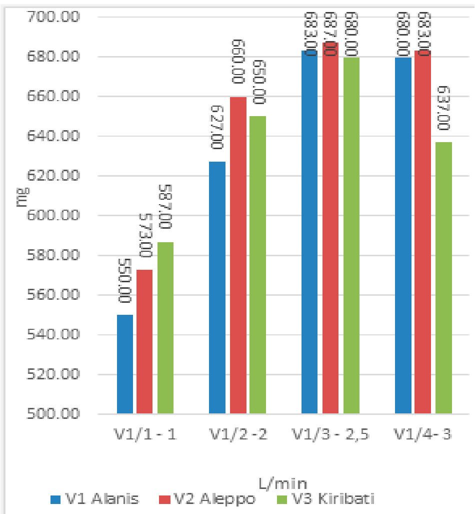


Figure 11. Nitrate content of lettuce plants

CONCLUSIONS

The aim of the study was to identify the best way to ensure the level of oxygen to obtain the best quality lettuce plants.

The results of the experiment are the followings:

1. Concerning the average mass of the lettuce plants:

-The largest average mass of the assortments was identified at the flow rate of 2.5 L/min,

-The lowest average mass of the assortments was identified at the flow rate of 1.0 L/min,

-Therefore, a flow rate of the nutrient solution higher than 2.5 L/min is not justified.

2. Related to the average length of the plant roots:

-The longest length of the plant roots was identified at the flow rate of 1.0 L/min,

-Withal, there is a significant relationship between the length of the plant root and the rate of drainage of the nutrient solution.

3. The impact of oxygen availability in the plants root zone: during the study was demonstrated that there is a direct connection between the increasing of the flow rate of the nutrient solution and the increasing of the concentration of oxygen. However, considering the above-mentioned results, a flow rate of the nutrient solution higher than 2.5 L/min is not justified.

4. The nitrate content of lettuce plants was in all the cases below the limit allowed by the quality standard of 2,000 mg / kg for lettuce crops in the greenhouse during the winter time and increased in the same time with the nutrient solution flow rate.

The lowest amount of nitrate content was evidenced for all lettuce varieties at a flow rate of 1.0 L / min.

The highest nitrate content was identified in lettuces where the solution flow rate was 2.5 L/min.

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THE EFFECT OF FERTILIZATION ON THE GROWTH PARAMETERS OF SEEDLINGS AND LETTUCE PLANTS GROWN IN THE NFT SYSTEM (NUTRIENT FILM TECHNIQUE)

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Abstract

Lettuce is one of the important vegetable crops being grown worldwide both in the open field and in greenhouses due to its nutritional benefit. Growing lettuce in hydroponic especially in the NFT system always gets attention from the growers and scientist communities, and for farmers, it challenges when it comes to selecting appropriate fertilizers and cultivars for growing to reach optimal growth and at the same time with high yield performance together with market preferences. The aim of the study was to identify the most favorable effect of fertilizers on the growth of the lettuce seedlings as well as the cultivars growth grown in the NFT system. The study was conducted in Hortinvest greenhouse using seven varieties of lettuce. Three types of fertilizers were used during the seedlings stage including Universol, Formulex, and Bio-Grow. We found the differences in the duration of plant emergence, vegetative growth of seedlings as well as varieties growth cultivated in NFT.

Key words: *Lettuce, seedlings, NFT, Fertilizer.*

INTRODUCTION

Vegetables play an important role as a source of food providing vitamins and other necessary nutrients for a human being. It was estimated that the world population will reach 9 billion by 2050 (Tilman et al., 2002), in this context, more food is needed especially vegetables to feed such a huge population while agricultural land will be decreased due to urbanization. Relying on conventional farming alone, cannot solve the problem of food shortage and environmental issues.

Hydroponics is commonly used in crop production practice worldwide, especially in developed countries with big-scale production. It is regarded as a high-tech cultivation system or the soilless cultivation in which plants basses on the mineral nutrient solution (Jones, 1982; Sardare & Admane, 2013; Becherescu et al., 2018; Phibunwatthanawong & Riddech, 2019). In the system, the nutrients are fed to the roots directly (van Os et al., 2019). The hydroponic system, it is divided into two systems (open and closed), the surplus nutrient solution is not collected for recycling in the open system. It is collected and recycled back into the nutrient tank in the closed system. There are three types of open systems for hydroponic based on water and nutrient distribution, it includes the Deep Flow Technique (DFT), Nutrient Film Technique (NFT), and the Aeroponic system (Kovácsné et al., 2019 and Göddeke et al., 2019). Hydroponic system by using a nutrient film technique (NFT) was first developed by Allen Cooper and his teamwork in the 1960 s at the Glasshouse Crops Research Institute in Little Hampton, UK. It is the type of growing the crops in the shallow film by using nutrients solution that flows near the roots, so the plant easily directly absorb nutrient from the solution (Hardeep et al., 2019). Currently, the worldwide cultivated area of vegetable production was about 3.5% growing under tunnels and greenhouses based on the hydroponic system by using floating system, NFT or aeroponic (Sambo et al., 2019). World vegetable production increased almost double between the year 2000 to 2019 from a volume of 682.43 million metric tons to 1130.2 million metric tons. The leading country producing fresh vegetables in 2019 was China with an amount of 588.26 million metric tons, followed by India, with 132.03 million metric tons, and the United States, with 29.29 million metric tons (Shahbandeh, 2021). In Romania, around 80% of vegetables come from imports and in 2019,

approximately 740000 tons of vegetables worth 516 million euros were imported (Drăghici et al., 2021).
Lettuce is one of the popular crops are being grown in hydroponics especially in the NFT system due to its nutritional values, contains a large variety of phytonutrients, fibres and other elements necessary to a proper functioning of the human body (Hoza et al., 2020). Lettuce belongs to the family of *Asteraceae* within the order *Asterales*. It has more than 1.620 genera and 23.600 species distributed worldwide (Petruzzello, 2018,).
The urban cultivation system, also known as the hydroponic system, is now seen as a viable solution to the limited land area suitable for agriculture. It is also an alternative option for increasing vegetable yield, nutrient, and water efficiency, environment friendly and can support vegetables year-round which are better opportunities for sustainable food supply in both developed and developing countries (Daniel, 2014, Chow et al., 2017). Cultivation of lettuce on NFT always challenging for the grower in terms of choosing the type of fertilizers to have a good healthy seedling and suitable cultivar with high yield performance due to abundant products available on the market. The objective of our study was to evaluate the effect of fertilizer on the growth of lettuce seedlings and cultivar in NFT system.

MATERIALS AND METHODS

The study was carried out in the greenhouse under the natural light condition at the Hortinvest, Research Center for Quality Control of Horticultural Produce, Faculty of Horticulture, UASMV, Bucharest, for one growing cycle from Dec 07, 2021, to Jan 30, 2022.
Seven cultivars of lettuce were used in the experiment including four cultivars of Lollo Bionda, Fast fall lettuce (V1) from Seng van company, and KKL (V2) from Kbal Koh, these two cultivars were the type of loose-leaf lettuce brought from Cambodia, and other two were from Romania, Lollo Bionda (V3) from Amia, Lugano (V4) from RZ. Another two types of Lollo Rosa lettuce – Carmesi (V5), Lollo Rosa (V6) from Holland Farming company, and one Chinese purple leaf (V7) which was a type of oak leaf lettuce from YingKe seeds company. All the lettuce seeds were sown on 07 December 2021 in the plastic tray (40 x 60 cm) filled with substrate from Plantobalt mixed with perlite in the proportion of 75% and 25%. The seeds were placed in the row covered by vermiculite. A week after sowing, the young seedlings were transferred into jiffy peat pellets and treated with different nutrient solutions from the beginning of sowing until the seedling stage, the solutions were used including Universol, which the composition of 18N-11P-18K+2.5MgO, Formulex, 2.3N-0.9P-3.4K, Ca 1.85, Cu 0.002, Fe 0.040, Mn 0.010, Mo 0.001, and Zn 0.0025 (%w/v), Bio-Grow, 4N-3P-6K, and control treatment was tap water. The nutrient solutions were mixed with water at the rate of 2g/l and applied daily to seedlings.

The seedlings were transferred into NFT when the plant had 3-4 true leaves about 20 days from the date of sowing. The experimental design was RCBD with 3 replications, five plants for each variety. pH and EC were checked on a daily basis by maintaining at a certain level, during the first week in the NFT, the EC was maintained at 1.2-14 mS/cm, while increased to 1.8-2.1 mS/cm until harvest, and pH was 6.0 during the growing cycle. The temperature and humidity were monitored in the greenhouse (Figure 1). CO_2 was maintained (550-650 ppm).
Data collected on seedlings stage including plant height, and the number of leaves per plant were recorded on the day transfers into NFT, and growth rate was recorded two times (10 days before transfers into NFT, and on the day transfers in the NFT system), the date of emergence true leaf was observed and recorded when the 50% of the seedling emerge true leaf.
In NFT, all data were determined at the harvesting stage. includes the number of leaves per plant, plant height, plant spread (measured manually), Leaf area using scanner, while fresh mass using an electronic scale, and dry mass were taken 1 gram of fresh leaves by cutting leaves into fine pieces dried at constant temperature 105°C for 24 hours. Statistical analysis was used STATISTICA, StatSoft software (version 10) to perform ANOVA analysis, and Tukey HSD was used to compare the significant difference of each dependent variable at $p \leq 0.05$, or 0.01 levels.

RESULTS AND DISCUSSIONS

The date emergence true leaf: The result obtained showed that the date of emergence of true leaf of the lettuce cultivars was 12-15 days counted from the date of sowing. Universol, Formulex, and water treatment had a similar date of emergence but Bio-Grow showed a delay in the emergence true leaf (Table 1).

Plant height: There was a strong interaction between fertilizers and cultivars over plant height at $p \leq 0.01$ (Table 2). Each fertilizer influences differently over plant height at $p \leq 0.01$. The plant height of the seedling used nutrient solution from Formlex was higher than other fertilizers, followed by Universol (7.22 cm and 7.11 cm). Nutrient from Universol was not significant with the control treatment (tap water), but the plant height was slightly higher than the control. There was a negative effect of using Bio-Grow while its value was lower than the control treatment (4.05 cm and 6.82 cm). For cultivars, the mean value across all fertilizer treatments were significance difference at $p \leq 0.01$, Fast Fall lettuce and KKL had a higher plant height than other cultivars (7.65 cm and 7.31 cm) followed by Chinese purple leaf, Carmesi (6.57 cm and 5.98 cm), while the plant height of Lollo Rossa, Lugano and Lollo Bionda was shorter than other varieties (5.60, 5.51 and 5.49 cm). There was a correlation between plant height and cultivars in control treatments (R^2= 0.2587) (Figure 2), and this correlation was higher in Universol nutrient solutions (R^2= 0.4183) (Figure 3), whereas in Formulex and Bio-Grow were R^2=0.2587 and R^2=0.2493) (Figures 4 and 5).

The number of leaves: There was a significant difference at $p \leq 0.05$ between the cultivar and the fertilizers, and also among the fertilizers types was different at $p \leq 0.01$ over the number of seedling leaves (Table 2). Universol, and Formulex had a greater number of leaves (3.69 and 3.60) than the control treatment, and Bio-Grow (3.36 and 3.17). For cultivars, there was a significant difference at $p \leq 0.01$. The cultivar which had a greater number of leaves was Chinese leaf purple, followed by Lugano and Carmesi (4.15, 3.70 and 3.65). While Lollo Bionda had a lower number of leaves than other varieties (2.90). There was a correlation between the number of leaves and cultivars in the control treatment but was very low (R^2=0.0773) (Figure 2), and this correlation continued to increase in Universol and Bio-Grow (R^2=0.2704 and R^2=0.6849) (Figures 4 and 5).

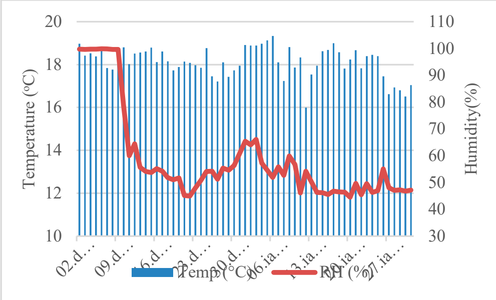

Figure 1. The temperature and relative humidity in the greenhouse

Table 1. The date of emergence true leaf of lettuces seedlings

Cultivar	Water	Universol	Formulex	Bio- Grow
V1	12	13	12	15
V2	12	12	12	15
V3	14	14	13	15
V4	13	12	13	14
V5	13	13	13	15
V6	14	13	13	15
V7	13	13	14	15
Mean	13.0	12.9	12.9	14.9

Table 2. The interaction of fertilizers and cultivars on the lettuce seedlings for plant height and the number of leaves

Cultivar (C)	Plant height (cm)	Number of Leaves
Fast Fall lettuce (V1)	7.65 ± 0.49 a	3.45 ± 0.14 bc
KKL (V2)	7.31 ± 0.55 a	3.25 ± 0.10 cd
Lollo bionda (V3)	5.49 ± 0.27 d	2.90 ± 0.07 d
Lugano (V4)	5.51 ± 0.22 d	3.70 ± 0.11 b
Carmesi (V5)	5.98 ± 0.21 c	3.65 ± 0.11 bc
Lollo rossa (V6)	5.60 ± 0.17 d	3.25 ± 0.10 cd
Chinese purple leaf (V7)	6.57 ± 0.35 b	4.15 ± 0.13 a
Fertilizer (F)		
Contol	6.82 ± 0.19 b	3.36 ± 0.10 b
Uniersol	7.11 ± 0.24 ab	3.69 ± 0.09 a
Formulex	7.22 ± 0.22 a	3.60 ± 0.10 a
Organic Grow	4.05 ± 0.10 c	3.17 ± 0.10 b
Cultivar (C)	**	**
Fertilizer(F)	**	**
C x F	**	*

n=20 for cultivar and n=35 for fertilizer.
Means and standard error followed by the same letters are not significantly different at p < 0.05 or 0.01

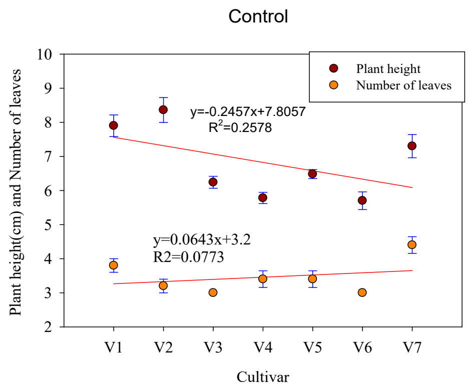

Figure 2. The correlation between cultivar, plant height and number of seedlings leaves in control treatment at seedling stage

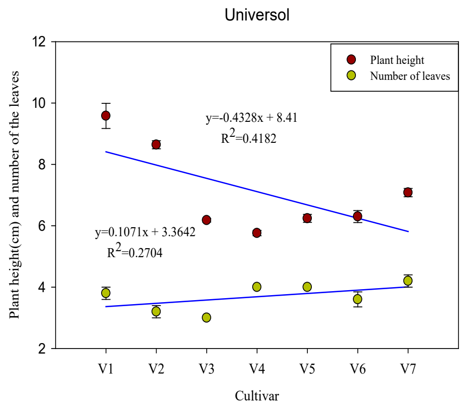

Figure 3. The correlation between the cultivar, plant height and number of seedlings leaves in Universol nutrient solution

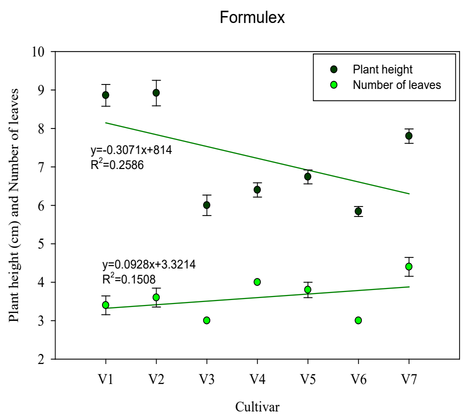

Figure 4. The correlation between the cultivar, plant height, and number of seedlings leaves in Formulex nutrient solution

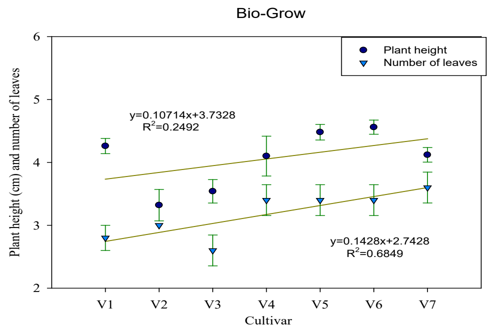

Figure 5. The correlation between the cultivar, plant height and number of seedlings leaves in Organic Grow nutrient solution

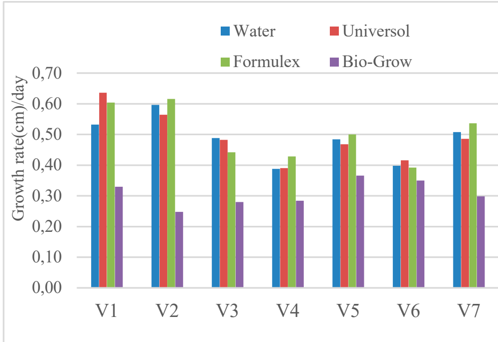

Figure 6. The growth rate of lettuce seedlings in different nutrient solutions

The growth rate of lettuce seedlings among cultivars in the control treatment, Universol, and Formulex was the same minimum value (0.39 cm/day) while the maximum value was (0.60-0.64 cm/day). In contrast, Bio-Grow had poor seedlings growth (0.25 -0.37 cm) compared to the other (Figure 6).

Plant height: The performance of the lettuce cultivars over plant heigh showed a significant difference at $p < 0.01$. V7 had a greater plant height (40 cm) followed by V1 and V2 (26.2 and 26.5 cm). The shortest cultivar was observed with V3 and V6 (15.9 cm and 16.9 cm) (Figure 7).

The number of leaves: There was a highly significant difference at $p< 0.01$ for the number of leaves among the cultivars. V7 was found higher in the number of leaves, followed by V6 and V4 (58, 35, and 33 leaves). V1 and V2 were lower in the number of leaves (25 leaves) (Figure 7).

The plant spread: There was also a highly significant difference at $p < 0.01$ for the leaves of the plant spread. V7 had a bigger plant spread followed by V1 and V2 (56.3, 41.2, and 39.4 cm) while the smaller plant spread was found with V6 and V3 (26.4 and 24.9 cm) (Figure 7).

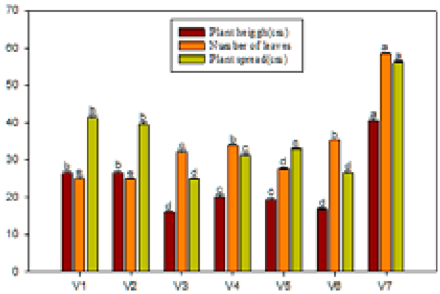

Figure 7. Plant height, number of leaves and plant spread analysis of lettuce cultivars grown in NFT system. Means and standard error followed by the same letters are not significantly different at $p < 0.05$, n=5

A correlation was also found between the plant height and the number of leaves (R^2=0.4474) (Figure 8), and the plant height with the plant spread had a strong correlation (R^2=0.9806) (Figure 9).

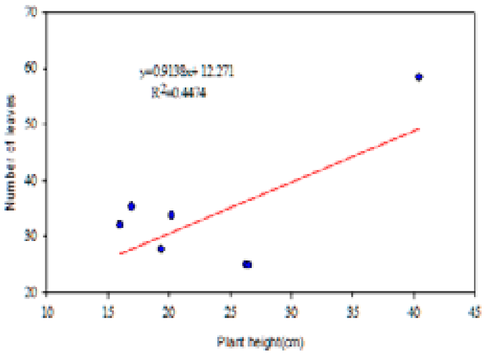

Figure 8. The correlation between the plant height and the number of leaves of lettuce cultivars

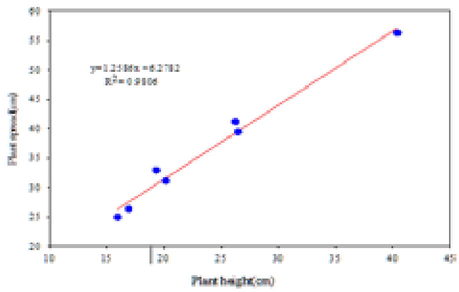

Figure 9. The correlation between the plant height and the plant spread

The differences of plant height, number of leaves and the plant spread of each cultivar possibly related to the characteristic of cultivars, this result supported Dahal et al., (2021).

Total leaf area: There was a highly significant difference at $p < 0.01$ for the leaf area of the lettuce cultivar. V1 and V2 had a bigger leaf (25.9 and 245.0 cm^2/leaf), V5 and V7 medium size leaf (192.5 and 187.7 cm^2/leaf), while V4, V3, and V6 had a smaller in leaf (150.1 137.0 and 127.5 cm^2/leaf) (Figure 10).

Fresh mass: The result from the analysis showed highly significant at $p < 0.01$ among the lettuce cultivars on the NFT system. V7 performed well with fresh weight (374.8 g/plant), followed by V1, V2 and V4 (192.5, 177.0, and 173.3 g/plant). V6 was observed lowest in fresh mass (134.5 g/plant) (Figure 10).

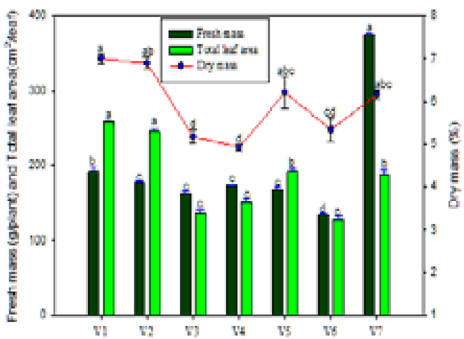

Figure 10. Fresh mass, total leaf area, and dry mass analysis of lettuce cultivars grown in the NFT system. Means and standard error followed by the same letters are not significantly different at $p < 0.05$. n=5 for fresh mass, and total leaf area and dry mass n=3

Dry mass (%): The highly significant difference level was found with dry mass at $p < 0.01$ for lettuces. V1, V2, V5, and V7 were not significant difference with the percentage of the dry mass was 7.01%, 6.89, 6.21, and 6.17%. In contrast, three cultivars including V6, V3, and V4 had a lower dry mass (5.34, 5.17, and 4.92%), respectively (Figure 10).

The total leaf area and plant height had a correlation with the value of R^2=0.220, while the fresh mass had a higher correlation with plant height R^2=0.8717 (Figures 11 and 12). There was a strong correlation found between the dry mass and the total leaf area R^2=0.9107 (Figure13).

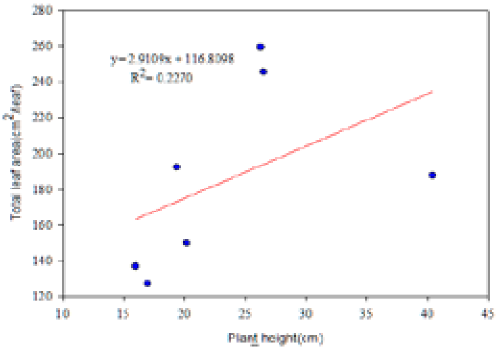

Figure 11. The correlation between the plant height and total leaf area of lettuce cultivars

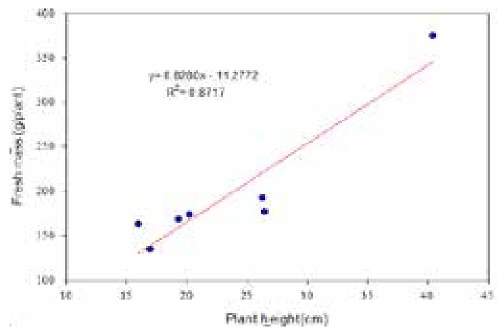

Figure 12. The correlation of plant height and fresh mass of lettuce cultivar

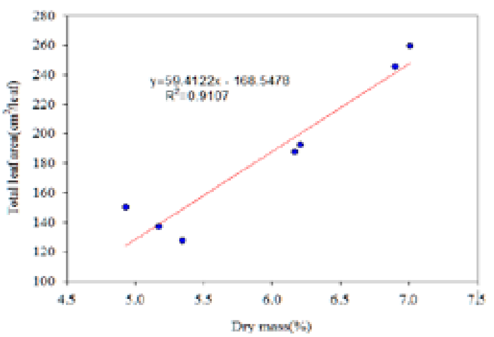

Figure 13. The correlation of dry mass and total leaf area of lettuce cultivar

CONCLUSIONS

Based on our result from the experiment on seedlings stage used different nutrients solution, there was the effect on the lettuces cultivar treated solution on the plant height, the number of leaves, date of emergence of true leaf and the growth rate. Formulex and Universol had a better performance on the seedling growth and emergence of true leaf but slightly different with using water while using Bio-Grow showed lower in plant growth and late emergence true leaf compared with the other treatment. Choosing the type of fertilizers for using during the seedlings might have a positive and negative effect on the growth of the plant. Further research should be carried out to investigate the type of fertilizers appropriate for use during the seedling stage.

For the experiment of lettuces grown on the NFT system, V7 had a very great performance in all parameters observed in both growth and the yield than other cultivars. V7 is a suitable cultivar for the NFT system, however, this cultivar is an oak leaf lettuce, the market preferences might be the influence factor for the grower in making decisions on type of cultivar to grow.

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PRELIMINARY RESULTS REGARDING INTEGRATED PEST MANAGEMENT METHODS OF ARTHROPOD SPECIES IN SWEET POTATO CROP – CASE STUDY – WIREWORMS

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Abstract

Wireworms, larvae of click beetles, Agriotes spp, are important pests of sweet potato. There is currently no curative treatment available to control wireworms and preventive treatments are mainly chemical. Therefore, is a need for a better understanding of the damaging factors to develop effective integrated control strategies and methods (IPM). This study aims to present the preliminary results on bait and pheromone traps used in a sweet potato crop to evaluate the densities of three major wireworm species in south-eastern Europe (Agriotes lineatus, A. obscurus, and A. ustulatus). Biological control with Metharizium anisopliae and Beauveria bassiana was applied. Agriotes ustulatus was the main species caught with pheromone traps, over 2400 specimens, while A. lineatus were 270 specimens, and A. obscurus 280. Statistical analysis was performed for evaluating the effectiveness of biological control with Metharizium anisopliae and Beauveria bassiana, the result demonstrating the viability of the method, the number and size of commercial sweet potatoes being significantly higher in the treated plot. The study offers perspectives to develop preventive and curative solutions for the sustainable control of wireworms.

Key words: *IPM, biological control, bait traps, pheromone traps.*

INTRODUCTION

The model of conventional agriculture involving the use of pesticides is questioned in terms of sustainability, environmental and human health (Wilson and Tisdell 2001; Tilman et al., 2002; Geiger et al., 2010). One of the biggest challenges for agriculture in the coming years will be to reconcile productivity with other components of sustainability, especially in order to achieve substantial reductions in pesticide use (Foley et al., 2011; Lechenet et al., 2014).

European Directive 128/2009/EC on the sustainable use of pesticides has made it mandatory to apply the principles of integrated pest management (IPM) in the European Union from 1 January 2014 (Barzman et al., 2015).

The first IPM principle recommends the prevention and suppression of pests by adopting crop rotation and other preventive agronomic strategies that can reduce the risk of pests and the need for plant protection measures. Therefore, in order to comply with Directive 128/2009/EC, farmers should make the most of agronomic solutions and research should support them in developing the best strategies (Furlan et al., 2020).

Particularly challenging is the control of soil-dwelling-pests because they are difficult to access. Even though these insects are mostly ‘‘out of sight’’ their feeding pressure on belowground parts of crops is of great economic importance (Hunter, 2001; Blossey and Hunt-Joshi 2003; Blackshaw and Kerry, 2008).

Elateridae, popularly known as click beetles, is a family of beetles that is distributed worldwide and includes about 9,000 species (Barsics et al., 2013).

The family includes 27 genera, but most of the significant arable crop pests belong to the genus *Agriotes*.

Agriotes are found mainly in the Holarctic and eastern areas and there are about 200 species worldwide.

Click beetles are characterized by a multi-annual life cycle, which differs dramatically between species. They can be divided into two

main groups. The first group consists of species with adults that do not overwinter, live a short period and lay eggs a few days after mating (*A. ustulatus, A. litigiosus*), (Furlan, October, 2004).
The second brings together species with adults that overwinter and live for months. They lay eggs for a longer period (*A. sordidus, A. brevis, A. lineatus, A. sputator, A. obscurus, A. rufipalpis* and *A. proximus*).
Adults emerge from the soil in the spring, usually from late March to early May. Females *A. obscurus* L., *A. sputator* L. *and A. sordidus lay* their eggs in May or June, below the soil surface (Miles 1942; Furlan, 2004) while the oviposition peak of *A. ustulatus* occurs in July and early August (Furlan, 1996).
The larvae of *Agriotes* spp. are popularly called "wireworms" due to their elongated body, covered by a strong chitinized, yellow-brown skin. In the last stage of development, the body can reach a length of 18-30 mm. It has 3 pairs of well-developed legs and mandibles.
Polyphagous pests, with the help of strong mandibles, dig into the tubers leaving small, round holes on the surface and narrow tunnels inside the tuber.
Although the attack does not affect the yield, it causes a considerable decrease in quality, which can make the tubers unmarketable even when the damage is relatively small.
In the specialized literature, various authors have conducted studies on methods of monitoring and integrated pest management of wireworms. The most relevant are listed in Table 1.

Table 1. Agriotes studies review

Reference	Study subject	Species/crop type
Furlan and Howard, 2021	Capturing distance of YATLOR pheromone traps for clic beetles *Agriotes (A. brevis, A. sordidus, A. litigiosus* and *A. ustulatus).* Male adults were released into the open field where the rate and distance at which they were recaptured were tracked. Catch rate was significantly affected by distance, species and wind direction and decreased as the distance increased. Most beetles were caught at short distances (up to10 m), in the first five days	Corn, soybeans, sugar beet, wheat, black field.
Furlan et al., 2001	Evaluation of the efficiency of YATLOR and VAR pheromone traps for *A. brevis, A. obscurus, A. lineatus, A. sputator, A. rufipalpis, A. litigiosus* and *A. ustulatus.* Investigating the relationship between catches in pheromone traps and the level of wireworm soil population	Soybeans, cereals
Furlan et al., 2020	The potential of a simple agronomic strategy, namely the proper timing of meadow ploughing to prevent wireworm attacks on maize and other arable crops in early stages	Corn, fodder plants
Staudacher et al., 2013	Reducing wireworm damage on corn plants by diversifying crops	Corn, wheat, legumes
Parker et al., 2001	Monitoring methods and agronomic methods applied to reduce the number of soil wireworms larvae Biological control methods The insecticidal effect of glucosinolates	Potatoes
Barsics et al., 2013	Identification of Agriotes species, life cycle description, optimal times for control measures application Correlation between the risk of attack with the number of wireworms caught in bait traps The advantage of using pheromone traps for monitoring but also in control Agrophytotechnical methods Biofumigation control with glucosinolates Biological control methods	
Furlan, 2007	Accurate monitoring of Agriotes species. Prediction of the degree of attack Agrofytotechnical control methods: crop rotation, synchronization of different tillage techniques depending on the ecology of the target *Agriotes* species, irrigation synchronization Biological control methods	
Parker, 1996	Monitoring methods for wireworms using cereal bait traps	Potatoes
Furlan, 1998	*Agriotes ustulatus* biology. Larval development, pupation, life cycle and practical implications.	

Vernon and van Herk, 2013	The importance of identifying the *Agriotes* species present in the crop. Methods of identification of *Agriotes* species. Biology and ecology of harmful *Agriotes* species. Description of the activity of harmful *Agriotes* larvae. Methods for monitoring *Agriotes* species and risk assessment in potato cultivation. Control methods: agronomic methods, biological methods, biotechnical methods.	Potatoes
MacKenzie, 2010	Agrophytotechnical methods for controlling wireworms (crop rotations, push-pull strategy, immobilization strategy)	Carrots, alfalfa, mustard, oats, buckwheat, barley, clover, flax, wheat.
Morales-Rodriguez et al., 2017	Monitoring methods of *Agriotes* larvae using 4 types of bait traps	Cereals
Furlan, 2014	Economic damage thresholds (correlations between the number of larvae belonging to different *Agriotes* species caught by different methods and the degree and intensity of the attack on maize plants)	Maize
Furlan et al., 2010	The role of biofumigation in wireworm control.	
Poggi et al., 2018	The influence of climatic factors, agronomic practices, identification of dominant *Agriotes* species and landscape characteristics in assessing the risk of wireworm attack.	Maize
Saussure et al., 2015	Management of damage caused by wireworms in corn crops using landscape-scale strategies.	Maize

MATERIALS AND METHODS

Integrated pest management

Integrated pest management is a systems approach to pest control that combines biological, cultural, and other alternatives to chemical control with the judicious use of pesticides. The objective of IPM is to maintain pest levels below economically damaging levels while minimizing the harmful effects of pest control on human health and environmental resources.

In 2021, a field experiment was established on a one-hectare organic sweet potato crop (*Ipomoea batatas*) in (Călărași county, southeast Romania) that harbored high densities of *Agriotes* larvae.

Agronomic methods

Economically these methods are the cheapest control methods. It prevents pest multiplication and attack. Significantly reduce the number of treatments required and it is used for long periods and in large areas.

In organic vegetal farms, biodiversity is a key factor for sustainable production. (Toncea, 2016).

Due to the multi-annual biological cycle of wireworms (Furlan, 2004; Parker and Howard, 2001), crop rotation is considered good agricultural practice for pest control, when it includes plant species that do not host *Agriotes*.

Diversity in plant cultivation is achieved through a combination of various and relatively long crop rotations (Table 2).

Table 2. Crop rotation used on the experimental field

Plot/ year	**2021**	**2022**	**2023**	**2024**	**2025**	**2026**	**2027**
1	Alfa-alfa	Alfa-alfa	Egg-plant	Peas– Oats/ Bro-ccoli	Sweet potato	Courgette	Alfa-alfa
2	Alfa-alfa	Alfa-alfa	Alfa-alfa	Egg-plant	Peas – Oats/ Bro-ccoli	Sweet potato	Courgette
3	Courgette	Egg-plant	Peas– Oats/ Bro-ccoli	Sweet potato	Courgette	Alfa-alfa	Alfa-alfa
4	Eggplant	Peas– Oats/ Bro-ccoli	Sweet potato	Courgette	Eggplant	Peas–Oats/ Bro-ccoli	Sweet potato
5	Peas– Oats/ Bro-ccoli	Sweet potato	Courgette	Alfa-alfa	Alfa-alfa	Alfa-alfa	Egg-plant
6	Sweet potato	Courgette	Alfa-alfa	Alfa-alfa	Alfa-alfa	Egg-plant	Peas–Oats/ Bro-ccoli

Tillage, sweet potato crop varieties used and planting time

The larvae migrate vertically through the soil depending on the interaction between the climate conditions and the soil texture. In Europe, they generally migrate twice a year, in spring and autumn, when there are abiotic favorable conditions in the upper layers of the soil (Jung et al., 2012; Parker and Howard, 2001), making them vulnerable to tillage during this period. Tillage and manual hoeing reduce the number of eggs and young larvae by mechanical damage. It brings them to the surface of the soil, where they are exposed to predation and desiccation (Table 3).

Table 3. Tillage practices used on the experimental field in 2021

Tillage date	Equipment used
15/03	Rotary tiller
29/03	Rotary tiller
26/04	Rotary tiller
17/05	Rotary tiller
07/06	Rotary tiller
25/06	Hoe (manual)

The varieties ROK1 and KSC Korea, are resistant to abiotic and biotic stress (developed by The Research and Development Station for Plant Culture on Dăbuleni Sands).

Respecting the optimal planting time (May 15-20) significantly reduces the attack of Agriotes larvae. On the experimental plot, the planting took place on May 25.

The delay of planting by 15-30 days causes massive decreases in tuber production and increases the degree of wireworms attack.

Wireworms generally have two intense periods of activity that can result in significant damage to the crop. Intense periods of activity occur from March to May and from September to October. Any delay in harvesting the crop brings an additional risk of damage to the tubers.

Physical-Mechanical methods

Bait traps

The basic principles of an integrated pest management program include pest identification through monitoring (Barzman et al., 2015).

Worm larvae are attracted to CO_2-producing sources, such as germinating seeds, plant respiration, and decaying plant material. From the large number of CO_2-producing baits tested, including fruit and vegetables (eg melons, carrots and potatoes), processed cereals (eg bran, oats and flour), germinated cereal seeds (eg wheat, barley) and/or other seeds (eg corn, sorghum) have been found to be most effective.

Bait traps made and used in accordance with (Parker, 1996) were deployed to estimate wireworm population densities from April to mid-May. The bait traps were placed on April 12, when the soil temperature (5 cm deep) exceeded 7^0 C.

On May 12, the baits were removed from the soil, on average, 3 larvae of wireworms were caught per trap which presents a high risk of economic damage.

Biotechnical methods

Pheromone traps are used for monitoring, but in large numbers, they can be useful in controlling pests by mass capture of adults.

Pheromone traps for *Agriotes ustulatus, Agriotes lineatus, Agriotes obscurus* males were purchased from the Csalomon Plant Protection Institute in Hungary and were randomly placed in the sweet potato crop.

The main characteristics of application of pheromone traps were as follows:

Table 4. Number of Pheromone traps and disposal time

Disposal time	Pheromone species	Number of pheromone traps
28/06	*A. ustulatus*	4
28/06	*A. lineatus*	2
28/06	*A. obscursus*	2

Biological methods

It involves the use of living organisms and the products of their biological activity in order to regulate pest populations.

Fungal preparations are holistic tools in sustainable agriculture.

Fungal preparations with enzyme extract from *Beauveria bassiana* and *Metarhizium anisopliae* (BMV certified organic product) were applied by drip on 12 rows 750 meters long and 1 row was left untreated to verify the efficiency of the biological treatment (Table 5).

Table 5. Method and application time of the biological treatment

Application time	Liters/hectare	Method of application
20/07	3	Drip system
26/07	3	Drip system
02/08	3	Drip system
09/08	3	Drip system
17/08	3	Drip system
26/08	3	Drip system

RESULTS AND DISCUSSIONS

The first step in wireworm management consists in assessing the risk of crop damage by monitoring the level of populations in place (Barsics et al., 2013). The day after the pheromones traps were installed an inspection was made (Table 6). The pheromones were changed after 30 days.

Table 6. Monitoring results after one day of the pheromone traps disposal

Time of inspection	Species/trap number	Male adults captured
29/06	*A ustulatus* 1	62
29/06	*A ustulatus* 2	55
29/06	*A ustulatus* 3	58
29/06	*A ustulatus* 4	61
29/06	*A lineatus* 1	8
29/06	*A lineatus* 2	12
29/06	*A obscurus* 1	9
29/06	*A obscurus* 2	8

The inspections were made at the following intervals by Table 7:

Table 7. Pheromone traps monitoring results

Time of inspection	Species/trap number	Male adults captured	Average caught/day
07/07	*A ustulatus 1*	490	61.25
07/07	*A ustulatus 2*	487	60.88
07/07	*A ustulatus 3*	468	58.50
07/07	*A ustulatus 4*	502	62.75
07/07	*A lineatus 1*	70	8.75
07/07	*A lineatus 2*	90	11.25
07/07	*A obscurus 1*	84	10.50
07/07	*A obscurus 2*	77	9.63
21/07	*A ustulatus 1*	862	61.57
21/07	*A ustulatus 2*	886	63.29
21/07	*A ustulatus 3*	863	61.64
21/07	*A ustulatus 4*	902	64.43
21/07	*A lineatus 1*	114	8.14
21/07	*A lineatus 2*	138	9.86
21/07	*A obscurus 1*	118	8.43
21/07	*A obscurus 2*	122	8.71
04/08	*A ustulatus 1*	643	45.93
04/08	*A ustulatus 2*	628	44.86
04/08	*A ustulatus 3*	682	48.71
04/08	*A ustulatus 4*	614	43.86
04/08	*A lineatus 1*	67	4.79
04/08	*A lineatus 2*	72	5.14
04/08	*A obscurus 1*	68	4.86
04/08	*A obscurus 2*	74	5.29
18/08	*A ustulatus 1*	402	28.71
18/08	*A ustulatus 2*	388	27.71
18/08	*A ustulatus 3*	396	28.29
18/08	*A ustulatus 4*	404	28.86
18/08	*A lineatus 1*	22	1.57
18/08	*A lineatus 2*	21	1.50
18/08	*A obscurus 1*	26	1.86
18/08	*A obscurus 2*	23	1.64
20/08	*A ustulatus 1*	1	0.5
20/08	*A ustulatus 2*	1	0.5
20/08	*A ustulatus 3*	1	0.5
20/08	*A ustulatus 4*	2	1
20/08	*A lineatus 1*	0	0
20/08	*A lineatus 2*	0	0
20/08	*A obscurus 1*	0	0
20/08	*A obscurus 2*	0	0

The final monitoring results of *Agriotes* adults caught were as follows:

Table 8. Final monitoring results and economic thresholds

Agriotes Species	Adults caught	Economic threshold
A ustulatus 1	2420	300
A ustulatus 2	2434	300
A ustulatus 3	2455	300
A ustulatus 4	2485	300
A lineatus 1	222	200
A lineatus 2	315	200
A obscurus 1	280	200
A obscurus 2	291	200

The monitoring, of the 3 major *Agriotes* species present in the field, between 28 of June and 20 of august shows that the economic threshold of 300 *A. ustulatus* adults caught per trap and 200 *A. obscurus* and 200 *A. lineatus* has been exceeded resulting in higher catches.
The verify the efficiency of the biological treatment a row was left untreated and a statistical ANOVA - single factor analysis was applied on the tubers' weight and number from 3 repetitions of 5 meters from the untreated row (V1) and 3 repetitions of 5 meters from one treated row (V2) (Table 9).

Table 9. Data used for ANOVA single factor analysis

	V1R1	V1R2	V1R3	V2R1	V2R2	V2R3
Plants number/5 m	15	17	16	13	16	13
Stems number/5 m	180	204	208	130	192	130
Fresh stem weight kg/5 m	7.3	7.5	7.7	6.5	7.5	6.5
Tubers total number/5 m	82	107	124	159	144	111
Tubers total weight kg/5 m	13.2	9.5	15.5	13.5	14	11.5
Marketable tubers number /5 m	32	5	13	54	47	44
Marketable tubers weight/5 m	6.2	1	2	10	7.7	8.3
Unmarketable tubers number/5 m	50	102	109	94	97	67

The statistical results demonstrate the *Metharizium anisopliae* and *Beauveria bassiana* fungus biological control efficacy.

CONCLUSIONS

The key moments in predicting the implement-tation of integrated control strategies are, first of all, the knowledge of the time of appearance of the adult population and the period of egg-laying, in order to prevent the multiplication of larvae and to increase the destruction of eggs and first larval stages.

Secondly, the knowledge of the periods in which the larvae feed actively.

Therefore, to protect crops and identify the right time for the application of control measures is important to know, the distribution of species, their life cycle characteristics, methods of population density prediction and economic damage thresholds.

The use in crop rotation of plants of the *Brassicaceae* family (glucosinolates in the composition of these plants have an insecticidal effect).

The use of pheromone traps in large numbers can significantly reduce the adult population and slow down the reproductive cycle.

Avoid the cultivation of plants sensitive to attack on a soil previously cultivated with perennials plants.

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EFFECTS OF SEVERAL ORGANIC FERTILIZERS ON GROWTH, DEVELOPMENT AND QUALITY PROPERTIES OF TOMATOES OBTAINED IN ORGANIC SYSTEM: A REVIEW

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Abstract

One of the main goals of today's agriculture is to produce healthy food and to increase the efficiency of plant production by protecting and conserving the environment. Organic tomatoes (Lycopersicon esculentum Mill.) represent one of the most valuable crops consumed widely either fresh or processed because for its sensory qualities, nutritional value and for its benefits on human health (reduce the risk of cardiovascular disease and certain types of cancer). Organic fertilizers are originated from plant and animal wastes and contain N, P, K and microelements. They are used to obtain the nutritional demands of plants for their growth and development, as well as improve the physical, biological and chemical properties in the soil. The objective of this study was to evaluate the effectiveness of organic fertilizers on quality characteristics (soluble solids contents, lycopene and carotenoids, titratable acidity, minerals and vitamins), on the growth and development of organically cultivated tomatoes based on the lasted valuable information.

***Key words**: physico-chemical characteristics, vegetable growth, biofertilizers, sustainable agriculture.*

INTRODUCTION

The world population is reaching 9 billion by 2050, mankind will be facing the challenge of increasing food and energy production from crops by at least 70% to account for the increase in world population by adopting more sustainable farming methods and systems in a climate and environmental change scenario. The nutritional, organoleptic and nutraceutical quality of food also needs to be enhanced for improving the health in rural and urban communities. The enhanced vegetables production both in quantity and quality, needs to mitigate the changing climatic environment characterized by reduced and uneven distribution of rainfall, of extreme temperature fluctuation, of climatic events like flooding, storm, and drought, which reduces yields.

The use of pesticides and synthetic fertilizers in the process of fertilization provoke many health problems and environmental pollution (deterioration of soil characteristics and fertility) and lead to accumulation of heavy metals in plants (Dinu et al., 2015; Mahmoodi, 2017; Iosob et al., 2020).

The objective of decreasing the negative impact of agriculture on human and environment heath is a global challenge of interdisciplinary research. Organic agriculture is a new approach of farming aimed to develop production technology to obtain durable stability of the environmental protection and human safety. The implementation of organic farming principles is a tool featured by capacity to replace old practices and inputs that endanger the human health and environment.

The development and large-scale application of friendly environmental inputs and practices are strongly recommended by the new organic regulation (EU) 2018/848 and strategies such as: Farm to Fork, Green Deal, and Plant Protein Plan that highlight the importance of the development of new tools and solutions with the ability to limit the harmful effects.

Food production may be further reduced by the shift into low input systems due to local and global environmental concerns requiring governments to enact and enforce regulatory instruments for sustainable perspective for resilient, efficient, and sustainable farming systems and land use.

There are plenty studies in the international literature that demonstrates the quality of organic vegetables (Chassy et al., 2006; Kapoulas et al., 2011; Hallman, 2012; Araujo et al., 2015; Abdelkader et al., 2021; Doltu et al., 2021).

Tomato (*Solanum lycopersicum*) is a flagship species in the *Solanaceae* family, one of the most important and suitable vegetable crops grown throughout the world for its high nutritive value, antioxidant properties and for its aids on human health (Meena et al., 2017; Dūma et al., 2018; Shehata et al., 2018). Tomato fruit has a high content of water (94.52%) and a low caloric value, but it is a good source of vitamins: A, C, K, B1, B2, B3, PP (Viskelis et al., 2015; Tieman et al., 2017) and minerals (potassium, phosphorrous, magnesium, calcium, iron, sodium, copper, zinc (Dūma et al., 2018; Murariu et al., 2021) and contain organic acids, sugars, dietary fibre, many phenolic compounds, and carotenoids - lycopene and b-carotene (Nour et al., 2015; Ali et al., 2020).

Globally, tomato is a major consumed fruit vegetable with per capita consumption of either fresh or processed type of about 21 kg in 2017 or around 19% of the total vegetable consumption per year (FAOSTAT, 2020). According to the Food and Agriculture Organization of the United Nations, in 2020, the area cultivated with tomatoes worldwide was 5,051,983 ha with a production of 186,821,216 tons, and in Europe, in the same year, the area harvested was 424,449 ha with a production of 22,810,698 tons.

In Romania, in 2020, the total area harvested with tomatoes was 22 470 ha with a production of 449 460 tones. Compared to the previous year, the production of tomatoes increased by 12 910 tones.

The tomatoes consumption is associated with decreasing risk of developing digestive tract, prostate cancer (Giovannucci et al., 1995; Maguer, 2000), chronic degenerative diseases such as certain types of cancer (Giovanucci, 1999) and cardiovascular diseases (Agarwa and Rao, 2000).

The application of organic farming practices and input imprints benefits on the environment: contributes to the increasing of biodiversity and resistance to disease and pests (improving the soil quality makes plants healthier), commits to soil conservation (cover crops, mulching, intercropping), contributes to the reduction of eutrophication and water pollution, reduced energy use and some benefits on human health (reduce air pollution, makes food healthier due to its nutritional value) (Djokoto, 2015; Muller et al., 2016). Organic farming largely relies on organic materials instead of chemical fertilizers, pesticides, herbicides, or other synthetics. High-efficiency organic fertilizers may increase crop yield, does not affect soil quality, making it a support tool balance long-term food security and environmental protection (Angadi et al., 2017; Cen et al., 2020).

The differences between organic and conventional management practices include: the use/ non-use of chemical pesticides; the use/non-use of chemical fertilizers; and the need for a long and varied crop rotation (Bengtsson et al., 2005).

The goal of this review analysis was to investigate and determine the potential impact and efficiency of organic amendments on quality essences and on the growth and development of organically cultivated tomatoes.

MATERIALS AND METHODS

This assessment is based on a comprehensive evaluation of materials available in the international literature. The information for this analysis was gathered from open access sources on the internet through sites like Google School, Google Academic, Free Full PDF, Research Gate, and Science Direct. To find work content, we utilized the following keywords and phrases such as "organic manure," "biofertilizer," "quality of organic tomatoes," "growth," and "development." This study comprised studies on organic fertilizer that were connected to effects (advantages and downsides) on organically cultivated tomatoes and were available as free full papers in English. According to the analysis of the selected documents, the results show that organic farming, fertilizer application, and their effects on the quality and quantity characteristics of organic tomatoes are extremely broad and extensive internationally. Two inclusion criteria were considered: thematic focus (referring to the effect of natural fertilizer on growth, development and quality parameters of organically tomato) and document type (journal and books chapters). Following these guidelines, 82 studies and experiments were chosen that have both positive and negative features, such as increased vitamin C content, soluble solids, titratable acidity, fruit per plant, and overall yield, as well as negative elements, such as the fact that some organic fertilizers have limitations.

RESULTS AND DISCUSSIONS

Biofertilizers have been recognized as a possible technique for achieving a more sustainable agricultural system by improving food output while also improving ecosystem functionality (Swapna et al., 2016; Mahanty et al., 2017). Ecological fertilizers contain living or inert microorganisms or bioactive components obtained from organisms such as bacteria, algae, and fungi that can aid in soil fertility and plant growth (Abdel-Raouf et al., 2012).
Bacteria or fungi capable of nitrogen fixation, phosphate solubilization, sulphur oxidation, plant hormone synthesis, or organic compound decomposition are commonly used as biofertilizers (Verma et al., 2018; Pirttilä et al., 2021). Rashid et al., 2015, demonstrate that by affecting the aggregation of soil particles, microbial biofertilizers play an important role in preserving soil fertility and improving its structure. Inoculation with biofertilizers boosted crop yield by 16.2 percent on average when compared to non-inoculated controls, according to a study by Schütz et al. (2018).
Bacterial fertilizers are appropriate for use in agricultural fields because of their high humic acid concentration and low hazardous element content.
Vegetable, mineral, and animal matter are used to make organic fertilizers. In this case, a short classification can be done:

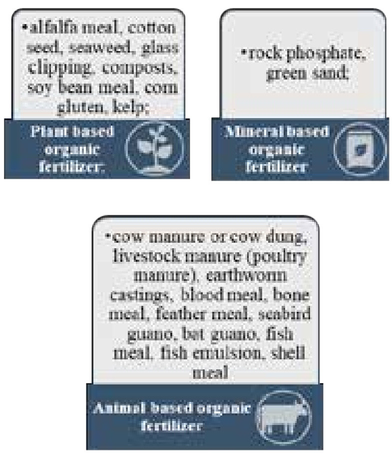

Figure 1. Classification of organic fertilizer

When biofertilizers are applied to seeds, plant surface or soil, living cells of different types of microorganisms from these products colonize the interior of plant and promotes growth by converting N and P from unavailable to available form (El-Yazid et al., 2007; El-Yazeid et al., 2011). Many researchers studied the role of organic manures and, also, shown that the use of compost in horticulture is beneficial because increase the quality, grown and yield of fruits (Adekiya, 2009; Khan et al., 2017; Bilalis et al., 2018). Youssef and Eissa, (2017) demonstrated that the combination of rock phosphate, feldspar, and rabbit manure with Microbine (Bio-N), Phosphorin (Bio-P) and Potassiumag (Bio-K) significantly enhanced vegetative growth, yield, and fruit quality of tomato plants.

The influence of organic fertilizer application on GROWTH and DEVELOPMENT of tomatoes plants

From planting to harvest, open-field tomato plants have a long production cycle that necessitates the use of supplemental fertilizer to maintain vegetative and reproductive growth (Choi, 2020). Natural fertilizers contain N, P, K and microelements in different rates depending to the source of the fertilizer. Organic fertilizer originated from molasses containing 7% N, 7% P and 7% K and from this point of view, Ulusu et al. (2017) demonstrated that the organic fertilizer from molasse increased the mineral and organic matter content of the soil while also developing the soil reaction for microorganism activity and represent a good alternative to synthetic fertilizers for tomato production under greenhouse conditions. Tiwari et al. (2016) discovered that foliar spraying tomato plants with vermiwash offers all of the necessary nutrients for growth, early flowering, and increased yield.
Organic fertilizers are environmentally friendly and provide nutritional requirements, suppress plant pest populations, and increase the yield and quality of agricultural crops (Tonfac et al., 2009; Souri et al., 2018; Laily et al., 2021). In a study conducted in Japan was concluded that organic fertilizer increased microbial biomass and enhanced nutrient circulation such as N and P circulation activity (Kai et al., 2020).
Nutrient is one of supporting factors of optimum growth and development of tomato plant (Tittarelli et al., 2017). Tomatoes require proper and sufficient nutrients for good fruiting and

subsequent quality, and as a heavy feeder of nitrogen, phosphorus, and potassium, they respond well to manure and fertilizer application (Gideon, 2012 and Singh et al., 2014).

In research conducted by Basilio, 2021 two biofertilizers composed of two different bacterial consortia were used in order to study their effects in the production and productivity of tomato plants: biofertilizer 1, constituted by the consortium *Bacillus* + *Pseudomonas*, and biofertilizer 2, constituted by the consortium *Azospirillum* + *Pseudomonas*. Biofertilizer 1 provide more benefits to the plant in greenhouse soils with higher levels of organic matter composed of higher concentrations of labile C fractions, whereas biofertilizer 2 provide more benefits to the plant in greenhouse soils with low organic matter and low total P content, resulting in a high P plant demand.

According to the findings of Mahmood et al. (2020) adding organic fertilizer to the soil and spraying garlic extract, whey, and bread yeast in tomato has increased the concentrations of N, P, and K in the soil and leaves, as well as a substantial improvement in plant dry weight, chlorophyll content, fruit number, fruit weight, and plant yield

El-Yazeid (2011) showed that growth parameters such as stem diameter, number of leaves, branches per plant, and leaf area/plant had significantly higher values when phosphate solubilizing microorganisms were combined with rock phosphate treatments.

Mutale-joan et al. (2020) studied the effects of 18 crude bio-extracts (CBEs) derived from microalgae and cyanobacteria on tomato plants (*Solanum lycopersicum*) at three different biomass concentrations: 0.1, 0.5, and 1 g L-1. The application of CBEs to tomato plants improved chlorophyll content, nutrient uptake, and, in many cases, root and shoot length and dry weight. According to Ordookhani et al., (2010) inoculating tomato plants with *Pseudomonas putida*, *Azotobacter chroococcum*, *Azospirillum lipoferum*, and a mixture of arbuscular mycorrhiza fungi (*Glomus lipoferum*, *Glomus mossea*, and *Glomus etunicatum*) increased lycopene antioxidant levels.

According to several studies, plots treated with a combination of organic and inorganic fertilizers produced more fruits per plant, heavier fruits per plant, and, ultimately, a higher total yield (Anwar et al., 2012; Ferdous et al., 2017). Similar, Laily et al., (2021) in a study conducted in Bangladesh obtained the highest yield due to fruit per plant and weight of fruit per plant from plants treated with chemical fertilizer in combination with organic fertilizer. Islam et al., (2017) reported that the application of mixed fertilizers, organic 2/3 + inorganic 1/3 produced the highest number of flower clusters, fruit clusters, fruit yield and plant height.

Heeb et al., (2006) sustained that the organic fertilizers have limited effects on plant growth and yield as compared to chemical fertilizer. Several researchers proved in their studies that higher yields were obtained from chemical fertilizer treatment (Mahmood et al., 2017; Turhan and Özmen, 2021).

The influence of organic fertilizer application on QUALITY PROPERTIES of tomatoes plants

Tomato (*Solanum lycopersicum*) is an adaptable and one of the most cultivated vegetables throughout the world for taste, colour and diversified use. Tomato fruits contains carotenoids which include β-carotene and lycopene. β-carotene is a pro vitamin and is responsible for orange coloration and lycopene has antioxidant properties and is responsible for redness (Sidhu, 2017; Dobrin, 2019; Salehi et al., 2019). Stoleru et al. (2020) sustain that the organic fertilization has a positive effect on the lycopene accumulation and antioxidant activity of tomato.

Some studies report better taste, higher vitamin C content, and higher levels of other quality-related compounds for organically grown tomatoes (Mitchell et al., 2007; Murtic et al., 2018; Peralta et al., 2020). Coppens et al. (2016) demonstrated that microalgal fertilizers improve the quality of tomato fruits through an increase in the sugar and carotenoid content. In a meta-analysis, Worthington (2001) concluded that organic crops contained more vitamin C, iron, magnesium, and phosphorus and significantly less nitrates than conventional crops. Similarly, Vinha et al. (2014) reported that the organic tomatoes were healthier than those produced by conventional practices. Ochoa-Velasco et al. (2016) compared the total antioxidant content of tomato fruits after biofertilization with *Bacillus licheniformis* and different nitrogen fertilizer doses. In general, they describe an improvement in tomato fruit quality in terms of total hydrophilic antioxidant compounds (vitamin C and total phenols) after inoculation with *B. licheniformis*, as well as a reduction in nitrogen dosing.

Soluble sugars (glucose, fructose and sucrose) and organic acids (mainly malic and citric acids) are major osmotic compounds accumulated in tomato fruit (Heuvelink, 2018). Pieper et al. (2008) studied the differences between production systems and identified that total and soluble solid were significantly higher and consistency was greater in organic tomatoes than in conventional ones.

Turhan (2021) exposed that the highest lycopene, total carotenoid, vitamin C contents and, also, fruit soluble solids and dry matter contents were obtained as reaction of application of organic fertilizers, especially from poultry manure.

Another study guided in Turkey, demonstrate that three different dosses (1, 3 and 5 g L^{-1}) of bacterial fertilizers which include *Azotobacter* spp., *Bacillus subtilis* and *Bacillus megatarium* affected the plants by increasing the mineral content (N, P, K, Ca, Mg) of fruits (Dursun et al., 2019).

Cyanobacteria can be used as a biofertilizer for enhance the qualitative and quantitative characteristic of tomatoes. In this regard Hussain et al. (2019) showed that the interaction of cyanobacteria with Marwa var. (a variety of tomatoes) gave the highest rate of yield traits, as well as total soluble solids, total acidity, vitamin C and fruit content of carotene and lycopene.

A two years experiment during 2013 and 2014 season demonstrate that the quality and quantitative parameters of tomatoes was higher when the biofertilizers was applied (Table 1). The biofertilizers used was *Azospirillum* sp. and *Azotobacter* sp. (nitrogen fixing bacteria) under the commercial name of Nitrobein and a mixture of P dissolving bacteria (*B. megatherium*) and N fixing bacteria *Azospirillium* sp. and *Azotobacter* sp. under the commercial name of Microbein (Mesallam et al., 2017).

Microalgae are a high potential source of biofertilizers and one of these microalgae is *Chlorella vulgaris*. Coban et al., (2020) investigated the effect of microalgae on nutrient saving in soilless greenhouse tomatoes. The algae used to have a major improvement impact on average tomato fruit production, expanded vitamin C levels, and increased mineral content P, Mg, and Na in tomato fruit. Furthermore, Suchitra et al. (2020) demonstrated that the foliar spraying of *Chlorella vulgaris*, as well as the combination of this algae with cow manure, have gained the highest soluble solids, total soluble sugar, ascorbic acid, total protein and moisture of tomato fruits.

Table 1. Effects of biofertilizer on quantitative and qualitative on fruit tomato during 2013 and 2014 season

Treatment	Effect of biofertilizers on fruit yield of tomato					
	Fruit weight (gm)		Fruits yield/plant (kg)		Fruits yield/fed (ton)	
	2013	2014	2013	2014	2013	2014
With biofertilizers	178.75	136.06	3.68	3.49	25.76	24.43
Without biofertilizers	154.06	132.94	3.49	3.30	24.43	23.10
Treatment	Effect of biofertilizers on quality of tomato					
	TSS%		Ascorbic acid (mg/100 g FW)		Titratable acidity (mg/100 ml juice)	
	2013	2014	2013	2014	2013	2014
With biofertilizers	3.42	3.68	17.06	22.69	0.96	0.86
Without biofertilizers	3.17	3.66	15.86	22.12	0.89	0.91

In a recent study Carricondo-Martínez et al. (2022) comparison between waste-derived amendments and inorganic fertilization on tomato yield, chemical and physical properties of tomato fruit was observed. The treatments with fresh crop residue, goat manure and composted vegetable waste showed significant lower yields compared with the inorganic fertilizer. In this experiment the highest value of fruit colour was obtained with conventional fertilization, but regarding to beta-carotene content, this was higher in organically cultivated tomato.

CONCLUSIONS

The reduction of chemical fertilizers in favour of products based on yard waste animal manure, products based on microorganisms or bioactive compounds, is a good alternative, not only to reduce the overuse of chemical fertilizers and pollution of the environment, but also to ensure food quality. On numerous occasions, green product application has been shown to improve crop output, reduce disease, and promote plant nutrient coordination.

In this review, we have discussed several examples of plant bacteria that, when used as biofertilizers, promote plant growth, increase yield, and improve some quality parameters (soluble solids contents, lycopene and carotenoids, titratable acidity, minerals, and vitamins) of organic tomatoes. On the other hand, we have described how the combination of some organic fertilizers contributes to preserving soil fertility and improving its structure.

It is also observed that there are a lot of studies comparing the amounts of secondary metabolites, growth and development of plant in organically farmed to those in conventionally grown vegetables.

Moreover, the application of fertilizer, whether organic or inorganic, boosted tomato growth, yield, and quality. It was discovered that combining organic and inorganic fertilizers was better than utilizing each separately.

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THE ORGANOLEPTIC QUALITIES OF SOME VARIETIES OF FRENCH BEANS

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Abstract

French beans are one of the most consumed vegetables in Romania, especially during fasting periods, but also worldwide, knowing that it has an important nutritional value. Due to its high protein content, many people who want to adopt an animal protein-free diet resort to eating bean-based foods. In the present study, four french bean genotypes were analyzed that showed differences in color, seed size and weight, but most importantly in taste.
From these, a control variety was chosen that presented white seeds, more precisely the 'Doina' variety, a variety approved by VRDS Buzau in 2020. Tastings were carried out followed by the completion of opinion polls, which found that the genotype preferred by consumers was the L3 genotype, a genotype that presents seed of red color. The purpose of this article was to find out if consumers are open to eating beans of a different color than the classic white.

Key words: *beans, genotype, 'Doina', VRDS Buzau.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important vegetable which is consumed worldwide for its edible seeds and pods because are a good source of protein (de Almeida Costa, da Silva Queiroz-Monici, Reis, & de Oliveira, 2006; Siddiq, Butt, & Sultan, 2011), has dietary fibre, starch (Osorio-Díaz et al., 2003), minerals, vitamins (Kutos et al., 2003), and beneficial nutraceuticals such as polyphenols (Wu et al., 2004), while containing little or no total fat, trans-fat, sodium and cholesterol (Drewnowski A, 2010; Raw P., 2012). Several studies have shown that regular consumption of beans can help lower total and LDL cholesterol and other risk factors for heart disease (Anderson et al., 1990; Bazzaro et al., 2011; Finley et al., 2007; Kabagambe et al., 2005). Bean intake has been associated with a decreased risk of breast, stomach, colorectal, kidney and prostate cancers in human and animal studies (Bobe et al., 2008; Dahm et al., 2010; Lanza et al., 2006; Thompson et al., 2012). Beans are high in natural antioxidants (Vinson et al., 1998). The color of the bean coat appears to affect the antioxidant capacity because this correlates with total phenolic content of the bean. Colored beans (red, brown or black) possess greater antioxidant activity than white beans (Madhujith et al., 2004). Furthermore, some of these antioxidant compounds are lost during typical preparation and cooking methods, although significant amounts of antioxidants still remain (XU BJ et al., 2008).

Dry beans are the second most cultivated species after soybean, from Fabaceae family (Gherase et al., 2020).

The popularity of the crop originates from the fact that it is relatively easy to produce, it is flavorful and versatile, and it is a good source of nutrition. Dry beans are one of the most consumed vegetables in Romania, especially during fasting periods, but also worldwide, knowing that it has an important nutritional value. Dry beans are usually prepared by soaking in water to imbibe the seed followed by cooking in a water-based broth either boiling or using a pressure cooker to shorten preparation time. With increasing urbanization, consumer preferences are shifting in favor of convenience foods and commodities, which require reduced food preparation time (Siddiq & Uebersax, 2012). The methodology of sensory profiling constitutes the basis of a

descriptive quantitative analysis, defining a product with the minimum number of words and with maximum efficiency, using a precise tasting sheet, which can be reproduced and is understood by all.
The objective is to define the sensory quality of the beans, establishing a protocol for the preparation of samples and a tasting sheet for the texture profile. The first methodological aspect is the formation of a tasting panel, the organization of sessions, the preselection of descriptive factors and the final list. It ends with a tasting sheet with the descriptive factors, in order of perception, and with a structured scale (Bourne, 1972; Blair, 1978; Meilgaard et al., 1987). The second methodological aspect is the training of the judges, with an evaluation of agreed criteria and the consistency and the ability of the team to reproduce results as well as their sensory evaluation of the varieties, by explaining the differences that exist between them (Drake, 1989; Mioche and Touraille, 1990).
Evaluating its individual traits it is possible for the breeders to get both total and detailed idea for the organoleptic properties of new lines and varieties. Sensory analysis is a significant element of the complex evaluation of bean quality in many investigations (Brewer et al., 1994; Mnkeni et al., 1995; Pevicharova and Poryazov, 2002). The aim of the present study was to assess the relationship between the total sensory evaluation and some characters of sensory and chemical analyses in for types of dwarf french bean and to estimate which of them have priority in the quality breeding of this vegetable crop.

MATERIALS AND METHODS

The Laboratory of Genetics, Breeding and Biodiversity from VRDS Buzau has a valuable germplasm collection of *Phaseolus* sp. grouped by type of plant growth and direction of use (Tănase et al., 2021).
The present study began in the first decade of May 2021, in the experimental field of VRDS Buzău, where the entire bean germplasm collection was cultivated.
The applied culture technology was specific to the field bean crop (Tanase et al., 2020).
Phenological, biometric and laboratory measurements were carried out during the vegetation period. The descriptors used were the one from UPOV guidelines.
Following this evaluation, four genotypes of beans were chosen to be analyzed from an organoleptic point of view, including the 'Doina' variety, a variety recently approved by VRDS Buzau, which was chosen in the current study as a control variety.
The choice of the four genotypes of beans was made based on criteria related to the size, but especially the color of the seeds. The genotypes were noted G1-white beans, G2-black beans, G3- red beans, G4- variegated beans (Figure 1).

Figure 1. Seeds detail

The aim of this study was to show that these four genotypes differ not only visually, through the criteria mentioned above, but also sensory (taste and texture). In this, regard tasting panel was made.
Our survey included 18 questions, in the first part was described the demographic profile of the respondents, followed by the main characteristics of the seeds and a comparison between them.
The tasting panel was formed by 16 respondents with ages from 18 to over 50 years old, both males and females, from rural and urban area. The resulted data were analysed with descriptive statistic methods.

The cooking and preparation conditions of the samples will be selected, recreating the conditions of consumption (Rousset-Akrim et al., 1995).
For the tasting, the four types of beans were prepared in the same way. 350 g of beans were used for each genotype, which was first soaked in water for 24 hours. After this time, the beans were boiled in four separate pots, but identical in material (stainless steel) and capacity (5 l), in two liters of water which was added a teaspoon of sodium bicarbonate, for thus avoiding packaging after consumption.
After the water boiled, the beans were left to boil for a period of 8 minutes, during which time the foam formed on top is removed, for the same reason that sodium bicarbonate was added. After 8 minutes the water was changed to a clean and cold one. Sodium bicarbonate was not added this time. The final cooking took 2 hours and 8 minutes, resulting in a total cooking time of 2 hours and 16 minutes.
Before to the tasting, the respondents were informed about the health benefits of beans.

RESULTS AND DISCUSSIONS

The seeds of the four genotypes of dwarf french beans, G1, G2, G3 and G4 were harvested at technological maturity, boiled and were share within our respondents.
The seeds were measured and weighed 3 times: at technical maturity, after soaking for 24 hours and after boiling.
After the boiling period, it was found that all genotypes kept the color of the seeds, except for the G4 genotype, whose seeds turned completely brown (Figure 2).
For a better understanding of respondents opinion and preferences on the Table 1 is presented their socio-demographic profile. The interviewed respondents were from 18 to more than 45 years old. From our study, 69.8% were women and 31.2% men. In 69.8% of the cases people were from the urban area and 31.2% of them from the rural area. Mainly are people with postgratuade studies (38.6%) and college (35.1%) studies.

Figure 2. Seeds detail of G4

Table 1. The socio-demographic profile of respondents

Variable	Consumers No.	Consumers %
Sex		
- Men	5	31,2
- Women	11	69,8
Area		
- Urban	11	69,8
- Rural	5	31.2
Age		
- 18-24	2	12.5
- 25-35	8	50.0
- 35-45	4	25.0
- Over 45	2	12.5
Studies		
- High school	4	25.0
- College	5	31.2
- Postgratuade studies	7	43.8

Sensorial analyses were organized in October 2021 for the four genotypes and the results are presented in the Table 2 for each criterion: seed taste and texture.
In terms of seed size 50% of respondents preferred G2 (Figure 3), a genotype that has the smallest seeds from this study. Specifically, before boiling the seed had an average length of 1 cm and a width of 0.65, and after the boiling it reached a length of 1.37 cm and a width of 0.67 cm.
The initial weight was 0.47 g and after boiling it increased to 1.54 g. On the other hand, G3 is the genotype of beans that had the largest dimensions, more precisely before boiling the seeds had an average length of 1.64 cm and a width of 0.78, and after boiling the length was 2.43 cm and the width 1.19 cm. The initial weight was 0.48 g and after boiling it increased to 1.48 g.

Table 2. Sensorial analyses of the seeds

Variable	Consumers No.	Consumers %
What genotype attracts you in terms of seed size?		
- G1	7	43.7
- G2	8	50.0
- G3	0	0.0
- G4	1	6.3
Are you reserved to try different colored been seeds?		
- Yes	4	25.0
- No	12	75.0
You would be willing to replace the classic bean with white seeds with beens that have different colored seeds?		
- Yes	12	75.0
- No	4	25.0
How often do you normally eat beans?		
- Rarely	5	31.2
- Fasting period	5	31.2
- Monthly	4	25.0
- Weekly	2	12.6
Given the amount of protein the beans contains, would you be willing to replace meat with bean in some cases?		
- Yes	12	75.0
- No	4	25.0

Figure 3. Seeds detail G2

Consumers were open to tasting all the bean genotypes, even though they normally ate only white beans. Only 25 % of them were a little reluctant to try them when they first saw the seeds.

75% of respondents would be willing to replace the classic white beans (Figure 4) with the genotypes of beans tasted in the current study (Table 3). There were also respondents who woud not give up the consumption of beans with white seeds, but rather would consume all the genotypes tried because they consider that the four genotypes tasted have different tastes.

Table 3. Respondents` preferences before tasting

Variable	Consumers No.	Consumers %
Which bean genotype do you like best in terms of seed taste?		
- G1	4	25.0
- G2	0	0.0
- G3	6	37.5
- G4	6	37.5
Which bean genotype do you like best in terms of seed texture?		
- G1	4	25.0
- G2	0	0.0
- G3	7	43.8
- G4	5	31.2
Taking into account all the analysed aspects, do you have a favorite bean genotype? If so, what is it?		
- G1	3	28.8
- G2	0	0.0
- G3	8	50.0
- G4	5	31.2
Which bean genotypes satisfied your tastes the last?		
G1	0	0.0
- G2	16	100.0
- G3	0	0.0
- G4	0	0.0
Will you recommend to other people to try beans with different colored seeds?		
- Yes	16	100.0
- No	0	0.0

Figure 4. Seeds detail of G1

At the same time, taking into account all the health benefits it has and the amount of protein found in beans, these people would in some cases replace meat with beans. Both in terms of texture and taste, G3 (Figure 5), beans with red seeds were preferred by consumers (43.8%), who said that its seeds are creamier and have a sweeter taste.

Figure 5. Seeds detail of G3

So, if in the first part of the questionnaire, being asked which genotypes of beans attracts them the most from a visual point of view, most respondents chose G2, things changed considerably when they tasted the four genotypes. Consumers were least satisfied with G2, while G3 was the most popular bean genotype, after analysing it from all points of view.

CONCLUSIONS

The respondents were delighted with the organization of the tasting they took part in, they were interested in finding out as about the health benefits of eating beans and also tasting other types of beans than those frequently consumed by them.

At the same time, the respondents had a positive feedback regarding the taste of the 'Doina' variety, comparing it with the beans frequently consumed by them.

In the future, we want to carry out more complex and larger tastings and why not to registration the genotype that was most liked by consumers, G3 for patenting and approval. They were pleasantly surprised by the different taste of the geotypes tasted in this study and showed a great openness to consume beans with seeds of different colors.

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PHENOTYPICAL RESEARCH CONCERNING CLIMBING BEAN SEEDS

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Abstract

Bean germplasm collection of Vegetable Research and Development Station Buzău, Romania, contain over 450 accessions. The maintenance of this collection must accomplish the following requirements: maintaining the variability of this species (Phaseolus vulgaris L.), creating new varieties and adaptability to the climate changes in what it concerns these accessions. In 2020, this collection was cultivated in green - house covered with polyethylene. This paper presents the comparative study of 10 climbing bean seeds accessions. The seeds of each accession were evaluated in what it concerns quantitative (100 seed weight, length, thickness and width) and qualitative characteristics (seed colour, brilliance, veining and shape). The seeds weight varied between 57.57 g (V_5) and 28.88 g (V_3). More than half of the seeds variants taken into study, presented white colour, except: V_1 and V_3 - different beige and V_5 presents 2 colours (half white, half dark red). The highest values regarding seeds length and width were registered at V_{10} (17.03 and 9.11 mm). All accessions presented are stabile and it presented stability during the last growing seasons.

***Key words**: Phaseolus vulgaris L. var. communis, germplasm colection, accessions.*

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) has been grown in Romania for over 400 years (Rădulescu, 1940; Olaru, 1982) on large areas.

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 collecting of local bean population from the main vegetable areas in Romania.

Bean germplasm collection of V.R.D.S. Buzău contains over 450 accessions.

Similar researches were made in Romania by Rădulescu I. M., Munteanu N., Stan N.; Leonte C.; Riviș I. and Nedelea G.; Giurcă D. M.; Danci O. and Madoșa E. (Rădulescu, 1940; Munteanu, 1985; Stan et al., 1993; Stan et al., 1995; Leonte et al., 2004; Riviș and Nedelea, 2008; Giurcă and Murariu, 2009; Danci O. et al., 2010; Madoșa et al., 2010; Madoșa et al., 2011).

The maintenance of this collection must accomplish the following requirements: maintaining the variability of this species (*Phaseolus vulgaris* L.), creating new varieties and adaptability to the climate changes in what it concerns these accessions.

MATERIALS AND METHODS

There were selected 10 accessions with undetermined growth and climbing habit (van Schoonhoven and Pastor-Corrales, 1994) from the V.R.D.S.

Buzău bean germplasm collection. Therefore, the experimental variants were the following:

V_1 – accession 31 BAA;
V_2 – accession 49 BE;
V_3 – accession 60 A;
V_4 – accession 60 CB;
V_5 – accession 93;
V_6 – accession 115 CA;
V_7 – accession 127 AA;
V_8 – accession 127 BD;
V_9 – accession 127 DBB;
V_{10} – accession 174.

The biological material (climbing accessions) was cultivated in green – house covered with polyethylene conditions, according to the common production technology recommended for this area by the specialty literature (Munteanu et al., 1989; Ciofu et al., 2003; Ruşti and Munteanu, 2008).

The determination of plants, pods and seeds characteristics was made according to U.P.O.V. guideline (2005), C.P.V.O. protocol (2009) and color scale (Genchev and Kiryakov, 2005).

This work presents the variability of the main seeds characteristics of 10 accessions of climbing bean from the germplasm collection.

For determination, there were used 10 seeds from each accession, according to *Handbook on evaluation of Phaseolus germplasm* (De la Cuadra et al., 2001).

Length determination was made in mm measured in parallel with the hillum, width was measured from the hilum to the opposite side and thickness was measured perpendicular on width in cross section.

According to Descriptor for *Phaseolus vulgaris* (IBPGR, 1982), van Schoonhoven A. (1994) and Debouck D. (2009) there were used 100 seeds mass (g) randomly chosen.

In order to determine seeds weight there was used an analytic scale (Partner WAS220/x), and for data analysis there were used values with 4 decimals.

There was used variation coefficient because it allows the direct comparison of data sets variation (Ireland, 2010).

The coefficient of variation (CV) in a single sample with observations is defined as CV=*s/m*, where *m* is mean and *s* is standard deviation (Forkman, 2009).

Variability appreciation according to the CV values (Munteanu and Fălticeanu, 2008; Giurcă and Murariu, 2009) was made this way: low variability (CV< 10%), mean (CV= 10 – 20%) and high (CV > 20%).

RESULTS AND DISCUSSIONS

The study was made in Romania (45°9′N and 26°49′E) in 2020. This paper presents the variability of the main seeds quantitative characteristics (100 seed weight, length, thickness and width) and qualitative characteristics (seed color, brilliance, veining and shape) of 10 accessions of climbing bean from the V.R.D.S. Buzău germplasm collection.

I Quantitative characteristics

Seeds weight (Table 1) varied from 28.88 g (V_3) to 57.57 g (V_5). The variability coefficient in what it concerns this characteristic, had the value equal to 24.61%; this shows a high variability for the experiment.

Table 1. Main quantitative characteristics of the seeds

Variants	100 seeds mass (g)	Seeds dimensions (mm)			Length/ Width	Width/ Thickness
		Length	Width	Thickness		
V_1	39.41 cd*	13.03 d	7.50 c	5.84 b	1.74	1.28
V_2	46.39 b	15.04 b	7.82 b	5.20 c	1.92	1.51
V_3	28.88 e	13.02 d	6.57 e	4.86 c	1.98	1.35
V_4	46.85 b	15.42 b	7.01 d	5.40 b	2.20	1.30
V_5	57.57 a	13.17 d	8.82 a	7.56 a	1.49	1.17
V_6	32.52 e	12.92 d	6.54 e	4.77 c	1.98	1.37
V_7	40.89 bc	14.20 c	7.91 b	4.76 c	1.80	1.66
V_8	33.89 de	12.88 d	6.47 e	5.38 b	1.99	1.20
V_9	31.48 e	12.77 d	6.79 de	4.84 c	1.88	1.40
V_{10}	56.39 a	17.03 a	9.11 a	4.84 c	1.87	1.88
Mean	**41.43**	**13.95**	**7.45**	**5.34**	**1.88**	**1.41**
CV%	**24.61**	**10.37**	**12.80**	**16.01**	**9.88**	**15.48**
Min	28.88	12.77	6.47	4.76	1.49	1.17
Max	57.57	17.03	9.11	7.56	2.20	1.88
	LSD 5% = 5.9386 LSD 1% =8.1444 LSD 0.1% =11.0854	LSD 5% =0.8377 LSD 1% =1.1488 LSD 0.1% =1.5636	LSD 5% =0.3210 LSD 1% =0.4402 LSD 0.1% =0.5992	LSD 5% =0.5871 LSD 1% =0.8051 LSD 0.1% =1.0958		

*Different letters between variants denote significant differences (Duncan's test, p<0.05).

According to van Schoonhoven A. (1994) five accessions have medium size (25 g to 40 g) and five accessions (V_5, V_{10}, V_4, V_2 and V_7) have large size (more than 40 g).

Seeds length (Figure 1) registered the maximum value at V_{10} (17.03 mm) and the minimum value at V_9 (12.77 mm).

The variability coefficient in what it concerns this characteristic for the studied accessions was 10.37 %, which shows a mean variability.

Width varied between 6.47 mm (V_8) and 9.11 mm (V_{10}), having a mean variability coefficient equal to 12.80% (mean variability).

Figure 1. The largest (V_{10}) and the smallest (V_9) length

A mean variability (16.01%) is also observed in what it concerns the thickness of the seeds studied. This characteristic varied between 4.76 mm (V_7) and 7.56 mm (V_5).
The highest values (Figure 2) in the experience was registered at V_5 (100 seeds mass, thickness) and V_{10} (length, width).

Figure 2. Seeds belonging to V_{10} and V_5

The variability coefficient for the three quantitative characteristics (length - 10.37%, width - 12.80 % and thickness - 16.01%) was mean.
Regarding seed length/width ratio (L/W) and seed width/ thickness ratio (W/T), the lowest values were recorded at V_5.
The biggest value of L/W was 2.20 (V_4) and of W/T - 1.88 (V_{10}).
A similar situation is described by Sinkovic et al., 2019: high variability of weight and mean variability of length, width, thickness, L/W and W/T. In that case the lowest value was at W/T 12.64% (mean variability); in this case the lowest value is at L/W (9.88% - low variability).

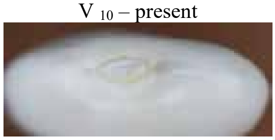
II Qualitative characteristics

More than half of the seeds variants taken into study presented white colour, except (Figure 3): V_1 and V_3 – which presented a different beige and V_5 which presented 2 colours (half white, half dark red).

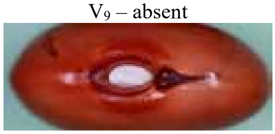
Figure 3. Seeds with 2 colors and different beige

Around the hilum many variants present a different color (Figure 4).

V $_{10}$ – present



V_9 – absent



V_1– present

Figure 4. Different color around the hilum

Seeds shape was determined by median longitudinal section (Figure 5).
According to U.P.O.V. guideline (2005), C.P.V.O. protocol (2009) and color scale (Genchev and Kiryakov, 2005: five variants had kidney – shaped (V_2, V_3, V_4, V_6 and V_{10}); three variants were elliptic (V_1, V_8 and V_9), V_5 was circular to elliptic and V_7 was rectangular.
According to Debouck 2009 almost all variants had a medium brilliance.
Regarding (CPVO) seed veining, biological material taken into studying present weak and medium intensity (Figure 5).

Figure 5. Medium veining at V_{10}

CONCLUSIONS

The highest values in the experience was registered at V_5 (100 seeds mass, thickness) and V_{10} (length, width).

The highest coefficient of variation was calculated for the 100 seed weight (24.61%) and the lowest for L/W (9.88%).

More than half of the seeds variants taken into study presented white colour, except: 2 variants which presented a different beige and V_5 which presented 2 colours (half white, half dark red).

Regarding shape of the seeds: five variants had kidney – shaped, three variants were elliptic, one – circular to elliptic and another was rectangular.

All accessions presented are phenotypic stabile and it presented stability during the last growing seasons.

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EXPERIMENTAL RESEARCH ON BIOINSECTICIDE ACTIVITY OBTAINED BY USING AN OLEIC EXTRACT FROM DWARF SILVER FIR ON SOME VEGETABLE CROPS

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Abstract

The article presents a series of experimental studies on the use of an oleic extract, produced by steam separation utilizing a prototype distillation equipment. The separation process is performed with the use of wet saturated steam under pressure, using as raw material twigs and buds of dwarf silver fir. Since the volatile extracts of aromatic plants do not bring a direct nutritional contribution to the seeds subjected to germination, the germination increase and the root growth of the plants can be obtained only from the exercise of the anti-microbial, anti-oxidant and insect-repellent effect. The use of essential oils in the protection of seeds and rhizosphere is an ecological method because it does not negatively affect the number and frequency of species of useful microorganisms. The nutritional relationship that is established between the seeds treated with volatile oils and the germination bed can be influenced by the changing proportions of microbial populations with beneficial effects on productivity.

Key words*: bioinsecticide activity, oleic extract, dwarf silver fir, vegetable crops.*

INTRODUCTION

The chemical fertilizer industry is known for its high energy consumption (especially methane gas) and for its potentially high environmental risk (Manea, 2016; Mircea, 2020; Nenciu, 2022). The decrease of global fuel resources and several environmental challenges are raising the pressure on manufacturing costs. This highlights the potential presented by some organic alternatives, such as biofertilizers, that can be produced from various plant extracts (Muscalu, 2018; Tudora, 2021; Nenciu & Vladut, 2021). Biofertilizers present the great advantage of being non-polluting for terrestrial flora and fauna. In addition to their basic soil fertilizing effect, they also show a significant role as an inhibitor of pathogenic fungi and bacteria that can affect agricultural crops. Organic farming is a growing industry in Romania, that has seen an upward trend in recent years, for both vegetable and animal production sectors (Butu, 2022; Nenciu, 2022). In the organic farming industry, product marketing is highly important. Only MADR-registered merchants can sell organic products through different market channels, including sales at the farm gate, retail stores, specialized stores, the online exchange market for organic products (Bougherra, 2015; Cardei, 2021), and seasonal marketplaces. One of the essential conditions for the development of organic farming is promoting the concept, in order to make consumers aware of the benefits of these products. The higher price for organic products is justified by the quality, which is guaranteed by inspection and a certification system (Tisserand, 2014).

The use of fertilizers/pesticides in organic farming must leave no residues of chemicals, according to European regulations. Therefore, identifying new substances and compounds with fertilizing / insecticide action represents a continuous challenge for the field of agricultural scientific research in the 21st century (Udayashankar, 2022).

The insecticide industry produces compounds that may have harmful effects on insect populations, generating odors, vapors, gases, smoke, heat, oils, soap, etc. Natural chemicals, on the other hand, can partially or completely replace these substances. For example, the strong smell of garlic, tobacco, rhubarb and other similar plants is repulsive to some insects. Hot peppers, alcohol, salt and other substances can burn or destroy several pests. The oils suffocate certain insects, while the soap or detergents added to the solution have the role of making the substances stick better to the leaves and stems.

An important direction in today's agriculture is the use of biofertilizers made from plant extracts, containing bioactive substances. These essential oils are produced from medicinal, aromatic, farmed, or perennial plants. The advantage of using bioactive substances to the detriment of synthetic chemicals include the reduced contamination of the aquatic and terrestrial environments, the suppression of insect populations resistant to classical control measures, and the lack of unfavorable human and animal impact.

The use of essential oils to preserve seeds and the rhizosphere is an environmentally friendly strategy since it does not reduce the amount or frequency of beneficial microorganisms. The nutritional interaction that develops between seeds treated with volatile oils and the germination bed can be altered by changes in microbial population levels. In some cases, the complex action of volatile oils stimulates the development of microbial populations capable of solubilizing nitrogen, phosphorus, and potassium from insoluble chemical combinations, thereby increasing the reserve of nutrients available to plants without the use of conventional fertilizers. The goal of the research for the action of fir essential oil was to find a balance between preventing pathogen growth and causing phytotoxic effects. The main components of silver fir essential oil are Limonen 70%, α-Pinen 10%, Mircen 2.8% (Anna Wajs, 2010) and its action is a repellent for nematodes (*Meloidogyne incognita*).

Volatile oils are the product of the extraction process of volatile aromatic compounds that are found naturally in different parts of plants. A typical volatile oil is a mixture of chemical compounds such as esters, oxides, aldehydes, phenols, alcohols, terpenes, etc., which provide the oil its special characteristics. These complex mixtures make the essential oil an effecttive agent with effective bioinsecticide action.

There are several methods of extracting volatile aromatic compounds from plants: cold pressing, steam distillation, solvent extraction, fat extraction, filtration, etc. Pure volatile oils are only those obtained by steam distillation or cold pressing, because only these methods maintain the therapeutic properties of the oils unaltered (Kopaczyk, 2022).

Most industrially produced volatile oils are obtained by extraction with water vapor. All components of a volatile oil have higher boiling points than water but have high volatility at temperatures below the boiling point and can be easily entrained by water vapor at a lower temperature than the boiling point. Separation is made easier by their insolubility in water and their distinct densities.

The migration of volatile oil from plant cells to the surface layers precedes its production. The length of this process is determined by the type of the raw material being used. Diffusion is easier on green plants than in dried plants, and in flowers rather than in roots, seeds, or wood.

Vapor entrainment is the most widely used process for obtaining volatile oils, where steam is generated using an ancillary installation. This ensures a uniform temperature and the possibility of an efficient control of the steam flow. Furthermore, the degradation of the volatile oil is avoided due to the direct contact of the plant with the superheated walls of the extractor. These are large-scale systems that can handle many tons of raw material.

The vegetal material is positioned at the top, being supported by a perforated metal basket. Steam production can be performed either with direct steam technology (less often used), or indirect steam. Direct steam is introduced to the bottom of the container through a distributor (perforated spiral). When heated with indirect steam, the thermal agent circulates in a jacket and ensures the vaporization of a quantity of water that is introduced in the driving vessel, together with the raw material. The plant material comes into contact only with the generated water vapors, which ensures a gradual and controlled heating. The separation is performed with a modified Florentine distiller vessel, in order to extend the residence time and ensure a better separation of the volatile oil.

In order to reduce the losses caused by the partial dissolution of the volatile oil in the floral water, in most cases the product obtained in the Florentine distiller vessel enters a recirculation cycle.

Silver fir or white fir (*Abies alba* Mill.), is a fir native to Europe, whose area is limited by the Pyrenees Mountains, north of Normandy, east of

the Alps and Carpathian Mountains, to southern Italy and northern Serbia. It is a massive, evergreen conifer, growing up to 40–50 m tall (rarely 60 m), with trunk diameter of up to 1.5 m.
The goal of the present research was to test several recipes, composed of active natural compounds, that could be successfully applied to improve the development of tomatoes plants. The present paper is oriented towards Silver fir extract, however, our extensive research consists in combining several volatile oils for stimulating the plants growth, or to be used as repellent for some pests.

MATERIALS AND METHODS

A Wet Saturated Steam Distillation Equipment (E.D.A.S.U.P.) was designed for the current experiment, in order to optimize the characteristics of the oil for the field of bio-insecticides. The wet saturated steam distillation equipment has the following main elements: Electric Steam generator (1), Pressure regulator (2), Distillation vessel (3), Distillation vessel holder (4), Connecting hoses between vessels and steam generator (5), Serpentine (6) Cooling vessel (7), Flexible hose emptying cooling vessel (8), Cooling vessel holder (9), Flexible hose for draining hot water (10), Volatile oil collection vessel (11), Hot water drain (12), Exhaust volatile oil (13) (Figure 1).

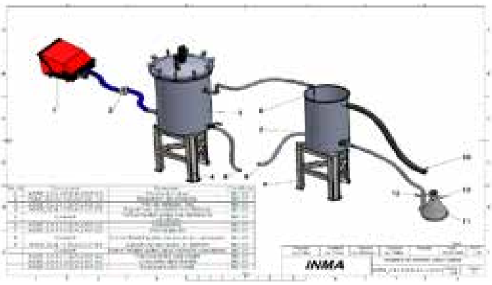


Figure 1. Wet saturated steam distillation equipment functioning under pressure **E.D.A.S.U.P**

Electric steam generator PULSE 7K, has supply voltage of 400 V, absorbed power 7000 W, degree of protection IPX5, supply flow of 3 l / min, max. supply pressure 6 bar.
Silver fir twigs were used to experiment the wet saturated steam distillation equipment, functioning under pressure E.D.A.S.U.P. (Figure 2).

Figure 2. Silver fir raw material in process and oil production

Since the volatile extracts of aromatic plants do not bring a direct nutritional intake to the seeds subjected to germination, the increase of germination and root growth of plants can be obtained only from the exercise of the anti-microbial, anti-oxidant and insect-repellent effect. Seeds prepared for sowing undergo a series of physical-mechanical processes that damage the morphological structures of the surface in order to achieve the required quality standards. Although these seeds possess high levels of purity and germination under controlled conditions, they may be susceptible to disease in the field due to phytopathogen colonization of injured tissue.
The protocol for preparing the mixture based on plant extracts has been prepared as follows: 0.2 g of agar is dissolved in 95 ml of distilled water at a temperature of 95-100ºC, then is cooled to room temperature, 5 ml of plant extract is added and stirred on a magnetic plate until homogenizes, while keeping away from the light.

Preparation of batches for testing the effect of plant extracts at different concentrations.
A batch of 1200 seeds is prepared (with its own unaltered microbiological load). The seeds are being mixed and divided into 24 batches of 50 seeds each (for small seeds e.g., peppers, tomatoes) or 48 batches of 25 seeds each (large seeds e.g., cucumbers). Petri vessel (90 mm x 16.2 mm, with ventilation) are being labeled from V1R1 to V6R4, or V6R8.
The seeds are displayed in the Petri vessel, then in the lid are inserted five disks of industrial paper (three layers) of cellulose.

Performing the treatment:
Four batches of 50 or 25 seeds are weighted each to determine the average mass, then is calculated the equivalent amount of mixture of 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm and

5000 ppm for the average seed mass. Next is added the test dose of the vegetal extract mixture to one side of the plate without touching the seeds. Then is added the amount of water needed for homogenization with the plant extract, which is mixed well with the seeds. The cellulose discs are being placed on top of the seeds, and the cellulose discs are soaked with 10 mL of water. After that enters into an incubation process at the optimum temperature, for three days.

Performing measurements and observations after the treatment:

- The seeds are being weighted;
- Germinated seeds are counted;
- The length of the root / hypocotyl is measured;
- Embryos are weighed;
- Observations are made on visible microbial activity;
- CIM (minimum inhibitory concentration) is determined.

RESULTS AND DISCUSSIONS

The results of experiments performed using wet saturated steam distillation equipment functioning under pressure (E.D.A.S.U.P), using as raw material silver fir are briefly presented in Table 1.

Figure 4 describes the treatment on tomato seeds with silver fir essential oil (*A. alba* Mill.). The test showed that at concentrations between 1000 and 3000 ppm, tomato seeds did not produce negative effects on root growth in the first four days compared to the untreated control sample. The presence of a substantial standard deviation in both control and sample measures indicated that the batch of seeds lacked morphological uniformity and structural integrity. In comparison to the other situations, the concentration of 3000 ppm was observed to enhance the formation of absorbent filaments. This result might be a form of drought adaptation for conifers, and it could be used in groundwater studies.

Table 1. Experimental setup and results obtained using Wet Saturated Steam Distillation Equipment Functioning Under Pressure

Parameters	Sample Silver fir
Batch mass [kg]	6
Air	27
Temperature [°C]	
- cooling water	26.7
- steam pipe at the outlet of the generator	128.1
- steam pipe at the entrance to the distillation vessel	95.2
- steam in the distillation vessel	80.2
Air humidity [% RH]	61.6
Test duration [min]	50
Volume of resulting products	
- oil [ml]	27.1
- floral water [l]	4.6

In the germination phase, a Gaussian response curve was found, with the peak at 200 ppm, depending on the concentration of the extract used. When seedlings were treated with silver fir essential oil at a dose of 2000 ppm (depicted in Figure 5), germination increased by 10% over the control sample, with the least standard variation across the observed tests. This indicates that the seed's morphological structure is influenced and vulnerable to microbial invasion, as depicted in Figure 3.

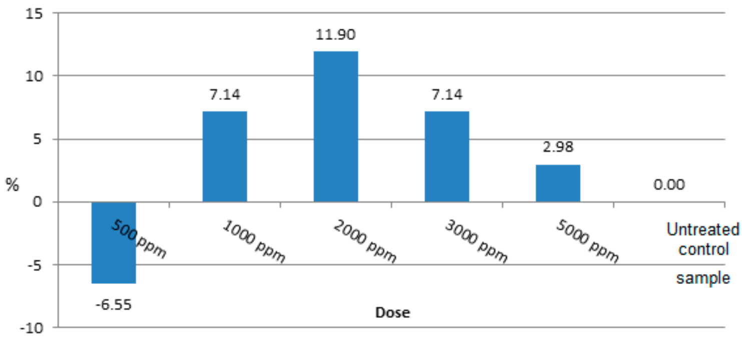
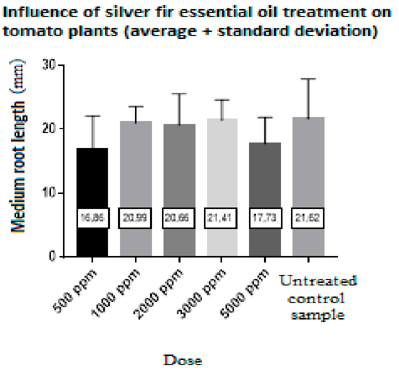
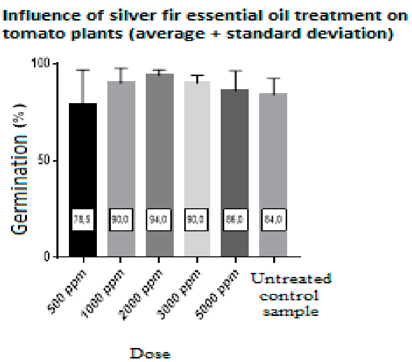


Figure 3. Evaluation of the growth and development of tomato plants in the early vegetative stages influenced by the treatment with silver fir essential oil



Alpha	0.05			
Dunnett's multiple comparison test	Averages difference	Statistical significance	Summary of significance	Adjusted value P
Untreated control sample vs. 500 ppm	4.764	No	ns	0.4614
Untreated control sample vs. 1000 ppm	0.636	No	ns	0.9997
Untreated control sample vs. 2000 ppm	0.9578	No	ns	0.9982
Untreated control sample vs. 3000 ppm	0.2132	No	ns	0.9999
Untreated control sample vs. 5000 ppm	3.894	No	ns	0.6378

Figure 4. Evaluation of silver fir essential oil effect on root development, after treating the tomato seeds. Statistical significance



Alpha	0.05			
Dunnett's multiple comparison test	Averages difference	Statistical significance	Summary of significance	Adjusted value P
Untreated control sample vs. 500 ppm	5.5	No	ns	0.8964
Untreated control sample vs. 1000 ppm	-6	No	ns	0.8617
Untreated control sample vs. 2000 ppm	-10	No	ns	0.4988
Untreated control sample vs. 3000 ppm	-6	No	ns	0.8617
Untreated control sample vs. 5000 ppm	-2	No	ns	0.9984

Figure 5. The influence of silver fir essential oil treatment on tomato seed germination. Statistical significance

CONCLUSIONS

Calculating the combined effect of root growth and germination of tomato seeds, when treated with silver fir essential oil, have been found that a dose of 2000 ppm can increase plant development by 11.9 %, compared to untreated control sample.

Our previous research shown that from the volatile plant extracts (oregano, silver fir, laurel, lavender) tested on tomato crops in the early vegetative stages, oregano extract had a negative influence on growth. Therefore, it is rather recommended for seeds fumigation for preservation (during storage periods), or as an organic herbicide. Silver fir extracts showed positive results and shall be associated with other nutritional extracts to initiate plant protection.

Biochemical content of the volatile extract of fir oil have been determined to possess high quantities of pinene and limonene, both of them having insecticidal, insect-repellent, and anti-microbial properties (Udayashankar, 2022).

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STUDY REGARDING THE STRUCTURE OF A RANCH ACCORDING TO THE CIRCULAR ECONOMY

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Abstract

The circular economy is the sole viable alternative of the linear economy, namely the classic model of output and input, where the raw materials are taken from nature, being used to manufacture the products, which afterwards are used and possibly eliminated as wastes - manufacture - use - throw pattern.
On the basis of this concept has been projected a logical scheme of the circular economy for the output activity developed at the farm made with European funds, fields crops farm and manufacturing industries of zootechnical by-products.
The impacts on the economic circularity are highlighted through specific indicators. For the present study it has been identified the possibility to evaluate the circular utilization rate and the material indent, considering the value of the primary materials used to get the final product.

Key words: *circular economy, logic scheme of the circular economy, circular utilization rate, material indent.*

INTRODUCTION

The circular economy is considered the single real alternative of the output and input classic model, where the raw materials are used to manufacture the products, which are exploited according to their value of utilization and further they become wastes. (Vişinescu et al., 2020).
According to the European Committee, the circular economy is *the economic space where* the value of products, materials and resources is maintained in economy as long as possible, the generation of wastes being minimized.
The concept of circularity is closely connected to that of the efficient use of natural resources during products life cycle, as well as wastes conversion into new resources for other industries (https:/ / bioeconomie.ro/2013/09/04/ primulpost/2013).
The circular economy is considered the only real alternative of the output and input classic model, where the raw materials are used to manufacture the products, which are exploited according to their value of utilization and subsequently become wastes.

MATERIALS AND METHODS

The rural economy revamping requires the organization of some complementary agricultural activities which valorise on zonal plan the raw agricultural products obtained, in order to practice a circular economy as a starting point in the practice of bioeconomy.
To exemplify a management model that applies the circular economy it has been elaborated the present study for a zootechnical farm made with European funds through the NRPD - EAFDR program, in the frame of an economic circuit which also includes an agricultural farm of field crops and different manufacturing industries of livestock by-products.
In the frame of this project, it is proposed to buy Romanian Bălţata bulls, breed which has the morpho-productive characteristics of Simmental breed, with improved peculiarities of strength and adaptation to the harsher environmental conditions of our country.
Farm's capacity will be of 100 heads. Growth and fattening period is of 12 months, the calves being purchased at a weight of at least 140 kg, the delivery being made at one year at the weight of 650 kg. The net number of bulls per

series which reach the end of growth for exploitation is of 98 (Feasibility study. Applicant: SC ECOVIT FARM INVEST SRL)

Study's objectives are determining the fodder need for raising bulls, the provision of fodders necessary from the agricultural farm and delivery of animal by-products to the processing industries.

The value expression of the products entering into this economic circuit makes possible the economic evaluation of the circular output activity, but also the calculation of indicators specific to the economic circularity.

The economic circularity is expressed through a set of indicators which can be determined at macroeconomic level. Among these we identified two indicators which can be determined on the basis of registering the value of circular production chain products and by-products.

RESULTS AND DISCUSSIONS

On the basis of the circular economy has been projected a logical scheme of the circular economy for the output activity developed in the ranch which includes the collaboration with field crops farm and the industries manufacturing the zootechnical by-products. (Valorificarea-subproduselor-din-industria-carnii: https://dokumen.site/,2018; https://www.scridoc.com/2021/04/deseuri-carne.html.Ce facem cu deşeurile de la carne, 2021).

Animal feed is made by manufacturing a part of the vegetal production obtained by the agricultural farm which receives in return the manure used to fertilize the arable land. (Lege nr. 22 din 17 decembrie 1971 pentru organizarea producerii şi folosirii rationale a resurselor de nutreturi. Publicat în Monitorul Oficial nr. 156 din 17 decembrie 1971) Furthermore, the field crops farm supplies also the straws necessary for animal bedding. From the ranch it is obtained the main product (carcass of the slaughtered animal), alimentary by-products, non-alimentary by-products and manure. The non-alimentary by-products are wastes, but they can be exploited according to the principles of circular economy in different industries, as it is shown in the circular economy's logical scheme (Table 1) (Visinescu, 2020).

The agricultural products quantities obtained on agricultural farm for supporting the feeding of fattened bulls have been calculated knowing the recipe for the concentrates type II and III, (Popa et al., 1980) as well as the processing yield of raw agricultural products: corn grain/corn cobs (about 75%), corn grain/corn flour (about 77%), good grain/bran (about 23%), sunflower seeds/grit (about 45%).

The recipes for the two types of concentrates used to bulls feeding contain also calcium, salt and mineral supplement (zoofort) (Popa et al., 1980).

The necessary fodder produced on the field crops farm is presented in Table 2.

From the sum of 851.790 lei being the newly created value on the ranch, a part represents the main product and the by-products exploited into the farm and another part represents the by-products value which enters into circularity.

The by-products resulted from the farm enter into circularity being taken over by other manufacturing industries. These reach the value of 50.884 lei.

To the ranch yields is added also the value of manure taken over from the ranch.

The valuation of total operating expenses is presented in Table 3 and the value of the products obtained on the circular output chain is presented in Table 4.

Setting in the economic impacts determined by the activities organisations, considering the bioeconomy principles conduct to important methodologic changes (https://agriculture.gouv.fr/ la-bioeconomie-nouvelle-vision-du-vivant, 2019).

Therefore, beside the economic efficiency set by specific indicators must be highlight the ecologic impacts materialized in decreasing wastes, diminishing the consumption of raw materials and materials by extending the life of products.

Table 1. The logical scheme of economic circularity

Input - concentrated fodder (corn grains and cobs, wheat, barley, sunflower) - green fodder (alfalfa, other green fodder) - grain straw - solid supplements (calcium, salt, zoofort)	**The farm livestock**	Outputs - carcase - food by products (head, organs, legs, tails, bones, blood) - non by products (skins, horns, hooves bones, blood, intestines, endocrine glands) - stable man	Inputs - food industry - carcase - food by products (skins, horns, hooves bones, blood, intestines, en docrine glands) Inputs Leather industry - rough skins Inputs By products manufacturing industry - horns, hooves bones, blood, intestines, endocrine glands. Inputs The pharmaceutical industry endocrine gland, bones, tendons
Outputs - concentrated fodder: corn grains and cobs, wheat, barley, sunflower, green fodder (alfalfa, other green fodder) - grain straw	**Farm for field crops**	**Inputs** **- manure**	
Input - concentrated fodder (corn grains and cobs, wheat, barley, sunflower) - green fodder (alfalfa, other green fodder) - grain straw - solid supplements calcium, salt, zoofort)	**The farm livestock**	Outputs - carcase - food by products (head, organs, legs, tails, bones, blood) - non by products (skins, horns, hooves bones, blood, intestines, endocrine glands) - stable man	Inputs - food industry - carcase - food by products (skins, horns, hooves bones, blood, intestines, en docrine glands) Inputs Leather industry - rough skins Inputs By products manufacturing industry - horns, hooves bones, blood, intestines, endocrine glands. Inputs The pharmaceutical industry endocrine gland, bones, tendons
Outputs - concentrated fodder: corn grains and cobs, wheat, barley, sunflower, green fodder (alfalfa, other green fodder) - grain straw	**Farm for field crops**	**Inputs** **- manure**	

Source: Vişinescu (2020), Scientific report no.3, - Partial results regarding the efficiency of the bioeconomy in the sustainable development of the rural area in the study area.

Table 2. The need for concentrated fodder produced on the farm for field crops

Agricultural product fodder	Required products -tons for					
	Type II concentrate		Type III concentrate		Total	
	Product agricultural	Fodder	Product agricultural	Fodder	Product agricultural	Fodder
Corn grain	12				12	
hominy		8.70				8.70
Corn cobs			23	23	23	23.00
Wheat	7		31		38	
Bran		1.50		6.75		8.25
Barley			10	10	10	10.00
Sunflower	7		10		17	
groats		2.40		4.50		6.90
Lucerne m.v.	12				12	
Alfalfa flour		1.80				1.80
Total	38	14.4	74	44.25	112	50.65

Source: Vișinescu (2020), Scientific report no.3, - *Partial results regarding the efficiency of the bioeconomy in the sustainable development of the rural area in the study area.*

Table 3. Estimation of total operating expenses

Nr.crt	Feed type	Quantity, kg	Value, lei
1	Hay	126,000	**126,000**
2	Concentrates		**69,109**
	-from the farm	50,650	66,115
	-mineral supplement	150	834
	-calcium	300	150
	-salt	300	270
	-zoofort	300	1740
3	Succulents (potatoes)	90,000	**54,000**
4	Pickled fodder	180,000	**41,400**
5	Straw for bedding	54,000	**4,320**
	Total feed		**294,829**
6	Water for drinking	1,440,000	7,200
7	**Acquisition of bulls**	100 heads	112,000
	Total operating expenses		**41,4029**
8	Drugs		3,120
9	Diesel fuel		5,800
10	Insurance		4,000
11	Cost of living labor		55,200
	Other expenses		**68,120**
12	**Total expenses**		**482,149**
13	**Annual depreciation**		**242,958**
	Annual expenses		**725,107**

Source: Vișinescu (2020), Scientific report no.3, - *Partial results regarding the efficiency of the bioeconomy in the sustainable development of the rural area in the study area.*

Table 4. The value of the products obtained in the circular production chain

Nr. crt	Income from livestock farm	The value, lei
1	Capitalization of the main product	800,906
2	Capitalization of by-products	50,884
3	Manure	24,650
4	Total	876,440

Source: Vișinescu, (2020), Scientific report no.3, - *Partial results regarding the efficiency of the bioeconomy in the sustainable development of the rural area in the study area.*

The setting in of these impacts might be obtained by identifying some indicators which can reach the circularity in economy (https://www.statistiques.developpement-durable.gouv.f r/i 2021):

1. Household expenses for maintenance and repair of products

Household expenditure (excluding vehicle maintenance) is marked by an increasing consumption of goods that consume resources and generate CO_2 emissions for their manufacture, transport and distribution. Prioritizing repairs over renewals prolongs the life of products and thus limits their replacement.

Monitoring consumer spending on resident dedicated to the maintenance and repair of household goods makes it possible to analyse the evolution of household practices in this area. Since 1960, per capita spending on household goods has increased. The increase was more intense in new goods purchases than in maintenance and repair costs.

2. ***Evolution of the volumes of waste deposited***

According to European Directive (EU) 2018/851 of 30 May 2018, the storage or incineration of waste without energy recovery is the least convenient solution as it loses significant resources that minimize the circular economy.

3. Incorporation of recycling raw materials in production processes

Recycled raw materials (MPR) result from waste that can be introduced into the production process by totally or partially replacing virgin raw materials thus saving natural resources. The circular rate of use of materials measures the share of waste recovered in the form of materials in the manufacture of a product. The circular utilization rate is defined as the ratio between the volume of raw materials in circular recycling and the total volume of materials used.

4. Number of companies and local authorities that have benefited from a functioning economy support system by state agencies

Functional economics refers to a new business model that seeks to replace the sale of a material good or a traditional service with the sale of the use of the good and useful effects (benefits to customers). It is a more economical model in terms of resources and pollution due to the decoupling between the production of goods and income and the optimization of the use of material resources and goods.

5. Number of industrial and territorial ecological approaches

Industrial and territorial ecology is a way of organizing inter-society based on the exchange of resources or the pooling of resources and services. The economic factors involved in this process can collaborate for the shared use of infrastructure, equipment (heating networks, tools or production spaces), services (collective waste management, business travel plans, etc.), materials (waste become resources for others) or jobs.

6. The European Ecolabel

The European Eco-label reflects the environmental quality of products and services at all stages of their life (manufacturing, use, transport and disposal). Obtained on the basis of a voluntary approach, it is the only label that guarantees the ecological quality of products in all EU Member States. A manufacturer may own one or more eco-labelled products related to one or more product categories.

7. Internal consumption of materials on resident

The demand for goods and services of domestic producers of goods requires the use of raw materials from the territory and the import of raw materials that cannot be produced locally. The sum of the volumes of materials extracted from the territory and imported, minus the volume of materials exported, is the domestic consumption of materials. This indicator is part of the provisions on the 2030 Sustainable Development Goals, defined by the United Nations.

8. Material productivity

Material productivity (EUR/kg) is the ratio between Gross Domestic Product (GDP) and Domestic Material Consumption (DMC). This indicator, which characterizes a more resource-efficient economic system, is part of the UN's 2030 Sustainable Development Goals. Trend Material productivity increased by 12% between 2010 (2.63 EUR / kg) and 2018 (2.96 EUR / kg), the target for 2030 being set at 3.42 EUR / kg. In 2007, before the 2008 crisis, material productivity was 2.28 EUR / kg.

9. Material imprint

The material imprint shows the amount of primary materials used to obtain the final product and includes both direct volumes of materials (material contained in manufactured products) and indirect volumes (materials not included in those products but necessary for their manufacture). This notion is analogous to the carbon or water footprint, the material footprint (domestic consumption in raw material equivalent) being an indicator established according to the methodology recommended by Eurostat.

10. Real estate repair and recycling

The indicator aims to identify the number of jobs associated with economic activities related to the circular economy. This includes activities related to "extension of use" and "recycling", ie reuse of goods, repair, waste collection and recovery of materials. These activities, on the whole, require more jobs per unit produced than in the economy (https://www.statistiques. developpement-durable.gouv.f r/i 2021).

In the present study has been identified the possibility to calculate the circular utilization rate (RUC) on the basis of the knowledge the value of raw recycling materials (VRC) and the total amount of the materials consumed in the production process (VMC):

$$\text{RUC} = \frac{VRC}{VMC} \times 100\ (\%)$$

It has been considered the raw recycling circular materials are the by-products taken over to be used in other production sectors, as well as the manure taken from the agricultural farm, whose value is of 75,534 lei (Table 4).

The total value of the materials consumed in the output process results from the total exploitation expenses and from the medicines cost, being of 417,149 lei (Table 3).

On the basis of the above-mentioned data has been calculated the circular using rate for the ranch:

$$RUC = \frac{75534}{417149} * 100 = 18\ \%$$

The circular using rate for the ranch provides the value share for the circular raw materials, included into the output processes of other economic sectors.

Another possibility to express the economic circularity is the material mark determination, considering the value of the primary materials used to obtain the final product. This includes both the value of the materials used in the output process, as the non-included materials into the final product, but which are necessary.

The value of the primary materials used to obtain the final product (Vm) are: bulls' cost, fodder, water for animals, medicines.

For a case study, according to data from Table 3, the value of those materials is, at follows:

- bulls cost	112,000 lei
- fodder cost	294,829 lei
- water for animals	7,200 lei
- medicines	3,120 lei
Sum :	417,149 lei

The total value of the product obtained (Vp) (main product and by-products) is of 876,440 lei.

Material print (Am) determined by the value expression of its components is:

$$Am = \frac{Vm}{Vp} = \frac{417149}{876440} * 100 = 47.6\ \%$$

The material print in value expression shows that the primary materials from the output process lend 47.6 % to obtain a new product and 52.4 % represents the new created value in the output process, in this case meaning the raising and fattening bulls.

CONCLUSIONS

The circular economy is the sole alternative of the classic model of output and consumption, where the raw materials are used to manufacture products which are used according to their value and, at the end, become wastes.

The circular economy means to recycle the materials in order to extend products life cycle.

Putting in practice the circular economy based on setting up a ranch made with European funds and on economic relations between this and the economic agents specific for a complete output circuit.

The highlighting of the economic circularity has been made by elaborating a logic scheme including the activities of the ranch, of the field crops farm and from specific technical industries.

The output process of the ranch has been set up according to the special recommendations and to the economic circularity principles.

Objectives of study: to determinate fodder need for raising bulls, to provide the necessary fodder from the agricultural farm and to develop the zootechnical by-products from the manufacturing industries.

The economic circularity is appraised through specific indicators in order to highlight the ecologic input materialized by prolonging the products life.

On a local economic scale, this study could calculate two indicators of the economic circularity.

The circular utilization rate of 18 % represents the value share of the recycled raw materials included into the output processes from other economic sectors.

The material mark in value expression shows that the output raw materials bring a contribution of 47.6 % to obtain the new product and 52.4 % represents the new created value through the output process.

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BEHAVIOR IN DIFFERENT CULTURAL SYSTEMS OF A GENETICALLY STABILIZED *CYMBOPOGON CITRATUS* (LEMONGRASS) GENOTY AT PGRB BUZAU

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Abstract

PGRB Buzau focused on acclimatizaion and breeding of new species that can be grown as vegetable crops in Romania. Since 2010, the Bank's specialists have been studying the species Cymbopogon citratus, accumulating a valuable genetic resource. Two genotypes with distinct phenotypic expressiveness have been genetically stabilized, G1 with strong anthocyanin coloration at leaves base and G2, the subject of this study has the entire plant with light green leaves. It was found that in greenhouse the species can be grown as a perennial and in the field, due to negative temperatures over winter, it requires protection or annual establishment of the crop. In terms of quantitative characters expressiveness, significantly higher values were recorded in the greenhouse, the maximum green mass yield obtained being 4182 g/plant of which 45% represents the production of edible stems. The species can be successfully cultivated both in greenhouse and in the field in Romania. No diseases or pests have been found to affect the crop and the species is appreciated for its strong lemon aroma and multiple uses as ornamental, medicinal, aromatic.

Key words*: aromatic, acclimatization, breeding, germplasm, lemon grass.*

INTRODUCTION

Cymbopogon citratus is native to Sri Lanka and South India, and is now widely cultivated in the tropical areas of America and Asia (Shah et al., 2011).

Lemon grass, popularly known as citronella grass is a member of the *Poaceae* family and belongs to the genus *Cymbopogon*. The genus *Cymbopogon* constitutes of approximately 140 species that show widespread growth across the semi-temperate and tropical regions of Asian, American and African continents (Ranade et al., 2015).

The history of this aromatic plant shows way it has travelled on almost all the continents of the world, from its area of origin, India, being then extended to America and Africa and later to Europe, conquering so many different nations with its aroma.

Lemongrasses (*Cymbopogon* spp., *Poaceae*) are a group of commercially important C_4 tropical grasses (Lewinsohn, E. et al., 1998).

"The properties which recommended the grass to the native gardener of India also contributes to its early introduction into the colonies of those European Powers which then had possessions in India. The lemon-grass was introduced into Jamaica most probably in 1739. From there it was soon spread to the other British islands in the West Indies as an elegant and powerful diaphoretic under the popular name of lemon-grass." (Otto Stapf., 1906).

The name *Cymbopogon* is derived from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the flower spike arrangement. *Cymbopogon citratus*, Stapf (Lemon grass) is commonly used in teas, soups and curries. It is also suitable for poultry, fish and seafood (Shah et al., 2011).

The plant belongs to the *Poaceae* family, having a pendulous habit, with lanceolate leaves, rough to the touch.

Lemon grass is a perennial monocotyledonous grass which can grow up to about 180 cm in height and 125 cm in width.

It grows in clusters. It has long, slender, drooping bright green leaves that measures from 1.3-2.5 cm in width and 92 cm in length. Leaves are simple with entire margins. Flowers grow on spikes (Ranade et al., 2015).

However, it has many bulbous stems that increase the clump size as the plant grows (O.A. Lawal et al., 2017).

The plant can multiply both by seeds and assesual by dividing the bush.

Lemongrass is generally propagated through seeds. Seed is mixed with dry river sand in the ratio of 1:3 and sown in the field at the rate of 20 to 25 kg/ha. Alternatively, seedlings can be raised in a nursery in one-tenth of the area of the main field and transplanted after 45 days (Joy et al., 2006).

The essential oils contained in this plant provide both a strong lemon aroma and multiple medicinal, therapeutic and culinary properties. As a vaporizer, the oil works as an effective panacea against bacteria, flu and colds (Shah et al., 2011). A strong lemon fragrance, a predominant feature of this grass, is due to the high citral content in its oil. The redolence of the oil enables its use in soaps, detergents and perfumes. It also finds an application in the pharmaceutical industry (Ranade et al., 2015).

Since the beginning of its cultivation, this plant has surprised with its sweet and distinct aroma. "This is a most delicate sort of fragrant Grass which being rubbed smells like Baume and Lime or Limon peel together. The Portuguese woman fume their children with it and give the Decoction of it with other things for Fevers and to strengthen weak stomachs; but the Natives use it not, which together with its growing in gardens on the Sea coast and not up the country, as I can yet observe, makes me think the Portuguese brought this from other parts and planted it here." (Petiver, 1702)

Lemongrass contains several important bioactive compounds which are useful in several health issues. These active compounds are normally found in the leaves (Olorunnisola, S. K. et al., 2014).

Among the many properties of this plant are: antiviral, anti-inflammatory, anxiolytic, anti-cancer, etc. (Figure 1).

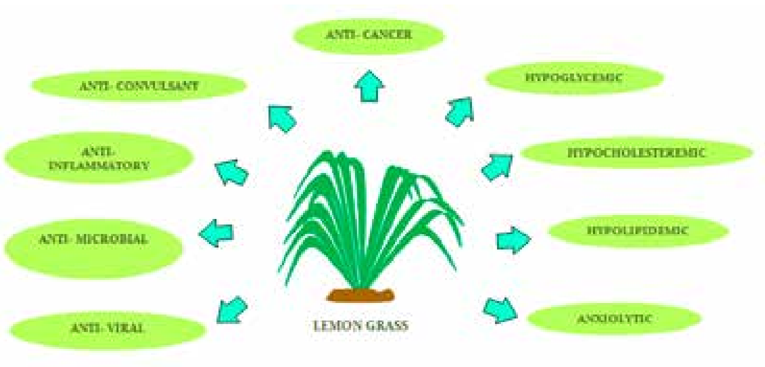

Figure 1. Activities shown by lemon grass that contribute to its therapeutic value (Ranade et al., 2015)

MATERIALS AND METHODS

Two genotypes were selected from the PGRB Buzau germplasm collection, G1 belonging to *Cymbopogon nardus* which has anthocyanin coloured leaflets at the base of the plant and G2, genotype which has all vegetative parts coloured green, belonging to *Cymbopogon citratus* (Figure 3)

G2 is genetically stabilized and is the subject of the present study. It was grown in two experimental variants, both under greenhouse-V1 S and open field-V2 C conditions.

Seedling production was carried out by seeding, even though the growth rate is much slower after germination and the germination capacity is low, below 80%. It is recommended, however, for a more accelerated rate of plant growth and much earlier crop establishment, to propagate plants by bush division. The plant behaves as a perennial in protected areas and as an annual in the field. Thus, seedling production can only take place in protected areas.

The research has three main objectives: acclimatization of the species to pedoclimatic conditions, improvement of the species in order to obtain genotypes with distinct phenotypic expression and development of culture technology, both in greenhouse and in the field for the improved genotype.

In order to develop the appropriate cultivation technology, several technological variants of crop establishment were used, both in terms of plant density per row and distance between rows. The research found that for this genotype, the optimal cropping pattern is 120 cm between rows and 50 cm between plants/row in greenhouse and 70 cm between rows and 40 cm between plants/row in the field. This cultivation technology provides the necessary surface area for nutrition and harmonious plant development, both in the root system and the aerial part. Manual and mechanical maintenance as well as harvesting can easily be carried out (Figure 2).

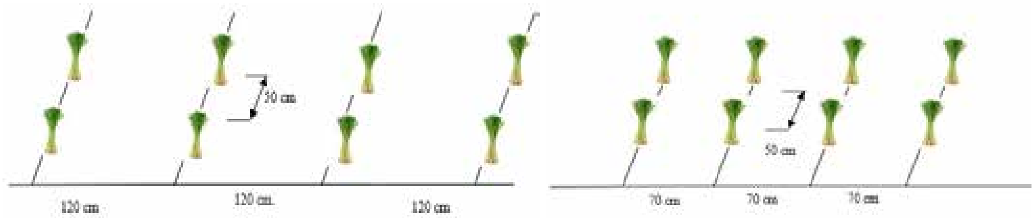

Figure 2. Greenhouse cropping scheme (left) and field cropping scheme (right)

Phenotypical and biometrical measurements of the main qualitative and quantitative plant characters were performed according to UPOV (https://www.upov.int/portal/index.html.en) and IPGRI descriptors (https://www.bioversityinternational.org/e-library/publications/categories/descriptors/).

Statistical calculations were performed using SPSS software, Pearson correlation coefficients were determined for the experimental variants, V1 S and V1 C as well as variance analysis by ANOVA test followed by DUNCAN test with 95% confidence interval and p-value < 0.05%.

Figure 3. G1 (left) and G2 (right)

RESULTS AND DISCUSSIONS

The plant was characterized qualitatively, phenotypically and quantitatively. According to the descriptors followed, the plant has a globular bush, lanceolate leaves, slightly spatulate, covered with glandular peristyles, rough and oriented towards the leaf tip (Figure 4). Leaf thickness is 0.2-0.3 mm on average. The average leaf vein thickness is 3-4 mm.

Figure 4. Plant and leaf details

Quantitative plant characteristics measured for the open field variant-V1C recorded significantly lower values compared to the average values recorded for the V2S variant, grown in protected areas, thus demonstrating the requirements of the plant for the respective growing environment (Table 1). In the case of leaf width and number of leaves/plant, the values for leaf width and number of leaves/plant were approximately similar and were not influenced by the growing medium.

The protected growing environment resulted in a vegetative mass of edible stems of 45% of the total plant mass, which is quite high for the potential of the plant.

Figure 5. Leaf/plant inserts

Table 1. Mean values of the main quantitative characteristics of the two variants

Characteristics	V1 S	CV%	V1 C	CV%
Plant height (cm)	202,2± 11,65 [a]	5,76	69,6±8,4[b]	12,08
Plant diameter-top (cm)	158,2± 5,91 [a]	3,74	79,2±10,4 [b]	13,13
Plant diameter -base (cm)	27,6± 2,73 [a]	9,88	21,4±2,7 [b]	12,40
Stem lenght (cm)	60,4± 11,89 [a]	19,69	29,8±5,9 [b]	19,84
Leaf lenght (cm)	104,4± 36,01 [a]	34,50	53,6±11,6 [b]	21,68
Leaf width (mm)	20,6± 7,94[a]	38,54	14,6±7,2[a]	49,50
Stem no./ plant (piece)	37,6± 4,88 [a]	12,99	28,4±5,6 [b]	19,62
Leaves no./stem (piece)	6,6± 1,851[a]	28,10	5,6±1,4[a]	24,22
Stem weight (g)	47,6± 4,18 [a]	8,77	28,8±5,7 [b]	19,93
Total weight of stems (g)	1690,2± 136,20 [a]	8,06	864,2±157 ,2 [b]	18,19
Total weight of leaves/plant (g)	2404,2± 128,09 [a]	5,33	1081,4±111,0 [b]	10,27
Leaves no./plant (piece)	253,4± 9,07 [a]	3,58	185±16,0 [b]	8,65
Plant weight (g)	4033,2± 95,74 [a]	2,37	2028,6±215,8 [b]	10,64

*letters represent Duncan test results with 95% confidence interval and p<0.05%; CV-coefficient of variation

Considering the correlations established between the quantitative characters determined in the open field variant, a maximum of 0.996 is obtained between plant height and stem length, which means a strong correlation between the two characters. Also, a high coefficient of 0.957 was obtained between total plant weight and leaf length. The same coefficient of 0.940 was obtained between total stem weight and leaf length as well as the number of leaves/plant, meaning that they are directly proportional in a positive trend (Table 2).

Table 2. Matrix of Pearson correlation coefficients for the main quantitative plant traits in V1 C

Characteristics	*H*	*D*	*Ls*	*Ll*	*Sno/pl*	*Lno/S*	*TwS*	*PlW*
Plant height (cm)-H	**1**							
Plant diameter -D	0,336	**1**						
Stem lenght (cm)-Ls	**0,974**	0,122	**1**					
Leaf lenght (cm)-Ll	0,600	0,215	0,565	**1**				
Stem no./ plant (piece)-Sno/pl	0,817	-0,147	**0,895**	0,754	**1**			
Leaves no./stem (piece)-Lno/S	**0,883**	0,063	**0,911**	0,839	**0,975**	**1**		
Total weight of stems (g)-TwS	0,641	0,224	0,607	**0,999**	0,779	0,864	**1**	
Plant weight (g)-PlW	0,635	0,277	0,587	**0,997**	0,746	0,844	**0,998**	**1**

In terms of establishing correlations between the main quantitative characters of plants grown in V1 S, the highest correlation coefficient of 0.999 was recorded between total stem weight and leaf length. A strong correlation was also recorded between total plant weight and total stem weight, resulting in a correlation coefficient of 0.998. A strong interdependence was observed

between total plant weight and leaf length, resulting in a coefficient of 0.997 (Figure 3, Table 3).

Table 3. Matrix of Pearson correlation coefficients for the main quantitative plant traits in V1S

Characteristics	*H*	*D*	*Ls*	*Ll*	*Sno/pl*	Lno/S	*TwS*	*PlW*
Plant height (cm)-H	**1**							
Plant diameter -D	**0,890**	**1**						
Stem lenght (cm)-Ls	**0,996**	**0,925**	**1**					
Leaf lenght (cm)-Ll	0,781	0,413	0,727	**1**				
Stem no./ plant (piece)-Sno/pl	**0,900**	0,627	0,865	**0,917**	**1**			
Leaves no./stem (piece)-Lno/S	0,867	0,670	0,847	0,858	0,771	**1**		
Total weight of stems (g)-TwS	0,723	0,397	0,677	**0,940**	0,765	**0,940**	**1**	
Plant weight (g)-PlW	0,653	0,241	0,588	**0,957**	**0,900**	0,673	0,817	**1**

Even though the mean values of the main characters were reduced in V1C compared to V1S, both growing environments give favorable results, and the plant can be grown in both protected and open fields. No diseases or pests affecting the crop were recorded throughout the growing season. The strong point of this species is its novelty for the range of aromatic plants cultivated in Romania.

Lemon grass has proven to be a multi-purpose plant, which can be cultivated both in the field and especially in protected areas to obtain superior edible stems.

CONCLUSIONS

A genotype belonging to *Cymbopogon citratus*, lemongrass, was introduced in the study from the PGRB Buzau germplasm collection.

The G2 genotype was cultivated in two experimental variants, both in open field and greenhouse conditions.

The plant behaves as a perennial in protected areas and as an annual in the field. Thus, seedling production can only take place in greenhouse.

Biometrical and phenotypical measurements of the main quantitative characters showed that the mean values of V1C were significantly different from those of V1S, the latter having much higher quantitative values.

The G2 genotype is genetically stabilized and represents a novelty for the range of aromatical and medicinal plants cultivated in our country, being able to be grown both in protected areas and in the field.

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COMPARATIVE STUDY OF DIFFERENT CULTIVARS OF LETTUCES IN UNHEATED POLYETHYLENE GREENHOUSE DURING WINTER-SPRING PERIOD

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Abstract

The aim of our study was to test some varieties of lettuce in unheated greenhouse (high tunnel) for the winter growing with November transplanting. The experiment was carried out in the period 2015-2016 in the experimental field on University of Forestry - Sofia (42° 7' N, 23° 43' E). Were selected 19 cultivars (16 Batavia and 3 Lollo types) with different requirements for the terms and conditions of cultivation. The seedlings were planted on the block method with four replications in the second ten days of November in polyethylene greenhouse. Until the time of harvesting, four surveys have been made on the percentage of rooted and dead plants. During the harvesting of production (second ten days of March - early April) were made biometric measurements (diameter and average weight per plant). Several cultivars for winter indoor cultivation were highlighted: 6 from Batavia, all of Lollo, and two from Batavia for outdoor. Regardless of recommendations given for each cultivar it needs they to be screened for each region, microclimate and growing period.

Key words*: Batavia type, Lactuca sativa, Lollo type, High tunnel, November transplanting.*

INTRODUCTION

One of the first fresh vegetables on the market, appearing early in the spring, is lettuce. The lettuce is cool season-hardy crop and develops at a temperature of 5-25°C. The optimal temperature for its growth and development is 16-18°C (Lorenz and Maynard, 1988; Kartalov et al., 2007).

Temperatures above 25°C cause the bolting. (Genkova, 2009) and at a temperature below 5°C growth of plants stops (Cholakov, 2009). Young and hardened plant (stage 7-8-th leaf) can withstand lowering the temperature to -5/ -6°C (Cholakov, 2009; Divina, 2016).

One way to year-round production of salads is their greenhouse production. An alternative to glass-greenhouses are polyethylene tunnel greenhouses without heating (Tüzel and Leonardi, 2009). They are economically viable to maintain and produce quality produce (Wells and Loy, 1993).

Salad is one of the first crops grown in a polythene greenhouse, along with warm-season tomatoes and cucumbers, to get off-season production (Lamont, 2005). In the US, the UK, Belgium and Germany lettuce rank as one of the main crops for cultivation in plastic tunnel greenhouses (Lamont, 2009).

The factors that affect the quality of lettuce are the growing season, the weather conditions and the variety (Koudela and Petříková, 2008). High tunnels protect salads from the unfavorable weather conditions encountered in their outdoor cultivation, thus affecting the time of harvesting, increasing the yield and quality of the produce, and thus these facilities are an alternative for creating early planting of lettuces (Santos et al,. 2009; Wallace et al., 2012).

It has been established that the salad growing season influences more on the yield and quality of the produce than the composition of the nutrient solution whose effect is not so well expressed (Fallovo et al., 2009). Date of planting also has a significant impact on yield (Sharma et al., 2009). In the winter production of leafy vegetables and the earlier date of planting, the length of the growing season is not reduced until the harvest, however seedling before the fall of the low winter temperatures and the occurrence of winter frosts leads to greater plants in the spring (Borrelli et al., 2013).

Lebeda et al. (2007) describe seven *Lactuca sativa* morphotypes, which cover seven main groups of varieties (including oil-bearing plants) that differ phenotypically (by Kristkova et al., 2008). In the Mediterranean region (Spain, Italy), as well as in the Middle East and North Africa, the main type is Rommaine (Cos) lettuce, in its different shapes and colors. In Northern Europe, both Cos lettuce and Batavia are popular. Stalk (asparagus) lettuce remains important in Egypt, the Middle East and China. All except iceberg found in red and green color of leaves (Ryder, 2002).

Two of the widespread types for Northern and Central Europe, which also enter in Bulgaria, are the Batavia and Lolo.

Type Batavia is characterized by open to strong heading; generally medium thick, rather strongly blistered leaves, predominately yellowish or medium green; leaf margin with weak to strong undulation (UPOV, 2017). In cold conditions not always have a clear position (DUS Test, 2016). It produces moderately dense heads with a crunchy texture and intermediate between iceberg and loose leaf types. Varieties are in red or green color (Divina, 2016). Lebeda et al. (2007) refers Batavia type to Crisphead lettuce (var. *capitata* L. nidus jäggeri Helm), together with Eissalat и Iceberg type (by Křístková et al., 2008)

Lollo type - it is non-heading; thin leaves with strongly undulated leaf margin. The plant as a whole shows mainly the undulating leaf margins. In general, strongly blistered leaves, blisters are rather small (UPOV, 2017). Forms tender leaves that are delicate and mildly flavored. Varieties come in green and red and green or purple color (Divina, 2016). Lolo type refers to Cutting lettuce (var. *acephala* Alef., syn. var. *secalina* Alef., syn. var. *crispa* L.) and this morphotype is extremely heterogeneous (Křístková et al., 2008).

In order for varieties to reveal their full potential and productivity, they need to be screened to check their adaptation in a given area. This should be done locally, across a broad range of contrasting environments to define and identify the most stable and well adapted varieties for a certain period of vegetation and environment (Dufault et al., 2006). Proper selection of varieties makes it possible to avoid bolting when growing at higher temperatures (Rader and Karlsson, 2006).

The aim of our study was to test and compare some of the offered lettuce cultivars in unheated polyethylene greenhouses (high tunnel) for the winter-spring growing season as November transplanting.

MATERIALS AND METHODS

The field experiment was conducted during 2015-2016 at the experimental field (42° 7′ N, 23° 43′ E and 552 m above sea level) of the University of Forestry, Sofia, Bulgaria. The soil type is fluvisol, slightly stony and slightly acidic ($pH_{(H2O)}$ 6.2). For the purpose of the experiment was used unheated polyethylene greenhouse (high tunnel) covered with a standard transparent strengthen and UV stabilized polyethylene.

Lettuce cultivars: They were selected and tested 19 cultivars of salads, 13 of them are Dutch, three Swiss, two Italian and one French. Selected cultivars refer to two types: Batavia and Lollo (16 are Batavia type, 3 are Lollo type). Of these, six are intended for growing outdoors. Origin, type of lettuce cultivars and recommended season and growing conditions are described in Table 1.

Seedlings were planted in the second ten days of November. The period of transplantation in the polyethylene tunnel was determined in order to use the final moment for planting in this type of cultivation facility for the Sofia region, in line with the ongoing climate change. The date of planting was 12.11.2015, which was fixed according to the weather conditions and the medium-term forecast for the month.

They were used previously produced seedlings and lettuces were grown on a flat surface, in a row, with a plant spacing of 25 cm. The experiment was performed in a blocking method with four replications, with 18 plants for each replication per cultivar. In the polyethylene greenhouse was used drip irrigation and all care during growing period were the same for all variants.

Table 1. Origin, type of lettuce cultivars and recommended season and growing conditions

Variants No.	Cultivars	Origin	Type	Season	Conditions
1.	*Maritima*	Netherland	Batavia	Spring-Summer-Autumn	outdoor
				All year round	indoor
2.	*Funride*	Switzerland	Batavia	Spring-Summer-Autumn For summer cultivation	outdoor
3.	*Kriska*	Italy	Lollo Bionda	Spring-Summer-Autumn	outdoor
				Autumn-Winter	indoor
4.	*Florine*	France	Batavia	Spring-Summer;Autumn-Winter	outdoor indoor
5.	*Funtasia*	Switzerland	Batavia	All year round without high and low temp	outdoor
6.	*Noisette*	Netherland	Batavia	All year round	outdoor indoor
7.	*Malice*	Netherland	Batavia	Spring/Autumn	outdoor
				Autumn-Winter-Spring	indoor
8.	*Fuzila*	Netherland	Batavia	Spring-Summer-Autumn	outdoor
9.	*Satine*	Netherland	Lollo Rossa	Autumn-Winter-Spring	indoor
10.	*Fanela*	Netherland	Batavia	Spring-Summer-Autumn	outdoor
11.	*Sementel*	Netherland	Batavia	Autumn-Winter-Spring	outdoor indoor
12.	*Frisady*	Netherland	Batavia	Spring-Summer-Autumn	outdoor
				All year round	indoor
13.	*Donertie*	Netherland	Batavia	Autumn-Winter-Spring	indoor
14.	*Jazzie*	Netherland	Batavia	Autumn-Winter-Spring	indoor
15.	*Aquarel*	Netherland	Batavia	Spring-Summer-Autumn	outdoor
16.	*Isi 45194*	Italy	Lollo Bionda	Autumn-Winter-Spring	outdoor indoor
17.	*Funfix*	Switzerland	Batavia	Autumn-Winter-Spring	outdoor
18.	*Ostralie*	Netherland	Batavia	Autumn-Winter-Spring	indoor
19.	*Hettie*	Netherland	Batavia	Autumn-Winter-Spring	indoor

During the growing period were conducted several plant surveys to account the percentage of rooted as well as the percentage of died plants until the time of harvesting.

Harvesting began in the second ten days of March and continued until the first ten days of April. During the harvesting were measured the plant diameter and the average weight per plant. The collected data were analyzed by ANOVA and were expressed as mean ± standard deviations. Post hoc analyses were conducted using Fisher's protected LSD test.

RESULTS AND DISCUSSIONS

When growing lettuces during the winter season, the period of planting seedlings and weather conditions it is important.

Generally months covering the experimental period (November '15 - April '16) were warmer compared to the average monthly temperatures in the 30-year period, reflecting global warming (Table 2).

From the time of planting until the end of November average daily temperatures were favorable for rooting of seedlings, considering that in unheated greenhouses they were better than outside.

It was found that at night the temperatures in the high tunnels are only 1 or 2°C higher than outdoor temperatures, while daytime temperatures are 10°C higher due to daytime solar radiation (Gent, 2002).

In December the outside temperature dropped, and in the middle of the month irrigation was stopped, thus the plants were prepared for wintering.

Extremely low outdoor temperatures were not recorded during this month, except for 3 days of the month (below -5°C), as in the two days are measured temperatures around the critical minimum for the lettuces in the early period of their development (-5°C), and at the last day of the month is measured at a temperature of -11°C.

January was the month with a negative average temperature, it is the only month with a lower temperature compared to '30 period. During this period the plants were not watered and were not covered with additional plant protection films. From the end of December to the end of January, temperatures were below the freezing temperature of lettuces.

Table 2. Average monthly, average minimum, average maximum and extreme monthly temperatures during the experimental period

Months	Anomaly compared to 30-year period	Monthly temperature (t °C)			Extreme monthly temperatures (number of days)				
		Average montly	Average minimum	Average maximum					
	t °C	M±SD	M±SD	M±SD	below -5 °C	-5/+5 °C	+16/+18 °C	+18/+25 °C	above +25 °C
November ’15	+3.7	8.7±4.1	3.6±4.4	14.7±5.1	0	21	9	8	0
December ’15	+1.5	2.1±3.3	-1.1±3.2	6.3±4.1	3	27	0	0	0
January ’16	-0.4	-0.9±6.5	-4.8±6.9	3.8±7.1	13	18	1	0	0
February ’16	+6.6	7.5±4.3	2.8±3.8	13.0±5.3	0	25	2	7	0
March ’16	+1.8	7.1±3.0	2.3±3.0	12.6±4.8	0	25	1	5	0
April ’16	+3.4	13.9±3.7	7.3±3.9	21.0±4.9	0	8	4	14	8

These low temperatures were the main limit for winter growth, which is confirming the findings of Gent (2002).

February was warm, daily temperatures triggered visible plant growth and irrigation was restored. Since mid-March, lettuces in the polyethylene greenhouse had reached the stage of harvest.

During the growing period was carried out periodic monitoring of the number of died plants. The first observation was at the beginning of December, three weeks after the seedlings were planted, to check the percentage of the rooted plants and their condition for wintering. 100% of rooting of all plants was reported (Table 3).

Table 3. Amount of rooted and survived after wintering plants (%)

No	Cultivars	Amount of plants (%)			
		2015	2016		
		04.12.	08.02.	11.03.	08.04.
1.	*Maritima*	100	99	53	49
2.	*Funride*	100	97	68	68
3.	*Kriska*	100	94	61	61
4.	*Florine*	100	88	69	69
5.	*Funtasia*	100	79	25	8
6.	*Noisette*	100	89	50	31
7.	*Malice*	100	92	57	53
8.	*Fuzila*	100	89	4	0
9.	*Satine*	100	86	81	81
10.	*Fanela*	100	75	43	42
11.	*Sementel*	100	100	53	51
12.	*Frisady*	100	90	47	47
13.	*Donertie*	100	97	88	86
14.	*Jazzie*	100	93	68	51
15.	*Aquarel*	100	93	40	32
16.	*Isi 45194*	100	94	92	92
17.	*Funfix*	100	79	21	15
18.	*Ostralie*	100	86	64	64
19.	*Hettie*	100	100	75	75

At the end of January, sunny weather was established and daily temperatures started to rise sharply (about 11-15°C), and at the beginning of February they reached around and above the optimum for the growing of lettuces (up to 18.8°C) and plants apparently began to grow and develop.

At the end of the first ten days of February, the second monitoring of the number of plants in the greenhouse was made to determine how many of them survived during the low temperatures in January. They were made two more monitoring in the polyethylene greenhouse - in March and April.

In February at three of tested in the polyethylene greenhouse lettuce cultivars - *Funtasia, Funfix* and *Fanela*, the number of plants has fallen below 80%, while variety *Fanela* reported a 25% death of plants. In March with less than 50% of plants were six cultivars, and in April the total number of cultivars with less than 50% plants were 8.

In descending order (from the most failed, to the least) these eight varieties rank as follows: ***Fuzila>Funtasia>Funfix>Noisette>Aquarel> Fanela>Frisady>Maritima***.

There are significant differences in the amount of plants remaining after their wintering:

F (3, 72) = 37.06 MSE = 307.06 p < .0001 at the .05 alpha level.

The causes of plant dying are complex - the season of cultivation (part of the varieties are not suitable for growing in winter), weather conditions (especially temperature), fungal diseases. Of the eight cultivars with more than 50% of the died plants, five are not recommended to be grown in the winter and are intended for open areas. In three of these losses of fungal diseases have been less than 10% and only *Fuzila* has a high percentage (36%) loss from fungal diseases (Table 4). Attention was also paid to the *Funride,* which, although according to its description is intended for outdoor production during the favorable months of the year (spring-summer-autumn), survived successfully in a polythene greenhouse and losses were 32%.

Table 4. Recommended growing periods and conditions for the cultivation of cultivars and the amount of died plants (%)

No.	Cultivars	Recommended for		Died plants %	
		Season	Conditions	Total	Fungal disease
1.	*Maritima*	All year round	indoor	51	-
2.	*Funride*	Spring-Summer-Autumn	outdoor	32	10
3.	*Kriska*	Autumn-Winter	indoor	39	-
4.	*Florine*	Spring-Summer;Autumn-Winter	indoor	31	-
5.	*Funtasia*	Without high and low temp	outdoor	92	4
6.	*Noisette*	All year round	indoor	69	-
7.	*Malice*	Autumn-Winter-Spring	indoor	47	6
8.	*Fuzila*	Spring-Summer-Autumn	outdoor	100	36
9.	*Satine*	Autumn-Winter-Spring	indoor	19	4
10.	*Fanela*	Spring-Summer-Autumn	outdoor	58	7
11.	*Sementel*	Autumn-Winter-Spring	indoor	49	11
12.	*Frisady*	All year round	indoor	53	-
13.	*Donertie*	Autumn-Winter-Spring	indoor	14	-
14.	*Jazzie*	Autumn-Winter-Spring	indoor	49	10
15.	*Aquarel*	Spring-Summer-Autumn	outdoor	68	8
16.	*Isi 45194*	Autumn-Winter-Spring	indoor	8	3
17.	*Funfix*	Autumn-Winter-Spring	outdoor	85	14
18.	*Ostralie*	Autumn-Winter-Spring	indoor	36	22
19.	*Hettie*	Autumn-Winter-Spring	indoor	25	-

During the harvesting of the produce, biometric data were collected - diameter and average weight of the plants (Table 5).

There are significant differences in the diameter of the plants $F\ (17,\ 161)=5.10$ $MSE = 4.53$ $p < .0001$ at the .05 alpha level, as well as the average weight per plant $F\ (16,\ 40)=2.97$ $MSE = 1581.23$ $p = .002$ at the .05 alpha level.

In several cultivars, the plants reached a diameter of between 25-27 cm. In descending order (from the largest diameter to the smaller), they are arranged in the following way: *Fanela>Malice>Hettie>Frisady>Noisette>Isi 45194.* Immediately after them come *Funride* and *Kriska*, whose diameter is close to 24 cm.

The average weight of a plant varies, depending on the type and cultivars, was from 92 g to over 230 g per plant. The largest weight per plant (220-230 g) had three cultivars that are arranged in descending order in the following way: *Frisady>Fanela>Malice*. Immediately after them was again *Funride,* with 217 g, followed by *Hettie* - 211 g. (Table 5).

Table 5. Mean value (M) and standard deviation (SD) of plant diameter and weight per plant

No	Cultivar	Plant diameter (cm)		Plant weight (g/per plant)	
		M±SD	LSD*	*M±SD*	LSD*
1	*Maritima*	23.33±1.72	c d e f g	151±34	C D
2	*Funride*	23.90±3.48	b c d e f	217±19	A B
3	*Kriska*	23.83±2.48	c d e f	204±32	A B C
4	*Florine*	22.25±1.66	f g h	194±48	A B C
5	*Funtasia*	20.33±4.51	h	0,00	
6	*Noisette*	24.71±1.80	b c d e	183±24	A B C
7	*Malice*	25.58±1.93	a b	226±64	A B
8	*Fuzila*	0		0	
9	*Satine*	22.00±1.54	g h	94±20	D
10	*Fanela*	27.33±3.20	a	228±67	A B
11	*Sementel*	22.92±2.39	e f g h	185±41	A B C

No	Cultivar	Plant diameter (cm)		Plant weight (g/per plant)	
		M±SD	*M±SD*	*M±SD*	
12	*Frisady*	24.91±1.64	b c d	231±34	A
13	*Donertie*	22.08±1.68	g h	163±17	B C
14	*Jazzie*	23.11±1.45	d e f g h	204±64	A B C
15	*Aquarel*	22.50±0.97	f g h	164±13	B C
16	*Isi 45194*	24.58±2.23	b c d e	153±38	C
17	*Funfix*	22.40±1.67	d e f g h	150±0	B C D
18	*Ostralie*	22.22±2.44	f g h	188±38	A B C
19	*Hettie*	25.10±1.52	b c	211±46	A B

*Means within a column followed by the same letter do not differ significantly based on Fisher's LSD post-hoc analyses at $p<.05$.

CONCLUSIONS

By making a comprehensive analysis of the results in individual cultivars can say several of them, which are for indoor cultivation, stand out: from type Batavia are *Hettie, Frisady, Malice, Jazzie, Florine, Sementel, Donertie. Cultivars Frisady, Malice, Jazzie* and *Sementel* are slightly more sensitive to extreme temperatures, which is expressed in a greater variation in the average weight per plant in *Malice* and *Jazzie*.

All three varieties of type Lolo showed very good results: *Kriska, Isi 45194* and *Satine*, as the last one is the type Lollo Rosso and had a very gentle and tender leaves, which affected the average weight per plant. The *Kriska* cultivar was slightly more sensitive to extreme temperatures than the other two but had a higher average weight per plant than the other two Lolo type cultivars.

Two of the cultivars, type of Batavia, which are intended for outdoor cultivation, also showed very good results when growing indoors in winter: *Funride* and *Fanela*, but the second one was a little more sensitive to extreme temperatures.

Regardless of the recommendations given in the cultivar descriptions, it is necessary to screen the offered cultivars, for each region, microclimate and growing period, which confirms the conclusions of the Dufault et al. (2006) and Maynard (2014)

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FERTILIZATION SYSTEMS EFFECT ON THE GROWTH AND PRODUCTIVE MANIFESTATIONS OF GREENHOUSE TOMATOES, CULTIVATED AS A SOIL CROP

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Abstract

The purpose of the study is optimization of fertilization with the greenhouse tomatoes. The experimental work has been brought out during the period 2018-2019, in the test field of department "Plant Production" in a polyethylene greenhouse, without heating, with two varieties tomatoes - Grando F_1 and Pink Rok F_1, grown as a soil crop. Three schemes of fertilization have been tested with five formulations fertilizers for fertigation: brought in as main fertilization /control/ and a combined scheme: fertilization in seven and three days during the vegetation on the increase rate of the vegetative mass and productivity of the plants.
The results from the tested fertilization schemes show a positive effect on the growth and productive manifestations with both tomato varieties in comparison with the control. It is statistically proven that the impact is bigger with the combined fertilization by YaraTera Kristalon Special (18-18-18); YaraTera Kristalon Lazur (20-05-10); YaraTera Kristalon Orange (6-12-36); YaraTera Kristaflex Yellow 12-32-11 and YaraLiva Calcinit, brought in in 3 days, in comparison with the fertilization in 7 days, which is expressed stronger for variety Grando F_1. The results about the number and mass of the fruit per plant are analogous. By the analysis carried out on the averaged data is established that variety Grando F_1 exceeds as per productivity variety Pink rok F1 by 11.1% (fertilization in 3 days) and 4.8% (fertilization in 7 days), as the differences are mathematically proven.

Key words: *Solanum lycopersicum L, polyethylene greenhouse, fertilization, growth manifestations, productivity.*

INTRODUCTION

The greenhouse vegetable farming is one of the branches of the agriculture in our country, which has potential to generate significant revenues from a unit of area. The technologies in this production are developing very intensively. The efficiency of the vegetable farming is in direct connection with the scientific researches in the field of fertilization. Different models for maintaining and perfection of the nutritional regime are continuously studied and created, alternative, ecologically sound solutions are introduced, which comply with the requirements of the contemporary agriculture (Sengalevich et al., 2007; Tringovska & Kanazirska, 2007; Arnaudov et al., 2007; Boteva et al., 2013; Kostadinov et al., 2013; Tringovska et al., 2014; Naskova, 2017).
Over the last years in the countries with developed agriculture is carried out research work for optimization of the nutritional regime of the greenhouse crops (Nakano & Akimasa, 2003; Pascale et al., 2004). The emphasis is put on perfection of the fertilization models, based on more effective usage of the nutrient substances and allowing decrease of the brought in quantities in the soil (Kolota & Osinska, 2000; Naskova, 2017). This is necessary and in relation with the super intensive character of the greenhouse production, in which are introduced and are grown contemporary hybrids with valuable economic qualities and very high biological potential.
Different formulations fertilizers are offered at the Bulgarian market. The right fertilization, conformed with the permanently changing varietal composition, imposes to be brought out the researches for their usage with the vegetable crops in greenhouses, including with tomatoes (Martins, 2010; Tringovska, 2012; Márquez-Hernández, 2013). The broadening of the researches in our country is necessary in

order to be optimized the nutritional regime by offering appropriate schemes for fertilization, guaranteeing the obtaining of stable yield of high quality, which can guarantee the competitiveness at the Bulgarian market.
The purpose of the study is to be determined the impact of fertilization systems on the growth and the productive qualities of the greenhouse tomatoes, grown as a soil crop with purpose optimization of the nutrition.

MATERIALS AND METHODS

The experimental work has been brought out during the period 2018-2019 in a non-heated polyethylene greenhouse of department Plant Production - Technical University Varna with two varieties greenhouse tomatoes - Pink Rok F_1 and Grando F_1. Three fertilization schemes have been tested with five formulations fertilizers for fertigation. The plants are grown as a soil crop.

Conditions of the experiment
Variety: Variety Pink Rok F_1 and Variety Grando F_1;
Sowing: 02-03.03. - peat-perlitic substrate (1vol.:10 vol.), in terrines with 160 clusters;
Pricking out: 01-03.04. - peat-perlitic substrate (1 vol.:10 vol.), terrines with 28 clusters
Planting: 18-20.04.
Harvesting: until 08-10.08.
Density of the crops: 3 plants/m^2
Combing (pruning): Phase 4-5^{-th} flowerhead
Harvesting until: 30.09.

Fertilization schemes:
1. Control - only main fertilization by mineral fertilizer YaraMila Cropcare (8-11-23), 60 kg/da.
2. Fertilization for the whole vegetation by mineral fertilizers - nitrogen 18 g per plant; phosphorus 12 g per plant; potassium 28 g per plant, as the fertilization is carried out in 3 days.
3. Fertilization of the whole vegetation by mineral fertilizers - nitrogen 18 g per plant; phosphorus 12 g per plant; potassium 28 g per plant, as the fertilization is carried out in 7 days.

Characteristics of the fertilizers:
YaraTera KRISTALON Special (18-18-18) - It is appropriate for crops and phases of their development, when it is necessary the proportion between the main nutrient elements nitrogen:phosphorus:potassium to be - 1:1:1;
YaraTera KRISTALON Lazur (20-05-10) - A specific proportion between the nitrogen, phosphorus and potassium for crops and phases of their development, which require high nitric nutrition;
YaraTera KRISTALON Orange (6-12-36) - A formula with low content of nitrogen and high content of potassium, it stimulates the ripening of the fruit;
YaraTera KRISTAFLEX Yellow 12-32-11 - With high content of phosphorus. It stimulates the faster and better root-taking of the agricultural crops after pricking out;
YaraLiva CALCINIT - it is applied in the initial stages of the crop development and during formation and growing of the fruit, it is against calcium deficiency.
The plants are cultivated in soil and in peat substrate (Klasman TS 3). The experiment is set out as per the method of the long plots in 4 repetitions, with six plants in a repetition, with size of the harvesting area 6 m^2.
The quantity of the mineral fertilizers is determined on the grounds of an agro-chemical analysis of the soil. The application of the fertilizers has been carried out at background main fertilization by YaraMila Cropcare. The main fertilization is one and the same for all variants.
The cares for the plants growing are as per technology for early spring greenhouse production, without heating. Earth bee *Bombus terrestris* has been used for pollination.
The soil from the experimental plot is leached chernozem with a dark humus horizon (FAO, 2000), medium reserved with mobile phosphorus and nitrogen and well reserved with potassium (Table 1). The reaction of the soil solution is neutral pH = 7.05; the content of humus is 3-4%, as it decreases in depth; total salt concentration 0.34 mS/cm.

Table 1. Agrochemical analysis of the soil before the main fertilization

pH	Hummus	EC mS/cm	Nutrients - ppm				
			N	P	K	Ca	Mg
7.05	3-4%	0.34	133.3	8.6	117.1	121.1	80.5

The main fertilization is one and the same for all variants, as for the purpose 60 kg/da mineral fertilizer YaraMila Cropcare (8-11-23) has been brought in.

Indexes of the study

Microclimatic conditions

Everyday at 8.00 AM and at 2.00 PM are registered:

- temperature of the air (ºC) – there has been reported minimum-maximum thermometer at height 5-6 leaf from the top of the plants.
- relative air humidity (%). Measured by a digital hygrometer HD8501H.
- temperature of the substrate (ºC). It is measured at depth 8 cm by a digital soil thermometer.

Agrochemical analysis of the soil. The content of the assimilable nutritional elements has been determined by soil analysis (before setting of the experiment) in a water extract 1:2 vol./vol. There have been determined pH – potentiometrically; total concentration of the soluble salts - as per electrical conductivity, nitrogen - by an ion selective analyzer; phosphorus - spectrophotometrically; calcium and magnesium - complexometrically.

Biometric analyses: Rate of the stem growing and leaves formation - in 15 days until the end of the vegetation of 6 marked plants from repetitions are measured – the height of the stem (cm) and number of the leaves per plant.

Productivity: At mass fruit-bearing are analyzed as per 6 plants of repetition for determination of: number of fruit - number/plant; mass of the fruit - g/plant;

Mathematical processing of the data: The results are processed by a multidirectional comparative analysis as per the method of Duncan,s multiple range test and T-test (SPSS software).

RESULTS AND DISCUSSIONS

Microclimatic conditions

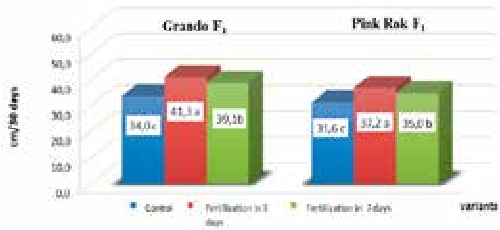
The registered data about the microclimatic factors show that during the period of bringing out of the experiments, the temperature conditions are close to the requirements of the tomato plants, while the relative air humidity in the afternoon hours in separate days is under the optimum for this crop.

Growth manifestations

The formation and the functional condition of the vegetative parts of the plants depend on many factors, including and nutrition. The balanced feeding of the plants is one of the main factors for impact on the growth manifestations and the productivity.

A summary index about the growth manifestations is the size of the formed fresh vegetative over-ground mass. It is proven about the reported period on the average that with variety Grando F_1 the plants have registered higher values of the growth rate of the stem with Variant 1 (fertilization in 3 days) (41.3 cm/30 days), the effect is weaker with Variant 2 (fertilization in 7 days) (39.1 cm/30 days), as the increase is respectively by 21.5% and 15.0% in comparison with the control (Figure 1).

The results are analogous and about variety Pink Rok F_1. The plants of Variant 1 have faster growth rate of the stem (37.2 cm/30 days), which is proven to differ from Variant 2 (35.0 cm/30 days), as the increase in comparison with the control is by 17.7% and 10.8%. The impact on the growth rate of the stems is proven to be weaker with the control variant.



a, b, c - Duncan`s multiple range test (p<0.05)

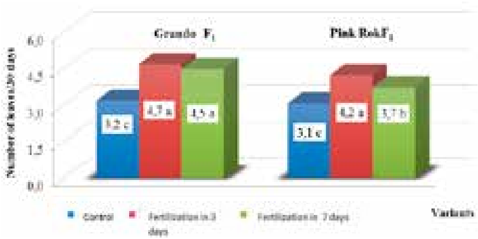
Figure 1. Stem growth rate (cm/30 days) - average for the period

In order to be clarified the meaning of the factor “Variety” on the growth rate of the stems is analyzed the impact of fertilization in comparison with the tested varieties. Statistically significant differences have not been established between the two varieties on the average for the period of study, the increase in comparison with the control is respectively by 11.0% and 11.7% (Table 2).

Table 2. Influence of fertilization on the growth rate of the stem, depending on the variety - average for the period (cm/30 days)

№	Variants	Variety Grando F_1	Variety Pink Rok F_1	Diffe-rence	Proof	%
1	Control	34.0	31.6	2.4	ns	107.6
2	Fertiliza-tion in 3 days	41.3	37.2	4.2	++	111.0
3	Fertiliza-tion in 7 days	39.1	35.0	4.1	++	111.7

The impact of the fertilization on the rate of leaves formation is established on the average for the period of study (Figure 2). The highest values of this index are established with variety Grando F_1 with fertilization in 3 days (4.7 pieces leaves/30 days), with close and unproved differences are the plants, the fertilized in 7 days (4.5 pieces leaves/30 days), the increase in comparison with the control is respectively by 46.9 and 40.6%.
Statistically proven are the differences between the variants of fertilization with variety Pink rok F_1, as the increase is by 35.5% (fertilization in 3 days) and 19.4% (fertilization in 7 days).



a, b, c - Duncan`s multiple range test (p<0.05)

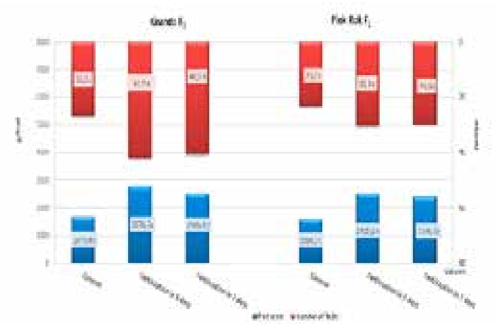
Figure 2. Rate of leaf formation - average for the period

On the average for the period of study the differences in the rate of leaf formation between the two varieties are proven with both variants of fertilization, stronger expressed with variety Grando F_1 (Table 3), as the increase is by 21.6% (fertilization in 7 days) and 11.9 % (fertilization in 3 days) in comparison with variety Pink rok F_1.
Proven differences between the two fertilization schemes have been established in comparison with the number and mass of the fruit on the average for the period.

Table 3. Influence of fertilization on the rate of leaf formation depending on the variety (number of leaves/ 30 days)

№	Varinats	Variety Grando F_1	Variety Pink Rok F_1	Diffe-rence	Proof	%
1	Control	3.2	3.1	0.1	ns	103.2
2	Fertiliza-tion in 3 days	4.7	4.2	0.5	+	111.9
3	Fertiliza-tion in 7 days	4.5	3.7	0.8	++	121.6

With Gradno F_1 variety the number of fruit varies from 26.6 up to 41.9 on the average per plant. Bigger increase is registered with Variant 1 (fertilization in 3 days), followed by Variant 2 (fertilization in 7 days), as the increase of the number of fruit in comparison with the control is respectively by 57.6% and 51.5% (Figure 3).
With variety Pink Rok F_1 the number of fruit varies from 23.2 up to 30.3 on the average per plant. As with Variant 1 (fertilization in 3 days) the number of fruit is the biggest, the increase in comparison with the control is by 32.9%, the effect is weaker of the fertilization with Variant 2 (fertilization in 7 days) - 27.2%.



a, b, c - Duncan`s multiple range test (p<0.05)

Figure 3. Mass and number of fruits per plant

The average mass of the fruit for variety Grando F_1 varies from 1675.0 g up to 2776.7 g/plant. It is proven that the biggest mass of the fruit is with Variant 1 (fertilization in 3 days), as the increase in comparison with the control is by 65.8%, while the increase of the fruit mass with Variant 2 (fertilization in 7 days), in comparison with the control is by 49.6% (Figure 3). Analogous are the data, which are obtained for variety Pink Rok F_1, as the effect in comparison with the control is expressed weaker (57.5% and 50,7 %).

Statistically significantly stronger is the impact of fertilization on the number and mass of the fruit with variety Grando F_1 (Table 4). Statistically significant differences between the varieties regarding the formed fruit per plant have not been established on the average for the period of the experiment.

Table 4. Influence of fertilization on the number of fruits of plants depending on the variety

№	Varinats	Variety Grando F_1	Variety Pink Rok F_1	Difference	Proof	%
1	Control	26.6	23.2	3.4	++	114.7
2	Fertiliza-tion in 3 days	41.9	30.3	11.3	+++	138.3
3	Fertiliza-tion in 7 days	40.5	29.6	10.9	+++	136.8

By the analysis carried out of the averaged data is established that variety Grando F_1 exceeds as per productivity variety Pink rok F_1 by 11.1 % (fertilization in 3 days) and 4.8% (fertilization in 7 days), as the differences are mathematically proven (Table 5).

Table 5. Influence of fertilization on the fruit mass of plants depending on the variety

№	Varinats	Variety Grando F_1	Variety Pink Rok F_1	Diffe-rence	Proof	%
1	Control	1675.0	1586.7	88.3	+	105.6
2	Fertiliza-tion in 3 days	2776.7	2499.6	277.1	++	111.1
3	Fertiliza-tion in 7 days	2506.4	2391.5	114.9	+	104.8

CONCLUSIONS

The applied fertilization schemes render positive impact on the formed fresh vegetative over-ground mass.

Higher values of the growth rate of the stem are registered with variety Grando F_1 with Variant 1 (fertilization in 3 days), the effect is weaker with Variant 2 (fertilization in 7 days), as the increase is respectively by 21.5% and 15.0% in comparison with the control.

On the average for the period of study the differences in the rate of leaf formation between the two varieties are proven and with both variants of fertilization, as it is expressed stronger with variety Grando F_1, as the increase is by 21.6% (fertilization in 7 days) and 11.9 % (fertilization in 3 days) in comparison with variety Pink rok F_1.

A positive effect has been established by the testing of the two fertilization schemes on the formation of fruit number per plant in comparison with the control. With both varieties tomatoes Grando F_1 and Pink Rok F_1 this is best expressed with Variant 1 (fertilization in 3 days). The fertilization impact on the number and mass of fruit with variety Grando F_1 is statistically significantly stronger.

It was established by the analysis carried out of the averaged data that variety Grando F_1 exceeds as per productivity variety Pink rok F_1 by 11.1% (fertilization in 3 days) and 4.8% (fertilization in 7 days), as the differences are mathematically proven.

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THE EFFECT OF TEMPERATURE AND STRATIFICATION TIMES ON SEEDS GERMINATION OF SOME *GLADIOLUS* SPECIES

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Abstract

In this paper, the germination of seeds from three species of the genus Gladiolus was studied: G. imbricatus, G. byzantinus and G. tristis. The seeds used had different origins: the seeds of G. imbricatus and G. byzantinus come from the plants from the Floriculture collection of at the Faculty of Horticulture ("Ion Ionescu de la Brad" Iasi University of Life Sciences), and the seeds of G. tristis come from specialized companies. The seeds were stratified at 4°C for 44, 58, 74, 86 and 98 days. The seeds were germinated in Petri dishes in the germination chamber at different temperatures to trigger the germination (13-14°C, 17-19°C and 20-22°C). Data obtained showed that the germination percentage depend of Gladiolus specie, temperature and number of days of stratification. Thus, at G. byzantinus, the best germination rate was obtained at the temperature of 13-14°C, after 58 days of stratification; in the case of 74 days stratified seeds, the germination temperature did not affect the percentage of germinated seeds. In G. tristis, germination was favored by temperatures between 17-19°C. The seeds of G. imbricatus germinatedin a reduced percentageonly after 98 days of stratification, but regardless of the temperature.

Key words: *germination, gladiolus, seed, stratification, temperature.*

INTRODUCTION

The *Iridaceae* Juss. family comprises a large number of herbaceous plants, perennials (geophytes), with underground organs rhizomes, corms and less often bulbs (Ciocârlan, 2009). The main center of origin of these plants is southern Africa, to which are added Central and South America, including the tropical part of Mexico and the Caribbean (Alves et al., 2011), so they are cosmopolitan, spreading from temperate regions.to the tropical ones (Ștefan and Oprea, 2007). The number of genera and species included in the family *Iridaceae*, varies, depending on different botanical classifications made over the time: approx. 2000 species distributed in 60-70 genera (Goldblatt et al., 2008, cited by Alves et al., 2011 and Raycheva et al., 2020), approx. 1800 species and 78 genera (Skrypec&Odintsova, 2020), approx. 1800 species and 92 genera (Ștefan & Oprea, 2007).

The *Gladiolus* L., with representatives studied in this paper, is one of the important genera of the *Iridaceae* family, considering from the ornamental point of view. The similarity between some species and the morphological variations determined by the environmental conditions have sometimes led to taxonomic confusions or uncertain delimitations, being treated, by many authors, as synonyms or placed in lower taxonomic ranks (Mifsud & Hamilton, 2013; Yetişen et al., 2014). According to some authors, the *Gladiolus* genus includes approx. 150 species (Reddy et al., 2019; Dahlgren, 1985 cited by Yetişen et al., 2014), followed by more than 250-260 species (Băla & Sala, 2021; Mifsud & Hamilton, 2013; Azimi, 2020; Sağiroğlu & Akgül, 2014) or even 311 species (Raycheva et al., 2020). Their origin is in different regions of South and tropical Africa, Asia, southern Europe and part of the Mediterranean basin (Cantor & Tolety, 2011; Mifsud & Hamilton, 2013; Reddy et al., 2019; Raycheva et al., 2020; Yetişen et al., 2014), many of them being spread throughout the world (Lewis et al., 1972, cited by Fernández et al., 2005).

Gladiolus species are petaloid monocotyledonous plants with oval or globular corms as underground storage organs, covered with several layers of fibrous tunics (De Hertogh & Le Nard, 1993; Cantor, 2016; Cantor & Pop, 2008). All species of gladiolus can be propagated sexually or asexually (Cantor, 2016), but seed production is higher than corm production (González et al., 2003). For commercial production, gladioli are vegetatively propagated by corms and cormels (Aljaser & Anderson, 2021), although this method is characterized by a low rate of propagation (each season a mother corm normally produces 1 to 2 new corms and several cormels). The production of corms and cormels is also severely affected by the high percentage of spoilage, during storage and the attack of *Fusarium* (Memon et al., 2016; Riaz et al., 2010). In recent years, numerous studies have been conducted to improve *in vitro* regeneration protocols, both in cultivated varieties of gladiolus and in wild species, in order to increase the rate of multiplication, to obtain free material from viruses and other pathogens (Kumar et al., 2018; Memon et al., 2016; Dutta Gupta & Prasad, 2010) and the conservation of rare wild species (Rakosy-Tican et al., 2012).

Sexual reproduction in gladiolus is important for breeders and to the recovery and maintenance of endangered germplasm (Fernández et al., 2005). Gladiolus species form winged or unwinged seeds (Mifsud & Hamilton, 2013). In general, seed-bearing plants bloom in the second year (Cohat, 1993, cited by Fernández et al., 2005) and do not always maintain the typical characteristics of the species or cultivar (Kumar et al., 2018).

For the wild gladiolus species, seed propagation is one of the important propagation pathways, due to its relatively good yield compared to corm propagation, especially when *ex situ* propagation is desired to restore threatened populations. For example, the vegetative spread of *G. imbricatus* is limited by the fact that in one season it, usually, forms only one new tuberobulb (Kostrakiewicz-Gierałt, 2017).

Studies on the germination of gladiolus seeds have been performed on both *G. hybridus* cultivars and wild species, in order to clarify a number of issues related due to the peculiarities of germination and to establish optimal seed propagation technologies. It is known that the germination of seeds of some plant species, especially perennials, is influenced by many factors, the literature focusing on issues related to seed origin, separate and simultaneous interaction of environmental factors, seed storage conditions, dormancy period, morpho-anatomical, physiological and biochemical features of the seeds etc. (Fernández et al., 2005).

Widespread in spontaneous plants, seeds dormancy appears as a mechanism of survival under various environmental conditions, specific to the areas of origin and is genetically imprinted. Entering or leaving seeds dormancy is influenced by external or internal factors (Toma & Jităreanu, 2007). Baskin & Baskin (2004) classified the types of seed rest in physiological, morphological, morphophysio-logical, physical and combined, ones. Some of the biological reasons for dormancy, listed by ISTA (1966, 1993), are the hard and imper-meable coating, the immature or latent embryo, the absence of the endosperm and the fleshy part of the fruit. The dormancy period varies from species to species (Olmez et al., 2007). The interruption of the seeds dormancy and the improvement of the germination is achieved by applying different treatments on the seeds: stratification, vernalization, physical, chemical, mechanical and hormonal treatments etc. (Toma & Jităreanu, 2007; Draghia, 2011; Basra et al., 2003; Afzal et al., 2004). Seed treatment before sowing is a known method and an effective way to increase the germination rate of seeds, as well as the growth rate in some species, for example, with small embryos or species with gradual seed development (Sivritepe, 2000). This treatment is also effective for better seed germination of vegetables and herbs (Arif et al., 2007).

Carpenter et al. (1991) showed that seed germination in *G. grandiflorus* was independent of light, but strongly in fluenced by temperature, themostsuitable being a constant of 20°C or an alternation of temperature between 20-25°C or 25-30°C. The same authors mention that reducing the humidity of the seeds and treating them for seven days, with temperatures of -20^0C, does not influence the germination.

Dutt et al. (2000) treated the seeds of some cultivars of *G. hybridus* with GA_3 solution (100, 150 and 200 ppm) and distilled water for 24 hours. Compared to distilled water treatment, the GA_3 treatments significantly increased seed germination rates and shortened the germination time.

In order to improve the germination rate and reduce the time required for germination at *G. alatus* seeds were applied by bathing for 48 hours, before sowing in KNO_3 solutions with different concentrations (1, 2, 3, 4 and 5%) and in distilled water, the control being untreated seeds (Ramzan et al., 2010). The best germination (over 80%) was obtained at the seeds kept in distilled water (92%) and only in 1% and 2% KNO_3 solutions. Hydration of the seeds in distilled water shortened both the minimum time required for germination of 50% of the seeds and the total time of germination. Similar experiments with *G. alatus* were performed by Mushtaq et al. (2012), who used KNO_3 treatments in concentrations of 0.25-5%, the better results were reported by treatments with distilled water and lower concentrations of KNO3 (0.25-0.75%) (Mushtaq et al., 2012).

The seeds stratification is also an efficient method of interrupting of dormancy and improving germination. The stratification ensures the good conditions for cold of the seeds and is achieved by an exposure to 1^0-5^0 (10°)C and humidity, a certain period of time, depending on the species.The experiments performed on *G. palustris,* both in the laboratory and in the field, indicated a better germination rate when the seeds were subjected to cold stratification schemes (Brunzel, 2010).

Seeds germination studies have been performed in other species of the Iridaceae family. For example, *Iris*s eeds are used to weaken the strength of the skin by mechanical action (Blumenthal et al., 1986). *I. ensata* seeds germinate in a proportion of approx. 94% if these are cold layered for 60 days (Xiao et al., 2010). In *Freesia* species it was found that the seeds germinate at temperatures between 15^0C and 18^0C, in about 21 days. In the Changjiang area of China, the seeds are sown directly in cold beds, which can be kept warm in winter (Long, 1996).

The objective of this paper was o determine the influence of temperature and stratification time on seed germination of some *Gladiolus* species from different geographical areas, including Romania.

MATERIALS AND METHODS

The study of seeds germination was performed on three species of the genus *Gladiolus*: *G. byzantinus, G. imbricatus* and *G. tristis*.

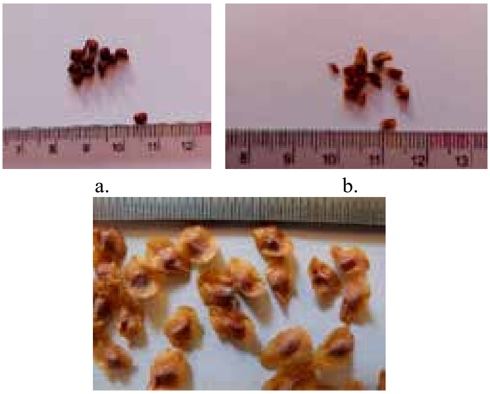
G. byzantinus Mill. it also appears with the synonymous name *G. communis* L. subsp. *byzantinus* (Mill.) A. P. Ham. However, the classification of the taxon as a subspecies of *G. communis* (Hamilton, 1976, quoted by Tison & Girod, 2014; Raamsdonk & Vries, 1989) is not supported by many authors, who do not recognize it as a distinct subspecies. Mifsud & Hamilton (2013) consider that *G. Byzantinus* is a sterile hexaploid (2n = 90), which is not found in nature and isjust a simple garden variety described by Philip Miller Hamilton (2013); Tison & Girod (2014) consider it is a polymorphic hybrid (*G.* x*byzantinus*), found, mainly, in the western Mediterranean basin, southern Spain and northern Africa, Italy and Sicily, reaching the coasts of Greece (Lopez-Espinoza et al., 2003). The plants form flowers talks of approx. 0.5-1 m tall, with bright purple-red flowers arranged unilaterally, which bloom in May-June.

G. imbricatus L., native to Central and Eastern Europe, the Mediterranean, the Caucasus and Western Siberia (Kose et al., 2019), is a declining species in Europe and on the red lists in many countries (Moora, 2008). In Romania it appears as a wild species, being reported in areas throughout Moldova, Muntenia, Transylvania, Banat, Oltenia, Maramureș (Oprea, 2005). It was cultivated *ex situ* from a local seeds populationin the Floriculture field at the Faculty of Horticulture in Iași. It grows up to 30-80 (100) cm in height and forms unilateral inflorescences with pink-lilac flowers and eeds of approx. 1.8 mg (Kose et al., 2019; Jõgarand Moora, 2008). *G. imbricatus* seeds weigh 1.8 mg (on average) and mature in August-September (Zelená, 1967; Goldblatt & Manning, 2008, cited by Kubíková & Zeidler, 2011), but in July-August in Iasi conditions. The seeds go through a dormancy that is interrupted during the winter (Chrtek et al., 2007, cited by Kubíková & Zeidler, 2011). Klimeš et al. (1997) characterized the limited vegetative spread, and the productions of corms are very small, respectively, one corm in a growing season (cited by Szczepaniak et al., 2016).

G. tristis L. is native to South Africa, but is widespread in areas of Australia and the coastal region of California. It has floral stems 60-70 cm high and inflorescences with 2-10 fragrant flowers, which bloom in spring (Gonzalez et al., 1998; Milandri et al., 2008).

The study of seed germination of the three species of *Gladiolus* were carried out in the period 2020-2021, in laboratory conditions, of

the Horticultural Research Center (former Fruit Research Laboratory "Prof. Dr. Gica Grădinariu") of the Faculty of Horticulture from "Ion Ionescu de la Brad" University of Life Sciences in Iasi Romania.
The seeds used in the experiment came from plants from the collection of the discipline of Floriculture (*G. byzantinus* and *G. imbricatus*) and from trade (*G. tristis*) (Figure 1).



a. b.

c.

Figure 1. Gladiolus seeds: a. *G. byzantinus*; b. *G. imbricatus*; c. *G. tristis*

The experiments were bifactorial, the experimental factors being represented by the stratification period, with five graduations (44, 58, 74, 86 and 98 days) and the germination temperature, with three graduations (20-22°C, 17-19°C and 13-14°C), resulting in 15 experimental variants (Table 1).

Table 1. The experimental scheme

Variant	Specification (no. days of stratification/ t^0C germination)
V_1	44 / 20-22
V_2	44 / 17-19
V_3	44 / 13-14
V_4	58 / 20-22
V_5	58 / 17-19
V_6	58 / 13-14
V_7	74 / 20-22
V_8	74 / 17-19
V_9	74 / 13-14
V_{10}	86 / 20-22
V_{11}	86 / 17-19
V_{12}	86 / 13-14
V_{13}	98 / 20-22
V_{14}	98 / 17-19
V_{15}	98 / 13-14

The seeds were stratified at a temperature of 4 - 6°C, starting on 11.10.2020, for different periods, according to the experimental scheme.
At the end of each stratification period, the seeds were placed in Petri dishes on a layer of filter paper moistened with 10 mL of distilled water, then placed in the germination chamber at the three temperature levels: 13-14°C, 17-19°C and 20-22°C (Figure 2).

a. b.

Figure 2. Stratification (a) and seeds germination (b)

The experiments were organized in a randomized blocks design with three repetitions; each repetition contains 100 seeds.
Observations and determinations on seed germination were performed daily, but in the summary tables, the data were entered every two days.
The dynamics of germination was determined from the beginning of germination. The total percentage of germinated seeds corresponded to the moment when, at 2-3 consecutive determinations, the same percentage of germination was obtained.
Germination rate is the percentage of seeds that have germinated overnight. Based on the individual rates, the average germination rate was calculated for each variant.
Germination rate (V_G) is the percentage of seeds germinated at a given date and was calculated according to the formula:

$$V_G = \frac{Gi}{n} (1), \text{ where:}$$

G_i = germination at a certain date;
n = number of days in which G_i germination was performed.
Germination rate (CV_G) is the rate of germination relative to the final germination of the seed and was calculated according to the formula:

$$C_{VG} = \frac{Gi}{GF \text{ x } n} 100, \text{ where:}$$

Gi and *n* have the same meanings as in the previous formula;
G_f= final germination.

The results of the final percentage of germination were compared to the average of the variants (considered control), and the interpretation was made using the analysis of the variance, with the LSD test (Saulescu and Saulescu, 1967).

RESULTS AND DISCUSSIONS

Gladiolus byzantinus

The observations on *G. byzantinus* (Table 2). indicate that seeds germination was influenced by the stratification period and less by the germination temperature. Seeds that were stratified 44 days had the lowest germination percentage, between 16 (V_1 and V_3) and 20% (V_2). With the increase of the stratification period, the percentage of germinated seeds also increased, so that at a stratification period of 58 days the percentage of germinated seeds reached 55% (V_4), 56% (V_5) and 51% (V_6). In the case of variants with stratified seeds 74 days (V_7, $V_8$and V_9), the percentage of germinated seeds was 74% higher than in the stratified 44 days and 38% higher than in the stratified 58 days. In variants with stratified seeds 86 and 98 days (V_{10}-V_{14}), the percentage of germinated seeds was over 96% (100% when the germination temperature was maintained at 17-19ºC).

The duration of germination was 11 days, for stratified seeds for 44, 58 and 74days (V_1 - V_9), seven days for stratified seeds for 86 days (V_{10}, V_{11} and V_{12}) and only five days for stratified seeds for 98 days (V_{13}, V_{14} and V_{15}). Differences were also found in the time taken from seed removal to stratification to germination: 12 days for 44 days stratified seeds (V_1-V_3); two days for 74, 86 and 98 days stratified seeds (V_7 - V_{15}); eight days for 58daysstratified seeds (V_4, V_5 and V_4) (Table 2).

Table 2. *G. byzantinus* germination dinamics

Var.	Date of removal from the stratification	Data/germinated seeds (%)																					
		05.12.	07.12.	09.12.	11.12.	13.12.	15.12.	17.12.	19.12.	21.12.	23.12.	25.12.	27.12.	29.12.	31.12.	02.01.	06.01.	08.01.	10.01.	12.01.	20.01.	22.01.	24.01.
V_1		12	12	14	14	14	16																
V_2	23.11.20	10	14	16	18	18	20																
V_3		8	12	12	14	14	16																
V_4							12	22	29	39	49	55											
V_5	07.12.20						10	19	28	38	51	56											
V_6							8	21	29	40	48	51											
V_7											10	24	54	78	84	90							
V_8	21.12.20										12	26	50	60	87	94							
V_9											14	20	50	64	83	86							
V_{10}																	10	18	82	96			
V_{11}	04.01.21																32	50	94	100			
V_{12}																	32	50	88	96			
V_{13}																					10	82	98
V_{14}	18.01.21																				32	94	100
V_{15}																					32	88	96

The dynamics of the germination rate in *G. byzantinus* (Table 3) indicate certain variations determined, in particular, by the duration of the stratification. Stratification for 44 days led to maximum values of the rate of emergence in the first day, respectively 12% in variants V_1, 10% in V_2 and 8% in V_3; towards the end of the period, regardless of the temperature, the germination rate was identical (2%). The 58 days stratified seeds had a relatively constant germination rate during the first nine days, and on the eleventh day the lowest rate (5-7%). The 74 days stratified seed variants (V_7-V_9) had high germination rates in the first days, with maximum values on the sixth day after germination, after which they decreased. Long-term stratification (86 and 98 days) resulted in a higher germination rate, indicating faster and more uniform germination. The maximum values were recorded on the fifth day from the beginning of germination on variants V_{10}-V_{12} and on the third day on variants V_{13}-V_{15}. The highest germination rate (72%) had the seeds of variant V_{13}, after two days from the beginning of germination and four days after they were removed from the stratification. In the case of these variants, in which the total percentage of germinated seeds was over 96%, on the last day of determinations, the germination rate dropped sharply to low values (6-16%).

Table 3. *G. Byzantinus* germination rate dynamics

Var.	Data/germination rate dynamics (%)																						Average
	05.12.	07.12.	09.12.	11.12.	13.12.	15.12.	17.12.	19.12.	21.12.	23.12.	25.12.	27.12.	29.12.	31.12.	02.01.	06.01.	08.01.	10.01.	12.01.	20.01.	22.01.	24.01.	
V_1	12	0	2	0	0	2																	2.7
V_2	10	4	2	2	0	2																	3.3
V_3	8	4	0	2	0	2																	2.7
V_4						12	10	7	10	10	6												9.2
V_5						10	9	9	10	13	5												9.3
V_6						8	13	8	11	8	3												8.5
V_7										10	14	30	24	6	6								15.0
V_8										12	14	24	10	27	7								15.7
V_9										14	6	30	14	19	3								14.3
V_{10}																10	8	64	14				24.0
V_{11}																32	18	44	6				25.0
V_{12}																32	18	38	8				24.0
V_{13}																				10	72	16	32.7
V_{14}																				32	62	6	33.3
V_{15}																				32	56	8	32.0

Germination rate and seed rate coefficient was analyzed in Table 4. The rate was different from one variant to another, as well as during the germination period.

In variants V_1-V_3, with stratified seeds 44 days, it is found that the highest values of velocity and coefficient of velocity were recorded at the first observations since the onset of germination, when the velocity was 4-4.7%, and the coefficient of velocity was between 23.3-25%. In the following days, the values were constantly decreasing, to values of 1.5-1.8% of the velocity and 9.1% of the coefficient of velocity. Stratification for 58 days (V_4-V_6) resulted in an increase in velocity, which was 6.3-7.3% at the beginning of germination and towards the end of the period 4.6-5.1%. And the coefficient of velocity was maintained at higher values, between 45.8% and 25.5%. Unlike the other variants, the stratification for 74days (V_7-V_9) was highlighted by a relatively constant level of velocity and coefficient of velocity, which oscillated, in most cases, between values of approx. 9-11%. The evolution of the two indicators in the case of 89 days stratified seeds (V_{10}-V_{13}) was characterized both by larger differences due to germination temperature and by day-to-day fluctuations, with alternative maxima and minima.

Thus, germination at temperatures above 20°C was slower in the first days, and the maximum velocity and coefficient of velocity were recorded on the fifth day. Instead, the temperature below 20°C led to lower fluctuations in velocity and coefficient of velocity but also with maximums on the fifth day. For the stratified variants 98 days, in the five days that the germination lasted, the analyzed indicators were at the maximum level on the third day (velocity between 20.7-29.3%, and the coefficient of velocity between 27.9-31.3%). On the last day, when germination is completed (96-100%), the velocity and the coefficient of velocity had very small variations (19.2-20%).

Table 4. *G. byzantinus* germination velocity and coefficient of velocity dynamics

Var.	V/Cv	Data/ velocity/coefficient of velocity dynamics																						Average
		05.12.	07.12.	09.12.	11.12.	13.12.	15.12.	17.12.	19.12.	21.12.	23.12.	25.12.	27.12.	29.12.	31.12.	02.01.	06.01.	08.01.	10.01.	12.01.	20.01.	22.01.	24.01.	
V_1	V	0.0	4.0	2.8	2.0	1.6	1.5																	2.4
	Cv	0.0	25.0	17.5	12.5	9.7	9.1																	14.8
V_2	V	0.0	4.7	3.2	2.6	2.0	1.8																	2.9
	Cv	0.0	23.3	16.0	12.9	10.0	9.1																	14.3
V_3	V	0.0	4.0	2.4	2.0	1.6	1.5																	2.3
	Cv	0.0	25.0	15.0	12.5	9.7	9.1																	14.3
V_4	V						0.0	7.3	5.8	5.6	5.4	5.0												5.8
	Cv						0.0	45.8	36.3	34.8	34.0	31.3												36.4
V_5	V						0.0	6.3	5.6	5.4	5.7	5.1												5.6
	Cv						0.0	31.7	28.0	27.1	28.3	25.5												28.1
V_6	V						0.0	7.0	5.8	5.7	5.3	4.6												5.7
	Cv						0.0	43.8	36.3	35.7	33.3	29.0												35.6
V_7	V										0.0	8.0	10.8	11.1	9.3	8.2								9.5
	Cv										0.0	8.9	12.0	12.4	10.4	9.1								10.6
V_8	V										0.0	8.7	10.0	8.6	9.7	8.5								9.1
	Cv										0.0	9.2	10.6	9.1	10.3	9.1								9.7
V_9	V										0.0	6.7	10.0	9.1	9.2	7.8								8.6
	Cv										0.0	7.8	11.6	10.6	10.7	9.1								10.0
V_{10}	V																0.0	6.0	16.4	13.7				12.0
	Cv																0.0	6.3	17.1	14.3				12.6
V_{11}	V																0.0	16.7	18.8	14.3				16.6
	Cv																0.0	16.7	18.8	14.3				16.6
V_{12}	V																0.0	16.7	17.6	13.7				16.0
	Cv																0.0	17.4	18.3	14.3				16.7
V_{13}	V																				0.0	27.3	19.6	23.5
	Cv																				0.0	27.9	20.0	24.0
V_{14}	V																				0.0	20.7	20.0	20.4
	Cv																				0.0	31.3	20.0	25.7
V_{15}	V																				0.0	29.3	19.2	24.3
	Cv																				0.0	30.6	20.0	25.3

Gladiolus imbricatus
The results obtained for this species have shown that the duration of statification has an important role in seed germination. According to the literature, the germination of *G. imbricatus* seeds takes place in cool and humid spring conditions, after periods of warm and cold stratification (Clothier, 2003; Kostrakiewicz-Gierałt, 2017).

Table 5. *G. imbricatus* germination dinamics

VAR.	Date of removal from the stratification	Data/germinated seeds (%)									
		07.12.	21.12.	04.01.	18.01.	01.02.	25.01.	27.01.	29.01.	31.01.	02.02.
V_1		0.0	0.0	0.0	0.0	0.0					
V_2	23.11.2020	0.0	0.0	0.0	0.0	0.0					
V_3		0.0	0.0	0.0	0.0	0.0					
V_4		0.0	0.0	0.0	0.0	0.0					
V_5	07.12.2020	0.0	0.0	0.0	0.0	0.0					
V_6		0.0	0.0	0.0	0.0	0.0					
V_7		0.0	0.0	0.0	0.0	0.0					
V_8	21.12.2020	0.0	0.0	0.0	0.0	0.0					
V_9		0.0	0.0	0.0	0.0	0.0					
V_{10}		0.0	0.0	0.0	0.0	0.0					
V_{11}	04.01.2021	0.0	0.0	0.0	0.0	0.0					
V_{12}		0.0	0.0	0.0	0.0	0.0					
V_{13}							6.0	20.0	27.0	34.0	40.0
V_{14}	18.01.2021							9.0	22.0	34.0	-
V_{15}								8.0	19.0	25.0	32.0

In our experience, the 44, 58, 74, and 86 days stratified seeds (V_1-V_{12}) did not germinate, regardless of the germination temperature to which they were exposed (Table 5). The 98 days statified seeds (V_{13}, V_{14} and V_{15}) germinated in a proportion of 32-40%, the maximum germination being obtained at temperatures above 20ºC (V_{13}), and the minimum at 13-14ºC, the temperature level directly influencing proportionally the percentage of germinated seeds. After removal from stratification, seed germination was triggered earlier (at seven days) by exposure to temperatures of 20-22°C, while at temperatures below 20°C (V_{14}-V_{15}), germination began after nine days (Table 5).

Table 6. *G. imbricatus* germination rate dynamics

VAR	Data/Germination rate dynamics (%)										Average
	07.12.	21.12.	04.01.	18.01.	01.02.	25.01.	27.01.	29.01.	31.01.	02.02.	
V_1	0.0	0.0	0.0	0.0	0.0						0
V_2	0.0	0.0	0.0	0.0	0.0						0
V_3	0.0	0.0	0.0	0.0	0.0						0
V_4	0.0	0.0	0.0	0.0	0.0						0
V_5	0.0	0.0	0.0	0.0	0.0						0
V_6	0.0	0.0	0.0	0.0	0.0						0
V_7	0.0	0.0	0.0	0.0	0.0						0
V_8	0.0	0.0	0.0	0.0	0.0						0
V_9	0.0	0.0	0.0	0.0	0.0						0
V_{10}	0.0	0.0	0.0	0.0	0.0						0
V_{11}	0.0	0.0	0.0	0.0	0.0						0
V_{12}	0.0	0.0	0.0	0.0	0.0						0
V_{13}						6.0	14.0	7.0	7.0	6.0	8.0
V_{14}							9.0	13.0	12.0		11.3
V_{15}							8.0	11.0	6.0	7.0	8.0

The highest germination rate was recorded nine days after interruption of stratification and germination of V_{13} seedlings and 11 days for V_{14} and V_{15} seedlings (Table 6). It is also observed that the temperature of 17-19°C shortened the germination period correlated with a higher average germination rate (14.7%) compared to the other two thermal levels, at which the average rate was 8.0% (Table 6). Germination velocity and coefficient of velocity (Table 7) were favorably influenced by more moderate germination temperatures below 20ºC, although the highest number of germinated seeds was 20-22ºC. The average values of the two indicators were higher at V_{14}, with the germination temperature of 17-19ºC, followed by V_{15} (13-14ºC) and, lastly, V_{13} (20-22ºC).

Table 7. *G. imbricatus* germination velocity and coefficient of velocity dynamics

VAR.	V/ Cv	Data/velocity/coeff. of velocity 07.12.	21.12.	04.01.	18.01.	01.02.	25.01.	27.01.	29.01.	31.01.	02.02.	Average V/Cv
V_1	V	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
	Cv	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
V_2	V	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
	Cv	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
V_3	V	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
	Cv	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
V_4	V	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_5	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_6	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_7	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_8	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_9	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_{10}	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_{11}	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_{12}	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_{13}	V						0.0	6.7	5.4	4.9	4.4	5.3
	Cv						0.0	16.7	13.5	12.1	11.1	13.3
V_{14}	V							0.0	7.3	6.8		7.05
	Cv							0.0	21.6	20.0		20.8
V_{15}	V							0.0	6.3	5.0	4.6	5.6
	Cv							0.0	19.8	15.6	14.3	16.5

Gladiolus tristis

From the analysis of the seed germination dynamics in *G. tristis* (Table 8), it is observed that the major influence on this process had the germination temperature, given that, regardless of the stratification period, at temperatures of 20-22°C, the seeds did not germinate. In the other two temperature variants, the final germination was between 78-86%. During the same stratification period, the best germination was recorded at 13-14ºC, respectively 2-4% more than at 17-19ºC. It can also be seen that the temperature of 13-14ºC caused a reduction time in germination time, especially at long layering periods (V_{12} and V_{15}).

The results obtained are similar to those reported by González and Lopez (2005), who stated that the optimal temperatures for seed germination in *G. tristis* var. *tristis* and *G. tristis* var. *concolor* are between 13-15°C.

Table 8. *G. tristis* germination dinamics

Var.	Date of removal from the stratification	Data / germinated seeds (%)																										
		02.12.	04.12.	06.12.	08.12.	10.12.	12.12.	16.12.	18.12.	20.12.	22.12.	24.12.	26.12.	28.12.	30.12.	02.01.	04.01.	06.01.	06.01.	08.01.	11.01.	13.01.	15.01.	17.01.	24.01.	26.01.	28.01.	30.01.
V_1		0	0	0	0	0	0																					
V_2	23.11.2020	8	24	55	58	71	79																					
V_3			15	33	55	68	81																					
V_4								0	0	0	0	0	0															
V_5	07.12.2020							10	26	54	60	70	82															
V_6									16	30	56	60	68	86														
V_7															0	0	0	0	0									
V_8	21.12.2020													10	26	54	60	70	78									
V_9															16	30	56	60	70	82								
V_{10}																					0	0	0					
V_{11}	04.01.2021																				10	30	70	80				
V_{12}																						20	40	82				
V_{13}																									0	0	0	0
V_{14}	18.01.2021																								10	26	66	80
V_{15}																										20	46	84

The germination rate differed between variants, but also within the same variant, during the germination (Table 9). In the studied variants, the maximum rate was reported on the fifth day after germination. Comparing the average germination rates from the variants with the same layering time, it is found that the values were higher at 13-15°C. Stratification for 86 and 98 days increased the average germination rate to 20-28%(Table 9).

Table 9. *G. tristis* germination rate dynamics

Var.	Data/ germination rate dynamics (%)																											Average
	02.12.	04.12.	06.12.	08.12.	10.12.	12.12.	16.12.	18.12.	20.12.	22.12.	24.12.	26.12.	28.12.	30.12.	02.01.	04.01.	06.01.	06.01.	08.01.	11.01.	13.01.	15.01.	17.01.	24.01.	26.01.	28.01.	30.01.	
V_1	0	0	0	0	0	0																						0.0
V_2	10	16	31	3	13	8																						13.5
V_3		16	18	22	13	13																						16.4
V_4							0	0	0	0	0	0	0															0.0
V_5							10	16	28	6	10	12																13.7
V_6								16	14	26	4	8	18															14.3
V_7														0	0	0	0	0										0.0
V_8													10	16	28	6	10	8										13.0
V_9														16	14	26	4	10	12									13.7
V_{10}																				0	0	0						0.0
V_{11}																				10	20	40	10					20.0
V_{12}																					20	20	42					27.3
V_{13}																								0	0	0	0	0.0
V_{14}																								10	16	40	14	20.0
V_{15}																									20	26	38	28.0

The rate of germination highlights the way in which the experimental factors analyzed ensure the germination of a certain number of seeds at a given time. The experimental data presented showed that the highest germination rate of the variants was recorded on the fifth day after the onset of germination (Table 10). In the variants with stratified seeds 44, 58 and 76 days (V_2, V_3, V_5, V_6, V_8, V_9), characterized by longer germination time (9-11 days), after this peak speed, the values were decreasing towards the end of the period. The variants with stratified seeds 86 and 98 days and which were germinated at 13-15°C (V_{12} and V_{15}) had the maximum velocity at the last determinations, respectively on the fifth day. Of all the variants, the highest germination rate (16.4-16.8%) had the stratified seeds 86 and 98 days, germinated at 13-15°C, and the lowest (7.2%) the seeds from variant V_2, stratified 44 days and germinated at 17-19°C.

The coefficient of velocity varied between 8.8-20% and recorded the highest values (20%) in the conditions in which the stratification was 86---98 days, and germination temperatures of 13-15°C (Table 10).

Table 10. *G. tristis* germination velocity and coefficient of velocity dynamics

Var.	V/ Cv	Data/velocity/coefficient of velocity																											Average
		02.12.	04.12.	06.12.	08.12.	10.12.	12.12.	16.12.	18.12.	20.12.	22.12.	24.12.	26.12.	28.12.	30.12.	02.01.	04.01.	06.01.	06.01.	08.01.	11.01.	13.01.	15.01.	17.01.	24.01.	26.01.	28.01.	30.01.	
V_1	V	0	0	0	0	0	0																						0
	Cv	0	0	0	0	0	0																						0
V_2	V	0	8	11	8.3	7.9	7.2																						7.07
	Cv	0	9.8	13.4	10.1	9.6	8.8																						8.62
V_3	V		15	11	11	9.7	9																						11.1
	Cv		17.4	12.8	12.8	11.3	10.5																						13
V_4	V							0	0	0	0	0	0																0
	Cv							0	0	0	0	0	0																0
V_5	V							0	8.7	10.8	8.6	8	7.5																7.23
	Cv							0	0	13.2	10.5	10	9.1																7.05
V_6	V								0	10	11.2	9	7.6	7.8															7.53
	Cv								0	11.6	13	10	8.8	9.1															8.75
V_7	V													0	0	0	0	0	0										0
	Cv													0	0	0	0	0	0										0
V_8	V													0	8.7	10.8	8.6	7.8	7.1										7.17
	Cv													0	11.1	13.8	11	10	9.1										9.17
V_9	V														0	10	11.2	8.6	7.8	7.5									7.52
	Cv														0	12	13.7	11	9.5	9.1									9.13
V_{10}	V																				0	0	0	0					0
	Cv																				0	0	0	0					0
V_{11}	V																				0	10	14	11.4					11.8
	Cv																				0	12.5	17.5	14.3					14.7
V_{12}	V																					0	13.3	16.4					14.9
	Cv																					0	16.3	20					18.2
V_{13}	V																								0	0	0	0	0
	Cv																								0	0	0	0	0
V_{14}	V																								0	8.7	13.2	11.4	11.1
	Cv																								0	10.8	16.5	14.3	13.9
V_{15}	V																									0	15.3	16.8	16.1
	Cv																									0	18.3	20	19.2

In order to highlight the extent to which the duration of the stratification and the temperature influenced the total percentage of germinated seeds, the statistical-mathematical processing, respectively the analysis of the variance was used. The evaluation considered both the cumulative action of the two experimental factors and their separate action. The cumulative action (Table 11) indicates a favorable influence, with very significant positive differences compared to the average, on variants V_7-V_{15} from *G. byzantinus* and only on variants V_{13}-V_{15} from *G. imbricatus*. In contrast, in *G. tristis*, the positive influence of the two factors was manifested in all variants, except those in which the germination temperature was maintained at 20-22^{0}C.The comparison of the results from the variants with seeds subjected to the same stratification duration allowed to highlight the individual influence of this experimental factor: 44 days, 58 days, 74, 86 days and 98 days. Negative differences compared to the average were in the stratified variants 44 and 58 days (*G. byzantinus* and *G. imbricatus*) as well as 74 and 86 days (*G. imbricatus*). The positive differences were determined by the stratification of 74 and 86 days (*G. byzantinus*) and 98 days (*G. byzantinus* and *G. imbricatus*). The non significant influence of the stratification duration was highlighted in *G. tristis* (Table12).

Table 11. The cumulative influence of experimental factors (temperature and stratification) on seeds germination

Var.	*G. byzantinus* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	*G. imbricatus* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	*G. tristis* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.
V_1	16	22.4	-55.3	000	0	0	-7.1	000	0	0	-54.3	000
V_2	20	28.1	-51.3	000	0	0	-7.1	000	79	145.5	24.7	xxx
V_3	16	22.4	-55.3	000	0	0	-7.1	000	81	149.2	26.7	xxx
V_4	55	77.1	-16.3	000	0	0	-7.1	000	0	0	-54.3	000
V_5	56	78.5	-15.3	000	0	0	-7.1	000	82	151.0	27.7	xxx
V_6	51	71.5	-20.3	000	0	0	-7.1	000	86	158.4	31.7	xxx
V_7	90	126.2	18.7	xxx	0	0	-7.1	000	0	0	-54.3	000
V_8	94	131.8	22.7	xxx	0	0	-7.1	000	78	143.6	23.7	xxx
V_9	86	120.6	14.7	xxx	0	0	-7.1	000	82	151.0	27.7	xxx
V_{10}	96	134.6	24.7	xxx	0	0	-7.1	000	0	0	-54.3	000
V_{11}	100	140.2	28.7	xxx	0	0	-7.1	000	80	147.3	25.7	xxx
V_{12}	96	134.6	24.7	xxx	0	0	-7.1	000	82	151.0	27.7	xxx
V_{13}	98	137.5	26.7	xxx	40	563.4	32.9	xxx	0	0	-54.3	000
V_{14}	100	140.3	28.7	xxx	34	478.9	26.9	xxx	80	147.3	25.7	xxx
V_{15}	96	134.6	24.7	xxx	32	450.7	24.9	xxx	84	154.7	29.7	xxx
$\bar{x}$	71.3	100.0	0.0	control	7.1	100.0	0.0	control	54.3	100.0	0.0	control
LSD 5% = 1.8; 1% = 2.4; 0.1% = 3.3					LSD 5% = 1.5; 1% = 2.0; 0.1% = 2.6				LSD 5% = 3.1; 1% = 4.1; 0.1% = 5.5			

Table 12. Seeds germination obtained under the influence variation of stratification duration

Stratif. (days)	*G. byzantinus* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	*G. imbricatus* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	*G. tristis* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.
44	17.3	24.3	-54.0	000	0	0	-7.1	000	53.3	98.2	-1.0	ns
58	54.0	75.7	-17.3	000	0	0	-7.1	000	56.0	103.1	1.7	x
74	90.0	126.2	18.7	xxx	0	0	-7.1	000	53.3	98.2	-1.0	ns
86	97.3	136.5	26.0	xxx	0	0	-7.1	000	54.0	99.5	-0.3	ns
98	98.0	137.5	26.7	xxx	35.3	497.2	28.2	xxx	54.7	100.7	0.4	ns
x	71.3	100.0	0.0	control	7.1	100.0	0	control	54.3	100.0	0.0	control
LSD 5% = 1.4; 1% = 2.1; 0.1% = 3.1					LSD 5% = 1.2; 1% = 1.8; 0.1% = 2.7				LSD 5% = 1.6; 1% = 2.4; 0.1% =3.6			

The study of the individual influence of temperature on the total percentage of germinated seeds (Table 13) was performed by grouping the results obtained at variants with the same thermal level: 20-22°C (V_1, V_4, V_7, V_{10}, V_{13}), 17-19°C (V_2, V_5, V_8, V_{11}, V_{14}) and 13-15°C (V_3, V_6, V_9, V_{12}, V_{15}). Compared to the average experience, the differences were non significant in *G. byzantinus* for 20-22°C and in *G. imbricatus* for 17-19°C and 13-15°C. Very significant positive differences were recorded at 17-19°C (*G. byzantinus* and *G. tristis*) and at 13-14^{0}C (*G. tristis*). The negative differences were at 13-14^{0}C (*G. byzantinus*) and at 20-22°C (*G. tristis)*.

Table 13. Seeds germination obtained under the influence variation of temperature

Temp. (^{0}C)	*G. byzantinus*				*G. imbricatus*				*G. tristis*			
	Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.
20-22^{0}C	71	99.6	-0.3	ns	8	112.7	0.9	x	0	0	-54.3	000
17-19^{0}C	74	103.8	2.7	xxx	6.8	95.8	-0.3	ns	79.8	146.9	-25.5	xxx
13-14^{0}C	69	96.8	-2.3	00	6.4	90.1	-0.7	ns	83.0	152.9	28.7	xxx
$\bar{x}$	71.3	100.0	0.0	control	7.1	100.0	0.0	control	54.3	100.0	0.0	control
	LSD 5% =0.8; 1% =1.4; 0.1% =2.6				LSD 5% =0.8; 1% =1.3; 0.1% =2.4				LSD 5%=3.0; 1%=4.9; 0.1%=9.2			

CONCLUSIONS

Germination of *G. byzantinus* seeds is mainly influenced by the duration of stratification and less by the germination temperature. At 74 days of cold stratification, the percentage of germinated seeds increases to over 80%, and at 86 and 98 days, germination reaches 96-100%, simultaneously with a decrease in germination time to 5 days (compared to 11 -14 days for short layering options).

The influence of stratification duration is evident in *G. imbricatus*, germination being triggered only at 98 days of stratification. Better germination is favored by temperatures of 20-22^{0}C.

In *G. tristis*, the factor with major influence on germination was temperature, at 20^{0}C the germination is blocked, regardless of the stratification duration. The higher germination rate and the shorter germination time was obtainedat 13-15^{0}C.

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INFLUENCE OF THE PLANTING SEASON AND CORMS SIZE ON THE *CROCOSMIA*, IN AGROCLIMATIC CONDITIONS OF IASI (NORTHEASTERN ROMANIA)

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Abstract

The genus Crocosmia, from the Iridaceae family, includes the plants with obvious potential both for garden decoration and as a cut flowers. This paper evaluated the influence of the planting times and the size of the corms on the some characters of ornamental interest of Crocosmia plants, in the agroecological conditions from northeastern Romania (Iași). The corms of Crocosmia 'Lucifer' from two size categories (3-5.9 g and 6-9 g) were used, which were planted in the experimental field in November and April.The observations and determinations made during 2018-2021 highlighted the fact that from the corms with a mass between 6-8 g, planted in autumn, they ensured the obtaining of plants with the highest number of flower stems and the most flowers on the stem. Regarding the height of the flower stems, the best results were recorded in corms with a mass between 3-5.9 g, planted in autumn. In the case of corms planted in the spring, the results were less significant, except for the number of shoots in plants resulting from large corms (6-8 g). The flowering period was longer to the plants obtained from corms of 3-5.9 g, planted in autumn.

Key words: *corm size, crocosmia, planting time, morphological characters.*

INTRODUCTION

The family *Iridaceae* (Juss.) is one of the largest families in Monocotyledonatae, being found in the most diverse natural habitats due to the high adaptability of its representatives. It includes over 2000 species distributed in 65-75 genera (Goldblatt et al., 2008). Among the genera belonging to the *Iridaceae* family, the best known in Romania are: *Iris, Gladiolus, Crocosmia, Crocus* and *Freesia*. *Crocosmia* is the genera of about seven species of perennials, identified in the pastures of South Africa (Armitaje, 1993).

Crocosmia is grown mainly for its attractive flowers and ornamental fruits (Armitage & Laushman, 2008), but can also be grown in landscaping or as a potted plant (Filios & Miller, 2010).

Crocosmias are perennial plants that have the corm as an underground organ. The tubular flowers, in shades of yellow, orange and red, numbering four to 20, are grouped in large, slightly arched spikes. They bloom from early summer to September (Brickell, 2011). The fruits are trilobate capsules (5-10 mm long), green to brown. Each plant can produce up to 14 new corms each year. They detach from the mother plant and begin to produce their own network of roots, being very easy to multiply.

In areas with cold winters, cormsshould be harvested in the fall (using the same technology as in sunflowers), stored at 4-9^{0}C and replanted in the spring (Armitaje, 1993).

The results of an experiment, organized in England by Brown, in 1967, show that crocosmia no longer has the same ornamental value when left in the same place for more than three years, so it is recommended to plant it annually or replant every 2-3 years (cited by Armitage &Laushman, 1990).

In Europe, research into the crocosmia crops period was conducted in Greece, near Athens, at the 'James Coey' cultivar (Armitage & Laushman, 1990). The results showed that the corms planted in January had a flowering period of 30 days, five and seven days shorter than for the plants in the corms planted in February and March. Armitage and Laushman (1990) also showed that delayed planting time resulted in a smaller number and lower quality of inflorescences. Plants from corms planted in January produced 1.3 inflorescences / plant, with a length of 25.7 cm, and the average for plants resulting from corms planted in February and March had 0.8 inflorescences with a length of 20.1 cm. Contradictory results were obtained by Żurawik et al., (2015), who planted the crocosmia cormson different dates (April 15, May 5, May 25), for staggering flowering period in three cultivars of *Crocosmia* ('Emily McKenzie', 'Lucifer' and 'Mars'). The longest flowering period was obtained in the plants resulting from the cormsplanted in March, in all three cultivars, this can be explained by the fact that crocosmia is a thermophilic plant and higher summer temperatures favor its growth (Goldblatt et al., 2004). Regardless of the cultivar, the corms planted on May 5 produced plants with the longest flower stems and the largest number of flowers.

At the *Tritonia crocata*, cultivated in Egypt, the effect of cold storage of corms and zinc foliar fertilization on the growth, flowering, productivity of corms and the composition of some chemical constituents was studied. The results indicated that all cold storage treatments tended to induce a constant and significant growth of flower, leaves and stems, increase in number of flowers, early flowering and increase the vase life of cut flowers, as well as corms production (El-Bably & Mahmoud, 2005).

The results of studies conducted on many species and varieties of geophytes in the family *Iridaceae* indicate that the size and quality of the flower stems and the yield of corms depend on the time of planting and the quality of the material with which the crop is established (Hetman et al., 2007; Ahmad et al., 2011; Zubair et al., 2013).

Thus, in *Gladiolus hybridus*, studies were carried out on varieties grown in different ecological conditions, in order to establish optimal variants of the size of the corms used in the establishment of crops or applied technologies. The authors report that the best results were obtained in the crops established with large corms, materialized by positive effects on early flowering, number of leaves per plant, height of flower stem, number of flowers/stem, flower size, duration of flowering, preservation of cut flowers, quantity and quality of production of new corms and cormels etc. (Alhajhoj, 2017; Amin et al., 2013; Bhat et al., 2009; Farid et al., 2002; Gowda, 1987; Mazzini-Guedes et al., 2017; Memon et al., 2009; Methela & Islam, 2021); Nafees et al., 2021; Pittu et al., 2017; Singh, 2000; Zaharia et al., 2018).

In *G. byzantinus*, observations and determinations performed in 2020 showed that corms with a mass > 10 g yielded plants with the highest number of shoots, flower stems and flowers. In the variants of the smaller corms, respectively, 7-9 g and <7 g, the results were lower, except for the height of the flower stems. From a phenological point of view, the onset of vegetation, the formation of flower stems, the onset and the duration of flowering were to the advantage of cormswith a mass > 10 g (Amișculesei et al., 2020).

Similar results were obtained for saffron (*Crocus sativus*), in terms of the positive correlations between the size of the corms and the number of flowers, the early and the duration of flowering (De Mastro & Ruta, 1993; Renau-Morata et al., 2012; Özel et al., 2017).

The aim of this study was to determine the effect of planting season and corms size on ornamental value in *Crocosmia* 'Lucifer'.

MATERIALS AND METHODS

This paper evaluates the influence of the planting period and the size of the corms on some ornamental characters of crocosmia plants (*Crocosmia* x *crocosmiiflora*) 'Lucifer', in the agroecological conditions in northeastern Romania (Iași).

Crocosmia 'Lucifer' is characterized by red-orange flowers, arranged 20-30 on flower stem with a height of approx. 40-60 cm.

Cormsof two size categories (3-5.9 g and 6-9 g) were used to set up the experimental crops, which were planted in the Floriculture field of the "Ion Ionescu de la Brad" University of Life Sciences from Iași, in November (2019 and 2020), respectively April (2019, 2020 and 2021).

The combination of the two experimental factors resulted in four experimental variants: V_1 - corms of 6-9 g, planted in autumn; V_2 -corms of 3-5.9 g, planted in autumn; V_3 -cormsof 6-9 g, planted in spring; V_4 - corms of 3-5.9 g, planted in spring.

The experiments were organized in randomized blocks design with three repetitions(20 plants/each repetition). The corms were planted in rows, at a distance of 15 cm between plants per row and 25 cm between rows. Crops established in the fall overwintered in the field without protection.

The observations and determinations made during 2018-2021 focused on: plant height, number of shoots per plant, number of leaves per shoot, number of stems per plant, stem height, number of flowers per plant, number of new corms. The results were compared to the average of the variants (considered control), and the interpretation was made using the analysis of the variance, with the LSD test (Săulescu & Săulescu, 1967).

The average monthly temperatures and the total monthly precipitations, which characterized the meteorological conditions of the experimental period (2018-2021), are presented in Figure 1 and Figure 2.

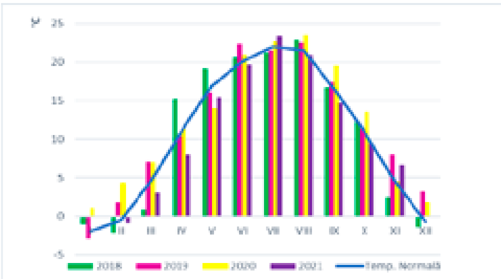

Figure 1. Average monthly temperatures (^{0}C)

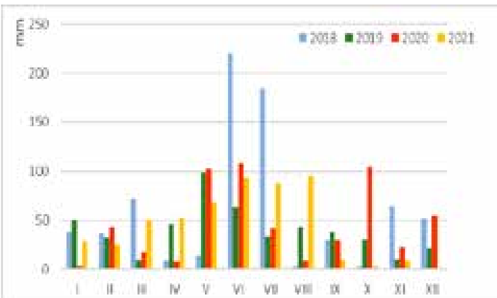

Figure 2. Total monthly rainfall (mm)

The average monthly temperatures recorded were higher in 2020, with large differences in January (1.1^{0}C; 3^{0}C higher than in 2019) and in February (4.3^{0}C; 2.5^{0}C higher than in 2019). The average annual temperature in 2020 was about 1.5^{0}C higher than in 2018 and 0.5^{0}C compared to 2019. Also, the precipitation regime varied during the analyzed period, especially in April and June of 2018 and 2020. The wettest year was 2018 (727.8 mm total annual), and the driest 2019 (478.7 mm total annual).

RESULTS AND DISCUSSIONS

The recording of data on the main phenophases was one of the objectives of the study and aimed to identify how crocosmia plants behave in experimental crops during the growing season. Phenological data refer to the onset of vegetation, the appearance of flowerstems, the beginning and end of flowering (Table 1). Phenology issues are also shown in Figure 4. Table 1 and Figure 4 include phenological data differentiated only by planting time and year of establishment of the crops, without taking into account the size of the corms, given that according to this criterion the differences they were very small and of no practical significance. The onset of vegetation was characterized by differences marked by both the planting season and the size of the corms. From table 1 it can be seen that in the crops established in the spring, the cormsstarted to grow three to six weeks later. The share of corms that started growing in vegetation was dependent not only on the time of planting, but also on the size of the corms (Figure 3). Thus, the corms with a mass of 3-5.9 g, planted in autumn (V_2), started in vegetation in a proportion of 94.57%, substantially surpassing all other variants. However, planted in the spring, the cormsof the same size category (V_4) had the lowest percentage of start in the vegetation (42.11%). The corms with a mass of 6-9 g, regardless of the planting season (V_1 and V_3), had approximately the same starting capacity in the vegetation, with slightly higher values when planted in autumn (V_1).

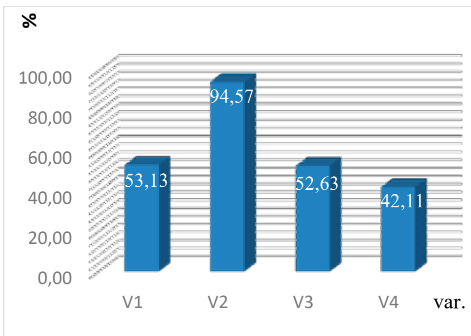

Figure 3. The corms started in vegetation (%)

Table 1. The main phenological data

The time of establishment	Date of establishment of the experiment	Starting in the vegetation	The appearance of flower stems	The beginning of flowering	End of flowering
Autumn	14.11.2020	23.04.2021	22.06.2021	07.07.2021	12 09.2021
	16.11.2019	08.04.2020	02.07.2020	08.07.2020	02.09.2020
Spring	24.04.2021	21.05.2021	21.07.2021	11.08.2021	25.10.2021
	06.04.2020	15.05.2020	11.07.2020	25.07.2020	17.09.2020
	02.04.2019	13.05.2019	07.07.2019	22.07.2019	15.09.2019

Also, from the analysis of the other phenological data (Table 1) we can highlight the differences generated by the planting season.

In spring crops, the late onset of vegetation led to delays in the emergence of flowerstems (with a maximum of 4 weeks in 2021) and the onset of flowering (with variations ranging from 17 days in 2020 to 35 days in 2021).

Flowering ended in the first half of September, about a week later in spring crops (Table 1).

The phenological diagram (Figure 4) better highlights the differences reported between the times to cultivate of the establishment of crocosmia cultures. The stage of full flowering, which characterizes the maximum period of decoration of the plants, had a shorter duration in the crops established in the spring. The exception was the year 2021, when the flowering lasted by approx. one week, probably due to a month's delay in flowering compared to the autumn crops.

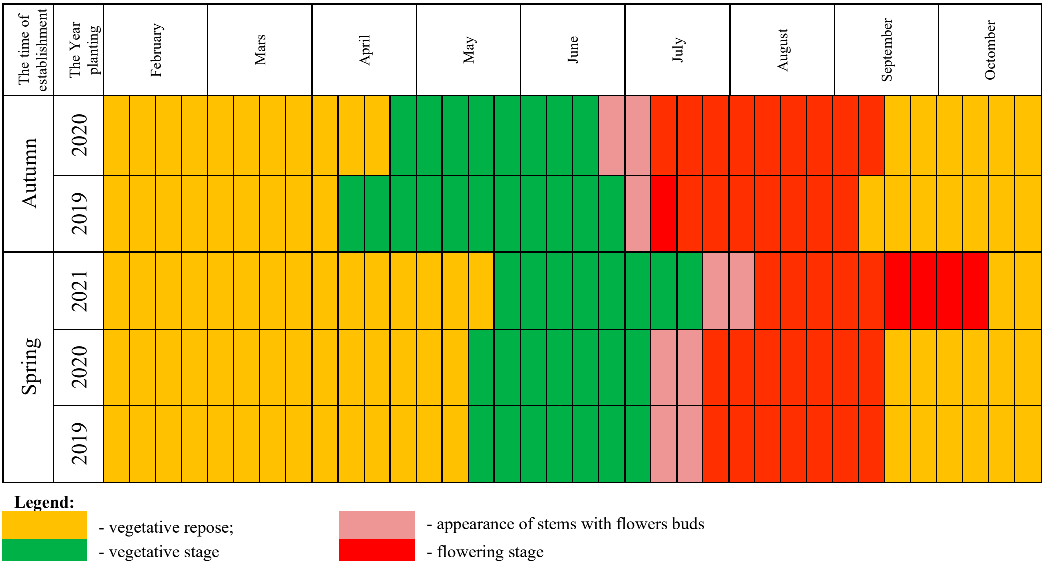

Figure 4. The phenological diagram of *Crocosmia* 'Lucifer'

The passage of each phenophase, from one stage to another, required acertain duration, with differences between the two or three years of vegetation.

In the crops established in autumn (Figure 5), from the start of vegetation until the appearance of flower stems were required 25 days more in 2020 compared to 2021, a difference that can be justified by the start of vegetation 15 days earlier in 2020, against the background of relatively high temperatures from January-March (with 3-4^{0}C above the normal area), followed by less favorable conditions: dry April (8.4 mm total precipitations), and May and June with excess humidity (102-108 mm total rainfall).

The extension of this period led to the shortening of the time until the beginning of flowering by nine days compared to 2021 (Table 1), so that, calendarically, in the two years, the opening of the first flowers took place on the same date (July 7-8). Flowering in autumn crops took place from the beginning of July until the first decade of September, but lasted 10 days longer in 2021 (Table 1, Figure 5), when July and August were characterized by a higher rainfall (88 mm in July and 95 mm in August), as opposed to 2020 (42 mm in July and 9.2 mm in August) (Figure 2).

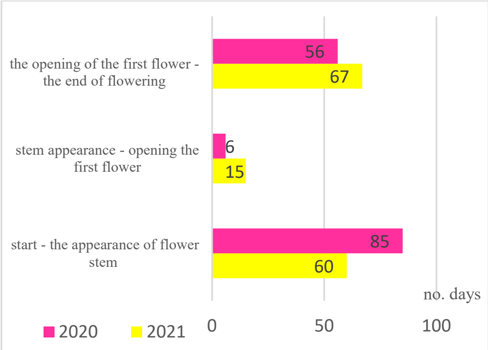

Figure 5. Duration of the main phenophases in the crops established in autumn (no. days)

In the case of plants from crops established in spring (Figure 5), it is found that the year 2021 is characterized by a longer duration of phenophases and a delay in their onset, compared to the other two years analyzed (2019 and 2020).

The gap recorded in 2021, may be the consequence of the establishment of crops with approx. three weeks later than in previous years, due to less favorable weather conditions (cold and rainy spring) (Figure 1, Figure 2).

In 2021, the latest flowering was recorded (August 11), both compared to the crops established in autumn and compared to those in the spring of 2020 and 2019 (Table 1, Figure 6).

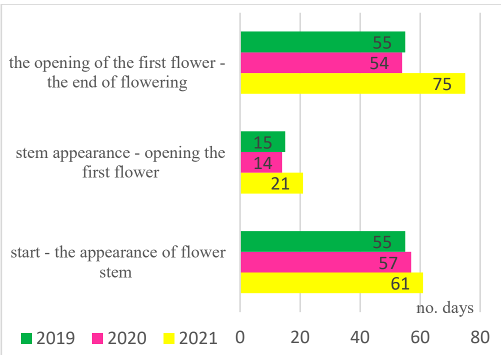

Figure 6. Duration of the main phenophases in the crops established in spring (no. days)

The morphological characteristics of the plants from the four experimental variants highlight both the aspects related to vegetative growth and flowering.

The *length of the leaves* varied from 66.5 cm (V_2) to 31.5 cm (V_4), the higher values being in the variants established in autumn, with very significant positive differences compared to the average (Table 2). Regarding the size of the corms, those of 3-5.9 g favored the growth in height of the leaves only in the conditions of the establishment of the autumn crops; instead, planted in the spring, formed the shortest leaves (31.5 cm).

Table 2. The length of the leaves

Var.	Absolute value (cm)	% from $\bar{x}$	Diff. from $\bar{x}$	Signif.
V_1	54.2	110.16	5	xxx
V_2	66.5	135.16	17.3	xxx
V_3	44.6	90.65	-4.6	000
V_4	31.5	64.02	-17.7	000
$\bar{x}$	49.2	100.0	0	control

LSD 5% =0.6 LSD 1%= 0.9 LSD 0.1%= 1.3

The *number of shoots per plant* had similar values to the variants established in autumn (2.2-2.6 shoots), regardless of the size of the corms, the differences from the average being insignificant (Table 3). In the case of plants planted in the spring, the size of the corms caused greater differences in the ability to germinate. From the corms of 6-9 g, formed three shoots/ plant, 25% more than the control (distinctly significant positive differences). The smaller corms (3-5.9 g) formed plants with the smallest number of shoots (1.8 shoots/plant).

Table 3. Number of shoots/plant

Var.	Absolute value	% from $\bar{x}$	Diff. from $\bar{x}$	Signif.
V_1	2.2	91.67	-0.2	ns
V_2	2.6	108.33	0.2	ns
V_3	3.0	125.0	0.6	xx
V_4	1.8	75.0	-0.6	00
$\bar{x}$	2.4	100.0	0	control

LSD 5% =0.3 LSD 1%= 0.4 LSD 0,1%= 0.6

The *number of leaves per shoot* was another feature analyzed to assess the vegetative growth of the crocosmia plants (Table 4).

Table 4. Number of leaves/shoot

Var.	Absolute value	% from $\bar{x}$	Diff. from $\bar{x}$	Signif.
V_1	6.2	98.41	-0.1	ns
V_2	6.8	107.94	0.5	x
V_3	6.2	98.41	-0.1	ns
V_4	6.0	95.24	-0.3	ns
$\bar{x}$	6.3	100.0	0	control
LSD 5% =0.5	LSD 1%= 0.7	LSD 0.1%= 1.1		

The values of the variants were close to the control (Table 4), andhavingthe non significant differences, except for the variant V_2, established in autumn with corms of 3-5.9 g, which recorded above average values and statistically assured differences (significantly positive).

The ornamental value of the plants was highlighted by the data on the number of flower stems/plant, the height of the flower stems (total and up to the level of the branches) and the number of flowers/stem.

The *number of flower stemsper plant* (Table 5) was favored by the establishment of autumn crops (V_1 and V_2) when, regardless of the size of the corms, the plants formed an equal number of flower stems (2.7), with values above that of the control (4), but with non significant differences. The influence of the corms size used for planting was evident in the variants established in the spring. The small corms, weighing 3-5.9 g, had the lowest capacity to form flower stems (1.8 stems/plant), and the differences from the control were significantly negative. Also, from the observations made, it was found that all the corms, regardless of season and size, formed flower stems.

Table 5. Number of flower stems/plant

Var.	Absolute value	% from $\bar{x}$	Diff. from $\bar{x}$	Signif.
V_1	2.7	112.5	0.3	ns
V_2	2.7	112.5	0.3	ns
V_3	2.4	100.0	0	ns
V_4	1.8	75.0	-0.6	0
$\bar{x}$	2.4	100.0	0	control
LSD 5% =0.4	LSD 1%= 0.6	LSD 0.1%= 0.9		

The *total height of the flower stems* (measured from the ground to the top of the main inflorescence) was strongly influenced by the time of establishment of the crops (Table 6). If the size of the cormsplanted in the autumn(V_1 and V_2) had a small influence on the increase in height of the flower stems, the spring season caused larger differences, to the detriment of the corms of 3-5.9 g (V_4). From the statistical analysis, there were differences compared to the control very significantly positive in the variants which were planted in autumn and negative in those with the planting season in spring (very significant in V_4 and distinctly significant in V_3).

Table 6. Total height of flower stems

Var	Absolute value (cm)	% from $\bar{x}$	Diff. from $\bar{x}$	Signif.
V_1	65.2	112.61	7.3	xxx
V_2	66.6	115.03	8.7	xxx
V_3	56.4	97.41	-1.5	00
V_4	43.4	74.96	-14.5	000
$\bar{x}$	57.9	100.0	0	control
LSD 5% =0.9	LSD 1%= 1.2	LSD 0.1%= 1.9		

The *height of the flower stems*up to the level of the branches (measured from the ground level to the insertion point of each branch) gives indications on the architecture of the flower stem and the possibility of separating the main stem from the branches, for use as a cut flower. In addition, the degree of branching of the flower stems can be highlighted.

The *height up to the first branch* varied from 32 cm (V_2) to 19.2 cm (V_4) and was influenced by both the time of establishment of the crops and the size of the corms(Table 7). Very significant positive differences were obtained in corms with a mass of 3-5.9 g, planted in autumn (V_2) and in corms with a mass of 6-9 g, planted in spring (V_3). In plants resulting from small corms, planted in spring (V_4), the first branch was formed close to the ground, at a height of approx. 19 cm.

The *height of the flower stem up to the second branch* was between 43.6-23.8 cm and registered very significant positive differences in the variants established in autumn and in the one established in spring, with large corms(Table 7). From the small corms, planted in the spring, the flower stemsformed the second branch at a low height (23.8 cm).

Regarding the *third branch*, it is observed that it was formed only in the plants from the variants established in autumn. The high height above ground level (45-50 cm) indicates the proximity to the top of the main stem, respectively at approx. 15-20 cm (Table 7).

Table 7. The height of the flower stems to the level of the branches

Variants	To the branch I			To the branch II			To the branch III		
	Absolute values (cm)	% from $\bar{x}$	Diff./ signif.	Absolute values (cm)	% from $\bar{x}$	Diff./ Signif.	Absolute values (cm)	% from $\bar{x}$	Diff./ signif.
V_1	29.8	103.11	0.9^{x}	39.0	105.12	1.9^{xxx}	44.9	187.08	20.9^{xxx}
V_2	32.0	110.73	3.1^{xxx}	42.0	113.21	4.9^{xxx}	51.1	212.92	27.1^{xxx}
V_3	34.6	119.72	5.7^{xxx}	43.6	117.52	6.5^{xxx}	0	0	-24^{000}
V_4	19.2	66.44	-9.7^{000}	23.8	64.15	-13.3^{000}	0	0	-24^{000}
$\bar{x}$	28.9	100.0	control	37.1	100.0	control	24.0	100.0	control
	$LSD_{5\%}$ = 0.7 $LSD_{1\%}$ = 1.1 $LSD_{0.1\%}$ = 1.6			$LSD_{5\%}$ = 0.8 $LSD_{1\%}$ = 1.1 $LSD_{0.1\%}$ = 1.7			$LSD_{5\%}$ = 0.6 $LSD_{1\%}$ = 0.9 $LSD_{0.1\%}$ = 1.4		

The number of flowers on the stem (Table 8) was influenced by the time of the establishment of the crops. The plants from the corms planted in autumn (V_1 and V_2) formed 36.5 and 36.8 flowers/stem, respectively, with 5.3-5.6more than the control.In the plants resulting from the corms planted in spring, the average number of flowers per stem was 28.5 and 23, respectively, the differences from the control being very significant negative.

Table 8. Number of flowers/stem

Var.	Absolute values	% from $\bar{x}$	Diff. from $\bar{x}$	Signif.
V_1	36.5	116.99	5.3	xxx
V_2	36.8	117.95	5.6	xxx
V_3	28.5	91.35	-2.7	000
V_4	23.0	73.72	-8.2	000
$\bar{x}$	31.2	100.0	0.0	control
LSD 5% =0.5	LSD 1%= 0.7		LSD 0.1%= 1.1	

In the Table 9 was determinated the production of corms obtained from experimental crops. Compared to the time of establishment, it can be seen that the autumn plantings reduced the capacity to form new corms, their number being 1.2-1.4. Depending on the size of the corms used to establish the crops, the highest yield was obtained for large corms, 6-9 g.

Table 9. Number of new corms

Var.	Absolute values	% from $\bar{x}$	Diff. from $\bar{x}$	Signif.
V_1	1.4	82.35	-0.3	ns
V_2	1.2	70.59	-0.5	0
V_3	2.3	135.29	0.6	xx
V_4	1.9	111.76	0.2	ns
$\bar{x}$	1.7	100.0	0	control
LSD 5% =0.4	LSD 1%= 0.5		LSD 0.1%= 0.8	

CONCLUSIONS

The size of the corms and the time of planting influenced most of the characters analyzed in the *Crocosmia* 'Lucifer'.

The highest proportion of corms in the vegetation (94.57%) was recorded in corms of 3-5.9 g, planted in autumn.

In the crops established in autumn, the smaller corms positively influenced especially the vegetative growth of the plants (leaf length and number of leaves, number of shoots). In the characters of flowers and inflorescences (number and height of flower stems, number of flowers), the results were approximately similar to those of plants obtained from corms of 6-9 g.

The establishment of crocosmia crops in the spring is justified only with the use of large corms.

Significant differences between planting seasons were recorded in the number of branches of the flower stems. The plants resulting from the corms planted in spring formed only two branches compared to those resulting from the corms planted in autumn which formed three branches on the stem.

The plants resulting from corms planted in autumn were characterized by the early stages of some phenophases (starting in the vegetation, the appearance of the flowerstems, flowering).

Corms with a mass of 6-9 g planted in the spring favored the production of new corms.

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THE INFLUENCE OF THERAPEUTIC HORTICULTURE ACTIVITIES ON QUALITY OF LIFE (QoL) ISSUES

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Abstract

It can be stated that during the last two years, the access and activities involvement in nature, public green areas or gardens were reduced or even suppressed by the crisis caused by the COVID 19 pandemic. However, in many countries' gardens were used in global public health crisis as a refuge to find peace of mind, pleasure, respite or relief. Therapeutic horticulture activities as gardening and people-plant interaction can offer them a meaningful time-spending outside. Several research studies indicates that this type of activities can increase people well-being and can reduce fatigue, attention disorder, anxiety, chronic stress or depression, and these long-term effects are frequently identified after an infection with coronavirus. The aim of present research was to analyse the influence of gardening activities in Quality-of-Life Issues like physiological, safety, social, esteem and self-actualization statements. In this regard, a questionnaire has been adapted including 15 statements to which respondents had to choose an answer from two possible options (affirmative or negative answer). The experimental study was conducted based on 27 people, who were involved in gardening activities for 3 days, 10 days or 6 weeks, depending on the group to which they belonged. The results indicated that all participants `enjoyed working outside`, most of them (96.3%) identified that `gardening is working with nature`. Even if 25 people stated that `the green area is a good place to meet people`, not everyone enjoyed working in group, because 63.0% of the said that they `enjoyed working alone`. Regarding the conducted gardening activities during the three study trials, most of them (96.3%) `wish to have the opportunity to attend regularly public horticultural therapy activities (gardening)`.

***Key words**: gardening; questionnaire; therapeutic horticulture; well-being.*

INTRODUCTION

Nature exposure can reduce the risk of disease due to chronic stress and improve physical and mental well-being (Abu Dabrh et al., 2022; Haller et al., 2019; Simson & Straus, 1997). Green areas improve the quality of social life, providing a suitable environment in which people can optimize their resources and adaptability (Kasey et al., 2021; Hartig et al., 2014).

Gardening activities are recreational, stress-relieving, relaxing, can influence the tryptophan concentration, induce a lower salivary cortisol level, and improve depressive symptoms and well-being (Mourão et al., 2021; Marcus & Sachs, 2013; Horowitz, 2012; Mynt et al., 2007; Sempik et al., 2005).

Trough the modern times, globalization, the powerful development of the constructed urban environments compared to the decrease in land of rural and natural areas, and the social and sanitary crisis caused by COVID-19 pandemic have resulted in the loss of human-nature contact and direct interaction (Sia et al., 2022; Rivas & Biana, 2021; Marsh et al., 2021; Chaudhury & Banerjee, 2020).

Therapeutic horticulture is a relatively new field of research that has required in recent years a scientific approach to the link between people and nature, in the sense of defining concepts and validating objective methods of measuring the physiological and psychological effects of gardening or activities and relaxation in nature on people (Sachs, 2019; Harris, 2017; Kent, 2015; Gonzalez et al., 2010). Also, the human-nature interaction is an important component in relieving symptoms at people with various health conditions and can have positive effects on their well-being and quality of life.

Evidence-based practice has shown that, for example, several Alzheimer's patients feel more comfortable in a garden where the whole landscape is visible, designing green areas without hidden or confusing zones, which can

cause forgetfulness or the feeling of being lost (Furness & Moriarty, 2006; Sempik et al., 2005; Simson & Straus, 1997).
This research can improve the information about gardens as spaces with benefits for health and wellbeing and highlight the positive influence of therapeutic horticulture activities on quality of life (QoL) issues even in public health crisis.

MATERIALS AND METHODS

The present research was an experimental study using pre-test assessed with the Beck Depression Inventory (Beck & Steer, 1987) and post-test survey of the influence of horticultural therapy activities on quality of life (QoL) issues (Buru et al., 2019), conducted among randomly selected volunteer subjects at University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
Starting with June 2019, a total of 27 surveys were engaged in therapeutic horticulture sessions for 3 days (2 man, 7 women), 10 days (1 man, 7 women) and 6 weeks (1 man, 9 women).
The age range of the volunteers involved in the study (4 men, 23 women) was from 19 and 32 years (Mean value - age = 21.48 years) and BDI score between 10 - mild to moderate depression and 25 - moderate to severe depression (Mean value - BDI = 15.07).
The research study was part of a wide `green care and maintenance` program in the university campus, based on the three TH sessions program types 3 days, 10 days and 6 weeks (excepting Saturdays and Sundays). Through these activities, volunteers practiced `plant-related activities through which they strive to improve their well-being through active or passive involvement` (Hitter et al., 2019; Adevi & Mårtensson, 2013; Kam & Siu, 2010; Gonzalez et al., 2009; Sempik et al., 2003) like seeding, planting, weeding and pruning.
At the end of the TH session, the participants were asked to complete the interpretation survey of the TH activity based on Maslow's hierarchy of needs is used (Desmet & Fokkinga, 2020; Maslow, 1943). The form and content of the material were adapted to serve the specific research study conducted, thus analyzing five existential categories: psychological side, security and personal integrity, social component, self-esteem and emotional sphere. Based on the result obtained by original version of this survey (Table 1) which was established using 24 questions (Waliczek et al., 1996), the current study was design by adjusting a form of 15 statements to test quality-of-life factors (Table 2).

Table 1. The influence of horticultural therapy activities on quality of life (QoL) issues survey (Waliczek et al., 1996)

Statement		Racial/ethnic background	Gender	City size	New York vs. Los Angeles
Physiological					
1	I like to work in the soil.	0.020*z	NS	NS	NS
2	I enjoy working outside.	0.000*	NS	0.031*	NS
3	I need the physical exercise.	NS	NS	NS	NS
4	I like the garden colors, smells, beauty.	0.038*	0.025*	NS	0.006*
5	Gardening is working with nature.	0.019*	NS	NS	0.010*
6	I like to work with my hands.	0.000*	NS	NS	
7	I feel healthier when I eat my own produce.	NS	NS	NS	0.016*
Safety					
8	I feel safe in the garden.	0.002*	NS	NS	0.001 *
Social					
9	It's a good place to meet people.	NS	NS	NS	0.018*
10	I enjoy helping others to garden.	0.011*		0.042*	0.000*
11	The gardens beautify my neighborhood.	0.000*	NS	NS	0.000*
12	I can share my produce with others.	0.004*	NS	NS	NS
13	My gardening experience helps others.	0.001*	NS	NS	NS
14	I care for my garden and community.	0.000*	NS	NS	0.034*
15	I enjoy working alone.	NS	NS	0.003*	NS
Esteem					
16	I can produce my own food.	0.000*	NS	0.002*	NS
17	I can create something of beauty.	0.009*	NS	NS	0.003*

18	Gardening makes me feel good about my own abilities.	0.018*	NS	NS	NS
19	My garden food tastes better than store-bought food.	0.003*	NS	NS	0.044*
20	I'm proud of my garden.	0.005*	NS	NS	0.005*
21	I can handle the work needed.	NS	NS	NS	NS
22	I can save money by gardening.	0.021*	0.019*	NS	NS
Self-actualization					
23	My garden gives me a feeling of peace.	NS	NS	NS	NS
24	I can teach my children to garden.	0.003*	NS	NS	NS

Nonsignificant (NS) or significant (*) at $p = 0.05$.

Similar studies were conducted by Marsh et al. (2021) and the obtained results shown that people found respite and other positive benefits for the physical, mental, and emotional challenges of COVID-19 through gardening. Some of respondent who contributed to study told that gardening can appear to be, almost the panacea to COVID- 19 or indeed to other major health crises. The survey structure developed for this research study is based on 15 questions, each with a single answer with Yes / No (affirmative / negative), and the choice of the right answer is influenced by the experience gained during the gardening program through therapeutic horticulture sessions. The estimated time required to complete the questionnaire was within 3-5 minutes, and this may be different depending on the respondents, as they managed to read the questions and choose the relevant answer for them.

Table 2. The influence of horticultural therapy activities on quality of life (QoL) issues survey (Buru et al., 2019)

Statement	Answer
1. I like to work in the soil.	Yes/No
2. I enjoy working outside.	Yes/No
3. I like indoor plants` colors, smells, beauty.	Yes/No
4. Planting is working with nature.	Yes/No
5. I feel safe during the planting time.	Yes/No
6. It's a good experience to meet people.	Yes/No
7. I enjoy helping others in the planting activity.	Yes/No
8. My gardening experience helps others.	Yes/No
9. I care for my plant and community.	Yes/No
10. I enjoy working alone.	Yes/No
11. I can create something of beauty.	Yes/No
12. I'm proud of my potting plant activity.	Yes/No
13. I can handle the work needed.	Yes/No
14. My plant gives me a feeling of peace.	Yes/No
15. I intend to do therapeutic horticulture.	Yes/No

RESULTS AND DISCUSSIONS

The survey was completed in the form of printed material, then the data were processed electronically. The first four questions belong to the category of psychological interpretations, and all respondents agreed in 96.3% with the positive statement of the first question `*1. I like to work in the soil*`.

Out of the total number of participants, only one person chose to apply a negative answer. All answers recorded at the next statement `*2. I enjoy working outside*` were 100% pointed out that the volunteers, during horticultural therapy activities, enjoy working outdoors.

Analyzing the collected date of 27 participants trough this survey, 92.6% of the respondent's state that they identify during the gardening activities the sensory elements of the ornamental plants, and only 2 participants denied this aspect. Also, most of the 26 people who took part in this experimental study consider that gardening is an activity carried out in nature, and 3.7% of them deny the statement.

The following statement `*5. I feel safe during the planting time*` highlights and evaluates the perception of safety in the green space, where it can be seen from the assessments that most of all people felt safe during the 3-, 10- or 6-

weeks sessions during (therapeutic horticulture) TH activities.

Thus, a few people (7.4%) stated that they do feel insecure, while most of those involved in the study give a positive answer.

In the next part of the survey, the following five questions are adapted to emphasize the social side of therapeutic horticulture activities `*6. It's a good experience to meet people*`, where analyzing the obtained results from the processing data, it can be counted that there are 25 affirmative answers and about 7.4% negative ones.

According to the results obtained next statement `*7. I enjoy helping others in the planting activity*`, those who undertake gardening activities in community spaces, are willing to offer help to others, registering 92.5% positive answers to the preliminary assessment. On the other hand, the help offered to others through the experiences and knowledge of the respondents decreases, obtaining a majority of only 85.2% which represents 23 positive respondents.

The last two statements are part of the category of the social element of gardening and highlight the strong sense of belonging to the local and community landscape after the completion of TH interventions. Analyzing the next one `*9. I care for my plant and community*`, it can be evidenced a great responsibility towards the green space of the volunteers involved in the research study, the majority of 96.3% having a positive answer. Under these conditions, the way in which the people involved in the experimental research want to participate in active TH is different, because 10 of the respondents prefer to work independently, respectively 63.0% of people like to work in organized/collective groups.

The next part `*11. I can create something of beauty*` defines the aesthetic side of gardening activities, where it can be seen that 92.6% of those involved in TH sessions express a positive opinion in this regard.

The following three questions assess participants' self-esteem after completing the plant-assisted TH intervention. The aesthetic side of the post-therapy green space is strongly highlighted by the results that confirm that most of all respondents evaluate and appreciate their work as a beautiful result. Thus, out of the total number of participants, the majority of respondents state that they are proud of the appearance of the green space in which they activated with over 96.3% positive answers. Therefore, this aspect of self-confidence, of the satisfaction perceived after the completion of TH activities, which always changes the appearance of green spaces, is a positive feeling among the participants.

For the current statement `*13. I can handle the work needed*` it turns out that in terms of the physical effort required to do the gardening activities, 8 volunteers faced difficulties, compared to 70.4% of all participants. Even though the average age indicates the involvement of a category of young people, they have encountered some physical issues.

The last two statements are evidencing the influence of TH activities on quality of life (QoL) issues assess the emotional side of the results of TH and gardening sessions. Thus, it can be observed that most people say that the green space offers a chance to relax, registering 100% positive statements. Therefore, this finding also validates the hypothesis that gardening activities can have recreational effects, beneficial to health (Marsh et al., 2021).

In the end, the last statement `*15. I intend to do therapeutic horticulture*` analyses the societal need to belong to a zone/community/group, and this time it is about the green space in which the individual spent a definite time by undertaking gardening activities. Therefore, results conclude with an affirmative answer of over 96.3% of the 27 respondents, the desire to attend with a predetermined recurrence public gardening activities in the future.

Based on the investigation made by measuring the influence of gardening activities on the quality of life, the results obtained following a survey with 15 questions were statistically analyzed to identify the relation between the studied items.

The results presented in the next figure show that regardless of the environment of origin, age, gender and period of therapeutic horticulture and gardening activities have a significant positive influence on the volunteer's well-being involved in this research study.

In this regard, there is a direct and positive relation between the answers of women from urban and rural areas, who answer in the affirmative way to the most questions regarding the favourable influence of gardening activities on well-being (r = 0.71).

The Pearson`s index value shows a significant positive correlation between the responses of women who followed TH sessions during 3 and 10 days (r = 0.84). Moreover, the analyzed data highlights a significant correlation between the answers of women who participated in TH sessions of 10 days and 6 weeks (r = 0.78), when they were asked about the importance of TH activities in their well-being. The correlation between the responses of men participating in TH sessions of 3 and 10 days (r = -0.15) is negative, and the Pearson`s index value (r = 0.02) in the case of men present in TH activities of 10 days and 6 weeks shows an insignificantly positive result and did not indicate statistical change.

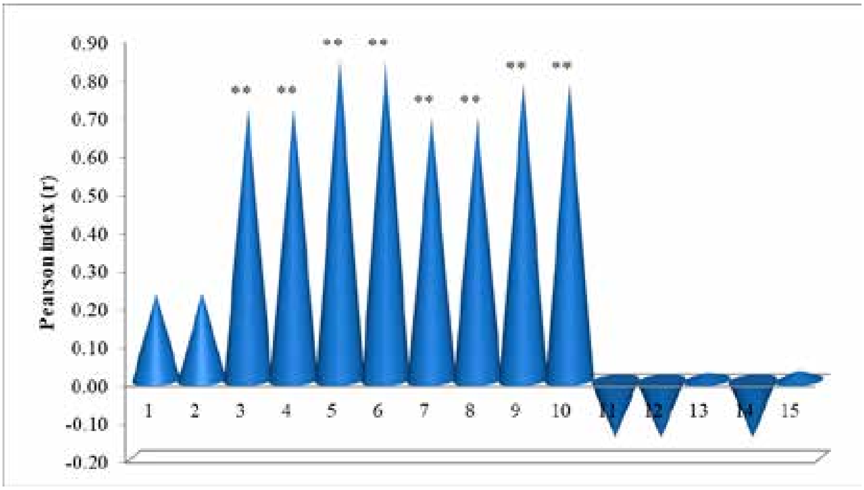

Figure 1. The influence of gardening activities on the quality of life expressed using the Pearson`s Index (15 cases, p 5% = 0.51, p 1% = 0.64, according to Ardelean et al., 2002)

According to the data obtained, the results shown in the Figure 1 evidence that there is an insignificant correlation (r = 0.23) between the answers of women and men of similar age (18-24 years old) which when asked about the joy of gardening, respectively between the answers women and men of similar age who consider gardening useful to those around them. There are no positive correlations between the answers of women from urban areas and men with the same background to most questions regarding the favourable influence of gardening activities on their well-being (r = 0.22).

CONCLUSIONS

The evaluation of the participants' perception after attending therapeutic horticulture (TH) sessions regarding the quality of life (QoL) following the gardening activities showed in large proportions positive answers regarding the psychological side, the security, from a social perspective, as well as in terms of self-esteem and emotional state. So, most people involved in different TH sessions enjoy spending time and gardening in the green area, facilitating also social interaction. An important aspect confirmed after the interpretation of these results is that gardening activities must be chosen according to the physical abilities of each participant.

Gardening activities can offer people the opportunity to be part of something meaningful and to rediscover their confidence, to improving their health and well-being.

The results obtained from the correlations indicate a possible link between gardening activities and respondents' well-being in most of the studied cases. Based on this finding, it can be stated the TH intervention is a possible alternative way to increase people wellbeing and help trough depression symptoms even after the 2-year pandemic period.

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THE ANALYSIS OF SOME ORNAMENTAL ROSE VARIETIES GROW IN THE GREEN SPACES FROM IAȘI

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Abstract

Species of the genus Rosa L. have importance for: creating green spaces, getting roses and their use as rootstock for cultivated varieties. The purpose of this paper is to highlight the possibilities of identifying potential ornamental species in the genus Rosa sp. The observations regarded six rose varieties: 'Queen Elizabeth', 'Golden Monica', 'Kardinal', 'Ingrid Bergman', 'Montana' and 'Diamond Jubilee'. There were studied six features defining the decorative value of these varieties, respectively: the foliage, the diseases resistance, the flowering intensity, the form of the flower, the colour of the petals and the odor.

Key words: *roses, leafage, flower, blossoming, perfume.*

INTRODUCTION

In the multitude of dendrological species which contain ornamental shrubs, spontaneous species of *Rosa* L. have a real importance for the design of green spaces, obtaining of noble roses, and many of them are utilised as parent stocks for cultivated sorts. (Mohan et al., 2010)
The conditions from Romania are generally favourable for this species.
One of the basic components of green spaces which assure an aesthetical aspect of localities and contributes to the welfare and good mood of people, which also assure a favourable working and living climate is represented by floral and roses landscapes designs (Wagner Șt., 2002).
Rose was considered from ancient times "Queen of flowers", due to its multiple qualities and particularly great wealth and beauty flowers, scented and with various colours and shapes (Zaharia D. et al., 2003, Wagner Șt., 2002).
The necessity for diversifying the assortments is a priority, having in view the diversity of biological material and the results obtained in the world. Literature shows, which in this genus are known over 400 species with more than 20,000 sorts (Bernardis R., 2011, Mohan et al., 2010, Zaharia D. et al., 2003, Wagner Șt., 2002).
They are characterized through a series of characteristics, among which we mention: the bush shape; 1 - the vigour; 2 - the leafage; 3 - the resistance to diseases; 4 - the shank and the floral peduncle; 5 - the blooming intensity; 6 -the rose bud shape; 7 - the flower shape; 8 -the durability of the flower in the field; 9 - the colour at opening; 10 - the colour at blossomming; 11 - the manner of petals fall; 12. - the perfume.

MATERIALS AND METHODS

Evaluation of the ornamental value of some rose types was made with the green spaces in the city of Iasi.
The research was done on six varieties of rose existing in Iași county green space: *'Golden Monica'*, *'Ingrid Bergman'*, *'Queen Elizabeth'*, *'Kardinal'*, *'Diamond Jubilee'*, *'Montana'*.
The 6 analysed types of roses in the green spaces in the city present the following characteristics:

1.*'Golden Monica'*variety

A Hybrid Tea class type. It presents vigorous shrubs, 80/50 cm, erect growth and fast redeployment. The leaves are green, medium and shiny. The flowers are medium-large and well developed. The buds are slim, with golden-yellow petals and a pleasant scent. They have good resistance against diseases. It is a type of rose for producing flowers cut in the field and unheated protected spaces (Figure 1a, 1b).

2.*'Ingrid Bergman'* variety

A Hybrid Tea class rose type. It has medium-vigour shrubs, 60/40 cm, compact and thick. The branches are erect, with medium leaves, dark green and with a skin-like aspect. The flowers have 30-40 large petals, velvety dark red in colour, slightly scented and presenting abundant inflorescence. They have good resistance against diseases. They are good for gardens and parks, in groups or massifs (Figure 2a and 2b).

3. *'Kardinal'* variety

A Hybrid Tea class rose type. This type has vigorous shrubs, 90/55 cm, with erect growth, thick branches and many thorns. The leaves are medium, dark green and shiny. The flowers are large, cardinal red in colour, slightly velvety and scented. They have good resistance against diseases. It is a type of rose for producing flowers cut in the field and protected spaces (Figure 3a and 3b).

4. *'Diamond Jubilee'* variety

A Hybrid Tea class rose type. It is a type of rose with medium vigour shrubs, 70/45 cm, and erect branches. The leaves are thick, dark green, semi-shiny. The flowers are large, with yellow-brownish petals, undated and pleasantly scented. They have rich inflorescence. They are easily attacked by fungus. They are used in massive or group plantations (Figure 4a and 4b).

5. *'Montana'* variety

A Floribunda class rose type. It presents medium-vigour shrubs, 70/45 cm, with semi-erect branches. The leaves are dark green and shiny. The flowers have 20-30 large petals, in groups of 5-7 flowers. They are red-orange in colour and have a faint scent. It is a type of rose resistant to diseases and cold weather. It is used in gardens and parks (Figure 5a and 5b).

6.*'Queen Elizabeth'* variety

A Floribunda class rose type. It presents vigorous shrubs, 120/60 cm, with erect and long branches. The leaves are large and shiny. The flowers are large and can be singled-out or in groups. The petals are pure, delicate pink and have a faint scent. It has good resistance to diseases. It is used alone, in groups, massifs or green fences (Figure 6a and 6b).

Figure 1a. *'Golden Monica'* variety

Figure 1b. *'Golden Monica'* variety

Figure 2a. *'Ingrid Bergman'* variety

Figure 2b. *'Ingrid Bergman'* variety

Figure 3a. *'Kardinal'* variety

Figure 3b. *'Kardinal'* variety

Figure 4a. *'Diamond Jubilee'* variety

Figure 4b. *'Diamond Jubilee'* variety

Figure 5a. *'Montana'* variety

Figure 5b. *'Montana'* variety

Figure 6a. *'Queen Elizabeth'* variety

Figure 6b. *'Queen Elizabeth'* variety

The observations were made in 6 periods, starting with the date of June 5th approximately every 2 weeks. In the end, we calculated the arithmetical mean for each characteristic in part and the total of points for the characteristics analyzed in each variety.
They consisted in studying 6 more important characteristics that define to the greatest extent the decorative value of these varieties and namely: 1: the leafage, 2. the resistance to diseases; 3. the blooming intensity; 4. the flower shape; 5. the colour of petals; 6. the perfume. The score a for each parameter studied was between 0-10 point and was given for each of the 6 periods analyzed (05.VI, 26 VI., 12.VII, 30 VII, 13 VIII, 01 IX).

RESULTS AND DISCUSSIONS

For the 6 varieties of roses analysis we drew up quality evaluation sheets that comprise the reliability characteristics and the data regarding which we made the observations (Tables 1-6).
The results regarding the studied characteristics are the following:
1. The leafage. This characteristic was appreciated according to the density of leaves on the sprouts, the colour, the shininess, the manner it maintains on the bush during the vegetation period, the maximum grade being 10. Among the varieties taken in the study the highest score was registered by the variety *'Queen Elizabeth'* and *'Golden Monica'* with 60 points , and the lowest score was registered by the varieties *'Ingrid Bergman'* and *'Diamond Jubilee'* with 48 points.
2. Resistance to diseases. This represents one of the most important aspects about the culture of roses. The most frequent diseases are the black spotting, (*Diplocarpon rosae*), the scab (*Phragmidium disciflorum*) and the mildew (*Sphaerotheca pannosa, var. rosae*), the maximum grade is 8. The tolerance to these diseases is determined by dense, puckered, dark green foliage and with a thick cuticle.
The very good results were registered by three of the analyzed varieties, having the maximum score of 48 points, with the exception of the variety *'Diamond Jubilee'* which has proven sensitive to diseases, registering 44 points.
3. The intensity of blooming. This characteristic represents one of the most important qualities of the varieties of roses. In the case of this character, the maximum grade is 10.
The varieties *'Ingrid Bergman', 'Kardinal', 'Queen Elizabeth', 'Golden Monica'* distinguished themselves with a maximum score of 60 points, followed by *'Diamond Jubilee'* variety, with 54 points. The minimum score was registered by *'Montana'* varieties, with 48 points (Wagner Şt., 1985).
4. The bud shape. It is a trait characterising all varieties of climbing roses; taking into account both the shape and the dimensions of the flowers.
The varieties *'Golden Monica'*, *'Ingrid Bergman', 'Kardinal', 'Queen Elizabeth'* and*' Diamond Jubilee',* distinguished themselves as having very beautiful flowers: registering a score of 54 points.
5. The colour at blossoming. It can be assessed rather subjectively according to the person executing the pre-operation. The maximum grade for this characteristic is considered 6, and the maximum grade is given to the varieties whose petals have an intense colour, well emphasized and that remains for one period of time (Wagner, Şt., 1985).
As regards the colour at flowering, the varieties *'Golden Monica', 'Ingrid Bergman', 'Kardinal', 'Montana'* and*' Queen Elizabeth'* distinguished themselves.
6. Perfume. It is also a much-appreciated characteristic, the maximum grade being 7. (Lammerts, W. E., 1951).
Of the 6 varieties analyzed, as regards the most perfumed one, we noticed the flowers of the *'Diamond Jubilee'* and *'Golden Monica'* varieties.
In the first variety the flowers are large, with yellow-brownish petals, undated and pleasantly scented, and the second variety with golden-yellow petals and a pleasant scent.
The quality evaluation operation of the rose characteristics offers us a general, subjective orientation, from some points of view regarding the decorative value of roses. Through this we can thus emphasize the very decorative varieties of roses (Miller N. F., 1962).

Table 1. The quality evaluation sheet for the *'Diamond Jubilee'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	8	8	8	8	8	8	48	8.0
2.	Resistance to diseases	8	8	8	7	7	7	7	44	7.3
3.	The blooming intensity	10	9	9	9	9	9	9	54	9.0
4.	The bud shape	9	9	9	9	9	9	9	54	9.0
5	The colour at blossoming	6	5	5	5	5	5	5	30	5.0
6	The perfume	7	7	7	7	7	7	7	42	7.0
Total points										**45.3**

Table 2. The quality evaluation sheet for the *'Golden Monica'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	10	10	10	10	10	10	60	10.0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	10	10	10	10	10	10	60	10.0
4.	The bud shape	9	9	9	9	9	9	9	54	9.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	7	7	7	7	7	7	42	7.0
Total points										**50.0**

Table 3. The quality evaluation sheet for the *'Ingrid Bergman'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	8	8	8	8	8	8	48	8.0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	10	10	10	10	10	10	60	10.0
4.	The bud shape	9	9	9	9	9	9	9	54	9.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	4	4	4	4	4	4	24	4.0
Total points										**45.0**

Table 4. The quality evaluation sheet for the *'Kardinal'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	7	7	7	7	7	7	42	7.0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	10	10	10	10	10	10	60	10.0
4.	The bud shape	9	9	9	9	9	9	9	54	9.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	1	1	1	1	1	1	6	1.0
Total points										**41.0**

Table 5. The quality evaluation sheet for the *'Montana'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations 05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX	Total points	Mean
1.	Leafage	10	9	9	9	9	9	9	54	9.0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	8	8	8	8	8	8	48	8.0
4.	The bud shape	9	8	8	8	8	8	8	48	8.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	2	2	2	2	2	2	12	2.0
Total points										**41.0**

Table 6. The quality evaluation sheet for the *'Queen Elizabeth'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations 05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX	Total points	Mean
1.	Leafage	10	10	10	10	10	10	10	60	10.0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	10	10	10	10	10	10	60	10.0
4.	The bud shape	9	9	9	9	9	9	9	54	9.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	1	1	1	1	1	1	6	1.0
Total points										**44.0**

CONCLUSIONS

The analysis of the behaviour of the 6 varieties, regarding the most important decorative characteristics emphasized the following aspects:

1. The most decorative leafage was ascertained in the *'Queen Elizabeth', 'Kardinal'* and *'Golden Monica'* varieties.

2. All the varieties analyzed, with exception for *'Diamond Jubilee'* variety that has proven to be sensitive to diseases, have proven most resistant to the attack of diseases.

3. The varieties *'Ingrid Bergman', 'Kardinal', 'Queen Elizabeth', 'Golden Monica* 'distinguished themselves through a great intensity of blooming.

4. The most beautiful flowers have proven to be those from the varieties *'Golden Monica', 'Ingrid Bergman', 'Kardinal', 'Queen Elizabeth'* and*' Diamond Jubilee'*.

5. As regards the colour of flowers the most valuable varieties distinguished *'Golden Monica', 'Ingrid Bergman', 'Kardinal', 'Montana'* and*' Queen Elizabeth'*.

6. The most perfumed flowers, of the four varieties analyzed have proven to be the flowers of the varieties *'Diamond Jubilee'* and *'Golden Monica'*.

7. From the point of view of the total score, the most valuable variety is *'Golden Monica'*, registering a score of 50.0 points, distinguishing itself through vigorous shrubs, 80/50 cm, erect growth and fast redeployment.

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PASSIFLORA QUADRANGULARIS GROWTH RESPONSE TO FERTILIZATION REGIMES IN CONTROLLED CLIMATE

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Abstract

Enriching the assortment of flowering plants in Romania by introducing exotic species in the context of contemporary climate changes is of real interest. Passiflora quadrangularis is a climbing plant cultivated for its fruits, nutraceutical properties, secondary metabolite content and ornamental value. This research was developed in order to validate the influence of foliar fertilizers on morpho-biometric traits. A bifactorial experiment was performed using the method of randomized blocks with three replicates. In this regard, the organic biostimulator Cropmax and the mineral fertilizer Nutricomplex 20-20-20 + M.E. were tested. Fertilization was carried out periodically during the vegetation period, in three doses: 0.05%, 0.1% and 0.2%. Determinations were made regarding the main morpho-biometric traits, the results being statistically interpreted. All treatments recorded higher values than the control variant (untreated), and the Cropmax biostimulator has a significantly positive effect on plants development.

Key words*: foliar fertilizers, morpho-biometric traits, Passiflora.*

INTRODUCTION

Passiflora genus is little known due to the diversity and complexity of species existing in nature (Lucarini et al., 2019) comprising over 750 species (Feuillet & MacDougal, 2007). Over 60 species produce edible fruits, but only some are used in the food industry (Thokchom & Mandal, 2017).

P. quadrangularis is a tendril-climbing vine with winged stem (Cantor et al., 2021) and woody stems at the bottom. The shoots can reach lengths of 18-50 m (Lim, 2012). The alternate leaves have an ovate or elliptical-ovate shape, are glabrous, with entire edges and a sharply acuminate peak. The long petiole has globular nectariferous glands (Narel Paniagua-Zambrana & Bussmann, 2020). The flowers are large, up to 12 cm in diameter, fragrant and pendulous. The fruits are very large (15-30 cm), ovoid-elongated or ellipsoid, yellowish green. Beneath the bark is found the fleshy, spongy, edible, juicy, sweet, white-yellow mesocarp (Lim, 2012).

P. quadrangularis or "giant granadilla", widespread in the tropics, is the most cultivated species after *P. edulis* in tropical America (Lucarini et al., 2019). The aryl (pulp) is the primary product that can be eaten fresh, with ice cream or processed into fruit juice, syrup or preserved nectar. The pulp can be eaten with the addition of sugar or honey (Lim, 2012). Before ripening, this fruit can be used as a vegetable. According to Morton, 1987, *P. quadrangularis* fruit has the following nutritional composition of the pulp, per 100 g product: water 94.4 g, protein 0.112 g, fat 0.15 g, crude fiber 0.7 g, ash 0.41 g, calcium 13.8 mg, phosphorus 17.1 mg, iron 0.80 mg, carotene 0.004 mg, riboflavin 0.033 mg, niacin 0.378 mg, ascorbic acid 14.3 mg. The nutritional value of aryl and seeds is: water 78.4 g, protein 0.299 g, fat 1.29 g, crude fiber 3.6 g, ash 0.80 g, calcium 9.2 mg, phosphorus 39.3 mg, iron 2.93 mg, carotene 0.019 mg, thiamine 0.003 mg, riboflavin 0.120 mg and niacin 15.3 mg (Lim, 2012).

According to Carranza et al., (2016), among the passion flower species in Colombia, *P. quadrangularis* is considered a priority by the Ministry of Agriculture and Rural Development, listed as a promising fruit for export, due to its acceptance as fresh fruit, juices and snacks. According to FAO, (2005) the fruit has a pleasant, sweet and at the same time slightly acidic characteristic aroma. The aroma of the fruit is mainly provided by the chemical compound geraniol (Montero et al., 2016).

For medicinal purposes, *P. quadrangularis* is used for the treatment of diabetes, strokes, burns, hypertension, arthritis, neuralgia and liver disease (Rodríguez et al., 2020) and has a cardiodepressant and a calming role (Osorio et al., 2000).

For growth and development, the Passion flower requires nutrition at all stages. Macronutrients N, K and Ca are absorbed in large quantities, followed by S, P and Mg. Among micronutrients, Mn and Fe are absorbed in the largest amounts, followed by Zn, B and Cu (Joy, 2010). The need for nutrients is related to the age and stage of development of the plants. According to Thokchom & Mandal, (2017), the approximate nutrient requirement for *Passiflora* is 150 kg/ha N, 100 kg/ha P and 200 kg/ha K.

In the study conducted by Ramaiya et al., 2021 the crop of *P. quadrangularis* was fertilized in the form of 250-50-80 kg/ha N-P_2O_5-K_2O or organic fertilizer, manure, 20 t/ha.

This study is part of a research plan for *P. caerulea* (Boboc Oros et al., 2021) and *P. quadrangularis* species. Growth and flowering in these species are dependent on the fertilization program. Thus, the present research aimed to establish the most advantageous fertilization program for *P. quadrangularis* to lead to the attainment of an optimal cultivation system for the circumstances in Romania.

MATERIALS AND METHODS

The research on the influence of the fertilization program on *P. quadrangularis* plants` growth and development was located in the greenhouse of the Department of Ornamental Plants, belonging to the Institute of Advanced Horticultural Research of Transylvania, of the University of Agricultural Sciences and Veterinary Medicine (UASVM) Cluj-Napoca and was began on April 22, 2019.

From a climatic regard, the greenhouse is placed, according to the W. Köppen system in the Dfclimatic region, described as the boreal climate with cold and humid winters (Bunescu et al., 2005). Plants were cultivated in a greenhouse with automatically controlled ecological conditions: natural light, 25/20°C day/night temperature and 60% relative humidity.

P. quadrangularis seedlings from two months old, obtained from rooted cuttings at the end of February 2019, represent the biological material of the present research. After the plants were transplanted to soil, were watered everyday in the first two weeks after transplantation, and after that period doubly a week.

Two fertilizers were tested: an organic biostimulator (Cropmax®) and a mineral fertilizer [Nutricomplex® 20-20-20 + microelements (M.E.)]. Cropmax® (Holland Farming, Groenekan, The Netherlands) is an organic growth biostimulant for all types of crops, contains amino acids, macro- and microelements, vitamins and polysaccharides. Cropmax® contains N (0.2%), P (0.4%), K (0.02%), Fe (220 mg/l), Mg (550 mg/l), Zn (49 mg/l), Mn (54 mg/l), Cu (35 mg/l), Bo (70 mg/l), Ca + Mo + Cb + Ni (10 mg/l), vitamins C and E, enzymes and carotenoids. The recommended concentration rate in greenhouse crops is 0.05 - 0.2%, every 7-10 days (Balint et al., 2018).

Nutricomplex® 20-20-20 + M.E (Trade Corporation International, Madrid Spain) is produced by high pure raw materials, supplemented with chelated micro-nutrients and it's free of chloride, sodium and carbonates. This fertiliser contains N (20% w/w), P_2O_5 (20% w/w), K_2O (20% w/w), Fe (0.06% w/w), Mn (0.04% w/w), Zn (0.02% w/w), Cu (0.01% w/w), B (0.02% w/w), Mo (0.003% w/w). In foliar application, the advised dosage is 250-400 g/hl. Nutricomplex® 20-20-20 (n.d.) (Boboc (Oros) et al., 2021).

A bifactorial experience was organized in randomized block method with three traits (Sestraș, 2019) as follows:

Factor A – the fertilizer:
a_1 – Nutricomplex
a_2 – Cropmax
a_3 – unfertilized (control)
Factor B – the dose applied:
b_1 – 0.05%
b_2 – 0.1%
b_3 – 0.2%

Fertilizers were performed every 14 days by foliar application. The treatments were accomplished from the first week after transplantation until the flowering. The results were compared to the control treatment, where the plants were sprayed with the same volume of water (without fertilizer). Tillage of plants was done using good agricultural practices.

Determinations regarding the main morpho-biometric traits have been performed to assess the quality of *P. quadrangularis* plant species. The morpho-biometric traits were: the average plant length, the average number of shoots per plant, the average number of internodes on the main stem, the average length of internodes (cm), the average number of leaves / plant, the stem diameter at 50 cm from the ground (mm) and the relative growth rate RGR. Regarding flowering and fruiting, were made determinations on the first node at which the flower on the shoot was occurred, the average number of flowers per plant, the flower diameter and the average number of fruit per plant. As different growth rates were observed during the growing season, four time intervals were established (22 April - 3 June, 4 June - 5 August, 6 August - 7 October, 8 October - 30 December) for which calculated the evolution of the relative daily growth rate (RGR). RGR was determined using the formula: RGR = W_2 -W_1/ T, where W_1 = first measurement, W_2 = second measurement, and T = the number of days between each (Boboc (Oros) et al., 2021).

Data were tested using the analysis of variance procedure, and the Duncan's Multiple Range Test (Duncan's MRT, $p<0.05$) was used as a post hoc test for comparison among treatment means.

RESULTS AND DISCUSSIONS

In order to determine the average growth of *P. quadrangularis* plants during the whole year after transplanting (April 22, 2019) (Figure 1), weekly determinations on plant growth were performed. At the end of 2019, the plants fertilized with Cropmax recorded average growths of 434.2 cm, those fertilized with Nutricomplex 368.6 cm, and the control variant 160.7 cm.

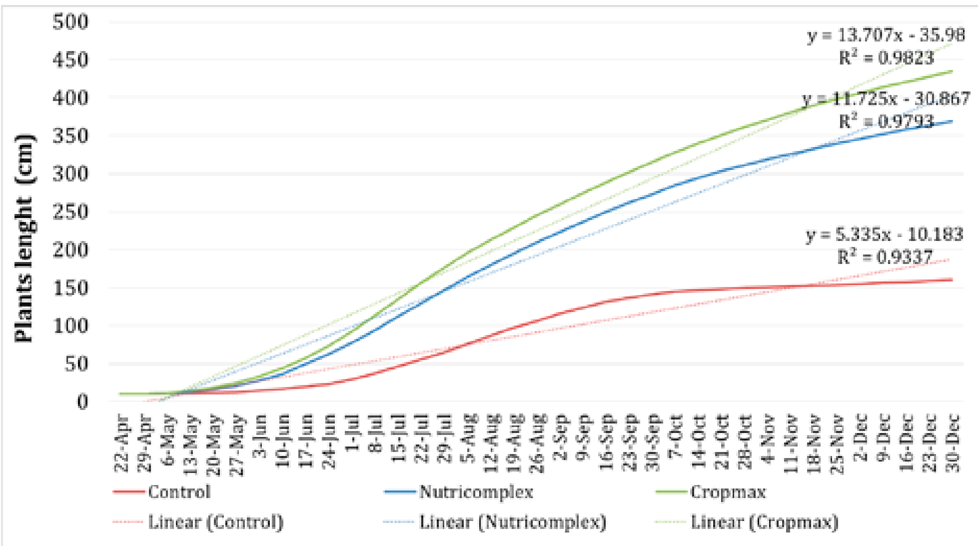

Figure 1. The evolution of average growths at *Passiflora quadrangularis* in 2019

Analyzing the evolution of average growths, different growth rates were found during the vegetation period, so four time intervals were established (April 22 - June 3, June 4 - August 5, August 6 - October 7, October 8 - December 30) for which has calculated the evolution of the relative growth rate (RGR). As shown in Figure 2, the Cropmax organic biostimulator favoured average growth rates for fertilized plants, superior to the Nutricomplex mineral fertilizer for each time interval analyzed (0.54; 2.61; 2.14; 1.29; 1.70).

According to Figure 3, a bifactorial analysis of RGR was performed for the experimental years 2019 and 2020. In 2019, RGR recorded the average independent value of fertilizer, of 1.25

and in 2020 by 1.11. The RGR values have not registered statistically significant differences.

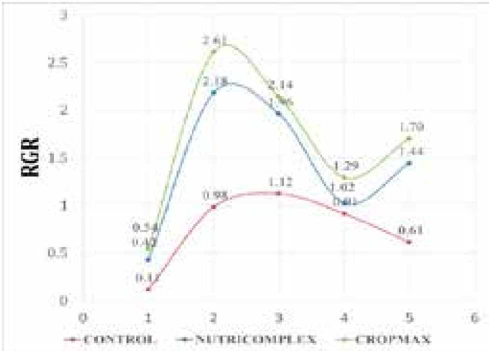

Figure 2. Relative growth rate (RGR) over time intervals in 2019 at *P. quadrangularis*

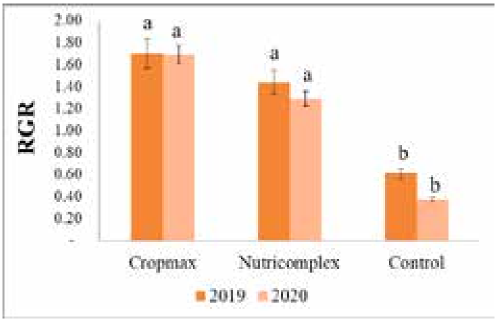

Figure 3. Results on the influence of fertilizer on relative growth rate in 2019 and 2020

A brief analysis of the information provided by RGR on the growth of *Passiflora* plants indicates that in the first year after planting, the growth was more pronounced.

Then, analyzing on time intervals, during the summer, in the interval 4.06-5.08 the increases were significantly superior to the other time intervals.

The application of fertilizers during the growing season to *P. quadrangularis* showed increases in the case of all morphological traits (Table 1) compared to the control variant (V_7). Regarding the average plant length, the longest shoots, of 475.27 cm ($v_{6)}$ were obtained on the lot fertilized with Cropmax 0.2%, and statistically significant differences were registered at variants V_1 and V_5-V_7. The average number of shoots varies between 8.23 (V_7) and 21.73 (V_6). Regarding the average number of internodes, the average values varied between 17.42 (V_7) and 37.80 (V_6). There were statistically assured differences between each experimental variant. The average length of the internodes varied between 8.44 cm (V_7) and 12.29 cm (V_6). The average number of leaves per plant varied between 73.33 (V_7) and 154.77 (V_6). The average stem diameter at 50 cm from the ground was between 6.69 mm (V_7) and 8.65 mm (V_6). Regarding the unilateral influence of the fertilizer on the morphological traits, there were statistically assured differences between the two fertilizers, the plants treated with Cropmax fertilizer demonstrating superior values to those treated with Nutricomplex for analyzed traits.

The decorative morphological traits (Table 2), regarding the flowering and fructification registered average values superior to the control variant (V_7). The first node at which the flower formed varied on average from 3.03 (V_6) to 6.44 (V_7). For this index, a lower average numerical value denotes a higher ornamental potential, with a higher number of flowers per shoot. Regarding the average number of flowers per plant, a maximum of 55.23 flowers were obtained when fertilizing with Cropmax 0.2%, the differences being statistically significant. The average diameter of the flower was between 10.37 cm (V_7) and 8.08 cm (V_6), the differences being statistically assured. The plants produced on average 16.73 (V_6) fruits per plant at the fertilization with the biostimulator Cropmax 0.2% and 13.33 at the plants fertilized with Nutricomplex 0.2.

The rate of fertilizer administered is directly proportional to fruit production. Plants fertilized with Cropmax produced a higher number of fruits compared to those treated with Nutricomplex, regardless of dose. Statistically significant differences were obtained between each experimental variant. Regarding the influence of the fertilizer on the decorative morphological characters, for the average number of flowers and the diameter of the flower there are superior differences, statistically assured when fertilizing with Cropmax. Regarding the average number of flowers and fruits per plant, Cropmax registers higher values. (on average 46.12 flowers and 11.22 fruits per plant) compared to Nutricomplex (which averaged 42.42 flowers and 8.94 fruits per plant).

Table 1. Results on the effect of fertilizer and dose applied on the morpho-biometrics traits

Var. no.	Fertilizer	Doze (%)	Morpho-biometrics traits					
			Plant length (cm)	Shoots number	Number of internodes on the main stem	Internodes lenght (cm)	Number of leaves / plant	Stem diameter (mm)
V_1	Nutricomplex	0.05	311.97 d	13.83 c	29.2 f	10.73 d	128.13 c	7.22 d
V_2		0.1	370.07 c	14.50 c	31.9 e	11.46 c	139.83 b	7.42 cd
V_3		0.2	395.9 c	16.23 bc	33.15 d	11.84 bc	150.83 a	7.54 c
		Average ($\overline{x}$)	359.31 B	18.88 A	31.42 B	11.34 A	139.6 B	7.39 B
V_4	Cropmax	0.05	369.33 c	16.03 c	34.28 c	10.78 d	142.63 b	7.60 c
V_5		0.1	437.47 b	18.87 b	35.90 b	11.93 ab	150.37 a	8.23 b
V_6		0.2	475.27 a	21.73 a	37.80 a	12.29 a	154.77 a	8.65 a
		Average ($\overline{x}$)	427.36 A	14.86 B	35.99 A	11.67 A	149.26 A	8.16 A
V_7	Unfertilized (Control)		146.73 eC	8.23 dC	17.42 gC	8.44 eB	73.33 dC	6.69 eC
DS 5%			25.89-30.3	2.66-3.00	1.09-1.22	0.38-0.43	6.62-7.46	0.22-0.24
DS 5% ($\overline{x}$)			28.22-28.79	2.33-2.38	1.94-1.98	0.33-0.34	8.92-9.10	0.36-0.37

Note: The difference between any two values followed by at least one different letter is significant. The average values followed by at least one different capital letter reflect the unilateral influence of the fertilizer.

Table 2. Results on the effect of fertilizer and dose applied on the morpho-decorative traits

Var. no	Fertilizer	Doze (%)	Morpho-decorative traits			
			The first node at which the flower occurred	Number of flowers / plant	Flower diameter (cm)	Number of fruits / plant
V_1	Nutricomplex	0.05%	3.63 b	35.37 d	8.33 ef	3.67 f
V_2		0.1%	3.23 c	42.70 c	8.83 de	9.83 d
V_3		0.2%	3.10 c	49.17 b	9.27 cd	13.33 b
		Average ($\overline{x}$)	3.36 A	42.41 B	8.81 B	8.94 A
V_4	Cropmax	0.05%	3.20 c	36.97 d	9.42 bc	5.43 e
V_5		0.1%	3.13 c	46.17 bc	9.87 ab	11.50 c
V_6		0.2%	3.03 c	55.23 a	10.37 a	16.73 a
		Average ($\overline{x}$)	3.12 A	46.12 A	9.88 A	11.22 A
V_7	Unfertilized (Control)		6.44 aB	8.67 eC	8.08 fC	0.47 gB
DS 5%			0.37-0.41	3.82-4.30	0.52-0.59	1.13-1.28
DS 5% ($\overline{x}$)			0.55-0.56	2.26-2.30	0.40	3.13-3.19

Note: The difference between any two values followed by at least one different letter is significant. The average values followed by at least one different capital letter reflect the unilateral influence of the fertilizer.

In the study carried out in parallel with the *P. caerulea* species, by (Boboc (Oros) et al., 2021), the fertilization with Nutricomplex and Cropmax influenced all morphological and decorative traits, the best results being obtained on the plants treated with 0.2% Cropmax.

In his research, Pacheco et al., (2017) made three types of fertilizers for *P. edulis* cultivated for fruit production: recommended mineral fertilizer, organic fertilizer represented by manure and the equivalence of recommended fertilization with potassium for *Passiflora* culture by double dose of organic fertilizer.

The fruits obtained from double-dose fertilized plants have maintained its commercial quality better and the single dose of organic fertilizer proved to be insufficient.

CONCLUSIONS

Applying foliar fertilizers to *P. quadrangularis* leads to more decorative, uniform and vigorous plants. The application of organic biostimulators is justified both for increasing the biomass of ornamental plants and in the case of an organic crop, justified by the important therapeutic and food properties.

Thus, in the first year after planting, the plants fertilized with Cropmax showed average growths of 427.36 cm, higher than the other experimental variants, and main decorative characteristics reached the best values when fertilizing with Cropmax 0.2% in both years after planting.

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RESEARCH ON THE INFLUENCE OF DIFFERENTIATED HYDRATION ON PHYSIOLOGICAL PROCESSES ON TWO LAWN MIXTURES

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Abstract

Today, in most countries, which have reached a very high level of industrialization, technicalization and urbanization, the conservation and creation of green areas is an important means of protecting man and his living environment. Green spaces are good for human health, not only by creating a favorable microclimate and a calmer environment, with cleaner and better oxygenated air, but also by influencing the neuropsychic state. Lawn is an essential and irreplaceable element for leisure and sports and is more than just grass. It is an indispensable landscape element with multiple values, through its silky texture, attractive appearance, green color, color that represents nature and life, freshness, rebirth, hope and vigor. The aim of the research was to study the influence of differentiated hydration on physiological processes in two lawn mixtures. For this purpose we used two types of lawn, the first, Turfline, composed of a mixture of 3 types of lawn seeds (65% Festuca arundinacea Starlett; 15% Lolium perenne Double; 20% Poa pratensis Geisha) and the second RPR Regenerating, namely Lolium perenne with regeneration by stolons, both irrigated daily and every three days. For the study of differentiated hydration on the two lawn mixtures, we determined the content of chlorophyll pigments in the leaves (SPAD units) and the rate of photosynthesis (%). The results of the research show that irrigation did not significantly influence the chlorophyll content of the two types of lawn. Regarding the photosynthetic capacity of the turf mixtures under study, it is observed that this has higher values in the case of RPR Regenerating, irrigated every 3 days, which indicates that this type of lawn is more resistant to drought than Turfline.

Key words: *lawn, chlorophyll, photosynthesis, irrigation, Festuca arundinacea, Lolium perenne, Poa pratensis.*

INTRODUCTION

In the garden, the lawn is an element of great decorative value, which creates an atmosphere of calm and tranquility, the large expanses of lawn introducing a note of solemnity and romance into the landscape.

The lawn is also of sanitary importance, due to its ability to help refresh the air, change the temperature and humidity. During the summer, on days with high temperatures, the lawn heats up much less than sand, gravel, brick, concrete, etc. And that's why the layer of air that comes in contact with the lawn is colder. Also, the leaves of the plants that enter the composition of the lawn, having a sticky composition, retain a large part of the dust particles, thus participating in the purification of the air. In general, the term "lawn" defines an area covered with grass, especially gramineae, which is subject to care and intended to perform certain decorative, recreational or sanitary functions.

In order to obtain the lawn, we may use species from the genera: *Agrostis, Cynosurus, Deschampsia, Festuca, Lolium, Phleum, Poa*, of medium and small size and which are thickened by pruning. Most of the time the grass species used for lawn are not grown alone, but in a mixture of at least 3, the composition being chosen according to their temperament, nutritional requirements, soil moisture, resistance to low temperatures, pruning, rapid growth, etc. [16].

For a sustainable and pleasant lawn, we need to know the species, the pedoclimatic conditions, the competitiveness of the species, the morphological and biological peculiarities (www.greenfieldsport.ro/intretinere_gazon.html).

On meadows, in parks and gardens, depending on the mental state and the character of the observed scene, the human being is prone to calm, daydreaming, vivacity, toning, receptivity, good mood.

Recreation in nature is increasingly adopted, representing at the same time an escape from the ordinary and a way to directly benefit from the beneficial actions of natural factors [5].

We should not have too many species in a lawn mixture as this involves difficulties in installation as well as in the exteriorization of the characters. It is therefore estimated that a mixture should contain 2-5 species (or varieties) [13].

When choosing the species or varieties that are part of a mixture, it must be done according to the desired objectives (aesthetic appearance, resistance to traffic, ease of maintenance).

The main species used for lawn are: perennial ryegrass (*Lolium perenne)*; red fescue *(Festuca rubra)*; tall fescue *(Festuca arundinacea)*; blue grass *(Poa pratensis)*; timothy grass *(Phleum pratense)*; common bent *(Agrostis capillaris)*; Bermuda grass *(Cynodon dactylon),* sheep fescue *(Festuca ovina).* These species are used for turf due to their adaptive capabilities.

Global climate change and increasingly extreme weather conditions show the need to look for ways to mitigate and reduce their negative effects on agriculture and to increase crop adaptability [9]. Lack of water and rising temperatures are becoming a growing environmental concern on grasslands, leading not only to reduced productivity but also to negative changes in ecosystem structure and carbon balance [7,14]. Growing perennials, such as grasses, increases soil carbon stocks due to their prolonged photosynthetic activity and higher root biomass [12].

Drought resistant plants are able to change their morphological characteristics and metabolic processes in order to survive periods of drought and to restore normal functioning after stress. A major effect of water deficiency is a reduction in the intensity of photosynthesis, which results from slower leaf development and premature senescence of leaves [15]. Drought stress limits the availability of CO_2, induces the loss of photosynthetic pigments, affects the activity of enzymes and also the activity of photosystem II [4]. In response to water deficiency, plants stimulate the production of reactive oxygen species (ROS) that cause membrane damage, protein degradation, enzyme inactivation and thus induce oxidative stress [18]. The stress caused by the water deficit affects the size of the tissues, the translocation of the assimilated and the portioning of the dry matter in the organs of the plants. However, the magnitude of the effects varies depending on the species, the stage of growth and also the duration and severity of the drought [2, 3].

Photosynthesis provides plants with energy and organic matter, with photosynthetic adaptation being a major component of water tolerance [18]. Photosynthetic light response curves that describe photosynthetic capacity, efficiency, and other parameters are commonly used to evaluate the performance of photosynthesis under environmental stress conditions [11]. Although closure of the stomata induced by high water potential is considered to be the main reason for the reduction of photosynthesis during short-term floods, the reduction of chlorophyll content may eventually lead to reduced photosynthetic capacity during long-term stress [6, 10].

MATERIALS AND METHODS

The biological material consisted of two types of lawn, the first, Turfline, composed of a mixture of 3 types of lawn seeds (65% *Festuca arundinacea* Starlett; 15% *Lolium perenne* Double; 20% *Poa pratensis* Geisha) and the second RPR Regenerating, namely *Lolium perenne* with regeneration by stolons.

The most important forage grasses, cultivated in the temperate zone are *Festuca arundinacea* and *Lolium perenne*. They are characterized by variable feed quality and productivity in optimal growing conditions, but also by different resistance to environmental stress, such as water scarcity. *Festuca arundinacea* has the ability to avoid water shortages with great potential for the development of a deep and extensive root system. This species is able to tolerate water deficiency, reprogramming its cellular metabolism in leaves and other organs. At the same time, the quality of fodder in *Festuca arundinacea* is not as good as in *Lolium perenne* even in optimal conditions of air temperature and soil moisture [8]. *Lolium perenne* is a species widely used not only as fodder but also as lawn grass in urban areas throughout Central and Western Europe [1, 3].

RPR Regenerating, namely *Lolium perenne* with regeneration by stolons, is a grass with a very high density that does not allow the appearance of weeds, the number one in tolerance of heavy traffic, strong and resistant, allowing several hours of play (https://www.barenbrug.biz/rpr).

The resulting stolons, sometimes called "runners", are buds that grow out of the axillary buds at the base of each plant. When an RPR plant finds an empty space in a damaged lawn, stolons will grow horizontally in that space and develop roots at the internodes. RPR develops a natural network of stolons, as a kind of net. This gives the RPR the highest tolerance to use. Instead of separate plants, as in traditional *Lolium perenne*, each RPR plant connects to other plants in the topsoil.

RPR brings strength and speed together in one species. This has the advantage that all the characteristics of a mixture are manifested in a single species. The seeds germinate quickly and can be used intensively after installing a dense lawn. Traditionally, strong species, such as Poa pratensis, germinate more slowly than Lolium perenne. In order to compensate for this in several lawn mixtures, slow-germinating species are combined with Lolium perenne. Despite the rapid germination and establishment of these mixtures, the tolerance to use is insufficient. RPR solves this problem by combining firmness and strength with speed of establishment and regeneration (https://www.barenbrug.com, https://dxgh891opzso3.cloudfront.net).

The aim of the research was to study the influence of differentiated hydration on two lawn mixtures (Turfline and RPR Regenerating).

For the study of differentiated hydration on the two lawn mixtures, we determined the content of chlorophyll pigments in the leaves (SPAD units) and the photosynthetic capacity of plants (%), in four phenophases, with the BBCH classification system to identify the stages of plant development. This classification system aims to find a common language for identifying the stage of the plant. The BBCH system was created by specialists from the world's leading pesticide producers: BASF (B), Bayer (B), Ciba-Geigy (C) and Hoechst-Shering (H). Identifying the main stages of development, the vegetative and generative phases, involve the use of this system. Each of these stages presents 5 stages of development encoded with numbers from 0 to 9, also each stage has 10 phenophases encoded with the same digits. The researches were determined in the development stages 22 (2 tillers visible), 29 (9 or more tillers visible), 33 (3 nodes detectable) and 45 (flag leaf sheath swollen (late-boot) (www.politicheagricole.it/flex/AppData/WebLive/Agrometeo/MIEPFY800/BBCHengl2001.pdf).

For experimentation we used two lawn mixtures: A_1 – Turfline and A_2 – RPR Regenerating, both irrigated daily (V_0) and at three days (V_1).

The total amount of chlorophyll (SPAD) in the leaves was determined using the portable chlorophyll meter SPAD-502 (Konica Minolta, Osaka, Japonia). This device determines the relative chlorophyll content by measuring the absorbance of a leaf in two wavelength ranges. The device measures the light absorbance of the leaf in the range of red light radiation close to IR. Using this principle, the chlorophyll meter calculates a numerical value, SPAD (single photon avalanche diode) which is directly proportional to the amount of chlorophyll in the leaf. With this device measurements were made at different stages of plant development, on healthy leaves.

The rate of photosynthesis was determined by the EARS Plant Photosynthesis Meter (PPM), which measures the use of photosynthetic light in plants. The measurement is based on chlorophyll fluorescence, a very weak optical signal emitted by the plant but which can be detected by the device. Due to its light weight, the instrument is very suitable for laboratory and field use. In addition, long measuring series can be performed automatically. For this reason, PPM has a wide range of applications in research, education and practice (www.groentennieuws.nl/article/88172/mini-fotosynthesemeter-voor-snelle-gewasanalyse/).

Already in the 1990's a number of interesting applications have been developed on the basis of measured photosynthesis yield. Provided these measurements are carried out at a fixed light level, they can predict the life of pot plants and cut flowers. They may also be used to judge the quality of green vegetables and fruit (https://silo.tips/download/the-photosynthesis-reaction).

Data represents mean and standard error. Statistical analysis of the data was performed using analysis of variance according to a three factor experiment (Lawn mix, watering, phenophase). Comparisons between averages were made using the multiple interval test. The meanings of the differences were represented on a letter basis, considering the values without common letters to be significantly different.

RESULTS AND DISCUSSIONS

Considering the analysis of the components of the variance in Table 1, we may observe that regarding the unilateral effects of the factors, only the mixture showed a real and strongly statistically influenced influence on the chlorophyll content of the plants. Also, the interaction between mixing and watering determined significant variations of this character, against the background of low and insignificant influences of the other sources of variation.

Regarding the rate of photosynthesis, there are small and insignificant individual effects of the three factors, lower than the chlorophyll content. As in the case of chlorophyll content, it is found that the interaction between mixture and watering showed a considerable and statistically assured effect on the rate of photosynthesis, in conditions of insignificant effects of other interactions but higher than in the case of chlorophyll.

Table 1. Analysis of variance for the effect of mixture, watering and phenophase on chlorophyll content and photosynthesis rate of lawn

Source of variation		Chlorophyll content			Photosynthesis rate		
	DF	SS	MS	F	SS	MS	F
Total	319	17768.46			37381.3	283.10	
Lawn mix (LM)	1	1672.62	1672.62	31.72**	283.1	58.70	2.40
Watering (W)	1	0.31	0.31	0.01	58.7	46.13	0.50
Phenophase (P)	3	116.32	38.77	0.74	138.4	948.80	0.39
LM x W	1	245.05	95.05	4.69*	948.8	4.03	8.04**
LM x P	3	2.33	0.78	0.01	12.1	19.30	0.03
W x P	3	1.91	0.64	0.01	57.9	5.13	0.16
LM x W x P	3	0.95	0.32	0.01	15.4	117.98	0.04
Erorr	304	15878.97	52.73		35866.8	283.10	

Taking into account the unilateral effect of the mixture, it is observed that the chlorophyll content (Table 2) recorded an amplitude of 4.58 with values between 31.72 SPAD units for RPR Regenerating (A2) and 36.3 for Turfline (A1). As such, Turfline plants showed a significant increase in chlorophyll content of approximately 14.4%. According to the analysis of the variance, we may observe the existence of an insignificant variation of the chlorophyll content between the two watering variants.

Table 2. The effect of mixture and watering on chlorophyll content (CC) photosynthesis rate (PR) of lawn

Lawn mix	Watering		CC(SPAD)
	V0	V1	$\bar{x} \pm s_{\bar{x}}$
Turfline (A1)	x 36.87 a	x 35.72 a	36.30±0.66
RPR Regenerating(A2)	x 31.21 b	x 32.24 b	31.72±0.44
$\bar{x} \pm s_{\bar{x}}$	34.04±0.60	33.98±0.58	34.01±0.42
Lawn mix	Watering		PR (%)
	V0	V1	$\bar{x} \pm s_{\bar{x}}$
Turfline (A1)	x 56.90 a	x 54.31 b	55.61±0.69
RPR Regenerating(A2)	y 55.34 a	x 59.64 a	57.49±0.99
$\bar{x} \pm s_{\bar{x}}$	56.12±0.88	56.98±0.83	56.55±0.60

CC- $LSD_{5\%}$=2.26; PR-$LSD_{5\%}$=3.38; Different letters indicate signifficant at p<0.05 (a,b, for LM; x,y, for W)

Given the combined effect of the mixture and watering, it can be seen that regardless of the watering treatment, the plants of the Turfline mixture registered a significant increase in chlorophyll content with increases between 10.79% for the daily irrigated variant (V0) and 18.13% for watering at 3 days (V1). The chlorophyll content in the plants of the two mixtures was not influenced by the watering treatment.

Regarding the rate of photosynthesis, it is observed that in the case of the daily watered variant, the plants of the two mixtures showed close values of this character, on the background of a reduced variation of 1.56%. Under the effect of 3-day watering treatment, the plants of the RPR Regenerating (A2) mixture recorded a significantly higher rate of photosynthesis by 5.33%. The rate of photosynthesis in Turfline (A1) plants was not significantly influenced by watering treatment. In the case of plants of the RPR Regenerating mixture, the application of watering at 3 days (V1) resulted in a significant increase of approximately 4.3% in the rate of photosynthesis.

Regarding the effect of phenophase, the chlorophyll content registered a reduced amplitude (1.64), with the limits from 33.13 in phenophase 22 to 34.76 for phenophase 45 (Table 3). Thus, the whole experience confirms that the stage of development of the plants did not significantly influence the chlorophyll content.

Given the effect of phenophase on the chlorophyll content of the plants in the two mixtures, there are small and insignificant variations of this character during the study, between 1.55 for Turfline and 1.72 for RPR Regenerating. In each phenophase, the plants of the Turfline mixture recorded a significantly higher amount of chlorophyll associated with relative increases between 13.32% for development stage 45 and 15.26% for stage 29.

Regarding the rate of photosynthesis, there are small and insignificant variations from one phenophase to another, based on amplitudes of 1.37% for Turfline plants and 1.93% for RPR Regenerating plants. Also, in each phenophase the composition of the turf mixture did not significantly influence the chlorophyll content, in the conditions of small variations between 1.37% for phenophase 45 and 2.45 for phenophase 29.

Table 3.The effect of mixture and phenophase on chlorophyll content (CC) and photosynthesis rate (PR) of lawn

Lawn mix	Phenophase				CC (SPAD)
	22	29	33	45	$\bar{x} \pm s_{\bar{x}}$
Turfline (A1)	x 35.38 a	x 36.26 a	x 36.62 a	x 36.93 a	36.30±0.66
RPR Regenerating(A2)	x 30.87 b	x 31.46 b	x 31.97 b	x 32.59 b	31.72±0.44
$\bar{x} \pm s_{\bar{x}}$	33.13±0.85	33.86±0.84	34.30±0.82	34.76±0.80	34.01±0.42
Lawn mix	Phenophase				PR (%)
	22	29	33	45	$\bar{x} \pm s_{\bar{x}}$
Turfline (A1)	x 56.40 a	x 55.95 a	x 55.03 a	x 55.05 a	55.61±0.69
RPR Regenerating(A2)	x 58.07 a	x 58.35 a	x 57.10 a	x 56.42 a	57.49±0.99
$\bar{x} \pm s_{\bar{x}}$	57.24±1.26	57.15±1.24	56.07±1.18	55.74±1.15	56.55±0.60

CC- $LSD_{5\%}$=3.20; PR-$LSD_{5\%}$=4.78; Different letters indicate signifficant at $p<0.05$ (a,b, for LM; x, for P)

Taking into account the interaction between watering treatments and phenophases (Table 4) it results that the chlorophyll content showed small and insignificant variations during the four phenophases, associated with amplitudes of 1.44 for the daily irrigated variant (V0) and 1.83 for the variant irrigated at 3 days. Regardless of the plant development stage, the watering treatment showed an insignificant influence on the chlorophyll content, on the background of some variations between 0.04 in phenophase 33 and 0.29 in phenophase 22.

Table 4. The effect of effect of watering and phenophase on chlorophyll content (CC) and photosynthesis rate (PR) of lawn

Watering	Phenophase				CC (SPAD)
	22	29	33	45	$\bar{x} \pm s_{\bar{x}}$
Daily (V0)	x 33.27 a	x 33.91 a	x 34.27 a	x 34.71 a	34.04±0.60
Every three days(V1)	x 32.98 a	x 33.80 a	x 34.31 a	x 34.81 a	33.98±0.58
$\bar{x} \pm s_{\bar{x}}$	33.13±0.85	33.86±0.84	34.30±0.82	34.76±0.80	34.01±0.42
Watering	Phenophase				PR (%)
	22	29	33	45	$\bar{x} \pm s_{\bar{x}}$
Daily (V0)	x 56.17 a	x 56.62 a	x 56.15 a	x 55.52 a	56.12±0.88
Every three days(V1)	x 58.30 a	x 57.67 a	x 55.98 a	x 55.95 a	56.98±0.83
$\bar{x} \pm s_{\bar{x}}$	57.24±1.26	57.15±1.24	56.07±1.18	55.74±1.15	56.55±0.60

CC- $LSD_{5\%}$=3.20; PR-$LSD_{5\%}$=4.78; Different letters indicate signifficant at $p<0.05$ (a, for W; x, for P)

The effect of the plant development stage on the chlorophyll content was slightly lower in the case of the Turfline mixture, on the background of an average rate of 0.037 SPAD and a more accentuated variation between the first two phenophases (Figure 1). In the case of plants of the RPR Regenerating mixture, the impact of phenophase on this character recorded an average value of 0.041 SPAD, associated with a relatively constant variation between phenophases. The high accuracy of these estimates is highlighted by the values of the coefficients of determination (0.9608-0.9935).

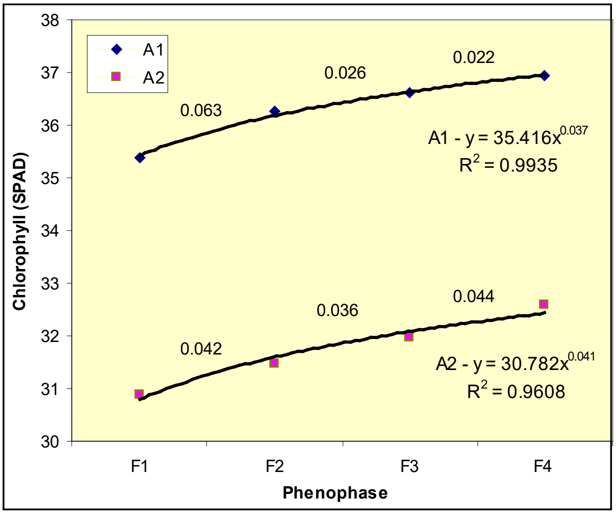

Figure 1. The variation of chlorophyll content during phenophases for different lawn mixture

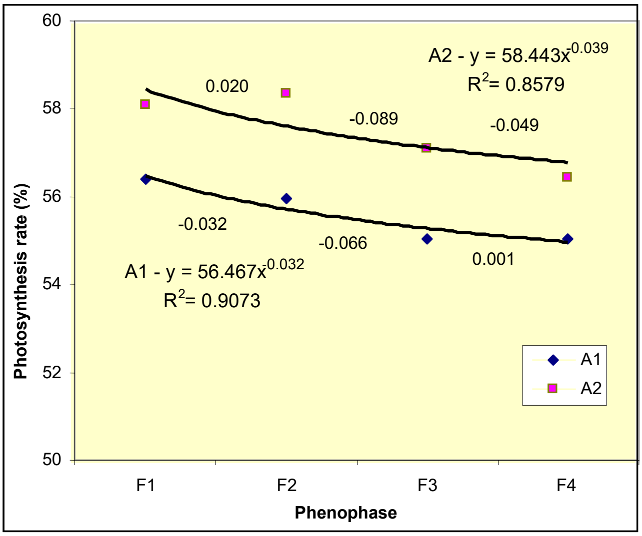

Figure 2. The variation of on photosynthesis rate during phenophases for different lawn mixture

On the background of a high accuracy (85.79-90.73%) it is estimated that the rate of photosynthesis has progressively decreased with the development of plants in the two mixtures. The respective variation was associated with average rates between -0.032% for the plants of the Turfline mixture and respectively -0.039% for the plants of the RPR Regenerating mixture (Figure 2). In both mixtures, the highest variation in photosynthesis rate is found between phenophases 29 and 33.

CONCLUSIONS

The results of the research show that irrigation did not significantly influence the chlorophyll content of the two types of lawn. Regardless of the plant stage of development, the watering treatment showed an insignificant influence on the chlorophyll content.

Turfline turf, irrigated daily, had a higher amount of chlorophyll compared to the 3-day irrigated version at all stages of development. In contrast, the RPR Regenerating lawn mixture had a higher chlorophyll content when watered at 3 days, compared to the daily irrigated variant, which indicates that RPR Regenerating is more drought resistant than Turfline.

Comparing the two lawn mixtures, the three-day irrigated RPR Regenerating recorded higher values in terms of photosynthetic capacity. In both mixtures, the highest variation in photosynthesis rate was found between phenophases 29 and 33.

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COMPARATIVE STUDY ON THE BEHAVIOUR OF *PLECTRANTHUS FORSTERI* AND *COLEUS BLUMEI* SPECIES GROWING ON THE GROUND AND IN VERTICAL SYSTEMS FOR GREEN FAÇADES IN THE CLIMATE OF NORTH-EAST ROMANIA

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Abstract

The flowering species Plectranthus forsteri and Coleus blumei, stand out with their compact foliage, particularly decorative and a high degree of coverage in a short time. This paper aims to study the behaviour of these species in vertical systems for green façades, in the climatic conditions of North East part of Romania. The vertical experiment was set up on the façades of an experimental module built specifically to test the strength and adaptability of several ornamental species in this system. The experimental structure was made of height levels that were applied on the four facades of the module oriented towards the four cardinal points. A ground control variant has also been set up. The study found that Plectranthus forsteri and Coleus blumei grown in vertical systems for green façades show high adaptability regardless of the cardinal orientation. On the ground, Plectranthus forsteri behaved well, creating a compact and uniform layer unlike Coleus blumei which had a more modest behaviour in the experience.

Key words: *Plectranthus, Coleus, green façades.*

INTRODUCTION

Plectranthus forsteri Benth. (syn. *P. coleoides* Benth.) and *Coleus blumei* Benth. (syn. *C. hybridus* Cobeau, Coleus x *Solenostemon scuttellarioides* (L.) Codd.) are two flowering species commonly used mainly as potted plants for both interior decoration and for the decorations of balconies and terraces in the warm season.

The *Coleus* species is also found in the seasonal decoration of parks and gardens, in borders, carpets, florals, arabesques (Toma, 2009). A study conducted in Bucharest identifies the species present in the decorative assortment of four out of six analysed parks (Mănescu et al., 2019). The classification of flowering species according to decorative features places the two species in the same category, those decorative by leaves, but they have other similar characteristics, such as a similar degree of branching, short-term coverage and moderate environmental requirements. *Pectranhtus forsteri* develops longer shoots (stems), about 100 cm, which makes this species an excellent plant for hanging pots, the edge of railings or other places where it can display its hanging port (Toma, 2009), once rooted, tolerates drought (https://plants.ces.ncsu.edu/plants/plectranthus-forsteri-marginatus/).

Coleus blumei is more compact, shoots usually have shorter lengths of about 40-60 cm, with less hanging port. Species is very sensitive to excess moisture and low temperatures. Both prefer sunny locations.

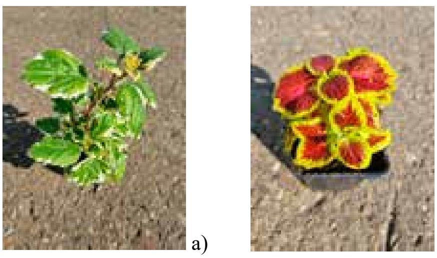
From a coloristic point of view, *Plectranthus* is presented only in shades of green and green with white-yellow (Chelariu, 2015), while *Coleus* offers a very varied range of colours pink, red, purple, green, yellow and various combinations between them (Toma, 2009). Its attractive foliage is of interest throughout the year (https://plants.ces.ncsu.edu/plants/coleus-scutellarioides/).

This causes the association of the two species to lead to floral arrangements with special decorative effects.

A growing concern among researchers is to find solutions to reduce the pollution installed in cities caused by excessive urbanization, which produces many negative effects on public health (Peschardt et al., 2012; Price et al., 2015). Expansion of green areas would substantially contribute to its reduction, reduce the urban island effect and contribute to raising quality of life (Chiesura, 2004; Pérez-Urrestarazu et al., 2016), but the lack of free construction land makes it remains, at this moment, a desideratum. One possible solution would be to extend these areas to the roofs and façades of existing buildings by creating green roofs and façades (Ghazalli et al., 2019). At the same time, they would make a substantial contribution to reducing aridity and beautifying densely built urban areas (Dascălu and Cojocariu, 2016).
In order for green façades to achieve their intended purpose, it is absolutely necessary to plant them with ornamental species that grow harmoniously, have a high degree of coverage, decorate for as long as possible and, of course, require little maintenance.
Therefore, in order to include plants in an assortment of decorative species that best meet the above requirements, they need to be tested in advance in local climatic conditions.

MATERIALS AND METHODS

The study material is represented by two flowering species *Plectranthus forsteri* (Figure 1a) and *Coleus blumei* (Figure 1b) decorative by leaves.



a) *Plectranthus forsteri* b) *Coleus blumei*

Figure 1. Study material - seedling

In order to establish the experiment, a uniform, branched seedling was used, purchased from local producers of flowering floricultural material. Plants were placed on the faces of an experimental structure built in order to study the behaviour of flower species planted vertically. Each face of experimental module was oriented towards a cardinal point.
Experience was established in the didactic field of the Floriculture discipline within the Faculty of Horticulture from ULS Iași and the research was carried out from the end of May when seedling was planted, until mid-October 2021.
Faces of experimental structure (Figure 2) consist of four equal, individual tiers arranged in layers. Total height is 2.40 m. Levels have been numbered from bottom to top, so that level 1 (P1) is at the bottom, closest to the ground, and level 4 (P4) at the top of the vertical module.

Figure 2. Experimental module - West and South façades - August 2021

Specimens of *Plectranthus forsteri* and *Coleus blumei*, respectively, were placed in groups, on each level, one below the other, on two adjacent, distinct columns. In the floral didactic field, a control variant was set up, planted on the ground, for each species. Other annual and perennial flowering species are also being studied on the experimental structure.
Levels of experimental module benefited from 25 litres of water each month. Control variant did not receive additional water supply. No fertilizers were used in any variant.

Figure 3. Control variant *Plectranthus forsteri* - August 2021

Figure 4. Control variant *Coleus blumei* - August 2021

Characteristics of seedling before planting had the following average values for *Plectranthus forsteri*: diameter = 13.40 cm; height 12.95 cm; number of shoots = 1.80, length of shoots = 13.75 and for *Coleus blumei* diameter = 11.00 cm; height = 8.65 cm; number of shoots = 1.47; shoot length = 8.68 cm.

After planting, observations were made at an interval of about 6 weeks, on the following parameters: plant diameter, plant height and number of shoots and their length.

After first measurement in July, *Coleus blumei* plants were shortened to about 10 cm.

For plants placed vertically, plant diameter was considered to be the distance measured between the extremities of the plant, parallel to the façade, horizontally, and the height of the plant was considered to be the distance from the façade to the extremity of the plant measured perpendicular to façade.

Photographic monitoring was also performed to perform a dynamic visual assessment of ornamental characters. Initial rooting rate was also calculated during the monitoring period (survival rate). Comparisons were made between levels, between façades of experimental module and façades of experimental module and the control variant. Anova Single Factor and Anova Two Way with Replication were used for statistical interpretation of the data.

RESULTS AND DISCUSSIONS

Annual flowering species have a rapid growth rate and maintain their aesthetic values for several months during a growing season (Draghia and Chelariu, 2011) and are therefore often used for the decoration of parks and gardens - grown on the ground or balconies and terraces - grown in pots and planters. It is precisely these qualities mentioned above that have attracted the attention of our research team to be studied in vertical systems, for green façades.

During the summer-autumn season of 2020, under similar conditions, the hybrid ‘Big’ of species *Begonia semperflorens* was studied (Cojocariu et al., 2020) and in the summer-autumn season of 2021, simultaneously with the species presented in this paper, species *Antirrhinum majus*, as well as a species with erect stems, with red leaves and flowers belonging to the species *Begonia semperflorens* (works in progress) were monitored.

All these mentioned species showed a very good adaptability to vertical planting in systems for green façades ensuring a constant decoration throughout the monitored period.

At the same time, during 2020, a series of ten perennial flower species were tracked (Cojocariu et al., 2022) whose behaviour has been studied to identify a wider range of ornamental plants that can be used with good results in various roofing systems for buildings in Eastern Europe.

In the same geo-climatic conditions of Iași city during 2014-2015 period, a study was carried out that proposes as a support for plants grown vertically a multifunctional modular prototype to be used in open spaces analyses the behaviour of three perennial flower species: *Sedum spurium*, *Sedum reflexum* and *Vinca major* (Dascălu and Negrea, 2016).

This paper deals with the vertical behaviour of flower species *Plectranthus forsteri* and *Coleus blumei*.

Calculation of initial degree of rooting and during observation period was performed comparatively both between the levels of experimental module and on variants (the façades of experimental module oriented towards a cardinal and control point).

In July, in the case of *Plectranthus forsteri*, percentage of rooting on the respective levels by variants was 100%. By levels, the survival rate was high, especially on levels 3 (P3) and 4 (P4) where, in October, rooting/survival rates were 94.73% and 100%, respectively (Table 1). Exception is level 1 (P1), where most of the plants were lost. Here, in October, only 61.53% of the initially planted specimens survived.

On cardinal and control guidelines, percentage of loss was small during the monitoring period.

Thus, in October, on experimental module, the lowest percentage of rooting/survival of 82.35% was registered on Eastern façade and the highest, of 88.23% on Western façade. Control variant did not lose any plants during this period (Table 2).

Table 1. *Plectranthus forsteri* - Percentage of rooting/ survival rate on levels

Level	JULY	AUGUST	OCTOBER
P1	100%	69.23%	61.53%
P2	100%	94.11%	88.23%
P3	100%	94.73%	94.73%
P4	100%	100%	100%

Table 2. *Plectranthus forsteri* - Percentage of rooting / survival rate on variants

Variants	JULY	AUGUST	OCTOBER
North	100%	85%	85%
East	100%	94.11%	82.35%
South	100%	87.5%	87.5%
West	100%	94.11%	88.23%
Control	100%	100%	100%

At the level, diameter of *Plectranthus* plants had a unitary development, within the same set of measurements. Towards the end of monitoring period this continued for the plants on levels 2 (P2), 3 (P3) and 4 (P4) where plant diameter received average values between 28.67 (P2) and 29.80 (P4). On level 1 (P1), plants developed less, where the obtained average value was 26.54 cm (Figure 5).

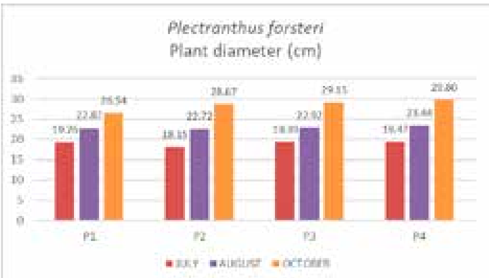


Figure 5. *Plectranthus forsteri* plant diameter dynamics on vertical module levels

Regarding height of *Plectranthus forsteri* plants (Figure 6), the lowest values were obtained on level 2 (P2) in all months in which observations were made. The highest values were reached by plants on levels 4 (P4) in October (27.11 cm) and on level 3 (P3) in August (20.79 cm). There were no significant differences in the same set of measurements.

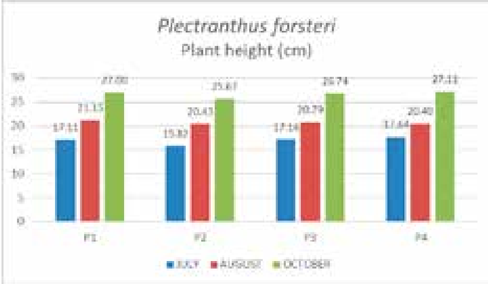


Figure 6. Height dynamics in *Plectranthus forsteri* on vertical module levels

Number of shoots per plant in June had from a statistical point of view, p *value* = 0.179635 > 0.05, equal averages per level, plants developing uniformly in terms of this parameter, but starting with August the number of shoots per plant recorded different average values (Table 3). Thus, plants on level 1 (P1) developed more shoots (3.0 in August and 3.25 in October respectively) compared to level 3 (P3) (1.83 in August and 2.56 in October respectively) and level 4 (P4) (2.00 in August and 2.17 respectively in October).

Table 3. Dynamics of number of shoots in *Plectranthus forsteri* on vertical module levels

Levels	JULY	AUGUST	OCTOBER
P1	2.43	3.00	3.25
P2	2.35	2.44	2.80
P3	1.79	1.83	2.56
P4	1.89	2.00	2.17

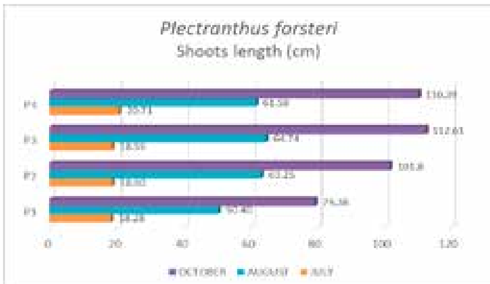


Figure 7. Dynamics of *Plectranthus forsteri* shoots' length on vertical module levels

In July and August, length of shoots per plant did not differ significantly in any of tiers. In October, on levels 2 (P2), 3 (P3) and 4 (P4), length of shoots obtained average values between 101.80 cm (P2) and 112.61 cm (P3). Also in October, on level 1 (P1) plants had an average shoot length of 79.38 cm (Figure 7).

Following Anova Two-Factor with Replication test, values of 1.72E-48 and 0.001565, respectively, were determined for p *value*, both lower than < 0.05, which indicates that both months in which measurements were made and cardinal orientation of the façade influenced the length of the shoots. In addition, interaction between these two factors (month and cardinal orientation) is also significant (p *value* = 0.005981 < 0.05).

Regarding diameter of *Plectranthus forsteri* plants on faces of experimental structure, there were no differences between the average values obtained in the same measurement.

Thus, in July they were in the range of 18.35 cm in the West and 19.96 cm in the South (Figure 8), in August in the range of 22.76 cm in the North and 23.48 cm in the West (Figure 9) and in October in the range of 28.20 cm in the North and 30.50 in West (Figure 10).

Diameter of plant, in control version, was larger than on the faces of vertical module, in each month of observations. It reached an average value of 25.39 cm in July, 35.56 in August and 44.00 cm in October.

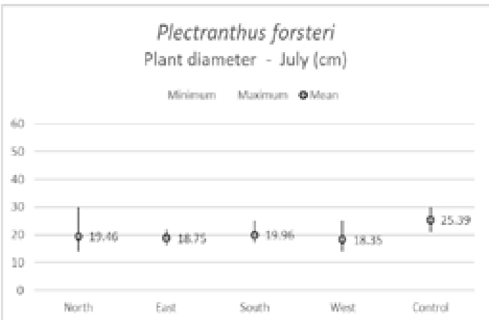


Figure 8. *Plectranthus forsteri* plant diameter on cardinal orientations and control - July 2021

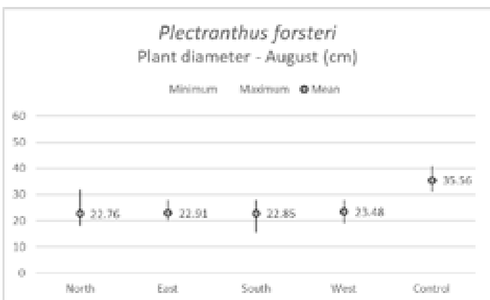


Figure 9 *Plectranthus forsteri* plant diameter on cardinal orientations and control - August 2021

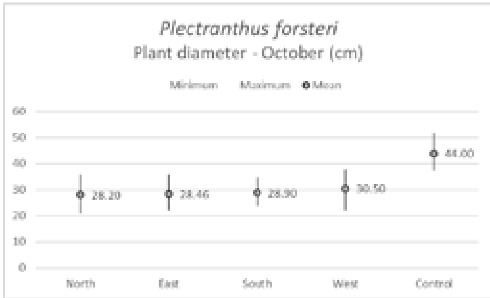


Figure 10. *Plectranthus forsteri* plant diameter on cardinal orientations and control - October 2021

In July, height of *Plectranthus* plants, on the faces, had close average values between 15.70 on North and 17.88 on East and different average values for control variant where a value of 25.75 cm was reached.

Starting from August, they developed differently in terms of height, both between faces of experimental module and between them and control variant. In August and October, the lowest values for this parameter, on the vertical structure, were obtained on Northern orientation (19.17 cm in July and 24.38 cm in August) and the highest on Western orientation (22.36 cm in August and 29.33 cm in October). The East and South orientations occupy the intermediate segment with 19.17 cm (August) and 20.50 (October) on East, respectively 20.48 cm (August) and 27.84 cm (October) on South. At control variant, height of plant reached an average value of 25.75 cm in August and 29.10 cm in October (Figure 11).

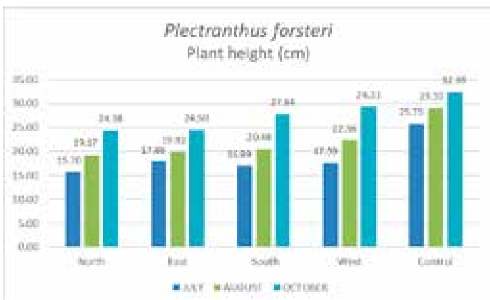


Figure 11. *Plectranthus forsteri* plant height - on cardinal orientations and control

These observations were statistically confirmed by Anova Single Factor test. Height of *Plectranthus forsteri* plant recorded equal average values on faces of experimental module

(p *value* = 0.29284 > 0.05) only in July. In August and October, the averages on cardinal orientations were different (August: p *value* = 0.041368 < 0.05; October p *value* = 0.041368 < 0.05). Compared to control variant, the averages were different in all three months (July: p *value* = 2.7E-10; August: p *value* = 1.16E-12 < 0.05, October: p *value* = 1.84E-12 < 0.05).
Number of shoots per plant had different averages both between cardinal orientations and compared to control variant in all the months in which observations were made (Table 4).

Table 4. Number of shoots in *Plectranthus forsteri* on cardinal orientations and control

Variants	JULY	AUGUST	OCTOBER
North	3.00	3.22	3.33
East	2.00	2.07	2.15
South	2.00	2.33	2.42
West	1.53	1.56	2.20
Control	2.29	4.07	4.93

On vertical structure, the highest number of shoots per plant was obtained on North façade (July: 3.00, August: 3.22, October: 3.33) and the lowest on West façade (July: 1.53; August: 1.56; October: 2.20) and East façade (July: 2.00; August: 2.07, October: 2.15). For control variant *Plectranthus* had the highest number of shoots per plant in August (4.07) and in October (4.93).
On the vertical module, length of shoots had a variable evolution during monitoring period. Thus, in July, no different values were registered on any of the faces (p *value* = 0.484353 > 0.05), these being in the range of 18.24 cm on Southern façade and 20.10 cm on Northern façade (Figure 12).

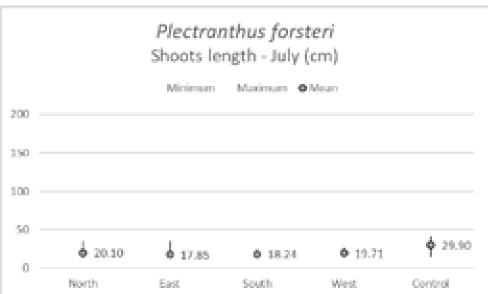


Figure 12. *Plectranthus forsteri* shoots length on cardinal orientations and control - July 2021

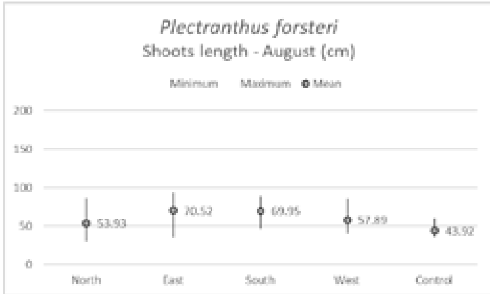


Figure 13. *Plectranthus forsteri* shoots length on cardinal orientations and control - August 2021

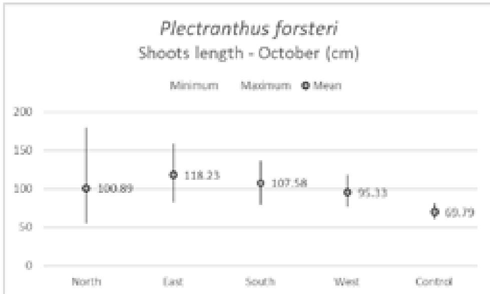


Figure 14. *Plectranthus forsteri* shoots length on cardinal orientations and control - October 2021

In August, the values were different (p *value* = 0.00272 < 0.05), ranging from 53.93 cm in the North to 70.52 cm in the East (Figure 13). In October, the averages become close again (p *value* = 0.063879 > 0.05), lengths of shoots gaining values in the range of 95.33 cm in the West and 118.23 cm in the East (Figure 14).
Between those four façades of the vertical experimental scheme and control variant, the average values are different in all three months in which observations were made (July: p *value* = 3.15E-08 < 0.05; August: p *value* = 2.05E-06 < 0.05; October: p *value* = 1.41E-06 < 0.05). Length of shoots, for control variant, had a moderate increase. If in July, length of shoots at species *Plectranthus* reached the highest average value of all variants - orientation and control - (29.90 cm) in August and October, this parameter recorded the lowest average values between variants (43.92 cm in August and 69.79 cm respectively in October). This development is justified by the culture system used.

Also, in the case of *Coleus blumei*, a high degree of rooting is recorded on the landings. In July, it was between 91.66% on level 3 and 100% on levels 1 (P1) and 2 (P2). Level 1 (P1), as at previous species, recorded the lowest percentage of rooting / survival, which was 75.0% in October (Table 5).

Table 5. *Coleus blumei* - Percentage of rooting/survival rate on levels

Levels	JULY	AUGUST	OCTOBER
P1	100%	83.33%	75.0%
P2	100%	91.66%	91.66%
P3	91.66%	83.33%	79.16%
P4	95.83%	95.83%	91.66%

Initially good rooting percentage and high survival rate were also obtained on cardinal orientations and control. Thus, in July, on experimental module, percentage of rooting varies between 95.83 on North, South and West orientations and 100% on East orientation. It can also be seen that this orientation was the most favourable for *Coleus blumei*, rooting/ survival percentage being, at the end of monitoring period, of 95.83%, the highest value recorded on vertical experimental structure. Similarly, as at previous species, control variant of *Coleus blumei* did not lose any plants during period in which observations were made (Table 6).

Table 6. *Coleus blumei* – Percentage of rooting / survival rate on variants

Variants	JULY	AUGUST	OCTOBER
North	95.83%	87.5%	83.33%
East	100%	95.83%	95.83%
South	95.83%	83.33%	79.16%
West	95.83%	87.5%	79.16%
Control	100%	100%	100%

On levels, in July and August, diameter of *Coleus blumei* plants had a unitary development. The average values were in July between 20.09 cm on level 2 (P2) and 22.40 cm on level 4 (P4) and in August between 23.84 cm on level 2 (P2) and 24.76 cm on level 4 (P4). In October, there are differences for plant's diameter. The biggest difference is between level 1 (P1) where diameter reached an average value of 29.14 cm and level 4 (P4) where the obtained average value was 31.75 cm (Figure 15).
To determine whether the two factors (month in which measurement was performed and the level on experimental module) influenced the diameter of plant, an Anova Two Factor with Replication test was performed.

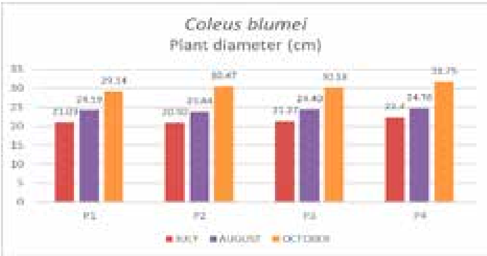


Figure 15. Dynamics of *Coleus blumei* plant diameter on vertical module levels

Thus, calendar month in which measurement was made influenced the diameter of plant, obtaining different average values per month (p *value* = 3.11198E-82 < 0.05). The averages obtained on levels were equal (p *value* = 0.099601017 > 0.05) which indicates that the level did not influence this biometric parameter. For the interaction between calendar month and level was determined p *value* = 0. 848120633 > 0.05 which shows that there is no interaction between those two factors.
Similarly, as in *Plectranthus*, plant height at *Coleus blumei* did not differ significantly in the same measurement. The average values in July were between 13.13 cm on level 2 (P2) and 14.80 cm on level 4 (P4) (Figure 16). In October differences were even smaller, of approx. 0.4 cm between levels.
The order of obtained values, from lowest to highest, is kept in each month in which measurements of biometric aspects were made. Plants on level 1 (P1) have the lowest height in all three sets of measurements. Followed by level (P2) and level 3 (P3) and, finally, with the highest height, the plants on level 4 (P4).

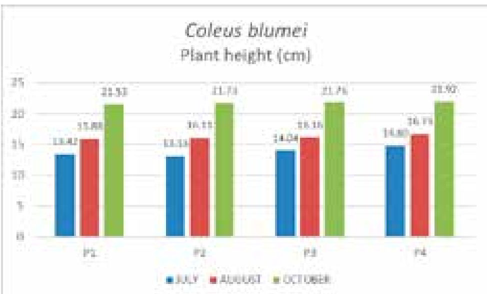


Figure 16. Height dynamics at *Coleus blumei* on vertical module levels

The variation in the number of shoots per plant was insignificant both between levels within the same measurement and between measurements (Table 7). Following Anova Single Factor test, this fact was statistically confirmed, with values higher than 0.05 for p *value* (July: p *value* = 0.216055; August: p *value* = 0.414283 and in October p *value* = 0.695675).

Table 7. Dynamics of number of shoots at *Coleus blumei* on vertical module levels

Levels	JULY	AUGUST	OCTOBER
P1	2.04	2.10	2.28
P2	2.13	2.36	2.41
P3	1.91	2.05	2.16
P4	1.65	1.96	2.14

Regarding length of shoots per plant at *Coleus* species, a p *value* = 0.003788 < 0.05 was obtained in July, which indicates a different development of the shoots per plant in that month. On level 4 (P4), shoots had the longest lengths, average being 18.06 cm. At the opposite pole are plants on level 2 (P2) where length of shoots reached an average value of 14.27 cm.

After measurements, in July, plants were shortened to about 10 cm from the plan of the façade. This pruning was beneficial because, later, development of the shoots of *Coleus blumei* plants on the vertical module was more uniform. In August, the average values for length of shoots received values between 22.05 cm (P1) and 23.14 cm (P4).

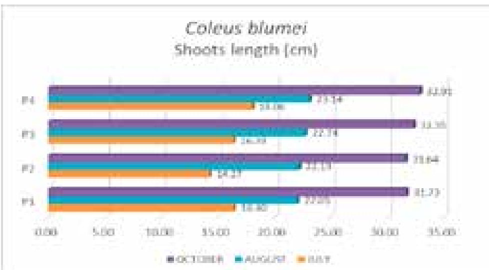


Figure 17. Dynamics of *Coleus blumei* shoots length on vertical module levels

Diameter of *Coleus blumei* plants on the faces of experimental module did not differ between the average values obtained in the same measurement. The lowest values were noted on North façade, and the highest on South façade in all three months in which observations were made.

The average values of diameters on faces of vertical module were in July, between 20.54 cm on North and 21.85 cm on South (Figure 18) in August between 23.65 cm on North and 25.40 cm on South (Figure 19) and in October between 28.70 cm on North and 32.11 on South (Figure 20).

At control variant, plant diameter had values close to the average values obtained on the faces of experimental module. If in July the average was 23.82 cm, higher than any value obtained on experimental structure, in October plant diameter had an average of 28.51 cm, lower than any value obtained on experimental structure in the same month.

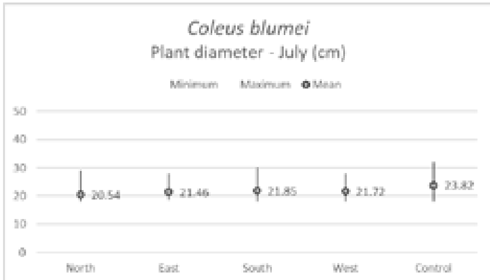


Figure 18. Diameter of the *Coleus blumei* plant on cardinal orientations and control - July 2021

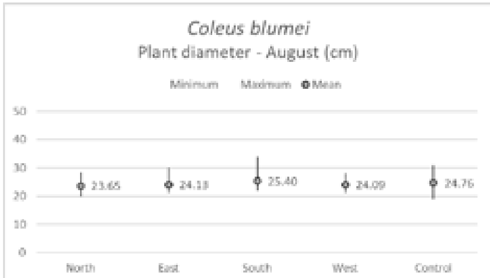


Figure 19. Diameter of the *Coleus blumei* plant on cardinal orientations and control - August 2021

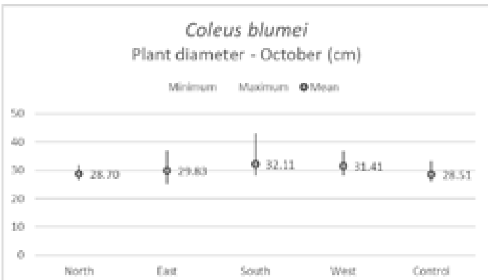


Figure 20. Diameter of the *Coleus blumei* plant on cardinal orientations and control - October 2021

There were no differences in height of *Coleus blumei* plants between faces of experimental module in any month in which observations were made. Statistically, the following values were obtained for p value: July (p *value* = 0.653596), August (p *value* = 0.894886), October (p *value* = 0.443128) higher than 0.05, which confirms the observations done previously. At control variant, plants had a higher height than the one on experimental module, in all monitoring months (Figure 21).

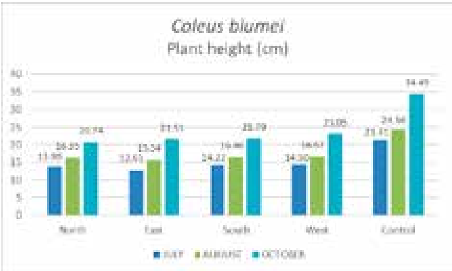


Figure 21. Height of *Coleus blumei* plant on cardinal orientations and control

Statistical analysis performed for all 5 variants (North, East, South, West and control) calculates a p *value* <0.05 (July p *value* = 5.18262E-09; August p *value* = 2.35326E-07; October p *value* = 2.56867E-09) which confirms that plants on the ground developed differently in height than those planted vertically.

The average number of shoots in *Coleus* plants on cardinal orientations and control didn't differ significantly between faces of vertical module or between them and control variant, regardless of month in which observations were made (Table 8). In July, on cardinal orientations, it received values between 1.78 (West) and 2.09 (North) and in October, between 2.00 (West) and 2.50 (North). The highest average value was obtained at control variant, in each month in which observations were made.

Table 8. *Coleus blumei* - Number of shoots on cardinal orientations and control

Variants	JULY	AUGUST	OCTOBER
North	2.09	2.33	2.50
East	1.92	2.04	2.09
South	1.96	2.30	2.42
West	1.78	1.81	2.00
Control	2.42	2.64	2.85

The order of the obtained values was kept by variants in all three sets of measurements. Thus, the lowest value was on West followed by East, South and North.

Between faces of vertical mode, in July and August, the average values for length of shoots don't register differences (July p *value* = 0.405428 and August p *value* = 0.873599, both values being > 0.05) (Figure 22 and Figure 23). In October, however, p *value* = 8.3E-21 < 0.05 indicating that length of shoots was different on cardinal orientations (Figure 24). The highest average value of 38.48 cm was obtained on Southern façade and the lowest on the Eastern one of only 28.99 cm.

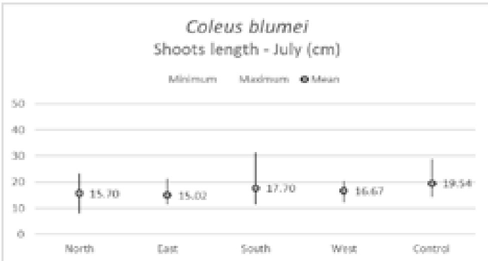


Figure 22. *Coleus blumei* shoots length on cardinal orientations and control - July 2021

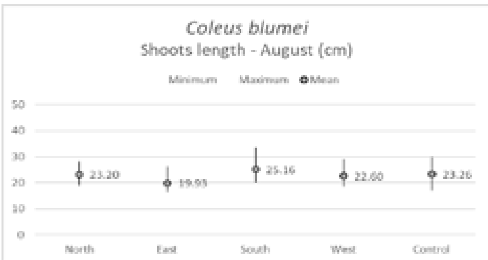


Figure 23. *Coleus blumei* shoots length on cardinal orientations and control - August 2021

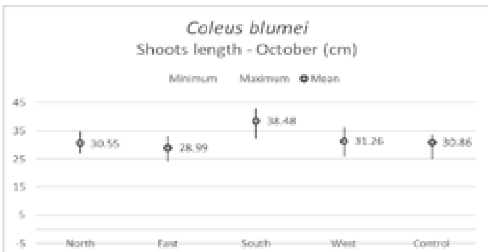


Figure 24. *Coleus blumei* shoots length on cardinal orientations and control - October 2021

At control variant, the obtained values were higher than those on vertical structure of 19.54 cm in July and 23.26 cm in August.

In October, however, the average length of shoots at control variant obtained an average value of 30.86 cm, lower than that obtained in Southern orientation, within the same set of measurements.

CONCLUSIONS

Plectranthus forsteri and *Coleus blumei* have shown high adaptability to vertical planting. They had a high initial rooting and a good survival rate, quickly covering the vertical surface. For *Plectranthus* species, levels 3 (P3) and 4 (P4), respectively Western orientation obtained the best results from this point of view and *Coleus* species on levels 2 (P2) and 4 (P4) respectively on Eastern orientation. At control variant, for both species, the rooting percentage and survival rate was 100% throughout the experiment.

In July and August, both on levels and on faces of experimental module, diameter of *Plectranthus forsteri* plants had a unitary development, within the same set of measurements. In October, there are differences between plants on levels 2, 3 and 4 compared to those on level 1 (P1), where their diameter is lower. A similar evolution was found in the case of *Coleus blumei*, except for October, when on level 4 (P4) plants grew more in diameter.

On the vertical module, length of shoots at *Plectranthus* species had a variable evolution during the monitoring period.

Position of *Plectranthus* plants in vertical system, as well as the hanging character of the plant, favoured growth in length of its shoots, especially on upper levels as well as on Eastern orientation. A similar evolution on levels but with smaller differences was also observed in the case of *Coleus blumei* species. For this, Southern orientation was the most favourable for the increase in length of shoots.

At control variant, *Plectranthus forsteri* had a good behaviour, creating a compact and uniform layer unlike *Coleus blumei* which recorded a more modest behaviour on the ground in the experiment.

Periodic visual evaluation showed that those two species studied maintained their aesthetic values throughout the experience.

At the same time, maintenance works were minimal from establishment until end of the season.

All these recommend these two species to be used in decoration of vertical surfaces. Also, the uniform appearance on ground of *Plectranthus* species recommends it to be used for compact floral borders.

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YIELD PERFORMANCES OF QUINOA FOR LEAVES UNDER IRRIGATION AND FERTILISATION REGIME

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Abstract

Quinoa is a pseudo-cereal native to South America, known mainly for seeds. In recent years, studies and research have begun to be done on leaves, as it is known that in the area of origin, some local populations used as vegetables. The aim of the research is to evaluate the effect of fertilisation and irrigation on the growth and production of two quinoa varieties (Vikinga and Puno), in order to introduce them on the Romanian economic market. The experience was organised in vegetation pots, in 42 variants, in the greenhouse. The obtained results show that the species is suitable for cultivation in protected areas, under the influence of factors: cultivar, fertilisation and irrigation. The highest amount of leafy mass was obtained by Vikinga variety under biological fertilisation irrigated with 75% of water from substrate capacity (SC) positively correlated with the leaf area and the number of leaves. The irrigation with a rate of 75 % of the substrat capacity obtained the best results, compared to the regimes of 50 % and 100 % of the substrate capacity.

***Key words**: quinoa, cultivar, fertilisation, irrigation.*

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is considered a pseudo-cereal, native from Latin America. The species still has a strong traditional imprint, even if new modern practices appear, due to studies conducted at the University of Colorado (USA) and in Europe (Pedersen at al., 2015; Mujica A., 2001).

The cultivation of the species was largely abandoned with the arrival of the Spanish conquerors, who replaced the quinoa plant with cereals brought from Europe (wheat and barley), much more productive at that time. The quinoa plant is currently grown throughout the Andean region, in the USA, in Europe, Asia and Africa (Bazile et al., 2016; Mazoyer et al., 2006).

Quinoa is a plant grown mainly for its edible seeds, with a high degree of digestibility (Asao et al., 2010). Also, the leaves can be eaten as a substitute for spinach, in various dishes, well known in the area of origin (Stoleru et al., 2021; Vitanescu, 2020).

The nutritional value of quinoa leaves is special, quinoa is a very interesting food, being a precious source of protein, vitamins and minerals (FAO, 1992).

According to the Food and Agriculture Organization of the United Nations (FAO), quinoa can assure the global food security due to its high nutritional qualities as well as tolerance to various abiotic stresses including salinity (FAO, 2013).

Due to the fact that it can be grown in the fields, as well as in tunnels and greenhouses, quinoa can ensures also a sustainable production throughout the year (Stoleru et al., 2022; Pedereson et al., 2020).

An intresting scientific papers highlight the unique nutritional value of quinoa leaves, both in terms of nutritional and anti-nutritional. One of this work brings to the fore the insignificant role of anti-nutritive substances (such as oxalates, saponins and trypsin inhibitors), content of three cultivars of quinoa (Titicaca, Puno and Vikinga), grown for its leaves and is subject to density of 7.7, 3.2 and 1.6 mil plants/ha and times of sowing by April 17. The content of precious minerals (Fe, Zn, Na and K) was significant depends on the cultivar compared to Mg and Ca, whose insignificant

values did not depended on treatments (Stoleru et al., 2019).
In this respect, the efforts of researchers are mainly focused on the following research directions, namely: drought resistance, salinity and the defence mechanisms of the quinoa species against abiotic stressors - drought and salinity.
Another research about abiotic stress such drought and salinity have been conducted to evaluate the effects of Biochar in relieving stress independent drought or salinity. The pots experience was conducted in the climate-controlled chamber to investigate the effects of Biochar on growth, physiology and yield of quinoa under independent and combined drought and salinity stress (Yang, 2020).
The results showed that Biochar, as an amendment to the soil, has the potential to improve the soil and alleviate the stress of drought and salinity (Yang, 2020).
A study was conducted in 2014 on the effect of organic and chemical fertilisation on the yield and quality of quinoa biomass and green amaranth biomass, intended for animal feed in the Mediterranean area, during the dry season bibliografie (Papastylianou et al., 2014)
The results shwoed the superiority of quinoa species over the green amaranth in terms of plant height and dry substance, while there were no significant differences in nutritional value and biomass between the two species.
Compost fertilisation showed higher values in terms of biomass quality in quinoa cultivation, while chemical fertilisation gave better results in amaranth cultivation (Papastylianou et al., 2014).
The aim of our research was to establish the upper and lower limits, which the quinoa species could tolerate without significantly affecting the growth and development, being subject to the influence of the irrigation and fertilisation factors.

MATERIALS AND METHODS

The research was carried out in a greenhouse of Iasi University of Life Sciences (IULS), Romania, during March 29 to April 07. The goal of study was to evaluate quinoa response to different regimes of fertiliser and irrigation doses under controlled conditions of temperature (16-18ºC/20-22ºC), humidity (70-75%/60-65%) and natural light (13/11 hours).
The biological material used was represented by quinoa seeds of two cultivars Puno and Vikinga. The seeds were kindly provided by Quinoa Quality ApS (Denmark) (Figures 1-3).

Figure 1. View of quinoa seeds

All plants were harvested at 35 days after sprouting (DAS) and all leaves were collected for further measurements and determinations.


Figure 2. View of quinoa leaves at the harvest time

Soil substrate was peat Kekkila® (300 l x 2) mixed with Orgevit® (3.00 kg/m^3) and Perlite® (3.00 kg/m^3).
Kekkila® is a substrate for seeding production, with pH adjusted to 5.5-5.9, with fertilised formula “starter” NPK 14-16-18 + ME.
Orgevit® is a fertiliser that can be used in organic crops and contains micro and macro-elements.
Perlit® results from volcanic rock with a granular structure and high porosity, produce rapid rooting of seedlings and seed germination, ensuring a harmonious development of plants.

The fertilisers used were represented by biologic fertiliser Micoseed MB® and chemical fertiliser KSC® II, in different quantities.
To test the influence of type of fertilisations on plant growth were used the following amounts:
- Biological fertilisation: 500 g/m^3 (F1); 1000 g/m^3 (F2); 1500 g/m^3 (F3) - Micoseed MB®;
- Chemical fertilisation: 1000 g/m^3 (F4); 2000 g/m^3 (F5); 3000 g/m^3 (F6) KSC®.

For the watering of the substrate, water was used in different percentages quantities 50%/75%/100% of substrate capacity.

Figure 3. View from the greenhouse of Iasi University of Life Sciences (IULS), Romania

The experience was organised in vegetation pots (2700 cm^3 capacity). Corresponding to the proposed factors resulted 42 variants, of 5 replicates, 8 plants for each repetition.
For statistical analyses the data are expressed as the means ± standard deviation (SD). The analysis of variance (ANOVA) was used to see the influence of cultivar, fertilisation and irrigation on the number of leaves, chlorophyll pigments, leaf surface and green leaf biomass of quinoa.
To determine the significant differences between treatments were established by using Tukey's post hoc test with a degree of confidence of 95% ($p \leq 0.05$), using a SPSS ver. 21.

RESULTS AND DISCUSSIONS

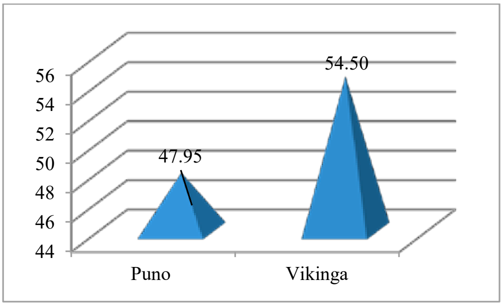
The results on the influence of the cultivar on the number of leaves, photosynthetic pigments, leaf surface and production are presented in Table 1.

Table 1. The influence of the cultivar on the morphological and photosynthetic indicators

Cultivar	No. of leaves	Pigments (CCI)	LAI (cm^2)
Puno	110.3 ± 2.6 b	11.9 ± 0.3 b	1454.5 ± 69.2 ns
Vikinga	120.5 ± 2.34 a	13.4 ± 0.2 a	1447.0 ± 43.8 ns

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns - nonsignificant).

The number of leaves is the character which recorder significant differences between the two variants, these being 110.4 leaves for the Puno and 120.6 leaves for Vikinga, the difference between the two cultivars is 10.2 leaves.
Also, the data from Table 1 show that the maximum value of the chlorophyll index recorded is 13.4 CCI in the case of the Vikinga cultivar and the minimum value is 11.9 CCI in the case of the Vikinga cultivar.
Regarding the study of influence of the cultivar on the leaf area, the data show that there are no significant differences, obtaining close value (1454.5 cm^2-1447.0 cm^2). Also, between the two cultivars there were no significant differences in production (47.95 leaves-54.50 leaves), as in Figure 4.



*The values represent the mean ± standard error.

Figure 4. The influence of the cultivar on the production

The results of the influence of the fertilisation regime on the number of leaves, photosynthetic pigments, leaf surface and production are presented in Table 2.

Table 2. The influence of the fertilisation on morphological and photosynthetic indicators

Fertilisation	No. of leaves	Pigments (CCI)	LAI (cm²)
NF	133.5 ± 6.5 a	10.1 ± 0.2 b	1597.3 ± 91.5 ab
F1	127.6 ± 3.2 a	9.9 ± 0.2 b	1723.3 ± 53.0 ab
F2	124.4 ± 1.4 a	9.9 ± 0.2 b	1908.3 ± 86.8 a
F3	116.7 ± 3.5 a	10.5 ± 0.2 b	1533.1 ± 64.1 b
F4	131.5 ± 3.7 a	15.2 ± 0.5 a	1613.2 ± 76.1 ab
F5	93.2 ± 3.9 b	17.1 ± 0.7 a	1062.3 ± 75.2 c
F6	81.2 ± 2.8 b	15.8 ± 0.9 a	718.0 ± 71.7 d

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant, V-Vikinga, 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)

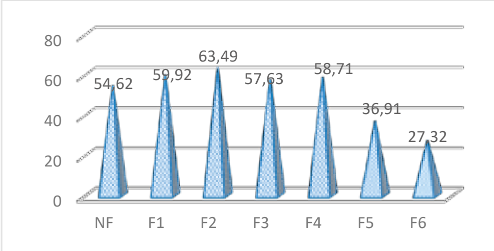
The effects of fertilisation on the growth of quinoa plants are shown in Table 2, where significant differences are observed between control and chemical variants, where the value varied from 133.5 leaves to 81.2 leaves, for the character number of leaves.

Also, higher values (127.6 leaves and 124.4 leaves) are observed for the biologically fertilised variants F1 - F2, compared to the chemically fertilised variants F5 and F6. There was a significant difference of 50.3 leaves between chemically fertilised F4 and F6 variants. It can be concluded that the plants suffer from a higher concentration of chemical fertiliser > 2000 g/m³ KSC I.

The data of Table 2, showed that in the case of chemical fertilisation F5, the photosynthetic pigments have the highest chlorophyll index of 17.1 CCI. We can also notice significant differences between biologically and chemically fertilised variants. Between biological variants F1-F3 there is a significant difference, also between the chemically fertilised variants F4-F6.

For the character of the leaf surface the values varied from 718.0 cm², in case of the variant F6, to 1908.3 cm², in case of the variant F2.

Regarding the production, the results presented in Figure 5, in the case of biological fertilisation with Micoseed MB®, variants F1-F3, recorded higher values compared with control. The maxim value obtained was 63.4g, of variant F2, due to the effect of microorganisms introduced into the crop substrate, this being also the maximum value registered within the experimental variants. Also, the variant F4 registered a higher value than the control.



*The values represent the mean ± standard error; 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 5. The influence of the fertilisation regime on the production

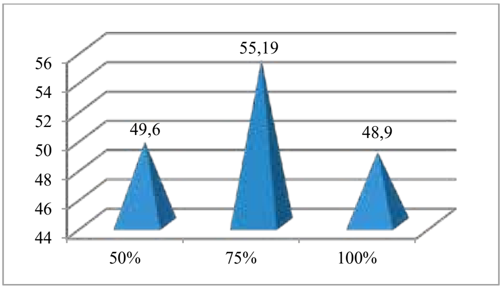
The variant F5 and F6 with the minim values of the measured biometric indicators- number of leaves, leaf surface and production, shows significant differences compared with all the other variants. This is explained by the fact that the concentration > 2000 g/m³ affects the growth and producion of quinoa plants.

The results on the influence of the irrigation factor on the number of leaves, photosynthetic pigments, leaf surface and production are presented in Table 3.

Table 3. The influence of the irrigation on morphological and photosynthetic indicators

Irrigation	No. of leaves	Pigments (CCI)	LAI (cm²)
50%	112.9 ± 2.7 ns	12.6 ± 0.2 ns	1666.0 ± 40.2 a
75%	118.9 ± 2.7 ns	12.3 ± 0.5 ns	1419.9 ± 68.1 b
100%	114.5 ± 2.7 ns	13.0 ± 0.3 ns	1266.4 ± 43.1 b

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns - nonsignificant); The values represent the mean ± standard error.



*The values represent the mean ± standard error; 50%, 75%, 100% irrigation regime

Figure 6. The influence of the irrigation regime on the production

The variants benefited from gradual irrigation starting from 50%, 75% and 100% of the substrate capacity (Figure 6).

For the character of the leaf surface, there are significant differences, the higher value of 1666.0 cm² recorded by irrigation variant of 50%, determines significant differences compared to the other two types of irrigation (75% and 100%). The results on the combined influence of factors (cultivar x fertilisation x irrigation) on the number of leaves, photosynthetic pigments, leaf surface and production are presented in Table 4.

The results on the combined influence of factors on the number of leaves, in the case of the quinoa species, we can notice that the results varied depending on the cultivar, fertilisation and irrigation regime, obtaining the following values for the number of leaves - from 62.00 leaves, in the case of the the chemical variant Vikinga - F6, to 144.60 leaves in the case of unfertilised Vikinga, with an irrigation rate of 75%.

Table 4. The influence of the combined factors on the number of leaves

Treatment	**Irrigation regime**		
Interaction	50%	75%	100%
P x NF	116.60 ± 11.58 abcdefg	118.00 ± 15.62 abcdefg	116.00 ± 10.70 abcdefg
P x F1	127.20 ± 5.61 abcdef	131.00 ± 7.91 abcde	120.20 ± 6.22 abcdef
P x F2	125.60 ± 3.98 abcdef	118.60 ± 2.99 abcdefg	112.40 ± 7.08 abcdefgh
P x F3	117.20 ± 2.85 abcdefg	121.20 ± 13.58 abcdef	116.40 ± 6.11 abcdefg
P x F4	117.40 ± 4.97 abcdefg	131.80 ± 6.68 abcde	140.80 ± 11.58 abc
P x F5	87.80 ± 5.94 defgh	83.80 ± 8.96 efgh	83.60 ± 8.53 efgh
P x F6	78.00 ± 18.94 fgh	68.20 ± 7.96 gh	86.40 ± 11.33 defgh
V x NF	161.60 ± 10.83 a	144.60 ± 11.71 ab	144.40 ± 10.21 ab
V x F1	131.00 ± 8.88 abcde	132.60 ± 6.82 abcde	123.80 ± 3.76 abcdef
V x F2	124.60 ± 2.99 abcdef	125.80 ± 6.97 abcdef	139.60 ± 9.68 abc
V x F3	112.40 ± 0.75 abcdefgh	132.40 ± 6.07 abcde	101.00 ± 20.53 bcdefgh
V x F4	134.20 ± 7.56 abcde	135.80 ± 4.85 abcd	129.40 ± 9.81 abcdef
V x F5	85.80 ± 7.00 defgh	126.40 ± 6.59 abcdef	92.20 ± 9.17 cdefgh
V x F6	62.00 ± 5.83 h	95.40 ± 3.93 bcdefgh	97.40 ± 11.12 bcdefgh

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant, V-Vikinga, NF – control, F1- 500 g/m^3 MB, F2 –1000 g/m^3 MB, F3- 1500 g/m^3 MB, F4 –1000 g/m^3 KSC I, F5 –2000 g/m^3 KSC I, F6- 3000 g/m^3 KSC I)

Table 5. The results on the combined influience of factors on the chlorophyll pigments (CCI)

Treatment	**Irrigation regime**		
Interaction	50%	75%	100%
P x NF	10.68 ± 0.80 defgh	9.84 ± 0.19 gh	9.96 ± 0.84 gh
P x F1	9.60 ± 0.32 gh	9.94 ± 0.16 gh	9.68 ± 0.52 gh
P x F2	9.72 ± 0.31 gh	9.44 ± 0.62 gh	10.92 ± 0.48 cdefgh
P x F3	10.78 ± 0.97 cdefgh	9.74 ± 0.31 gh	9.74 ± 0.45 gh
P x F4	14.82 ± 1.55 abcdefgh	13.80 ± 1.77 bcdefgh	14.20 ± 1.08 abcdefgh
P x F5	15.10 ± 0.53 abcdefgh	14.96 ± 1.61 abcdefgh	15.32 ± 1.59 abcdefgh
P x F6	14.32 ± 0.55 abcdefgh	12.74 ± 3.24 bcdefgh	15.00 ± 1.44 abcdefgh
V x NF	9.42 ± 0.24 h	10.46 ± 0.91 fgh	10.30 ± 0.38 fgh
V x F1	10.02 ± 0.77 gh	10.00 ± 0.44 gh	10.72 ± 0.62 cdefgh
V x F2	9.76 ± 0.73 gh	10.36 ± 0.54 fgh	9.76 ± 0.52 gh
V x F3	10.5 ± 0.34 efgh	9.74 ± 0.37 gh	12.54 ± 0.93 bcdefgh
V x F4	15.22 ± 0.84 abcdefgh	16.16 ± 0.59 abcdefg	17.22 ± 1.96 abcde
V x F5	19.18 ± 1.35 ab	17.44 ± 1.48 abc	20.82 ± 1.60 a
V x F6	17.40 ± 1.35 abcd	18.76 ± 3.09 ab	16.88 ± 1.98 abcdef

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant, V-Vikinga, NF – control, F1- 500 g/m^3 MB, F2 –1000 g/m^3 MB, F3- 1500 g/m^3 MB, F4 –1000 g/m^3 KSC I, F5 –2000 g/m^3 KSC I, F6- 3000 g/m^3 KSC I)

The physiological character represented by the chlorophyll pigments varied from 9.42 CCI, in the case of control Vikinga cultivar, with on irrigation rate of 50%, to 20.82, in the case of chemically fertilised Vikinga cultivar, F5 variant, with on irrigation rate of 100%.

Table 6. The results on the combined influience of factors on the leaf surface (cm²)

Treatment	Irrigation regime		
Interaction	50%	75%	100%
P x NF	1983.40 ± 261.95 abcd	1704.00 ± 209.22 abcdefghi	1079.20 ± 238.09 defghijkl
P x F1	2489.60 ± 127.93 a	1602.4 ± 200.96 abcdefghij	1202.00 ± 144.96 cdefghijkl
P x F2	2462.80 ± 138.37 ab	1560.80 ± 147.83 abcdefghij	1806.20 ± 214.55 abcdefg
P x F3	2072.60 ± 210.16 abc	1312.80 ± 268.70 cdefghijkl	1313.80 ± 93.48 cdefghijkl
P x F4	1959.40 ± 277.8 abcde	1603.60 ± 147.47 abcdefghij	1428.20 ± 58.87 cdefghijk
P x F5	1579.20 ± 144.71 abcdefghij	775.60 ± 186.08 ijkl	719.40 ± 106.77 jkl
P x F6	825.00 ± 282.63 hijkl	486.60 ± 63.77 l	579.40 ± 144.7 kl
V x NF	1700.60 ± 89.19 abcdefghi	1546.80 ± 91.58 bcdefghij	1570.20 ± 227 abcdefghij
V x F1	1698.60 ± 105.65 abcdefghi	1818.60 ± 55.42 abcdefg	1529.00 ± 148.97 cdefghij
V x F2	1814.00± 113.58 abcdefg	1716.20 ± 139.62 abcdefgh	2090.20 ± 103.31 abc
V x F3	1535.40 ± 30.16 bcdefghij	1868.00 ± 161.19 abcdef	1096.20 ± 227.09 defghijkl
V x F4	1636.60 ± 54.78 abcdefghij	1423.00 ± 82.48 cdefghijk	1628.60 ± 235.68 abcdefghij
V x F5	986.00 ± 109.10 fghijkl	1419.20 ± 100.17 cdefghijkl	894.40 ± 175.35 ghijkl
V x F6	580.80 ± 85.82 kl	1042.20 ± 104.74 efghijkl	794.00 ± 234.86 hijkl

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant, V-Vikinga , NF – control, F1- biologic/ 500 g/m³ MB, F2 – biologic/1000 g/m³ MB, F3- biologic/1500 g/m³ MB, F4 – chemical /1000 g/m³ KSC I, F5 – chemical /2000 g/m³ KSC I, F6- chemical/3000 g/m³ KSC I)

Regarding the influence of the factors on certain morphological characteristics, the differences can also be observed, from 486.60 cm², in the case of the chemical Puno variant F6, with the irrigation rate of 100%, to 2489.60 cm², in case of chemical Puno variant F1, with the irrigation rate of 50%.

The values of the leaf surface decrease gradually with the increase of the quantity of water administered for the three irrigation regimes.

The conclusion is that the irrigation regime is negatively correlated with the leaf area.

Table 7. The results of the technological factors on the yield (g)

Treatment	Irrigation regime		
Interaction	50%	75%	100%
P x NF	47.42 ± 5.19 abcdefgh	47.34 ± 10.56 abcdefgh	45.48 ± 3.82 abcdefgh
P x F1	58.22 ± 2.91 abcdefg	63.00 ± 6.80 abcdef	48.41 ± 2.86 abcdefgh
P x F2	61.87 ± 3.81 abcdef	64.75 ± 3.79 abcde	54.05 ± 5.78 abcdefg
P x F3	57.74 ± 1.76 abcdefg	57.73 ± 9.99 abcdefg	64.14 ± 5.23 abcde
P x F4	53.20 ± 3.03 abcdefg	60.08 ± 3.60 abcdef	50.98 ± 3.05 abcdefgh
P x F5	35.41 ± 3.59 cdefgh	31.06 ± 7.25 efgh	35.00 ± 6.12 cdefgh
P x F6	24.26 ± 9.22 gh	17.65 ± 4.46 h	29.26 ± 9.94 fgh
V x NF	54.61 ± 2.93 abcdefg	64.81 ± 4.54 abcde	68.08 ± 9.90 abcd
V x F1	59.42 ± 2.44 abcdef	74.90 ± 2.21 a	55.61 ± 6.60 abcdefg
V x F2	63.94 ± 5.53 abcde	67.10 ± 5.53 abcd	69.21 ± 4.83 abc
V x F3	59.18 ± 2.56 abcdef	71.55 ± 6.82 ab	35.45 ± 11.48 cdefgh
V x F4	67.00 ± 1.53 abcd	63.78 ± 3.62 abcde	57.23 ± 5.94 abcdefg
V x F5	34.35 ± 4.53 defgh	50.50 ± 4.97 abcdefgh	35.16 ± 7.12 cdefgh
V x F6	17.82 ± 4.24 h	38.35 ± 3.57 bcdefgh	36.54 ± 13.96 cdefgh

*The values represent the mean ± standard error. The lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - represents the highest value; ns – nonsignificant, V-Vikinga , 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 production, only the aerial part of the plant was studied (the edible part). The values varied from 17.82 g in the case of the chemical Vikinga cultivar, variant F6 with the irrigation rate of 50%, to 74.90 g, in case of the Vikinga cultivar – F1, with the irrigation rate of 75%. The cultivar that obtained the best results is Vikinga, as in the case of the researches done by Vitanescu (Vitanescu et al., 2019).

In terms of chlorophyll pigments and production it can be notice that 100% irrigation regime favored positively.

CONCLUSIONS

1. Vikinga is the cultivar that obtained the best results, but the differences are not significant compared to Puno, in terms of production, which means that the species is suitable for leaves cultivation.
2. The highest yield of quinoa leaves was obtained from chemical fertilisation, under the influence of the fertilisation regime, followed by the biological, which is recommended for the sustainable crops.
3. The irrigation at a rate of 75% of the substrate capacity obtained the best results. In the case of overirrigation the results obtained were much lower than in case of 50% and 75% irrigation regime. It can be concluded that the species has mechanisms of resistance and adaptation to water stress.
4. The combined factors Vikinga, chemical fertilisation 500 g/m^3 with 75% of the substrate capacity gave the best results.
5. The result regarding all the factors shows that the specie is suitable for cultivation in protected areas.

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ANATOMICAL AND PHYSIOLOGICAL CHANGES IN NEEDLES OF *PINUS NIGRA* J.F. ARNOLD REVEAL URBAN TRAFFIC AIR POLLUTION DRIVEN EFFECTS

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Abstract
The presence of trees in the urban area has many advantages for improving the environmental conditions and implicitly for the urban health and wellbeing. The urban environment, in turn, can be a polluting factor that can reduce trees health and physiological performances, due to various specific environmental constraints, including pollutants resulting from street traffic emissions. In this study, the changes of needles anatomical and physiological characteristics of the Pinus nigra species grown in urban area (Dendrological Park of the University of Agronomic Sciences and Veterinary Medicine from Bucharest and Road-Side urban trees) were quantified in order (1) to establish the effect of vehicles pollution on needles anatomy, (2) to establish the effect of vehicles pollution on needles gas exchanges, and (3) to evaluate the impact on assimilatory pigments content. We found that growth conditions have had affected all needle's analyzed attributes. Our results can be added to the existing ones as regard as the P. nigra vulnerability under urban environments and its use as a bioindicator of urban pollution.

Key words*: leaf, Pinus nigra, urban pollution.*

INTRODUCTION

The transformation of natural and agricultural land into urban areas is increasing every year, leading by all the related attributes the environmental degradation in relation to plant growth and increase plant stress (Czaja et al., 2020), as well as to the exacerbation of the climate change caused to urbanization (Hamdi et al., 2020; Ehrnsperger, & Klemm, 2022). As early as ten years ago, Solecki & Marcotullio (2013) point out that maintaining urban biodiversity levels may be key not only to urban residents, but also to global biodiversity in an urbanized future. Thus, the presence of trees in urban areas may be one of the strategies that can help strengthen the global response to climate change (Parsa et al., 2019; Lüttge & Buckeridge, 2020).

The ecological assessment of urban spaces is based on vegetation, which serves as an indicator of environmental conditions and ecological processes, acting both as bioattenuators and as bioindicators (due to their ability to absorb and degrade pollutants) (Jain et al., 2022). Plants are the ones that influence the environment and which in turn are influenced by it (Onete et al., 2010).

The presence of trees in the urban environment influences by multiple ways the microclimate (Leuzinger et al., 2010; Millward & Sabir, 2011; Gratani et al., 2016), can perform multiple ecological functions, can provide beneficial services for human well-being (Gerstenberg et al., 2016; Hall & Knuth, 2019; Machar et al., 2019; Fineschi & Loreto, 2020) and counteracting unfavorable conditions (Pace et al., 2021). If the right selection of trees species, proper planting and management of plants are ensured, urban biodiversity can grow and contribute to biological richness. In addition, trees are particularly suitable as indicators for prevailing environmental conditions (Lüttge & Buckeridge, 2020) and help to attenuating poor air quality etc. (Millward & Sabir, 2011; Sicard et al., 2018; He et al., 2020; Klingberg et al., 2022). The ability of trees to improve air quality in urban areas has also been demonstrated by measuring the accumulation of 32 poly-cyclic aromatic hydrocarbons (PAHs) in leaves of *Quercus palustris* and needles of *Pinus nigra*. A significant accumulation of low molecular mass

L-PAH (mainly gaseous) and high molecular mass PAHs (mainly particle-bound) was recorded in the 3-year-old pine needles, compared to the one-year-old ones, the pine proving superior to the oak from this point of view (Klingberg et al., 2022).
Moreover, the selection of species of urban greening must be done with attention with a view to also control and mitigate their pollution effects (Lyu et al., 2021).
There are many studies on the effects of pollution on plants, both as regard as the morpho-anatomical, as well as physiological and biochemical changes, as very well have already synthesized by Uka et al. (2017). For *Pinus sylvestris* species, Lin et al. (2001) shows a reduction in stomata density at both leaf surfaces by assuring a high concentration of carbon dioxide, although the number of stomata rows did not change significantly. Also, Fedorchak (2020) have highlighted the significant impact of industrial and vehicle exhaust emissions on assimilatory pigments in *Picea abies* and *Picea pungens*. It was suggested the possible involvement of the assimilatory pigment complexes of these species as bioindicators of air pollution status, available throughout the year.
Biomonitoring of the environment is a major concern nowadays (Hutnik et al., 2014; Juranović-Cindrić et al., 2018; Nikolić et al., 2019; Schulz et al., 2019; Trstenjak et al., 2020). In this regard, the species used include conifer needles, lichens, mosses, bees, and their by-products, as well as snails and have been widely used in recent research (AL-Alam et al., 2019). As the authors summarize, those of conifers, which are usually kept for at least five years, are well suited and provide information on the emission of pollutants for longer periods (due to waxy leaves and increased affinity for polar compounds). It is worth noting that the most studied species are *Pinus pinea* and *P. nigra*, whose geographical distribution is particularly large and whose needles have proved to be the most examined.
Given the above, an additional challenge for practitioners is the selection of the most suitable tree species for the urban parks of the future, in relation to regional and local climatic conditions, as well as considering their degree of tolerance to stressors (Brune, 2016). For the optimal arrangement of green infrastructure, so that cities stay healthy and thriving, and to mitigate the effects of climate change, the principle of "the right plant in the right place and with the right management" must be considered (Hasan et al., 2017; Ferrini et al., 2020; Petrova, 2020; Velasco-Jiménezet et al., 2020) that will be translates into a better quality of life and reduction of urban pollution (Dobrescu & Fabian, 2017).
In the context of these concerns, the aim of this study was to characterize the effects of urban traffic (and not only) on *Pinus nigra* needles (1) anatomy, (2) gas exchanges and other relevant associated indicators and (3) assimilatory pigments content.

MATERIALS AND METHODS

Experimental site and biological material used

The studies were carried out during the year 2021, on the black pine *Pinus nigra* J.F. Arnold needles collected from trees grown in the Bucharest area, Romania. The needles taken from the trees growing in the Dendrological Park (DP) of University of Agronomic Sciences and Veterinary Medicine (UASVM) from Bucharest and, respectively, that of the Road-Side trees (RS), along Ion Mihalache Boulevard were analyzed in parallel. For anatomical observations, needles formed in the current year were used (CYN), and for physiological one's needles formed in the previous year, i.e. one-year-old needles (PYN) and needles formed in the current year (CYN) were analyzed.

Pinus nigra needle anatomy analysis

Acicular leaves from 1-year-old branches, taken at in June, were analyzed. Anatomical observations and measurements were carried out on cross-sections in the middle area of the leaves. The sections were clarified with chloral hydrate and stained with carmine-alum and iodine green, according to the classical method (Morlova et al., 1966). The following anatomical needle traits were measured: E = Epidermis; C = Cuticle; H = Hypodermis; M = Mesophyll; Rc = Resin canals; En = Endodermis; Tt = Transfusion tissue; Vb = Vascular bundles; X = Xylem; Ph = Phloem.

Observations, measurements, and photographs were taken with the LEICA DM 1000 LED optical microscope, LEICA DFC 295 video camera and LEICA S 8 APO stereomicroscope, using 4x, 10x lenses, existing in the endowment of the Research Center for Studies of Food Quality and Agricultural Products (UASVM Bucharest).

In situ analysis of gas exchanges at the needles level

The values of needles gas exchanges were measured *in situ* with the help of the portable system for photosynthesis (LCpro-SD ADC BioScientific) equipped with an infrared gas analyzer, immediately after the randomly detachment of those from the sun exposed exterior branches (2 m above the ground) of three individual trees, in environmental conditions specific to spring (late March), early summer (June) and early autumn (September), 2021. As Robakowski and Bielinis (2017) found, the rate of photosynthesis in detached 1-year-old *Abies alba* needles was not significantly different from that of those attached. Parallel determinations were performed for those DP and RS trees (6 needles randomly selected - n = 3), between hours 10:00 AM - 12:00 AM, for the following physiological indicators: the intensity of photosynthesis (A) (µmol CO_2 $m^{-2}s^{-1}$), the intensity of transpiration (E) (mmol H_2O $m^{-2}s^{-1}$), the respiration rate - R (µmol CO_2 $m^{-2}s^{-1}$), the stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$), and the intercellular concentration of carbon dioxide (CO_2i) (µmol CO_2 L^{-1} air). Along with these, the value of the photosynthetic photon flux density (PPFD) was recorded (data are not shown). Based on the obtained data, other indicators associated with gas exchanges were calculated, respectively: the instantaneous water use efficiency (WUE) (A/E), intrinsic water use efficiency (iWUE) (A/g_s), the quantum yield (φCO_2) (A/PPFD), as well as the instantaneous carboxilation efficiency (ICE) (A/ CO_2i).

The assimilating pigments content of the needles

The Arnon spectrophotometric method was used for the quantitative determination of assimilating pigments. On briefly, the needles were taken from the tree, placed in Eppendorf's tubes and transported quickly to the laboratory, where the extraction in acetone 80% (Lichtenthaler, 1987) has been done. Then, the absorbance of the clarified extracts was measured using the spectrophotometer (Helios Alpha Thermo Scientific) at three wavelengths (663 nm to determine chlorophyll *a* - Chl *a*; 646 nm for chlorophyll *b* - Chl *b* and 470 nm for carotenoids). Afterwards, the content of assimilating pigments was calculated by using the specific formulas reported by Lichtenthaler (1987). The obtained values were finally expressed as mg $100g^{-1}$ fresh weight (FW).

Statistical analysis

The reported data of all parameters represent the mean ± standard error (SE). Statistically significant differences between the mean values were assessed using one-way analysis of variance (ANOVA). Then, the paired-samples Student T test (2-tailed) were accomplished and the significant differences among variants have been considered at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Pinus nigra needle anatomy

The leaf anatomy data of *P. nigra* are shown in Table 1 and these results are according to those reported in the literature (Andrei, 1978; Georgescu et al., 1999; Alvarez, 2009; Dörken & Stützel, 2012).

Very strongly statistically significant differences are observed ($P < 0.001$) in the case of needles collected from Street Trees for epidermis (23.4 µm), cuticle (4.40 µm), transfusion tissue (585.37 µm) and phloem (61.41 µm) compared to the values characteristic of those taken from the Dendrological Park trees (15.33; 2.87; 435.27 and respecttively, 35.79 µm). There were also significantly higher values ($P < 0.01$) for the vascular bundles (RS -126.59 vs. DP - 103.01 µm), as well as for the hypodermis ($P < 0.05$) (RS – 34.07 vs DP-16.29 µm). In the case of mesophyll, resin canals and xylem, there were no statistically significant differences ($P > 0.05$).

In a cross section, the *P. nigra* leaf has a semicircular contour, with a flat upper face being formed by the epidermis, the mesophyll and the central cylinder (Figure 1a, Figure 1 b, and Figure 1c).

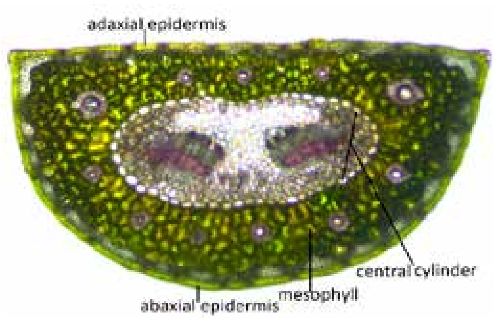

Figure 1a. Transversal section on the leaf of *Pinus nigra*

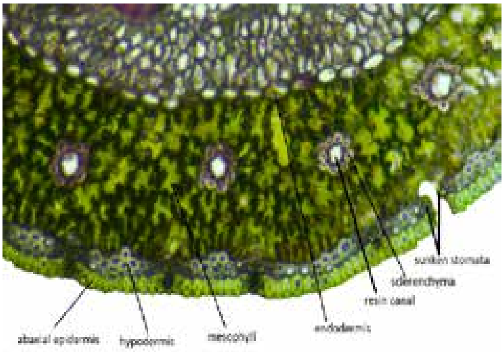

Figure 1b. Transversal section, detail of the leaf anatomy of *Pinus nigra*

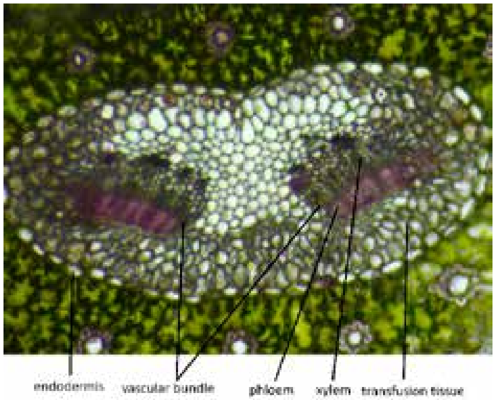

Figure 1c. Transversal section, detail of the central cylinder of the leaf of *Pinus nigra*

From the analysis of the literature, tissue thickness differs depending on the area and climatic factors (Tiwari et al., 2013; Crivellaro & Schweingruber, 2015). The epidermis is made up of cells with thick, low lumen walls in the shape of the letter X, covered by a layer of cuticle. The hypodermis consists of 1-3 layers of cells, with lignified, sclerified walls, being thicker in the two edges of the leaf, while mesophyll consists of septate cells.

Table 1. Anatomical characteristics of current year needles (CYN) of *Pinus nigra* from Dendrological Park (DP) and Road-Side (RS) trees. Mean value ± Standard Error. N=7

	Specification	*Pinus nigra* DP	*Pinus nigra* RS	P value
Thickness (µm)	E = Epidermis	15.33± 0.56	23.43± 1.48***	<0.001
	C = Cuticle	2.87±0.21	4.40±0.21***	<0.001
	H = Hypodermis	16.29±0.98	34.07±5.65*	<0.05
	M = Mesophyll	113.34±10.37	119.97±.89ns	>0.05
	En = Endodermis	18.07±0.77**	13.83±0.97	<0.01
	Tt = Transfusion tissue	435.27± 14.21	585.87±12.39***	<0.001
	Vb = Vascular bundles	103.01±1.84	126.59±4.95**	<0.01
	X = Xylem	67.66±2.38ns	65.17±2.46	>0.05
	Ph = Phloem	35.79±2.59	61.41±2.62***	<0.0001
Diameter (µm)	Rc = Resin canals	30.70±2.55ns	25.57±1.25	>0.05

Resin canals are also present, and their size is influenced by the number of secretory cells and sclerenchyma fibers (Ghimire, 2014). In the middle of the leaf there is the central cylinder surrounded by a primary endoderm, under which is the transfusion tissue and, the xylem and the phloem are located. Between the conducting fascicles there is a parenchyma comprised of parenchymal cells.

Stomata are located below the external level of the epidermis, having a large substomatal chamber (Figure 1c). Stomata number was proved to be significantly lower at a high carbon dioxide (CO_2) concentration (Lin et al, 2001; Domec et al., 2015). Nikolić et al. (2019) showed that for *P.nigra* (trees with shorter needles) a higher stomatal density was observed as against other 11 conifers studied species. Besides, the size of the needles as well as pollen vitality were also correlated with the distance from the heavy traffic.

Gas exchanges at the needles level at field site and associated indicators

The obtained results regarding the gas exchanges specific to the two studied variants and their seasonal dynamics are presented in Table 2.

Maximum photosynthesis rate (A) (5.66 μmol CO_2 m^{-2} s^{-1}) was determined in June, for previous year leaves (PYN), in the conditions of the Dendrological Park (DP) and at the opposite pole are the specific values for the leaves of the same age (1.11 μmol CO_2 m^{-2} s^{-1}), determined in September, but grown in the environmental conditions specific to street traffic (RS). We also noticed the maximum values of A during the early spring (RS - 5.37 and DP -5.01 μmol CO_2 m^{-2} s^{-1}, respectively), when the transpiration rate and stomatal conductance had low values, with non-significant differences between the two sites.

Needles transpiration (E) and stomatal conductance (g_s) also declined with needle age. In addition to the obviously lower values of A in the case of SR tree leaves, there is also a decrease in the process rate as the age of the needles increases.

Table 2. Seasonal course of photosynthesis rate (A), transpiration rate (E), stomatal conductance (g_s), intercellular carbon dioxide concentration (CO_2 i), respiration rate (R) and other associated parameters of *Pinus nigra*: previous year needles (PYN) and current year needles (CYN) from Dendrological Parc (DP) and Road- Side (RS) trees. Data are the Mean values ± Standard Errors. N = 3

March		June				September			
PYN		PYN		CYN		PYN		CYN	
DP	RS	DP	RS	DP	RS	DP	RS	DP	RS
Photosynthesis rate (A) (μmol CO_2 m^{-2} s^{-1})									
5.01 ±1.06	5.37 ±1.32^{ns}	5.66 ±0.21**	2.00 ±0.12	4.83 ±0.82^{ns}	3.58 ±0.59	4.34 ±0.15*	1.11 ±0.10	4.59 ±0.03*	2.88 ±0.09
Transpiration rate (E) (mmol H_2O $m^{-2}s^{-1}$)									
1.42 ±0.24	1.57 ±0.26^{ns}	4.28 ±0.63*	1.20 ±0.19	5.73 ±0.95*	2.19 ±0.28	2.22 ±0.03*	1.29 ±0.01	2.47 ±0.05**	1.66 ±0.04
Stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$)									
0.079 ±0.017^{ns}	0.075 ±0.018	0.082 ±0004**	0.029 ±0.003	0.152 ±0.024^{ns}	0.115 ±0.005	0.051 ±0.013^{ns}	0.046 ±0.01	0.07 ±0.01^{ns}	0.055 ±0.013
Intercellular carbon dioxide (CO_2) concentration (CO_2 i) (μmol CO_2 L^{-1} air)									
325 ±8.55	294 ±14.80	303 ±0.9	329 ±4.3	331 ±24.5	377 ±10.2	241 ±0.1	309 ±35.5	241 ±40.5	262 ±34.4
Respiration rate (R)(μmol CO_2 m^{-2} s^{-1})									
2.62 ±0.41	4.54 ±0.65*	3.42 ±0.25*	2.28 ±0.09	14.01 ±0.55***	9.81 ±0.54	5.92 ±0.14	8.03 ±0.19^{ns}	4.97 ±8.34	8.34 ±0.57*
Water use efficiency (WUE)(A/T) (μmol CO_2 m^{-2} s^{-1}/ mmol H_2O $m^{-2}s^{-1}$)									
3.528	3.420	1.322	1.667	0.843	1.635	1.955	0.860	1.858	1.735
Quantum yield (φCO_2) (A/PPFD) (μmol CO_2 m^{-2} s^{-1}/ μmol photons $m^{-2}s^{-1}$)									
0.0042	0.0044	0.0047	0.0017	0.0040	0.0030	0.0036	0.001	0.0038	0.0024
Instantaneous carboxylation efficiency (ICE)(A/CO_2i) (μmol CO_2 m^{-2} s^{-1}/(μmol CO_2 L^{-1} air)									
0.0154	0.0183	0.0187	0.0061	0.0146	0.0095	0.0180	0.0036	0.0190	0.0110
Intrinsec waster use efficiency (IWUE) (A/g_s) (μmol CO_2 m^{-2} s^{-1}/ mol H_2O $m^{-2}s^{-1}$)									
63.412	71.60	69.024	68.966	31.776	31.13	85.098	24.13	65.571	52.36
Values have been compared in paired for the same leaf age and different growing conditions. Differences significance is specifically marked at a probability of P<0.05 (*), P<0.01 (**), P<0.001 (***) and P>0.05 (non-significant – ns).									

Except for the March data, there is a decrease in the PYL of about 1.3 times, from June to September (DP), and about 1.8 times, respectively, in RS conditions. The trend was similar for CYN, except that the decrease was slower: 1.11 times for those in the park, and 1.24 times for those on the street.

As regard as the intercellular concentration of carbon dioxide (CO_2 i) the values were between 241μmol CO_2 L^{-1} air for the PYN, September, DP and respectively 377 μmol CO_2 L^{-1} air for CYN, June, RS. It is observed that except for the spring period, in all other cases, for the leaves of the RS trees the determined values are higher, compared to the values measured for the leaves of the trees in the park, if values are compared in pairs. Respiration rate varied between 2.28 μmol CO_2 m^{-2} s^{-1} (June, PYN, RS) and 14.01 μmol CO_2 m^{-2} s^{-1}) (June, CYN, DP). Significantly higher values were recorded for street trees: 4.54 μmol CO_2 m^{-2} s^{-1} (March, PYN) and September (CYN) (8.34 μmol CO_2 m^{-2} s^{-1}) as against the respiration rate in DP conditions.

Maximum values of WUE have been calculated in March (3.528 – PYN, DP), while during the seasons, the values decreasing were noticed till 0.843 (June, CYN, DP), in close relation with the specific highest transpiration rate. Moreover, the φCO_2 was highest in June (0.0047) (PYN, DP), while at the opposite pole was the value of 0.001 (September, PYN, RS), in relation with the highest carbon assimilation rate, and the lowest one, respectively.

Concerning the ICE, excepting the early spring period, when the differences between the two site were low, thereafter, at the pollution site the values were evidently less than at the park site. For instance, the lowest one was 0.0036 (PYN, September, RS), as against 0.0180 (PYN, September, DP).

As a measure of the potential water cost to maintain a given rate of photosynthesis per unit leaf area the IWUE was calculated. A maximum value was obtained in autumn (85.098) for PYN, DP, respectively the lowest one (24.13) in the case of PYN, RS for the same season.

The results agree with those obtained by Kinerson et al. (1974) in *Pinus taeda*, respecttively the highest A at those of one-year, during the late spring, corresponding to the greatest growth of the shoot, and of course, an obvious need for assimilated carbon for growth support in developing shoots and needles. Also, A declined for CYN as these began to mature (Ellisworth, 2000). For *A. alba*, Robakowski and Bielinis (2017) pointed out that with needle age, the structural adaptations that take place will influence too the rate of physiological processes, including an increase in internal resistance to CO_2. Thus, the rate of A decreased by 2 μmol CO_2 m^{-2} s^{-1}, while CYN recorded the highest A values (12 μmol CO_2 m^{-2} s^{-1}). Also, the rate of E, the WUE, as well as the g_s had lower values as the age of the leaf increased. On the other hand, at *P. sylvestris*, González-Rodríguez et al. (2019) emphasized an obvious decrease from late-spring till summer, reaching close to zero, in the case of A and E, respectively.

Regarding the possibilities of water conservation and increasing the WUE, according to the data obtained by Salazar-Tortosa et al. (2018), A values for *P. sylvestris* was maximum in June ((4.3 μmol CO_2 m^{-2} s^{-1}), had been similar those of other *Pinus* species, then a sharp decrease took place from June to July (2.85 μmol CO_2 m^{-2} s^{-1}). In the case of *P. nigra* and *P. halepensis* there were no significant differences between periods. Values of g_s have had a similar trend. Therefore, for *P. sylvestris* there were recorded the maximum iWUE values for both analysis data. Tomášková et al. (2017) state that *P. nigra* which grows well in southern Europe, the Mediterranean area and northern Africa, requires a continental climate compared to *P. sylvestris* and behaves better in extreme conditions. It can reduce the daily amount of E by 21% in one-year- needles and up to 50% in those of two years. And the studies conducted by Forner et al. (2018) highlighted the fact that the highest intra- and inter-annual plasticity in terms of WUE was observed in *P. nigra*, which maintained a higher water saving strategy. Naturally, the genus *Pinus* includes different species, with different needles lengths and with specific characteristics, which impact their anatomical traits (Wang et al., 2019). On this topic, additional research was conducted by Kuusk et al. (2018) on the characteristics of young (primary) and adult (secondary) (heteroblasts) needles in Mediterranean pines *P. halepensis*, *P.pinea* and *P.nigra*. There was highlighted that in the adult leaves, the fraction of supporting tissues increased positively with the known classification of drought tolerance of species (*P. halepensis* > *P. pinea* > *P. nigra*). In all species and ages of needles and plants, a negative correlation was observed between mesophyll volume fractions and structural tissues, showing a trade-off between investments in biomass and different needles functions. It is emphasized the ecological advantage of the juvenile morphophysiotype is to maximize the carbon gain when setting up plantations, while the characteristics of the adult needle improve the tolerance to environmental stress of plants stabilized at the final site.

An increase in the level of air pollution caused by heavy traffic can change the behavior of plants in terms of their ability to CO_2 assimilation, which has repercussions on other morphological, physiological, and biochemical characteristics.

Thus, changes in leaf thickness can affect stomatal movements, can lead to a decrease in g_s and therefore can reduce the process of A. The deposition of dust on the surface of the leaf decreases the intensity of the E and as an immediate consequence will be the increase of the internal temperature of the leaf (Singh și al. (2017). On *Lagerstroemia speciosa* trees on roadside, Singh et al. (2017) highlighted: a reduction of A with 36.7%, of E with 42.14%, of g_s with 66.85%. Also, an increase of stomatal resistance by 212.2%, of leaf thickness with 40.54%, as well as WUE with 9.4% have been noticed. In the same time, increased of lead (179.31%) and proline content (15.61%) were recorded as against to the trees exposed to less traffic area.

The assimilating pigments content of the *P.nigra* needles

Figure 2 shows the quantitative variability of the different categories of assimilating pigments. As expected, the highest values were recorded for chlorophyll *a* (Chl *a*), followed by chlorophyll *b* (Chl *b*) and carotenoids (C+X), respectively.

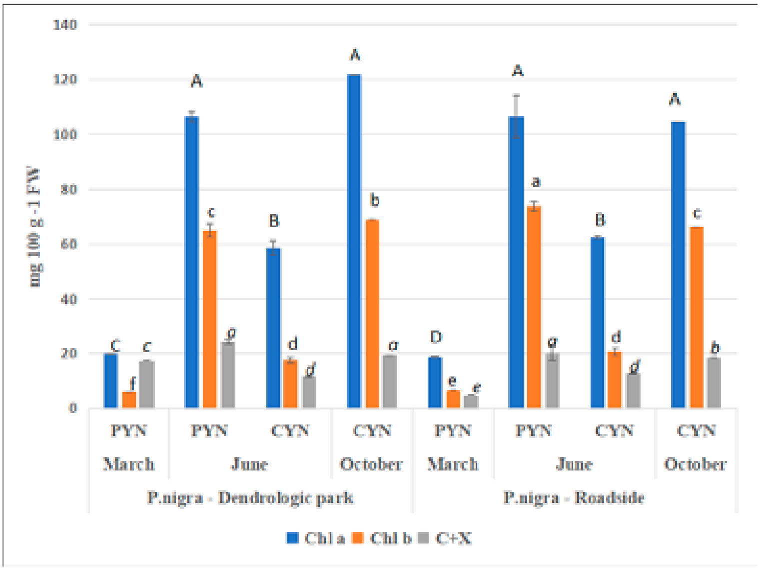

Figure 2. Assimilating pigments variation in *Pinus nigra* needles
Legend: Chl a= chlorophyll a; Chl b= chlorophyll b; C+X= carotenoids; PYN= previous year needles; CYN= current year needles. Columns are means ±standard errors. Different capital letters signify significant differences for Chl a; lowercase letters mean significant differences for Chl b. Italic lowercase letters mean significant differences for C + X. P <0.05.

From the point of view of statistical analysis, the comparison of the data was performed separately, by categories, starting from the highest value. We note that in the case of Chl *a*, the highest values were determined in autumn, for those formed in the PYN (121.68 mg 100 g $^{-1}$FW), while the minimum values of 18.77 mg 100 g $^{-1}$FW were also characteristic for PYN, at the beginning of spring, and the differences were statistically significant (P <0.001). If we compare the values for those trees in the DP and those on RS, we notice that at the beginning of summer, for PYN and CYN, respectively, there were no statistically significant differences (P> 0.05). But, it is obvious that between needles of different ages, the value differences are statistically significant.

At the same time, in the spring weather for PYN collected from the park, the values are significantly higher, compared to those specific to leaves taken from street trees ($P < 0.01$).

The maximum value of Chl *b* (73.85 mg 100 g $^{-1}$FW) was recorded in June at PYN, RS, while the minimum value (6.19 mg 100 g $^{-1}$FW) was calculated at the beginning of spring, for PYN, DP ($P < 0.001$). We also observed significantly higher values for PYN taken from RS trees (6.67 mg 100 g $^{-1}$FW) in March, compared to those in the DP (6.19 mg 100 g^{-1} FW) ($P < 0.001$). In early spring, higher values for PYN were recorded both in DP (64.83 mg 100 g $^{-1}$FW) and in RS (73.85 mg 100 g^{-1}FW), conditions, but statistically significantly higher for RS ($P < 0.05$). For CYN, at the beginning of the summer the values were lower for both studied variants and there were no statistically significant differences ($P > 0.05$). Data obtained in autumn, for CYN, in the case of both studied sites were significantly higher as against in June, and, in addition, for DP the value of 68.76 mg 100 g^{-1}FW, was significantly higher than 66.25 mg 100 g $^{-1}$FW, determined for needles taken from street trees.

The content of carotenoid pigments was between 24.24 mg 100 g^{-1}FW (needles from the previous year, June, DP) - 4.76 mg 100 g $^{-1}$FW (needles from the previous year, March, RS). In June, there were no significant differences for the two studied variants. In autumn, however, the amount of carotenoid pigments decreased significantly for leaves taken from SR trees, from 19.53 mg 100 g $^{-1}$ FW (DP) to 18.53 mg 100 g $^{-1}$FW (RS) ($P < 0.001$).

González-Rodríguez et al. (2019) studying the species *Pinus canariensis* mentions that the maximum values of Chl content were recorded in spring (578 μmol m^{-2}), then decreased during the year, reaching minimum values in winter (a decline of 15 %). The recovery of Chl was easily carried out the following spring, with significant changes for Chl *b*, and not for Chl *a*, respectively an increase of them with the decrease of the temperature. For C+X, the same tendency was noticed, with minimum value determined in spring. Their content increased throughout the seasons and that is interesting is the higher content of β-carotene during the winter and no recovery in the following spring. As regard as the Chl fluorescence, the maximum values of quantum efficiency of photosystem II (PSII) photochemistry (F_v/F_m) were measured during late spring and early summer (0.82) and a markantly reduction was noticed in autumn and winter (0.65).

In the case of *P. sylvestris* plants exposed to technogenic pollution, Kalugina et al. (2018) noticed several changes, such as: the amount of Chl *a*, Chl *b* and C+X decreased by the mass of a needle by 23, 40 and 42%, respectively. At the same time, as a protective reaction, there was an increase in the amount of ascorbic acid in the needles by 48%; the amount of water-soluble phenolic compounds by 29%; of the report Chl *a* /Chl *b* by 35%; the ratio between green and yellow pigments by 40%; and the level of water and alcohol soluble proteins, by 40 and 30%, respectively.

For the leaves of *P. abies* and *P. pungens* from industrially polluted places and exposed to exhaust fumes, the Chl *a* content decreased by 27.2% and 25.0%, respectively, and the Chl *b* content by 17.9% and 20.0% since May, until September, compared to the control. At the same time, the carotenoids content increased by 26.1% and 24.0%, respectively, as a protective measure. Also, the total Chl decreased by 24.4% and 23.6%, respectively; the Chl *a* / Chl *b* ratio with 11.4% and 12.3%; the ratio (Chl [*a* + *b*] / carotenoids) decreased by 30.1% and 38.0%, respectively (Fedorchak, 2020).

There are genetic differences between sensitive and pollution-resistant pine trees, and it is necessary to exploit the cultivation potential of the latter, both by exploring the native plant material and by enriching the genetic diversity (Chudzińska et al., 2014). Moreover, research on *Pinus pinaster* carried out by Acquaviva et al. (2012) highlighted some physiological indicators (e.g. antioxidant metabolites, heat shock proteins 70 and hemoxygenase) as being useful to monitor environmental contamination in a region and to better understanding the mechanisms implied in plant's protection and stress tolerance.

Recently, the ability of *P. nigra* to remove pollutants from the environment in urban area have been noticed (Przybysz et al., 2018; Petrova, 2020). In fact, since ten years ago, following studies conducted by Sawidis et al. (2011) in three European cities (polluted and unpolluted areas) for *P. nigra* and *Platanus orientalis*, black pine has proven to be a good accumulator of heavy metals, especially in the bark, and as such has shown its increased efficiency as a bioaccumulator of urban pollution, even though both species can be considered for comparative studies of this type.

Thus, searching for and planting trees with higher pollutant removal efficiency could be crucial to improving the quality of the urban environment.

Last but not least, the various local efficient and reasonable government regulation policies to control heavy traffic (consecutive and intermittent policies) have proven to be effective to alleviate vehicles pollution emissions continuously (Sun et al., 2022). At the same time, the promotion of the public transport can have beneficial effects on reducing urban air pollution for local residents, given the major contribution of passenger cars to the emission of particulate matter (PM1).

CONCLUSIONS

Based on the findings of the present study, it may be concluded that environmental conditions significantly ($P<0.05$) affect the *P.nigra* needles anatomy and physiological behavior, too. These differences are related also to seasonal adaptation of the foliage.

Urban traffic caused a significant increase ($P <0.05$) in the values of the thickness of the epidermis, cuticle, transfusion tissue, vascular tissues and hypodermis of *P. nigra* leaves, compared to the values determined for the leaves collected for the trees in the Dendrological Park.

Specific gas exchanges (photosynthesis rate, transpiration rate, stomatal conductance and intercellular carbon dioxide), but also their associated indicators (the instantaneous water use efficiency, intrinsic water use efficiency, the quantum yield, as well as the instantaneous carboxilation efficiency) were negatively affected by the urban traffic.

In addition, our study indicates that with the aging of pine needles, the structural adaptations and assimilatory pigments evolution occur. Also, changes induced by pollutants, influence physiological functions of the needles.

These results provide valuable information for the management of *P.nigra* species by using relevant anatomical and physiological indicators with a view to monitor environmental contamination in a region and for better understanding the mechanisms involved in plant protection and stress tolerance. It is necessary to exploit pollution-resistant species and apply management practices focused on both the use of native biological material and the improvement of genetic diversity (Chudzińska et al., 2014).

In order to improve the ecosystem services destined to regulate the urban vegetation, the search and planting of trees with increased capacity to remove pollutants from the environment can be an essential way to improve the quality of the urban environment (Petrova, 2020).

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THE IMPORTANCE OF BOTANICAL SURVEY AND PROPER MANAGEMENT IN THE PROTECTION OF RARE, ENDANGERED OR VULNERABLE SPECIES OUTSIDE OF PROTECTED AREAS - WORKING EXAMPLE: *HYACINTELLA LEUCOPHAEA* (K. KOCH) SCHUR

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Abstract

Botanical assessment is an important tool for many scientific works which provides information that allows the accomplishing of different evaluations on plant diversity and on anthropogenic actions; it could assist human activities on ecological reconstruction or on projects of green infrastructure. Hyacinthella leucophaea is a species of Pontic-Balkan origin, listed on the National Red List of plants from 1994 and considered rare in Romania. Through our botanical surveys and reviewed literature, we added new records on distribution of Hyacinthella populations in our country. Populations in a good state can maintain this status through proper management of the land, which would reduce the pressure of external factors.

Key words*: botanical survey, country distribution, grassland management, Hyacinthella leucophaea.*

INTRODUCTION

Assessing plant species or the botanical surveying of a vegetal community is a basic operation in order to correctly identify a vegetal association and to separate it from others in the field (Chifu et al., 2014). This is the first stage, also, in the mapping of vegetation from a research area (Ivan & Doniță, 1975; Cristea et al., 2004). The mapping of vegetation through finding in territory vegetal communities' priority to preservation have allowed to establish 32 habitats of communitarian interest that overlap on the Natural Park of Serra da Estrella (Portugal) and to define their management and preservation strategy (Meireles et al., 2009).

Floristic assessment allows to create vegetation databases (Attorre et al., 2014).

The data from botanical surveying will show the complexity of a plant community trough species richness, species diversity and structural relationships between them. An extensive inventory of vascular plants was performed in 4 years of field studies by Grădinaru (2021) to evaluate the impact of wind farms construction on composition and diversity of flora in two protected areas in Romania. Data collected allowed the rating of the floristic composition as an important indicator in assessing the specific richness of vascular plants and the dynamic stage of plant communities. It was also possible to establish the bioformes and lifespan of plant species, the geoelements, and the plant preferences for ecological factors which induce the distribution of plant populations in existing habitats. This complex assessment has highlighted the response of plant communities to precise anthropogenic actions as well as the applicable management strategies that can mitigate the effect of construction of wind farm on vegetation cover.

However, there are other uses of data result from botanical survey, such as the biodiversity rating, evaluation of species and habitats state of conservation, estimation on alien species rate of dispersion (Doroftei et al., 2005). V. Ciocârlan (2006) presenting 2 newly identified and 40 rare plant species in Romanian flora, most of them from Dobrogea, accentuated the danger of rarefaction and disappearing of some species under the strong intervention of human activity on vegetation cover. As a result of an inventory of flora species and habitats undertaken on the territory of Fântânița-Murfatlar site of communitarian interest, Petrescu (2015) had identified 15 threatened plant species, of national interest and had delimited four habitats, all of them of communitarian interest. Nine plant associations, evaluated by the author for their current natural state, showed slight to medium perturbations due to anthropic activity. A botanical survey conducted in a protected area near Neajlov River, in the South of Romania had enriched the previous list with 50 new species, added to those discovered in former studies. The existing risk of disappearance of this area as anthropic impact grows in intensity was one of the conclusions resulted from this study (Onete et al., 2016). Subsequently, in research undertaken on crop fields in Oltenia area, deploying botanical surveying methods, a number of 25 species of invasive and potentially invasive alien plants have been identified, three of them being considered the most harmful and widespread - *Sorghum halepense*, *Xanthium italicum* and *Ambrosia artemisiifolia* (Răduțoiu & Bălănoiu, 2021). In 2021 Georgescu et al. reported *Humulus scandens*, an invasive even transformer species, from the Dâmbovița River banks, at 40 km South of Bucharest. Known as an ornamental plant, *H. scandens* was observed as a naturalised species in N. America and Europe in the late nineteen century. First recordings about this species as a naturalised one in our country are found in a paper dating from 1969.

Through the information provided, the assessment of plant communities could assist other domains such as the achievement of green infrastructures by interconnecting natural areas with those designed by man to preserve biodiversity in urban areas (Talal & Santelmann, 2019).

The botanical surveying additionally allows the finding of new chorology records of rare and periclitated plants. In his first tome of Illustrated Flora of Romania V. Ciocârlan (1988) indicated adjacent to description of *Gymnospermum altaicum*, a rare species in our country, that he did not find the in 1976, during his field researches. Following the botanical surveys performed in North Dobrogea in 2005 Doroftei & Mierlă record this species from areas specified by the reference literature and from other adjacent zones.

The aim of this paper is to emphasise the importance of botanical assessment through a work example on *Hyacinthella leucophaea* populations presence in our country.

H. leucophaea is a species of Pontic-Balkan origin, listed on the National Red List of Vascular Plants from Romania (Oltean et al., 1994, Sârbu et al., 2007) and considered rare in Romania (Sârbu et al., 2013). *H. leucophaea* is part of the sl. Jurineo arahnoideae-Euphorbion stepposae (Dobrescu, 1971) Coldea et Sârbu in Coldea 2012 (cl. Festuco-Brometea Br.-Bl et R.Tx in Br.-Bl.1949), typical associations for the xerophytic grasslands encounter mainly in the eastern part of our country. These grasslands contain a characteristic and constant number of Pontic-continental, Pannonic and Meridional elements that differentiate them from those of Central Europe (Chifu et al., 2014). The paper indicates: a) a review of the references on the distribution of the *Hyacinthella leucophaea* in our country; b) new records of distribution of H. leucophaea following our botanical surveys; c) an assessment of the anthropogenic activities with impact on the *H. leucophaea* populations development in the new recorded sites.

MATERIALS AND METHODS

The main sources of this paper are the reviewed literature on *Hyacinthella leucophaea* species distribution in our country and the field data collected during botanical surveying performed in two localities from Prahova and Constanta counties.

In Prahova County *Hyacinthella leucophaea* plants assessment was carried out near the Marginea Pădurii village, Jugureni commune, located at the base of Istrița Hill, part of Curvature Subcarpathians (Ghinea, 1996, 1997). The Sarmatian Istrița limestone (Frunzescu et al., 2010) permitted installation of a chalk grassland type as well as *Hyacinthella leucophaea* populations.

The second reporting site is located on the hills near Murfatlar town and Basarabi-Murfatlar Cave Complex (Constanța County). A small, xerophytic grassland cover a steep slope with South exposure with many rare plant species that are also reported from the Fântânița-Murfatlar - Site of Community Interest, placed at 2 km South from our assessment zone.

The botanical survey has taken place during March and April 2021 and 2022.

RESULTS AND DISCUSSIONS

Hyacinthella leucophaea is a geophyte, bulbous plant of 7-25 cm tall; two-three (four, rarely) leaves basal and unequal (the inferior one wider), erect and coriaceous, shorter than or equal with floriferous stem; cylindrical raceme with campanulate flowers, shortly bracteolate; perigone pale-blue; perigone lacinias twice shorter than the tube; stamens with violaceous anthers; valvicide capsules, wider than longer; spring (March-April) - flowering (Zahariadi, 1966) (Figure 1).

Figure 1. *Hyacinthella leucophaea* - Basarabi-Murfatlar populations (March, 2022)

Distribution of the *Hyacinthella leucophaea* (K. Koch) Schur populations in our country (review of the references):

Bacău County (Oprea, 2005): Onești, Perchiu hill (Burduja et al., 1971).

Brașov County: between Hărman and Sânpetru; Brașov, on the Tâmpa and Stejărișul mic Hills (Zahariadi, 1966), Cetății and Lat Hill (Morariu & Ularu, 1979); Lempeș Hill (Heltmann, 1973).

Buzău County (Dihoru, 2015): Breaza, Istrița hill (Morariu, 1978); Subcarpathian basin of Slănicul de Buzău (Ciocârlan, 1968); Buzău Plain (Șerbănescu et al., 1962); Buzău county - silvosteppe to hill (Dragu, 1986); Aldeni, Beceni on Balaurul hill (Ciocârlan, 1968; Oprea, 2005).

Botoșani County (Oprea, 2005): Ștefănești (Mititelu et al., 1993)

Constanța County: Mulfatlar in the Serplacula valley, Târgușor, Mircea Vodă (Zahariadi, 1966); Adamclisi, Gura Văii (Negrean et al., 2002), Hagieni (Ștefureac, 1970); Dumbrăveni, Esechioi (Parincu, 1997); Fântânița-Murfatlar (Bavaru, 1970); Cheia (Horeanu, 1973); Constanța, valea Siminocului, Tîrgușor, Mircea Vodă, Gura Dobrogei, Sidorman, Mangalia (Morariu, 1978); Medgidia (Prodan, 1935); Palazu Mic (Sârbu et al, 2003); Techirghiol – grasslands (Făgăraș et al, 2008).

Dolj County: Calafat, Craiova (meadow) (Morariu, 1978).

Galați County (Oprea, 2005): Pechea, Vârlezi (Morariu, 1978); Bazinul Chinejii (Sârbu, 1977).

Hunedoara County: Hunedoara on Căpruța hill, Găvojdia on Cazanăș hill (Morariu, 1978).

Iași County : Șorogari (Petrescu, 1916) ; Valea Lungă (Mititelu, 1974); mal lac Chirița (Oprea, 2005).

Mehedinți County: Dudașul Schelei, Oglănicului valley (Morariu, 1978); in quarry grasslands, between Schela Cladovei and Cracul Găioarei (Roman, 1974).

Olt County: Obârșia on Braniștea Catârilor (Morariu, 1978).

Prahova County: Tohani hill (Morariu, 1978).

Sibiu County: Mediaș (Morariu, 1978); Moșna (Drăgulescu, 2003).

Suceava County (Oprea, 2005): Frumoasa on the southern slopes (Mititelu et al., 1970); Ponoare, Liteni (Mititelu et al., 1989).

Tulcea County: Beștepe hill, Măcin (Morariu, 1978); Culmea Pricopanului (Ștefureac, 1970); Agighiol hills (Grădinaru, 2021).

Vaslui County: Bârlad on the Dealu Mare and in Crâng, Zorileni (Mititelu et al, 1989); Movila lui Burcel reservation (Dobrescu et al, 1982); Mânjești, Tanacu - Dealul Glodului reservation (Vițalariu, 1968), Satu Nou-Solești on Poștei hill (Dobrescu, 1974); Rateș Cuza (Oprea, 2005); Mitoc (Horeanu & Vițalariu, 1992).

Consequently to our botanical survey, we may add two new mentions on list of *Hyacinthella leucophaea* distribution in our country:

Prahova County: Marginea Pădurii village, Jugureni commune; coordinates: N - 45.099321;

E - 26.428394; on the south-western slope of a plateau, at an altitude of 510 m (Figure 2).

Figure 2. South-western slope of Marginea Pădurii plateau (March, 2022)

Constanța County: Murfatlat town, on the Tibișir hill, at the back of Basarabi-Murfatlar Cave Complex; coordinates: N - 44.167092; E – 28.4055005; on a rapid slope with south-western exposition (Figure 3).

Figure 3. South-western slope of Tibișir hill (Octobre, 2021)

Festuco-Brometea class comprises secondary xerophilous and meso-xerophilous steppe grasslands, many of them located on the rough ground where a strong influence of anthropogenic factors could degrade them (Chifu et al., 2014).

On the southwestern slope of the Marginea Pădurii grassland can be found elements of Festuco-Brometea association, on a limestone substrate that appear frequently on the surface. Late in March and in April, together with *Hyacinthella leucophaea* can be seen plants of *Adonis vernalis, Carex caryophyllea, Potentilla cinerea, Pulsatilla montana* subsp. *dacica* species, and then plants of *Anthericum ramosum, Muscari neglectum* (Figure 4), *Anthyllis vulneraria*, or *Trifolium montanum* species.

Figure 4. *Muscari neglectum* (Marginea Pădurii southwestern slope - July, 2021)

Figure 5. *Hyacinthella leucophaea* (Marginea Pădurii - March, 2022)

On the plateau, due to specific ecological conditions, the Festuco-Brometea association intersect a grassland with species from Arrhenatheretalia order; this type of grassland also cover the opposite slope with North-East exposition. The entire surface is maintained by repeated mowing during the growing season. The meadow is not used for grazing. Grassland burning at the end of the vegetative resting season does not affect *H. leucophaea* population (Figure 5).

The second recording of *H. leucophaea* is for a grassland near the Basarabi-Murfatlar Cave Complex that covers a small area, located on a very steep slope; here can be seen species of the *Agropyretum pectiniformae* (Prodan, 1939) Dihoru 1970 association some of them also found in the Fântânița - Murfatlar reservation (Petrescu, 2015). The vegetal cover is slightly perturbed and are not signs of anthropogenic actions. Among identified plant species there are populations of *Crocus pallasii* observed late in autumn (Figure 6), *Echinops ritro* subsp. *ruthenicus, Satureja coerulea* or *Asparagus verticillatus.*

Figure 6. *Crocus pallasii* (Basarabi - Murfatlar October, 2021)

Together with *H. leucophaea* in March can be seen individuals of *Carex caryophyllea* and *Gagea pusilla* plants.

CONCLUSIONS

Botanical assessment is an important tool for many scientific works, providing information that permits to accomplish different evaluations on plant diversity and on anthropogenic actions; it could assist human activities on ecological reconstruction or on projects of green infrastructure.

Through our botanical surveys and reviewed literature, we added new records on distribution of Hyacinthella populations in our country.

The assessment of anthropogenic actions shows a low perturbation of vegetative cover on new recorded sites for Hyacinthella populations.

Places that shelter populations of rare or threatened species outside the protection zones are important biological reserves for endangered species and fragile ecosystems.

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MICROPROPAGATION OF ORNAMENTAL GESNERIACEAE SPECIES AND GENETIC UNIFORMITY ASSESSMENT OF *IN VITRO* PLANTS USING SCOT MARKERS

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Abstract

A micropropagation protocol via direct shoot organogenesis from leaf explants of four commercial cultivars of ornamental Gesneriaceae was established in this study. The shoot induction was successfully achieved on Murashige and Skoog (MS) media supplemented with 0.2 mg L^{-1} indole-3-acetic acid (IAA) and 0.5 mg L^{-1} benzylaminopurine (BA). In proliferation stage, the effects of two combinations of PGRs (V1-0.2 mg L^{-1} IAA + 0.2 mg L^{-1} BA and V2-0.2 mg L^{-1} NAA +1 mg L^{-1} BA) on shoot number and length were examined for each species. The results suggest that PGR's combinations significantly influenced shoot proliferation in all analyzed species and among the treatments 0.2 mg L^{-1} NAA in combination with 1 mg L^{-1} BA was the most effective for in vitro shoot multiplication. The in vitro rooting percentage was 76.86-96.66% and was species-dependent. In vitro-raised plants showed a very high rate of survival (92.59-95.24%). The genetic fidelity between the selected vitro-plants and mother plants were confirmed by SCoT markers and, therefore, the propagation method proposed in this study could be applied for commercial purposes as well.

***Key words**: Sinningia speciosa, Kohleria hirsute, Streptocarpus hybridus, Saintpaulia ionantha, genetic fidelity.*

INTRODUCTION

Over the years, the commercial propagation of new species and varieties of ornamental plants was determined by the improvement of decorative traits of the plants, mainly the color and shape of flowers, using classical breeding methods together with modern biotechnological techniques (Davies & Schwinn, 2006; Burchi et al., 2010; Zhao and Tao, 2015).
In addition, obtaining the varieties with a long shelf life of flowers was also a very important objective of plant breeders, as this characteristic of ornamental plants was usually considered a key feature for assessing their value on flower market (Serek et al., 2006; Noman et al., 2017).
Gesneriaceae is known as a family of flowering plants that includes approximately 3540 species (Christenhusz & Byng, 2016) originated from tropical and subtropical areas of Afro-Eurasia and America (Weber, 2004). Many of these species are grown worldwide as potted plants and among them *Sinningia speciosa*, *Kohleria hirsuta*, *Streptocarpus x hybridus* and *Saintpaulia ionantha* are highly valued by plant growers for their colorful and showy flowers.
Sinningia speciosa, known as gloxinia, is a tuberous plant native to Brazil with a spectacular, single or double flower corolla, which presents a variety of colors and patterns (Macas-Palacios et al., 2015).
Kohleria hirsuta, the wooly kohleria, is a species of flowering plants in the family Gesneriaceae originated from northern South America with very attractive dark olive green and hairy leaves. The tubular-shaped hairy flowers have a spectacular spotted throat with bright colors (especially orange and red) and bloom all year round (Kvist & Skog, 1992).
Streptocarpus x hybridus, commonly known as Cape primrose, is an indoor plant native to Afro-mountainous areas of Central, East and South Africa, including Madagascar and the Comoro Islands (Nishii et al., 2015). The long leaves (25-40 cm) of Streptocarpus form a rosette and the trumpet-shaped flowers appear on long peduncles. Flowering is from spring to early fall and the spectacular flowers last a long time on the plant (Cantor et al., 2004).

African violets, with their new botanical name Streptocarpus sect. *Saintpaulia ionanthus* (Nishii et al., 2015), are attractive houseplants native to tropical East Africa, Tanzania, and Kenya (Harrison, 1999). Due to the high demand for this species, there are many varieties of African violets available on the market of ornamental plants that are characterized by different colors (from white, pink, red to purple) and flower shapes (Ghorbanzade & Ahmadabadi, 2014; Buta et al., 2015).

According to previous reports, the Gesneriads have been propagated mainly by seeds and leaf cuttings from mature plants, but in this case, only a limited number of plants have been regenerated over a long period of time (Preece, 2003). Therefore, the development of valuable techniques that allow the rapid multiplication of these plant species in a short amount of time and limited space can be an important benefit for the plant material production of these ornamental plants (Maghami, 2003; Kozak et al., 2007; Ioja-Boldura & Ciulca, 2013; Hârţa et al., 2018).

As a modern method of large-scale plant propagation, tissue culture has been used for rapid multiplication of ornamental species (Rout et al., 2006). However, the effectiveness of the protocols used was strongly influenced by the species (genotype) and also by *in vitro* culture conditions, such as culture media, plant growth regulators (PGRs) and explant type (Debergh & Maene, 1981; do Valle Rego & de Faria, 2001; da Silva, 2013; Datta et al., 2017; Faleiro et al., 2019).

Moreover, another important objective of tissue culture is to obtain true-to-type plants, due to the fact that during tissue culture somaclonal variations might occur (Mujib et al., 2013; Eeckhaut et al., 2020). Thus, the evaluation of the genetic fidelity of micropropagated plants with mother plants using the Start codon targeted (SCoT) markers can be a valuable method to confirm the uniformity of *in vitro* raised plants at molecular level.

Due to the high ornamental value of Gesneriads, the trend of growers in recent years have been to produce high quality planting material for the flower market. In this context, the objectives of this research were (i) to determine the influence of plant growth regulators (PGRs) on the in vitro proliferation of four varieties of Gesneriaceae and (ii) to evaluate the genetic fidelity of micropropagated plants with the mother plants after the 6th successive subculture.

MATERIALS AND METHODS

The plant material used in this study was represented by four hybrid cultivars of *Gesneriaceae*: Avanti Blue (*Sinningia speciosa*), Carnival (*Kohleria hirsuta*), Shannon (*Streptocarpus x hybridus*) and Ivona Specta Adele (*Saintpaulia ionantha*). The mother plants were selected based on flower characteristics and were purchased from two certified nurseries from North Wales, UK. These mother plants were used as explant sources for the initiation and establishment of the *in vitro* culture and were grown as potted plants in greenhouse conditions at 23 °C (Figure1).

To initiate *in vitro* culture, young leaves from mother plants were collected and then were washed with running tap water using a magnetic stirrer to eliminate all the dust and impurities. Thereafter, the leaves were cut in fragments of approximately 10 x 10 mm in size under a laminar flow hood in aseptic conditions and were sterilized with 20 % commercial bleach ACE solution (Procter & Gamble, Romania; <5 % active ingredient) for 20 min followed by triple-rinse with sterile deionized water. The explants were then aseptically inoculated into glass test tubes (11.5 cm x 2 cm ø) containing 5 mL of sterile Murashige & Skoog (1962) medium (MS) supplemented with 2% (w/v) sucrose, 0.2 mg L^{-1} IAA and 0.5 mg L^{-1} BA and gelled with 0.6% agar (Sigma-Aldrich Inc). The pH of culture medium was adjusted to 5.9 before autoclaving at 121°C for 20 min. The cultures were grown in culture room at 24 ± 1°C under 16/8 h light/dark cycle. Light was provided by white fluorescent lamps.

After 56 days of culture on the initiation medium, the regenerated shoots from leaf explants were further multiplied at 21 days intervals by two passages on MS medium supplemented with 0.2 mg L^{-1} IAA and 0.5 mg L^{-1} BA for ensuring the stock of plants for subsequent in vitro multiplication experiments.

After *in vitro* culture establishment, in the proliferation stage, adventitious shoots were divided and individually transferred to 720 ml (v/v) culture jars containing MS medium supplemented with two variants of PGRs: V1- 0.2 mg L^{-1} IAA+0.2 mg L^{-1} BA and V2- 0.2 mg $L^{-}1$ NAA+1mg L^{-1} BA and solidifed with 0.6% Plant agar (w/v). Adventitious shoots were subsequently transferred in aseptic condition to culture jars and cultured (30 days/subculture) at 24 ± 1°C under fluorescent white light (33,6 μmol m^{-2} s^{-1}) conditions with a photoperiod of 16/8 h light and dark cycles. The average multiplication rate and shoot length were recorded after the 6^{th} successive subculture.

After the 6^{th} successive subculture, the proliferated shoots were individually separated and then were rooted on aseptic conditions using ½ MS medium supplemented with 1 mg L^{-1} IAA. The mean values of rooting percentages were calculated after 35 days.

The rooted plants were thereafter subjected to acclimatization process in laboratory conditions for 6 weeks. For this, the plantlets were taken out from the culture jars, rinsed carefully with sterile deionized water and then planted into transparent plastic containers with lids filled up with humidified perlite. After 42 days, the plantlets were individually transplanted to plastic pots with a diameter of 6 cm and filled up with a potting mixture made of peat, vermiculite and perlite (2:1:1) and hardened in greenhouse conditions. The mean values of the survival rate were recorded after 21 days of growth in greenhouse.

To assess the genetic fidelity of micropropagated plants with mother plants, DNA was isolated from both the mother plant and seven in vitro-raised plants of each species that were randomly selected after 21 days of growth in greenhouse. The harvested leaves were dried, grounded into fine powder (TissueLyser II, Qiagen, Germany) and then kept at 4°C until the genetic analyses were performed.

The extraction of total genomic DNA (gDNA) was performed using a CTAB (cetyl trimethylammonium bromide)-based protocol described by Lodhi et al. (1994) and improved by Pop et al. (2003) and Bodea et al. (2016).

The quality and concentration of DNA were determined with a NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, USA). Prior to SCoT analysis, DNA samples were diluted to 50 ng μL^{-1} using sterile double distilled water.

Two SCoT primers were used for each analyzed cultivars to confirm the genetic uniformity of vitro-plants with the mother plant. These primers generated scoreable fragments in all analyzed samples, and to ensure the reproducibility of the results all PCR reactions were repeated twice.

For SCoT analysis, PCR reactions were carried out with a total volume of 15 μL consisting of 3 μL gDNA, 5.6 μL distilled H2O for the PCR reactions, 2.5 μL GoTaq Flexi Green buffer, 2.5 μL MgCl2, 0.25 μL dNTP mix (Promega, USA), 1 μL SCoT primer (GeneriBiotech, Czechia), and 0.15 μL of GoTaq polymerase (Promega, USA). The PCR temperature cycling conditions were: (a) initial denaturation at 94°C for 5 min, (b) 35 cycles of denaturation at 94°C for 1 min, annealing at 50 °C for 1 min and elongation at 72°C for 2 min, and (c) the final elongation step of 5 min at 72°C. Separation of the amplified products was performed by electrophoresis on 1.6% agarose gels (Promega, USA) stained with RedSafeTM Nucleic Acid staining solution (iNtRON Biotech, South Korea) in 1X TAE (Tris-acetate-EDTA buffer), at 100V and 176mA for 2,5-3 hours. The electrophoretic profiles were visualized in UVP Biospectrum AC Imaging System (UVP BioImaging Systems, Germany).

Data analysis

The *in vitro* experiments were carried out in a completely randomized design (4 replication x 5 inoculums/ each treatment). One-way ANOVA was performed to check the differences between the experimental variants. When the null hypothesis was rejected, Duncan test ($p<0.05$) was used to determine the differences between the means. The values shown are means ± S.E.

SCoT gel images were analyzed using TotalLab TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) to determine the number and the range size of the amplified bands. Intensity of the bands were not considered while scoring.

(a) (b) (c) (d)

Figure 1. The commercial cultivars used in this study for tissue culture initiation: Avanti Blue-*Sinningia speciosa* (a), Carnival-*Kohleria hirsuta* (b), Shannon-*Streptocarpus x hybridus* (c) and Ivona Spectra Adele-*Saintpaulia ionantha* (d)

RESULTS AND DISCUSSIONS

Shoots induction and proliferation

The induction of adventitious shoots from leaf explants of four cultivars of *Gesneriaceae* was done using MS medium supplemented with 0.2 mg L^{-1} IAA and 0.5 mg L^{-1} BA. Our findings are consistent with previous reports that highlighted the high capacity of *in vitro* shoots proliferation of Gesneriad plants from leaf explants (Naz et al., 2001; Afkhami-Sarvestani et al., 2006; Shukla et al., 2012). Another type of explant, such as shoot tips was used to initiate the in vitro culture of *Kohleria amabilis* (Kozak et al., 2007), but in this study, *Kohleria hirsuta* shoots were successfully initiated from leaf fragments. On the other hand, we observed that the proliferated epidermal cells produced shoots directly, without callusogenesis.

In this study, MS medium with two different combinations of plant growth regulators (PGRs) were tested to choose the best variant of culture medium. The PGRs combinations significantly affected shoot proliferation in all Gesneriad cultivars (Table1).

The results show that the addition of citokinins (BA) and auxins (IAA and NAA) in MS medium had a stimulating effect on the rate of multiplication of the adventitious shoots (Table 1). As shown in Table 1, among the treatments, MS medium supplemented with 0.2 mg L^{-1} NAA and 1mg L^{-1} BA proved to be the best variant for shoot proliferation and significantly improved the number of shoots/explant for Avanti Blue (14.97 ± 0.35) and Carnival (18.15 ± 0.31). On the same variant of the culture medium (V2), non-significant differences were recorded between Shannon (16.71 ± 0.19) and Ivona Spectra Adele (16.91) cultivars.

Our results are in agreement with those reported by other authors (Park et al., 2012; Ghorbanzade and Ahmadabadi, 2014; Macas-Palacios et al., 2015) who stated that MS medium containing BA in concentrations between 0.2 to 2.0 mg L^{-1} and NAA (0.1-0.2 mg L^{-1}) led to the highest efficiency of shoot regeneration per explant. High shoot regeneration rates were observed for all varieties, indicating the genotype dependence of the protocol used (Figure 2).

The data presented in Table 2 show also that the longest shoots (4.15 cm) were recorded at *Streptocarpus x hybridus* cv. Shannon on MS medium containing V2-PGRs combination, while the shortest (1.33 cm) were recorded at *Sinningia speciosa* cv. Avanti Blue proliferated on MS medium containing V1-PGRs combination. Regardless of the PGRs variants used, significant differences were observed between the three varieties cultured on the same PGRs combination as shown in Table 2.

Our results show that for all analyzed cultivars, V2-PGRs (MS + 0.2 mg L^{-1} NAA+1mg L^{-1} BA) proved to be the most effective for shoots proliferation. The recorded value of the average length of the shoots was 1.67 cm for *Sinningia speciosa* cv. Avanti Blue. Compared to our results, Park et al. (2012) reported that the highest value of shoot length (1.7-1.8 cm) was obtained when the MS medium was supplemented with 2.0 mg L^{-1} BA+0.1 mg L^{-1} NAA, and either 7.0 mg L^{-1} $AgNO_3$ or 50 mg L^{-1} putrescine. In addition, Chae et al. (2013) reported that the highest value of shoot length (1.27 cm) was recorded when the regeneration medium (MS +2.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA) was supplemented with 5.0 mg L^{-1} STS (silver thiosulphate).

Table 1. The influence of PGRs on the rate of multiplication of proliferated shoots (number of shoots/inoculum) of the analyzed cultivars of *Gesneriaceae*

Cultivars	Multiplication rate	
	V1 MS+0.2 mg L^{-1} IAA+0.2 mg L^{-1} BA	V2 MS + 0.2 mg L^{-1} NAA+1 mg L^{-1} BA
Avanti Blue	9.46 ± 0.23 B*	14.97 ± 0.35 D
Carnival	9.96 ± 0.32 C	18.15 ± 0.31 F
Shannon	7.21 ± 0.21 A	16.71 ± 0.19 E
Ivona Spectra Adele	9.31 ± 0.23 B	16.91± 0.28 E

*The values shown are means ±SE. Different letters indicate significant differences between the treatments according to Duncan's test.

(a) (b) (c) (d)

Figure 2. *In vitro* shoot proliferation of *Gesneriaceae* cultivars: Avanti Blue (a); Carnival (b); Shannon (c) and Ivona Spectra Adele (d) on MS medium supplemented with 0.2 mg/L^{-1} NAA and 1mg/L^{-1} BA after 6th successive subculture

Table 2. The influence of PGRs on the length of proliferated shoots of the analyzed cultivars of *Gesneriaceae*

Cultivars	Shoot length (cm)	
	V1 MS+0.2mg L^{-1} IAA+0.2 mg L^{-1} BA	V2 MS + 0.2 mg L^{-1} NAA+1mg L^{-1} BA
Avanti Blue	1.33 ± 0.01 A	1.67 ± 0.02 C*
Carnival	1.54 ± 0.02 B	1.94 ± 0.01 D
Shannon	3.74 ± 0.02 E	4.15 ± 0.01 F
Ivona Spectra Adele	1.53 ± 0.02 B	1.86 ± 0.02 D

*The values shown are means ± SE. Different letters indicate significant differences between the treatments according to Duncan's test.

The results of this study show that also for *Kohleria hirsuta* (cv. Carnival) the MS medium supplemented with the V2-PGRs combination was the best option for the proliferation of shoots. The average length of the shoots (1.94 cm) was significant higher compared with that obtained by Kozak et al. (2007) at *Kohleria amabilis* (0.45 cm) grown on hormone-free MS medium.

Moreover, the results of this research support our previous findings (Hârţa et al., 2018) regarding the influence of cultivars and different combinations of PGRs on shoots regeneration of *Streptocarpus x hybridus*. Thus, the variant of MS medium supplemented with PGRs combination that included BA and IAA was found to be more effective in producing the higher number of rooted shoots (5.03) than the one supplemented with NAA and TDZ (4.5).

On the other hand, a few research groups reported that the addition of PGRs, such as zeatin (Daud et al., 2008), BA (Sunpui & Kanchanapoom, 2002) and TDZ (Mithila et al. 2003, Shukla et al., 2012) on the culture media of commercial varieties of *Saintpaulia ionantha* played a key role in the regeneration of shoots. For example, Daud & Taha (2008) reported that regeneration of African violet shoots from floral explants was stimulated when MS medium was supplemented with 1 mgL^{-1} BA and 2 mgL^{-1} NAA. Ghasemi et al. (2012) observed that the longest Saintpaulia shoots induced from the leaf disc (with an average of 7.6 mm) belonged to media containing 0.2 or 0.5 mgL^{-1}BA along with 0.5 mgL^{-1} IBA, while the shortest belonged to the media containing only 0.5 mgL-1 BA, (with an average of 2.8 mm). In this study, MS + 0.2 mg L^{-1} NAA+1mg L^{-1} BA was the best option for the proliferation of Ivona Spectra Adele cv. shoots. These results indicated the important role of auxin (NAA) in promoting both number and length of the regenerated shoots.

Rooting and acclimatization of vitro-plants

In vitro rooting of multiple shoots was recorded on ½ MS+1mg L^{-1} IAA for all of the four cultivars of *Gesneriaceae*. The mean values of the percentage of rooting of the shoots and also the survival rate (recorded after 21 days of growth in greenhouse) are presented in Table 3. The results summarized in Table 3 show that ½ MS supplemented with IAA stimulated the rooting of regenerated shoots for all four cultivars of Gesneriaceae. Moreover, there were significant differences between cultivars in term of *in vitro* rooting percentage (Avanti Blue 76.86 % vs. Carnival 85.66 %).

It is worth mentioning that roots induction and elongation is one of the most important steps for the successful production of in vitro regenerated plants (Ghorbanzade & Ahmadabadi, 2014). In the case of the analyzed cultivars of Gesneriaceae, the rooting process was successful.

Regarding the acclimatization process, it is noteworthy that more than 92% of *in vitro* raised plants of the four analyzed cultivars survived (Table 3). Rooted plants were successfully transferred in greenhouse, followed by normal plant growth and flower development.

Table 3. The rooting percentage and survival rate (%) calculated for each of the four Gesneriaceae cultivars. The values shown are means ±SE

Species	**Cultivars**	***In vitro* rooting** (%)	**Survival rate** (%)
Sinningia speciosa	**Avanti Blue**	76.86 ± 0.08 A*	95.24 ± 0.03 A
Kohleria hirsuta	**Carnival**	85.66 ± 0.06 B	94.93 ± 0.02 A
Streptocarpus x hybridus	**Shannon**	94.33 ± 0.06 C	95.05 ± 0.03 A
Saintpaulia ionantha	**Ivona Spectra Adele**	96.66 ± 0.03 C	92.59 ± 0.03 A

*Different letters indicate significant differences among the treatments according to Duncan's test.

Evaluation of genetic uniformity of acclimatized plants

In order to confirm whether or not somaclonal variation was detectable in *in vitro* raised plants, SCoT markers were employed to analyze the genetic fidelity of seven randomly selected acclimatized plants from each species, as well as their corresponding mother plants. Both SCoT primers used generated clear and scorable bands for all samples analyzed (Table 4).

Genetic analysis with SCoT markers led to a total of 10, 20, 11 and 19 monomorphic bands for Avanti Blue, Carnival, Shannon and Ivona Spectra Adele cv. respectively (Table 4).

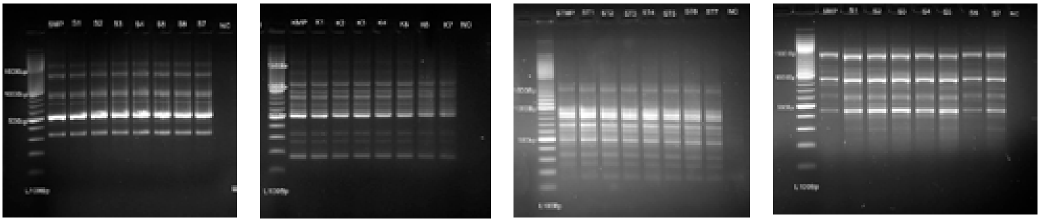
Figure 3 shows that *in vitro* raised plants have the same banding patterns as those of the mother plants, indicating their uniformity at DNA molecular level. Each SCoT markers generated monomorphic bands and their length ranged between 528 and 2036 bp. Moreover, no genetic variability was observed in any of the analyzed cultivars.

Despite the many advantages of clonal propagation through tissue culture techniques, the occurrence of somaclonal variation is a serious limitation in the practical applications of plant micropropagation (Soliman et al., 2014). In fact, the variability detected in regenerated plants from tissue cultures is a combined effect of genetic and epigenetic variations that may occur during different stages of *in vitro* culture protocols and genetic heterogeneity of explant cells (Bhojwani & Dantu, 2013). The genotype, explant type, the combination and concentrations of PGRs, the number of the successive subcultures and methods used for regeneration may also influence the genetic stability of *in vitro* raised plants (Martin et al. 2004). Even so, in this study, no genetic variability was observed between mother plants (MP) and the seven randomly selected acclimatized clonal plants of each *Gesneriaceae* cultivars and provided from 6th successive subculture (Figure 3).

Our results support previous findings reported by other authors refers to the fact that SCoT molecular markers were effective and gave reproducible results in evaluation of genetic fidelity of *in vitro* raised plants at *Dendrobium nobile* (Bhattacharyya et al., 2014) and *Chrysanthemum morifolium* (Nasri et al., 2018). The findings of our study further suggest that SCoT molecular markers are useful in assessing the genetic stability of *in vitro* propagated *Gesneriaceae* cultivars.

Table 4. Sequences of SCoT primers and the number of monomorphic bands generated in the analyzed Gesneriaceae cultivars

Primer name	**Primer sequence 5'-3'**	**Number of monomorphic bands**				Range size of bands (bp)
		Avanti Blue	Carnival	Shannon	Ivona Spectra Adele	
SCOT16	ACCATGGCTACCACCGAC	5	9	5	9	528-1712
SCOT25	ACCATGGCTACCACCGGG	5	11	6	10	201-2036
Total bands		10	20	11	19	-



(a) (b) (c) (d)

Figure 3. PCR banding patterns obtained with SCoT16 primer at *Gesneriaceae* cultivars: Avanti Blue (a); Carnival (b); Shannon (c) and Ivona Spectra Adele (d). Lane L-100 bp DNA step Ladder; SMP, KMP, STMP and SPMP - mother plant for each species; L1-L7 SCoT profile of acclimatized vitro-plants; NC-negativ control

CONCLUSIONS

As conclusions, the combinations of PGRs used in this research stimulated the shoot induction and direct shoots regeneration in devoid of the callus phase, considering that direct regenerated plants may exhibit greater genetic stability than those produced *via* callus. Based on ScoT molecular markers analysis, the genetic uniformity of the regenerated plants with the mother plants were confirmed and, therefore, the propagation method presented in this study could be applied also for commercial purposes.

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MYCORRHIZATION OF *CORYLUS AVELLANA* L. AND *QUERCUS ROBUR* L. SEEDLINGS WITH *TUBER AESTIVUM* VITTAD.

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Abstract

Mycorrhization could be a valuable tool in order to produce a remarkable and a high economical interest mushroom. Corylus avellana L. and Quercus robur L. are known as mycorrhizal hosts for Tuber aestivum Vittad. (Truffle). Cultivating truffles, is important to know the necessary pH level of the soil, on which the mycorrhization occurs. The selected tree species are known to have a high percentage of inoculation level under the effect of truffle spore inoculum. In the study, seedlings of the previously mentioned species were subjected to truffle infection at three soil pH levels (7.3, 7.5, 7.7). During the experiment two different weight of truffle and two inoculation methods (soil-inoculation and inoculation with suspension) were tested. Our results show that significant changes were obtained at 7.5 pH level, when the weight of the truffle added to the roots influenced the mycorrhization level in a positive way, and also inoculation method significantly influenced the T. aestivum appearance. In conclusion, our data suggests that Common hazel and European oak mycorrhization occurred, yet it is important to ensure the necessary growth requirements for a greater and higher quality production of truffles.

Key words*: bonitation score, inoculation pH, symbiosis, truffle.*

INTRODUCTION

Tuber is a major genus fungus which is producing hypogeous fruit bodies, commonly known as truffles (Paolocci et al., 2004). Besides being used in medicine, wild edible fungi have always been very popular in many national cuisines (Boa, 2004). Some of them can be cultivated, while many other can only be harvested in forests. One of the few cultivated fungi are the truffles. Also, it is mentioned that some of the species from this genus are economically important mushrooms, because they are producing fruits with a characteristic aroma and flavour profile (Paolocci et al., 2004; Gryndler et al., 2014; Shah et al., 2020).

The summer truffle (*Tuber aestivum*) is one of the most popular and commonly used truffle species in almost all European countries, it is the second most cultivated truffle worldwide (Murat, 2015). 90-95% of the gathered and cultivated truffles belong to this species (around 10 tons/year). *Tuber aestivum* is the only one that can be effectively cultivated in Transylvania.

Truffles are ascomyceteous fungi that belong to several families in the order Pezizales of the subphylum Pezizomycotina within the phylum Ascomycota (Kirk et al., 2008).

These ascomyceteous fungi have a subterranean mode of existence (Læssøe & Hansen, 2007). Other basidiomycetes can also have underground fruiting bodies but only species in the genus *Tuber*, *Tuberaceae*, are actually considered the true truffles (Jeandroz et al., 2008). This genus contains 180 to 230 species worldwide (Bonito et al., 2010; Bonito et al., 2013), 30 of them are present in Europe (Clemmensen et al., 2013), however only a few have markable properties and social value (Zambonelli et al., 2016).

The *Tuber*'s entire life cycle occurs in soil, according to Todesco et al. (2019) soil parameters (temperature) and water availability could have the greatest influence on the growth of the truffles. The most favourable soil type for the *Tuber* genus are the soils with a pH level of 7.5, with a Ca-Mg proportion of 25-55, K-Mg of 0.3-2.1, C-N of 13 and humus content of 12% (Wedén, 2004).

85-90% of angiosperms, 100% of gymnosperms and 70% of pteridophytes are known to have mycorrhizal associations with different species

of fungi (Takács & Vörös, 2005). All species of truffles live in this special relationship with their host plant. Truffles can only be cultivated if they have this mutual symbiotic association with a list of species of specific trees like *Quercus* sp., *Corylus* sp., *Picea* sp., *Populus* sp. (Győrfi, 2010).
In the mycorrhizal association, the host plant's roots are colonized by the fungus, either intercellularly as in arbuscular mycorrhizal fungi, or extracellularly as in ectomycorrhizal fungi (Johnson et al., 1997).
For many years, truffles were thought to only form ectomycorrhizas, however in recent research they found that truffles can also form arbutoid mycorrhizas (Iotti et al., 2014) and endomycorrhizas with orchids (Selosse et al., 2004).
The species of truffles that can be found in Romania can develop mycorrhizal associations with various species of trees with the exception of *Mattirolomyces terfezioides,* which can only have this type of connection with *Robinia pseudoacacia.* The most important species from economical point of view can mostly be found with: *Quercus* sp., *Coryllus avellana, Picea abies, Populus* sp., *Salix* sp., *Carpinus betulus, Fagus sylvatica, Pinus nigra* and *Tilia* sp. The truffle's choice of host plant depends mostly on the tree's environmental needs, primarily climate and soil conditions (Benucci et al., 2012).
From these host plants the first two seems to work best with the truffle.
Quercus sp. is a common tree in Europe. It can reach different heights from 1-2 m to almost 45 m. The species that can be found in Romania tend to reach the height of 35-40 meters (Fitter, 2004).
Corylus avellana can also be commonly found in Europe. It reaches a height of 3-8 meters, it is a typical shrub (Rushforth, 1999).
Truffles have a high economical value which is why it was very important to find the most efficient method of cultivation. This proved to be a difficult task not only because this genus has a complex life cycle that involves a symbiotic relationship with the host plant but also because it is entirely underground, therefore involves a complex relationship with soil microorganisms too.
In the present study we aimed to investigate the percentage of mycorrhization influenced by the substrate pH level, we tried to find the best host plant for the truffle and also to find out which is the best method to infect the host plants with truffle in order to produce the highest quality and quantity of truffles.

MATERIALS AND METHODS

In this research we selected two of the most commonly used host plants: *Quercus robur* and *Corylus avellana.*
Quercus robur commonly known as European Oak can be found in many Transylvanian forests so the acorns can be easily gathered or harvested.
In Transylvania *Corylus avellana* (Common hazel) is not that widely used as a host plant but it is still a favourable species because it goes to seed faster than the common oak. It needs more water, has a dense root system which benefits the truffle production.
The research was carried out in Târgu Mureș, Mures county.
In October in the first year of the experiment we gathered 8.8 kgs of acorns from the forest and bought 4 kgs of hazelnut. To prevent the infection of blackleg we merged the acorns in water for 2.5 hours in a temperature of 41°C. After that we dried the seeds for a short time and merged them again in a solution of Sodium dichloroisocyanurate for 20 minutes and washed-out the remains of any kind of chemicals.
For the hazelnuts were also used a solution of Sodium dichloroisocyanurate against bacteria and other infections but only for 10 minutes then again, were washed-out the remains. After this treatment we stored the seeds in a fridge until planting.
The substrate used in the experiment was a combination of peat moss (70%) and perlite 30%. The substrate was sterilized with a high-pressure steam for 2 hours. Before sterilization the planting medium had a pH of 3.53 and 19°C, so to reach the desired pH level were substantially added limestone powder, and adjusted the pH levels to 7.3, 7.5, and 7.7.
Hazelnut seeds were sown in a tray by two different methods to add the truffles. In the first method we grated the truffles and added it directly to the medium. The other method was to make a truffle suspension that we merged the seeds in. The suspension contained 30% of truffle grating, 30% clay and 40% water. One

half with 1.5 grams and the second half with 2 grams. pH level was monitored every 10 days, each time at 20°C, because the pH level changes with temperature so it is important to always make the measurements at the same temperature. A solution was made of lime so can maintain the pH level at the desired level, was measured 3 months straight. After 2 months the pH level reached a constant state, so it was reached the pH level that benefits the production of *Tuber aestivum.*

After cold treatment both selected plant's seeds (acorns and the hazelnut) were germinated in sterile perlite.

In the first part of the research, soil inoculation was used to infect these seedlings with truffle. In the second part the seedlings were merged directly in a truffle suspension. Two different quantities of truffle were used: 1.5 g and 2 g.

The Institut National de la Recherche Agronomique (INRA) worked-out a system to grate the level of infection. This system is called bonitation and it uses a six-degree scale to measure the bonitation level (Chevalier & Grente, 1973). Under a stereo microscope the mycorrhization branching was determined (Figure 3). The different levels are:

0 - if the mycorrhizal connection can't be seen at all;
1 - if the mycorrhizal connection reaches 10%;
2 - if the mycorrhizal connection reaches 20%;
3 - if the mycorrhizal connection reaches 30%;
4 - if the mycorrhizal connection reaches 40%;
5 - if the mycorrhizal connection reaches 50%.

Data were analyzed using Past 4 statistical software (Oslo, Norway). Data were tested for normality of errors and homogeneity of variance. All data were normally distributed. The significance of the differences between the treatments was tested by applying 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 $p < 0.05$.

RESULTS AND DISCUSSIONS

Truffles are significant for the fauna, because they serve as nutrition for the soil micro-fauna and also for some mammalian species (Schickmann et al., 2012; Özderin, 2020).

The hazelnut's mycorrhization with soil-inoculation under different pH levels and with different quantity of truffle shows different results. Significant changes were only found at the pH level of 7.5. At 7.3 the infection level reached 20% with both quantity of truffle. At 7.5 and with 2 grams of truffle the mycorrhization was not successful. Also, at 7.7 the mycorrhization wasn't successful neither with 1.5 g, nor with 2 g.

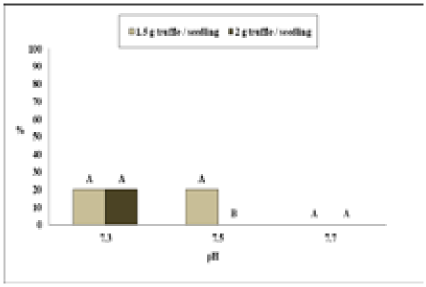

Figure 1. Mycorrhization percentage under soil-inoculation at different pH levels and quantity of truffle at hazelnut seedlings. Different uppercase letters indicate the significant differences between the grams of the truffles, according to Tukey test ($p = 0.05$)

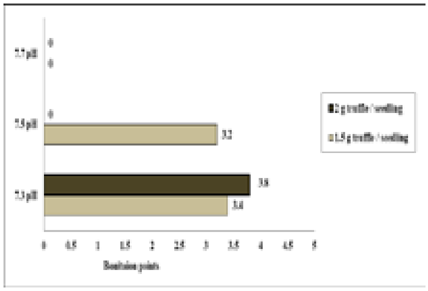

Figure 2. Bonitation level under soil-inoculation at different pH levels and quantity of truffle at hazelnut seedlings

It can be observed that on the Figure 1 the soil-inoculation at 7.3 with 1.5 grams of truffle reached the bonitation level of 3.4, while the inoculation with 2 grams reached the level of 3.8. The bonitation level at the pH level of 7.5 and with 1.5 grams of truffle was 3.2.

In a research Csorbainé Gógán Andrea (2011) found that merging the host plant's seeds in a suspension is more effective than the soil-inoculation, so we tried this method as well. Because the significant changes were observed at a pH level of 7.5, we used this pH level in the next part of the research.

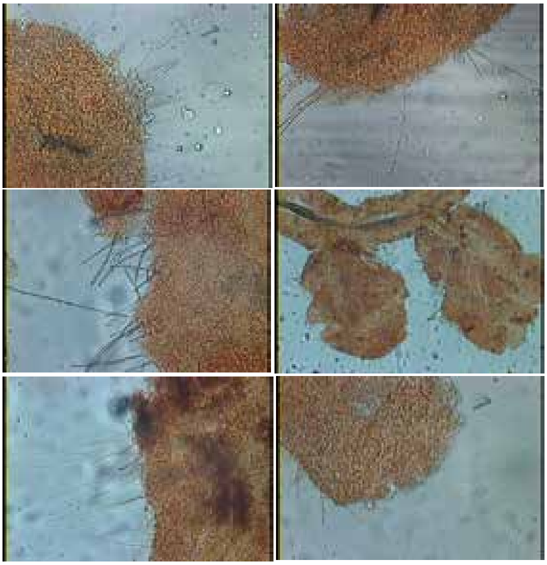

Figure 3. Mycorrhizal structure/branching under a stereo microscope in the roots of the selected host plants

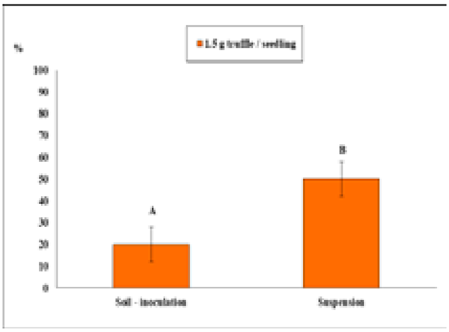

Figure 4. Percentage of soil-inoculation and infection with suspension at hazelnut seedling with a quantity of 1.5 grams of truffle. Different uppercase letters indicate the significant differences between the two inoculation methods, according to Tukey test ($p = 0.05$)

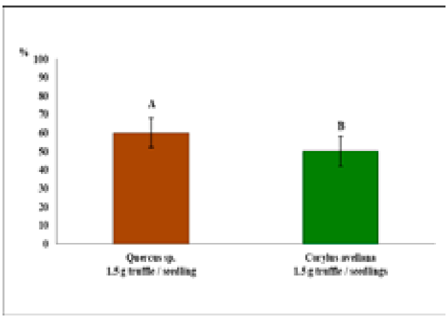

Figure 5. Infection level with suspension at both oak and hazelnut seedlings with a quantity of 1.5 grams of truffle. Different uppercase letters indicate the significant differences between the two host plants, according to Tukey test ($p = 0.05$)

Examining the results of the two different types of infection methods (Figure 4) at the hazelnut seedlings with a quantity of 1.5 grams of truffle was found significant changes between soil-inoculation and infection with suspension.

The infection percentage with soil-inoculation remained at 20% while with suspension the infection level reached 50%.

This shows a significant increase. At the infection with the truffle suspension, we can see a significant difference at the oak and hazelnut seedlings. The hazelnut seedlings reached the infection level of 50%, while the oak seedlings reached the level of 60%. According to Özderin, 2020 mycorrhization occurred on 50% of the pecan seedlings, the remaininged ones were not successful, because of contamination caused by inoculation, seedlings, water and even by the environmental factors.

CONCLUSIONS

In the production of high quality of truffle, it is very important for the fungi to form the mycorrhizal connection with the host plant. It can be seen from Figure 5 that while *Corylus avellana* also shows great results, the best from the two most commonly used hosts, *Quercus sp.* shows the best results with the infection level of 60%. This result suggests that *Quercus sp.* is the best host with these conditions.

Out of the three different pH levels was found that the best for truffle production is the level of 7.5 which can be seen on Figure 1 and Figure 2. Regarding the quantity of truffle, 1.5 grams showed the best results.

Out of the two methods of infection the most efficient is the infection with the truffle suspension.

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PERCEPTION AND ASSESSMENT OF FRUIT TREES VISUAL QUALITY DURING SPRING IN PUBLIC GREEN SPACES AND PRIVATE GARDENS

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Abstract

Fruit trees are valuable components in the ecosystem, having both aesthetic and utilitarian functions. This study focuses on assessing the aesthetic aspects of fruit trees during spring, using the expert based paradigm and the perception based paradigm. The Scenic Beauty Estimation method is used to measure the visual quality, but also five landscape parameters: vitality, harmony, fascination, naturalness and colour diversity. Such studies are important for better urban planning, fruit trees proving to increase the visual quality in public green spaces and private gardens, through its decorative elements, such as flowers. The integration of fruit trees in the landscape it is a necessity in creating a sustainable landscape.

Key words: *fruit trees aesthetic, landscape aesthetic, visual quality, urban planning.*

INTRODUCTION

In a fast urbanization process and society development, public green spaces, but also private gardens offer opportunities for landscapers by designing recreational spaces, experiencing nature by planting trees (Ma et al. 2021). The first step in creating a sustainable urban ecosystem is to establish harmony relations between design and the environment around us (Bulut et al., 2010). Landscape visual quality appreciation involves the inventory and evaluation of various visible attributes, with the aim of planning, designing and managing the landscape (Palmer & Hoffman, 2001). Urban vegetation reduces heat absorption, increases sun protection, acts as a barrier against pollution, improves air quality, masks the noise (Semeraro et al., 2021; Roy et al., 2012), thus having a positive impact on the quality of human life. In the urban ecosystem dynamic, fruit trees contribute to the diversity of the landscape, but also to the development of urban horticulture concept, having both an aesthetic and a utility function, through fruit production (Melinescu & Cosmulescu, 2021). The aim of the study is to determine the visual effect that fruit trees can generate in the landscape, during spring season, by their presence and absence, in order to improve urban planning.

MATERIALS AND METHODS

Based on both the expert paradigm and the perception-based paradigm (Daniel, 2001; Asur et al., 2020; Gungor & Polat, 2018), in order to achieve the aim, a series of 10 photographs with fruit trees from public green spaces, as well as from private gardens, were taken in Craiova, a city located in the southwest of Romania (44°19'0.01"N, 23°48'0.00"E).
The photos were taken during spring season. The 10 landscapes with fruit trees were coded as follows: LFS1 - LFS10, and the other 10 landscapes without fruit trees as follows: LWFS1 - LWFS10. The visual effect analysis of the fruit trees was performed through a questionnaire that focused both on the evaluation of the landscape with fruit trees and on the evaluation of the landscape without fruit trees. Adobe Photoshop CC 2018 was used to remove the fruit trees from the 10 original photos. In the questionnaire, participants were asked to rate the landscape according to their visual preference, but also according to 5 descriptive parameters: vitality, harmony,

fascination, naturalness and color diversity. The evaluation was performed using the Likert scale from 1 to 7, 1 representing the lowest value and 7 the highest value (Bulut et al., 2010). The determination of the visual quality index (VCI) was performed using the Scenic Beauty Estimation - SBE method (Daniel & Boster, 1976), one of the most popular methods in such studies. SBE has three components: descriptive - simply shows the characteristics of the environment, assessment - measures the quality of landscapes and preference - shows the subjective preference of those who observe the landscape (Mo et al., 2021). The questionnaire was completed by 84 people, including 49 specialists in landscaping, horticulture, environment and 35 students in the same fields.

The statistical data were analyzed in IBM SPSS Statistics 28.0.

RESULTS AND DISCUSSIONS

The mean visual quality index was determined for each landscape, both with and without fruit trees. No significant differences were observed between the visual quality index resulting from the expert paradigm and the one resulting from the perception-based paradigm, which shows that the visual perception is unitary, so the total visual quality index was calculated, compared to the 84 respondents. (Table 1 and Table 2).

Table 1. The visual quality index of the landscape with fruit trees in the spring season

Landscape variant	**The visual quality index resulting from the expert paradigm**		**The visual quality index resulting from the perception-based paradigm**		**Visual quality index total**	
	VCI Mean	SD	VCI Mean	SD	**VCI Mean**	**SD**
LFS1	5.55	1.40	4.91	1.97	**5.28**	**1.68**
LFS2	5.16	1.63	4.60	2.25	**4.92**	**1.92**
LFS3	5.67	1.42	5.28	1.56	**5.51**	**1.48**
LFS4	5.79	1.48	5.14	1.97	**5.52**	**1.72**
LFS5	5.08	1.70	5.00	2.04	**5.04**	**1.84**
LFS6	4.79	1.70	4.71	2.03	**4.76**	**1.84**
LFS7	5.06	1.76	4.71	2.16	**4.91**	**1.93**
LFS8	5.73	1.44	5.51	1.72	**5.64**	**1.55**
LFS9	6.08	1.23	5.97	1.44	**6.03**	**1.32**
LFS10	5.34	1.53	5.14	1.81	**5.26**	**1.65**
	N: 49		N: 35		**N: 84**	

In the case of the original landscape, with fruit trees, the highest value was recorded by LFS9, VCI = 6.03, a landscape that, in addition to fruit trees, also benefits from a decorative element - a Japanese kiosk, followed by LFS8, VCI = 5.64, a landscape in which fruit trees are grouped (Figure 1), both belonging in the category of public green spaces. The lowest value was recorded by LFS6, VCI = 4.76 (Table 1), a landscape from a private garden with a small size fruit tree, which shows that the size of the fruit tree is a factor in determining visual quality.

Table 2. The visual quality index of the landscape without fruit trees in the spring season

Landscape variant	**The visual quality index resulting from the expert paradigm**		**The visual quality index resulting from the perception-based paradigm**		**Visual quality index total**	
	VCI Mean	SD	VCI Mean	SD	**VCI Mean**	**SD**
LWFS1	4.30	1.89	4.74	1.73	**4.48**	**1.83**
LWFS2	3.40	1.81	3.22	1.88	**3.33**	**1.83**
LWFS3	4.38	1.93	4.85	1.61	**4.58**	**1.81**
LWFS4	4.04	1.95	3.97	1.74	**4.01**	**1.85**
LWFS5	2.20	1.69	2.00	1.49	**2.11**	**1.60**
LWFS6	3.02	1.82	2.80	1.34	**2.92**	**1.63**
LWFS7	1.81	1.49	1.65	1.23	**1.75**	**1.38**
LWFS8	3.20	1.83	3.05	1.79	**3.14**	**1.81**
LWFS9	4.34	1.77	4.94	1.62	**4.59**	**1.72**
LWFS10	3.61	1.81	3.80	1.67	**3.69**	**1.74**
	N: 49		N: 35		**N: 84**	

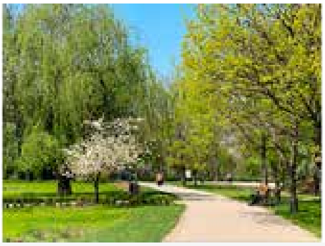
The most significant value, in the case of the landscape without fruit trees, was LWFS9, VCI: 4.59 (Table 2), thus, even in the absence of fruit trees, it remains the preferred landscape in terms of visual quality, hence the importance of landscape elements and how they are integrated into the landscape. A high value was also recorded in the case of LWFS3, VCI: 4.58. The lowest values of the landscape without fruit trees, were recorded in the case of LWFS7, VCI: 1.75, LWFS5, VCI: 2.11 and LWFS6, VCI: 2.92 (Table 2), landscapes from private gardens, showing that once the fruit trees disappear, the fence and unsightly surroundings can be seen, we can affirm that fruit trees, by their size, through the decorative elements, blur the unsightly landscape around even when they are solitary place.

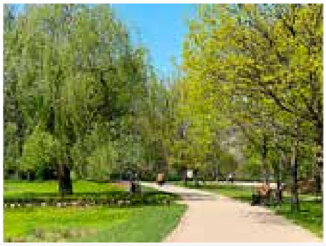

LFS1 (VCI: **5.28)**


LWFS1 (VCI: 4.48)

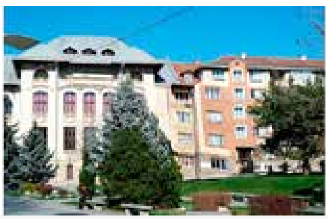

LFS2 (VCI: **4.92)**


LWFS2 (VCI: 3.33)

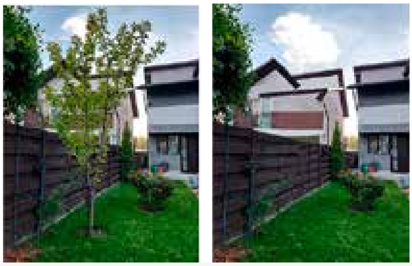

LFS3 (VCI: **5.51)**


LWFS3 (VCI: 4.58)


LFS4 (VCI: **5.52)**

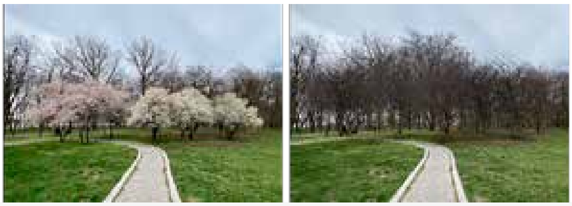

LWFS4 (VCI: 4.01)

LFS5 (VCI: **5.04)** **LWFS5 (VCI: 2.11)**

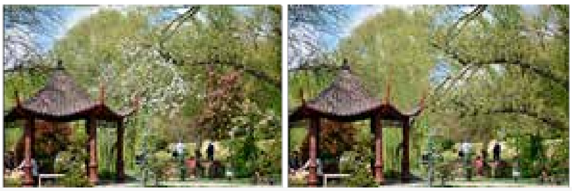


LFS6 (VCI: **4.76)** **LWFS6 (VCI: 2.92)**

LFS7 (VCI: **4.91)** **LWFS7 (VCI: 1.75)**



LFS8 (VCI: **5.64)** **LWFS8 (VCI: 3.14)**



LFS9 (VCI: **6.03)** **LWFS9 (VCI: 4.59)**

LFS10 (VCI: **5.26)** **LWFS10 (VCI: 3.69)**

Figure 1. Original landscape with fruit trees (left) and edited landscape without fruit trees (right)

There are significant differences between the two types of landscapes, which show that fruit trees have a positive effect on improving the visual quality of the landscape. Similar studies (Lisandru et al., 2016; Bulut et al., 2010) indicate the same effect of fruit species in the landscape. The fruit trees, in spring, decorate through flowers, completing the landscape, so the visual perception is stimulated by their diverse color, complementing the rest of the surrounding vegetation, as well as architectural elements.

A series of relationships were established between visual preference and 5 landscape descriptive parameters: vitality, harmony, fascination, naturalness and color diversity, both in the landscape with fruit trees (Table 3) and in the landscape without fruit trees (Table 4). The results of the Pearson correlation demonstrate a relationship between all these elements. In the case of the landscape with fruit trees, we notice a strong significance between the visual preference and landscape vitality and fascination (0.903), but also between the harmony and fascination (0.940) and harmony and naturalness (0.912). Harmony can be described as the way in which the elements of the landscape integrate, while the vitality of the landscape is generated by light and color, and the naturalness of the landscape implies a more sustainable planning and a limited human intervention on vegetation.

Table 3. The relationship between visual preference and the 5 descriptive parameters of the landscape with fruit trees

	Visual preference	Landscape vitality	Landscape harmony	Landscape fascination	Landscape naturalness
Landscape vitality	0.903**				
Landscape harmony	0.900**	0.904**			
Landscape fascination	0.903**	0.890**	0.940**		
Landscape naturalness	0.876**	0.878**	0.912**	0.897**	
Color diversity	0.810**	0.843**	0.852**	0.868**	0.842**

**Correlation is significant at the 0.01 level (2-tailed).

Table 4. The relationship between visual preference and the 5 descriptive parameters of the landscape without fruit trees

	Visual preference	Landscape vitality	Landscape harmony	Landscape fascination	Landscape naturalness
Landscape vitality	0.935**				
Landscape harmony	0.936**	0.948**			
Landscape fascination	0.926**	0.935**	0.949**		
Landscape naturalness	0.898**	0.912**	0.918**	0.898**	
Color diversity	0.860**	0.855**	0.858**	0.896**	0.820**

**Correlation is significant at the 0.01 level (2-tailed).

In the case of the landscape without fruit trees, we find also a positive relationship between landscape harmony and fascination (0.949), as well as between the visual preference and landscape vitality (0.935) and harmony (0.936). We notice a small increase in the significance of the descriptive parameters in relation to the visual perception of the landscape without fruit trees. The human eye tries to better identify the characteristics of the landscape, thus outlining a clear visual perception. The fruit trees, through the decorative elements, visually stimulate the viewer, so the descriptive parameters can be identified more easily. The landscape fascination can be described as the attractiveness that the viewer perceives (Liu, et al., 2021), thus it is recommended to collaborate with a landscaper in creating a landscape as attractive as possible, especially for private gardens, but also for public green spaces. Visual preference also has a significant relationship with color diversity. During the spring, the fruit trees decorate with white, red, dark or light pink flowers, colors that influence the visual perception, but also the mood. The pink color balances the green color and has a calming effect on the viewer, generating clarity, while the white color generates the effect of light distribution, being preferred in landscapes placed in areas with high temperatures (Ender et al., 2016), these can be assets of the fruit trees, the color of the flowers, white, recommending them in areas with high temperatures. The color red is the most easily distinguished by humans, due to the human sight structure (Li et al., 2017), so we can create an element of impact in the landscape by using fruit trees with red foliage, such as *Prunus cerasifera nigra*.

Fruit trees positively contribute at the improvement of the landscape, increasing the visual quality, out of the 10 landscapes, both in public green spaces and private gardens, in 9 of them fruit trees have made a very significant contribution at improving the landscape, only a single landscape recorded a significant contribution, LFS1 - LWFS1, 0.235 (Table 3). Fruit trees are among the first to enter in vegetation, thus masking the unsightly crown of trees which have not yet entered in the vegetation stage, increasing the quality of the landscape.

CONCLUSIONS

The study creates an overview of the landscape visual quality influenced by the presence or absence of fruit trees in spring, thus fruit trees generate a more attractive landscape, both in public green spaces and in private gardens, they can be used in groups or solitary. There is a wide range of fruit species, but also a multitude of ways to integrate them into the landscape, with many crown types, different types of habitus, but also a complex decorative power throughout the entire year. In the process of landscape design, human perception must be taken into account, both to improve the visual quality of the landscape, but also to improve the lives of residents, the vegetation has the power to generate a number of mental and physical benefits. Fruit trees not only contribute to breaking the monotony of the landscape, but, through their utilitarian function, fruit production, they generate economic benefits, so in terms of creating an ecological landscape, the integration of fruit is a necessity.

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BEHAVIOUR OF ORNAMENTAL SPECIES *NIPPONANTHEMUM NIPPONICUM* IN CROPPING CONDITIONS FROM SW AREA OF ROMANIA

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Abstract

Nipponanthemum nipponicum (Franchet ex Maxim.) Kitam., originating in Japan, is part of the hemicryptophyte perennial group. It is a valuable plant and it is noticeable by the fact that it blooms in a period when most of the flowering species end their period of decoration (September-November). In our country N. nipponicum is not known as an ornamental plant and has not been mentioned in any scientific work. As a result, in this paper we have proposed the study of the behavior in culture in order to introduce this species into the assortment. In Craiova city conditions, the flowering was observed at the beginning of October (4.10.) and the decoration duration was between 25 - 36 days. Analyzing the behavior of N. nipponicum plants under different cultivation conditions, it is observed that the plants grown in the sunny exhibition recorded the best results for all the analyzed parameters.

Key words*: assortment, diversification, Nipponanthemum nipponicum, ornamental plant*

INTRODUCTION

Landscapes and urban green space plays a critical role for keeping our cities attractive and healthy (Roy, 2019). Hemicryptophyte perennial plants are characterized by rich flowering and bright colors. They are largely used in the summer-autumn decorations because most plants flower in June - October. The systematic position of *Nipponanthemum* have also been the subjects of much debate (Shen, 2021). *Nipponanthemum* were classified into subtribe *Leucantheminae*, but all were later shown to belong to subtribe Artemisiinae according to molecular phylogenetic data (Sanz, 2008; Shen, 2021).

The genus *Nipponanthemum*, family Asteraceae, consists of only one species, *N. nipponicum* (Franch. ex Maxim.) Kitam, formerly considered part of *Chrysanthemum* (Kitamura, 1978; Koyama, 1995). It is separated from *Chrysanthemum* by morphological characters (Uehara, 2012), and the morphology of chromosomes (Nakata et al., 1995). There is a form with pink flowers, *N. nipponicum* roseum, but the base species is generally preferred, which was used by American horticulturist Luther Burbank to produce *Leucanthemum* x *superbum* (Shasta Daisy).

N. nipponicum (Franchet ex Maximowicz) Kitamura, syn. *Chrysanthemum nipponicum* (Franchet ex Maxim.) Sprenger, *Leucanthemum nipponicum* (Franchet ex Maxim.), Bull. (Garcia et al., 2004), common names "Nippon daisy" or "Montauk daisy"**.** Is a shrubby perennial of 20-60-70 cm tall (-100), native to coastal regions of Japan, and distributed on the rocky slopes near seashores of Pacific side (Gleason & Cronquist, 1991; Hong et al., 2015), but cultivated as an ornamental in other regions (Tosca et al., 1999). The leaves have dimensions of 20-75 (-90) × 12-15 (-20) mm, serrated edges, rarely entire. Unlike other chrysanthemums, the leaves of *N. nipponicum* are dark green, glossy, even leathery, which makes it attractive even before it starts flowering. The flowers are arranged in calatidium inflorescences, white ligulate florets and yellow tubular ones, typical of the *Asteraceae* family. Several authors mention that it blooms between September-November, a period in which most flowering species end their decoration period (Ellis, 2000; Fell, 2007).

The sources consulted show that *N. nipponicum* grows well in full sun, but also tolerates partial shade. Requires fertile, well-drained soil. It is a plant tolerant to drought, frost, salts and has great disease resistance; it blooms in autumn

and is also decorative through its leaves, all positive attributes for plantation in urban locations (Ellis, 2000).
Nipponanthemum can be propagated by seeds, cuttings, or division (Fell, 2007). *N. nipponicum* requires little maintenance. It may be cut back hard in spring to maintain a compact habit. Plants may be divided in spring every three years to maintain the vigor of the plant (Ellis, 2000). *N. nipponicum* can be used for the decoration of green spaces, as isolated plant or in groups, mixed borders, in rock gardens and xeriscaping, but also as cut flowers or as container plants.
In the literature, information on the ecological requirements, multiplication and culture technology of the perennial species *N. nipponicum* is very little or missing. In Romanian literature *N. nipponicum* was mentioned by Vidrașcu (2002). He specifies the plant is part of the decorative collection, unique in Romania, of the "Anastasie Fătu" Botanical Garden from Iași. In his study, Vidrașcu highlights the resistance against *Peronospora tanaceti* Gaum of the species.
The data presented in this work refer to the *N. nipponicum* plants behavior in the town of Craiova and it considers two differentiators: the age of the plants and the different cultivation conditions.

MATERIALS AND METHODS

The behavior of the *N. nipponicum* species was analyzed in the 2018-2019 period, in the experimental field of the Floriculture, Faculty of Horticulture of the University of Craiova. This is located in Craiova's "Al. Buia" Botanical Gardens. The sector is placed on a flat field with a clay-sandy soil and is protected by air flows. The temperate climate of Craiova is characterized by very hot, even scorching summers and more gentle winters. The average annual temperature varies between 10.5 and 11.5^{0}C and the amount of rainfall a year is on average 550 mm. The climate type is forest-steppe with abrupt winter to summer changes and slower summer to autumn transitions. It has Mediterranean influences which are typical in the Romanian fields. The above demonstrate the location poses a favorable environment for cultivating *N. nipponicum*.
In the first stage of the study we followed the behavior of the *N. nipponicum* in different light exposures (V1 - full sun, V2 - half-shade, V3 - shade). The biological material contained of uniform, two years old plants (Figure1).

Figure 1. Two-year-old plants in the experimental field

In the second stage of the study we followed the behavior of the *N. nipponicum* plants of different ages. The biological material was represented by different age plants: N1 - young plants, (obtained from cutting rooting), N2 - two years old plants and N3 - mature plants (> three years), from the Faculty's existing collection. Cuttings were obtained in the greenhouse of the Faculty. The recorded temperatures were between 20-25^{0}C while the soil humidity was maintained by permanent watering. Cuttings were transplanted after rooting in June.
The observations made in the 2018-2019 period focused on: average plant height, average leaf length, average leaf width, average flower diameter, start in vegetation, the appearance of flower buds, the blooming, the maximum flowering and the end of flowering.
The phenophases of the vegetation season, from the start of the vegetation until the end of flowering, were recorded using Biologische Bundesanstalt, Bundessortenamt and CHemical industry (BBCH) scale (Table 1), used by other researchers for various plant species (Meier, 1997; Stratópoulos et al., 2019; Cosmulescu & Scrieciu, 2019; Stanciu et al., 2021), through observations on leafing and flowering in the year 2019.

Table 1. Description of flowering phenophases recorded based on the BBCH code

No. of phenological phase	Phenological phase	BBCH Code	Description of BBCH Code
F0	Bud bursting	09	Buds show green tips
F5	Inflorescence occurrence	59	Separation of floral buds
F6	Beginning of flowering	60	The first flowers have opened
	Full flowering	65	At least 50% of flowers are open, the first petals fall
	End of flowering	69	The end of flowering: all petals have fallen

The following determinations were made: the number of days from the start of vegetation until the appearance of flower buds, the number of days from the start of vegetation until the beginning of flowering, the number of days from the beginning of flowering until the end of flowering, respectively the duration of flowering.

RESULTS AND DISCUSSIONS

Existing studies do not cover or are seldomly discussing ecological requirements, propagation or the culture technologies used for the *N. nipponicum* species. However, from the existing studies we can summarize that *N. nipponicum* develops well in full sun, but tolerates half-shade as well. The soil can have just average in fertility, as long as it is very well-drained.

Behavior of the N. nipponicum plants in different growing conditions

The time of phenological stages varies depending on species, on age and on the local climate within a species (Stanciu, 2021). In Table 2 one can observe that *N. nipponicum* behaves differently depending on the growing conditions. The onset of vegetation was the earliest recorded in the plants cultivated in full sun (18.03.2019), followed by the plants cultivated in half-shade (23.03.2019) – five days later. For the plants grown in full shade (07.04.2019), the onset of vegetation was last observed 20 days after the ones grown in full sun.

The first time the buds were noticed was on 19.09.2019, only for the plants cultivated in full sun. The blooming began on 04.10.2019, 15 days after the floral buds were observed. The flowering period was between 4.10.2019 - 7.11.2019 in the autumn season, only for the plants grown outdoors in full sun.

The number of days between the start of vegetation until the buds emergence was 192 days. The number of days between the start of vegetation until blooming started was 207 days. The decorative period, more specifically the number of days from start to end of the blooming period, was 35 days.

Table 2. Blooming phenology of *N. nipponicum* in different cultivation conditions

Cultivation exposure	Bud bursting (09)	Inflorescence occurrence (59)	Beginning of flowering (60)	Full flowering (65)	End of flowering (69)	Number of days 09-59	Number of days 09-60	Duration flowering 60-69 (days)
Full sun (V1)	18.03.2019	19.09.2019	4.10.2019	21.10.2019	7.11.2019	192	207	35
Half-shade (V2)	23.03.2019	-	-		-	-	-	-
Shade (V3)	07.04.2019	-	-		-	-	-	-

Physiologically, light has both direct and indirect effects. It affects on metabolism directly through photosynthesis, whilst indirectly through growth and development (Zhang et al., 2011). According to Kozlowska (2011) light has the strongest effect on the development of inflorescence buds and then on the formation of inflorescences.

Table 3 presents the effects of different light expositions on plants growth and development. The average plant height recorded the highest values (V1-36.5 cm) for the plants cultivated in full sun exposure, whilst the plants cultivated in shaded areas recorded the lowest values of this parameter (V3-18.2 cm) (Table 3).

The average leaf dimensions registered highest values in full sun exposure (V1-6.9 cm length, V1-5.3 cm width), whilst for the plants cultivated in half shade and shade, the recorded values were significantly lower, between 4.6-5.3 cm length and 1.5-1.7 cm width.
The average diameter of the inflorescence for the plants cultivated in full sun was 17.25 cm, while the average length of the floral stem was 15.5 cm.
Plants respond to shading by either acclimation or by avoidance. The process of acclimation is typically achieved by increasing the leaf area (Evans &Poorter, 2001). Avoidance involves a repositioning of leaves (Ballare, 1999). Low light intensity inhibits plant growth and productivity by depressing gas exchange (Gregoriou et al., 2007). For herbaceous perennials like *N. nipponicum*, light is an important factor affecting plant growth, morphology, and developmental changes. Cultivation in half shadow, but especially in full shadow reduced the vegetative growth and in some cases blooming did not happen. In conclusion, *N. nipponicum* for landscaping in full sun exposition presents a good growth habit, flowering and an attractive aspect of foliage.

Table 3. Cultivation conditions influence on the main morphological characteristics of the *N. nipponicum* plants

Cultivation exposure	Height of plants (cm)	Length of leaf (cm)	Width of leaf (cm)	Diameter of heads (cm)	Floral stem length (cm)
Full sun (V1)	36.5	6.9	2.1	7.25	15.5
Half-shade (V2)	30	5.3	1.7	-	-
Shade (V3)	18.2	4,6	1.5	-	-

Behavior of the N. nipponicum depending on the age of the plants

Table 4 presents the data on vegetation starting and blooming phenology based on the plant age. For mature plants aged more than three years old and for two years old plants, the vegetation start was recorded on 18.03.2019. For the young plants, obtained from rooted cuttings was recorded on 26.06.2019.
For both mature and two years old plants the buds appeared between 17.09.2019 - 19.09.2019. For the young plants the buds appeared 8-10 days later, on 27.09.2019. The blooming started between 01.10.2019 - 4.10.2019 for mature and two years old plants and on 16.10.2019 for young plants (with a 12-15 days difference).
The blooming period was between 1.10.2019 - 5.11.2019 for mature plants, and between 4.10.2019 - 7.11.2019 for two-year-old plants. The young plants, bloomed in the first year of cultivation between 16.10.2019 - 7.11.2019.
The number of days between the beginning of the vegetation and the buds' appearance was between 94 (for young plants) and 195 days (for mature plants). The number of days between the vegetation start and the blooming start was between 107 days (for mature plants) and 200 days (for two years old plants). The decorative period and the number of days from the start until the end of the blooming was between 22 days for the young plants (N1) and 35 days for the mature plants (N3).

Table 4. Blooming phenology for *N. nipponicum* depending on the age

Plant age	Bud bursting/ plantig (09)	Inflorescence occurrence (59)	Beginning of flowering (60)	Full flowering (65)	End of flowering (69)	Number of days 09-59	Number of days 09-60	Number of days 60-69 (duration flowering)
N1	25.06.2019	27.09.2019	16.10.2019	28.10.2019	07.11.2019	94	113	22
N2	18.03.2019	19.09.2019	04.10.2019	21.10.2019	07.11.2019	185	200	34
N3	18.03.2019	17.09.2019	01.10.2019	23.10.2019	05.11.2019	183	197	35

N1 - young plants; N2 - 2 years old plants; N3 - mature plants (> 3 years)

As we observe in Table 5, the average height of the plants, recorded the highest values at N2 (2 years old plants - 46.5 cm) followed by the N3 (mature plants> three years - 40.6 cm).
The young plants recorded the lowest average height (N3-32 cm). Regarding the average size of the leaves recorded the lowest value at N1 (6.5 cm, 2.1 cm, respectively), and the highest value was recorded at N2 (7.3 cm and 2.5 cm, respectively).
The average diameter of heads was between 5.7 cm (N3 - mature plants (> 3 years) and 7.25 cm (N2 - 2 years old plants). The average length of the flower stalk also recorded the highest value in plants at the age of 2 years (N2-16.3 cm).
The lowest value of this parameter, in correlation with the average plant height, was recorded in young plants (N1-12.7 cm).
In conclusion, the two-year-old *N. nipponicum* plants performed best in the cultivation conditions in the city of Craiova.
It is also worth mentioning that the young plants, resulting from rooted cuttings, bloomed in the first year of cultivation.

Table 5. The main morphological characteristics of *N. nipponicum* plants depending on the age of the plants

Plant age	Height of plants (cm)	Length of leaf (cm)	Width of leaf (cm)	Diameter of heads (cm)	Floral stem length (cm)
N1 - young plants (from cutting rooting)	32	6.5	2.1	6.8	12.7
N2 - 2 years old plants	46.5	7.3	2.5	7.25	16.3
N3 - mature plants (> 3 years)	40.6	6.8	2.3	5.7	15.5

CONCLUSIONS

N. nipponicum excel for the late blooms and general hardiness, it is ideal for combination planting and can enrich the assortment of perennial flowering plants.
The *N. nipponicum* is suitable for sunny places and can be introduced to the ornamental plant design patterns in places similar to Craiova ecological conditions.
The flowering was observed at the beginning of October (2.10-4.10) and the decoration duration was between 25-36 days.
N. nipponicum did not bloom in shade and semi-shade conditions, but stood out through its beautiful foliage throughout the vegetation period.
Analyzing the behavior of *N. nipponicum* plants, it is observed that the plants grown in the sunny exhibition recorded the best results for the analyzed parameters.
The 2-year-old plants performed best in the cultivation conditions in the city of Craiova. In the technology of growing montauk daisy, spring cutting to regenerate plants and dividing the plants every three years can influence inflorescence height and decorative capacity.
It is also worth mentioning that the young plants, resulting from rooted cuttings, bloomed in the first year of cultivation.

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RESEARCH ON MODULAR HORTIVOLTAIC SOLUTIONS

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Abstract

The present paper aims to document and identify some possible modular hortivoltaic solutions, which efficiently combine horticultural production with the generation of photovoltaic electricity on smaller spaces than usual in agrivoltaic applications. This initiative involves the development of structures to support photovoltaic panels, under which horticultural species can be grown in containers of different sizes and whose irrigation is carried out in a significant proportion with the help of rainwater collected from the surface of photovoltaic panels. At the same time, an assessment will be made of the potential of green energy generated by the photovoltaic panel modules with the presentation of some species that can be cultivated for utilitarian and ornamental purposes. The solutions resulting from the synergy generated by the combination of photovoltaic panels - green roofs will be able to be generalized and recommended in a wide range of situations, thus responding to the current needs of resource reuse and combating climate change.

Key words*: Agrivoltaics, hortivoltaics, container gardens, carbon reduction, rainwater reused, terraces.*

INTRODUCTION

Agricultural production combined with the production of electricity using photovoltaic panels forms the essence of the agrivoltaic system concept, first mentioned at the Fraunhofer Institute in Germany (Goetzberger & Zastrow, 1982).

By raising the supporting structure of the photovoltaic panels by about 2 m and increasing the distance between the rows to about 3 times the height of its modules, they obtained a uniform radiation on the ground, allowing the movement of agricultural machinery under the structure.

A pergola-like solar sharing system was developed and patented in Japan (Nagashima, 2005), and later the effects on a corn crop were studied (Sekiyama & Nagashima, 2019).

At Lavalette, near Montpellier, France, an experimental structure consisting of photovoltaic panels installed 4 m above the ground was built and the shadow effect generated by solar panels placed at different distances between rows was studied (Dupraz et al., 2011).

In Montpellier, the effects of precipitation intensity at various angles of inclination of solar panels were studied (Elamri et al., 2018), but also the yields of lettuce crops with observations on plant morphology and physiology (Marrou et al., 2013).

A study from the University of Arizona showed that plants grown under solar panels 3.3 meters above the ground and a 32° incline (latitude of the University of Tucson, Arizona) can lower daytime air temperatures by more than 1°C. the temperature rises during the night by 0.5°C, the temperature at the level of the solar panels was lower by 9°C, and the soil dried more slowly between waterings.

Regarding the species tested, compared to the plants grown in the field, the chiltepin pepper (*Capsicum annuum* var. *glabriusculum*) produced a quantity of fruit 3 times higher, the jalapeño pepper (*Capsicum annuum* var. *annuum*) had a production with 11% more small, but consumed 65% less water, while cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) - had a 100% higher yield using the same amount of water (Barron-Gafford et al., 2019). The period analysed in this study was 1990-2010. A study on a plot near the University of Hohenheim concluded that celery can be considered a suitable crop for cultivation under the solar panels of an agrivoltaic system

(Weselek et al., 2021). Other studies highlight the advantages of photovoltaic systems such as: dual land use (Mavani et al., 2019; Santra et al., 2017), their extended potential (Dinesh & Pearce, 2016), opportunities (Guerin, 2019), and perspectives on markets and communities (Pascaris et al., 2021).

There are many other ongoing studies in Germany, the Netherlands, Africa and Asia, but the results of the research have not yet been published.

However, before installing an agrivoltaic system, both the construction legislation (whether building permit is required) and the trade regulations, subsidies and tariffs applied in each country must be carefully studied (Vollprecht et al., 2021).

MATERIALS AND METHODS

There are many terraces of blocks of flats that are unoccupied and which, at least in theory, could house both plants and fotovoltaic panels for energy production.

With the help of a well-known application - Google Maps - it is relatively easy to identify which are the favourable locations, respectively the surfaces facing south, southwest or southeast, unoccupied by other buildings or installations (antennas, generators, air conditioning units). Also, with the help of the application we can approximate with sufficient precision the dimensions of the terraces so as to sketch the location of some hortivoltaic systems.

Thus, we identified several buildings with horticultural potential in the UASVM Bucharest campus. We also built a schematic diagram of a hortivoltaic system consisting of several photovoltaic panels, the supporting structure, the container garden in which both the plants and the concrete slabs will be placed to stabilize the structure, as well as and the rainwater tank from which the plants will be irrigated mainly.

Some species of utilitarian and ornamental plants that have been grown in containers in different conditions of temperature, humidity and light in the botanical garden of the USAMV Bucharest campus will be exemplified.

Each hortivoltaic garden and terrace has its peculiarities of orientation towards the sun and the ability to take on additional loads generated by the forces of weight and wind, and the set of structures will be analyzed by a structural engineer who at the end will give its approval for their installation.

RESULTS AND DISCUSSIONS

The smallest module contains 2 x 2 = 4 photovoltaic panels, but can reach 4 x 3 = 12 panels, an area easy to organize, install and maintain. The minimum height of the structure is 1.2 meters, and the maximum can exceed 2.7 meters.

In Figure 1 we have a module 12 (M12) - under the 12 photovoltaic panels (0.4 kW each, dimension - 1 x 2m) there is a 500 litre tank that collects rainwater and sends it to the 16 containers with a volume of 130 litres through a pump (12V) and drip tubes. The 130-liter containers can contain 4 pieces of 10-liter containers or 1 piece of 50-liter container, and those that contain the vertical pillars of the support structure have concrete slabs inside for stabilization, over which 10-liter containers are placed. An M12 can generate a maximum of 12 x 0.4 Kw = 4.8 kWh of electricity every sunny hour and can accommodate 16 x 4 = 64 plants in 10 litre containers.

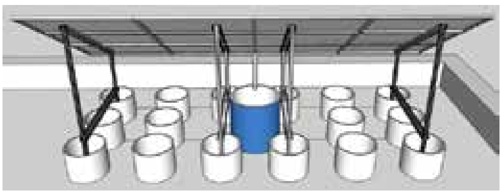

Figure 1. The M12 module contains 12 photovoltaic panels, 64 plants and can generate up to 4.8 kWh in every hour of full sun

In Figure 2 we have a photo with the positioning of the 10 litre containers in a 130 litre container.

The 130-liter containers protect the plant containers from excessive heat or frost, and depending on the engineering strength calculations, concrete slabs are provided at the bottom to stabilize the entire structure of the photovoltaic modules.

Figure 2. The M12 module contains 12 photovoltaic panels, 64 plants and can generate up to 4.8 kWh in every hour of full sun

A 10 litre container is also suitable for growing onion sets. Red, yellow and white onion varieties were planted (Figure 3).

Figure 3. *Allium cepa* 'Red Baron'

Container substrate contains an organic fertilizer in pellets which is particularly suitable for fertilizing horticultural crop. The optimal supply of nutrients and humus of the organic fertilizer is produced from chicken manure. In addition to organic matter, it contains all essential nutrients and it has a high content of organic matter which is essential for the maintenance of container soil fertility. The organic matter also improves the moisture retaining capacity of the substrate. It gradually releases its nutrients during the growing season of the plant.
In Figure 4 we can see *Salvia officinale*, *Lactuca sativa, Petroselinum* spp., *Levisticum officinale*. Containers containing these species will be placed in the sunniest areas.

Figure 4. Green leaves vegetables and herbs

In figure 5 we have *Lophantus anisatus*, a plant resistant to environmental conditions, with honey, therapeutic and culinary properties (Costache & Vînătoru, 2017). It is suitable for cultivation in the sunnier areas at the edge of photovoltaic modules.

Figure 5. *Lophantus anisatus* in September and March, respectively, after wintering outside without frost protection

Figure 6. shows easy to grow and low maintenance *Heuchera* spp. They grow well in containers, because the substrate is not too wet or too dry. Partial shade suits well heucheras, so is a good plant to grow under photovoltaic panels.

Figure 6. *Heuchera* spp.

Figure 7 shows 4 species of ferns, one of which is of the genus *Asplenium.*
Research has shown that *Asplenium nidus* Linn. has high rates of CO2 and HCHO removal, raises relative humidity and reduce temperature (Ying-Ming & Chia-Hui, 2015)
These features that reduce air pollution and carbon footprint, which are important for indoor cultivation, along with proven shade and cold tolerance on the outside, recommend ferns for growing in containers under photovoltaic panels. In the year 1990, dairy cows represented 59.46% of the cattle livestock and in the year 2010, they registered just 53.73%

Figure 7. Ferns in partial shade

In Figure 8 we can see an example of a terrace with M12 modules, arranged to allow the access of the maintenance staff but also of the eventual visitors. The spaces between the modules must also consider the latitude at which the panels are installed, the orientation towards the sun, the wind speed in the respective area, the existing obstacles on the terrace, as well as the strength calculations of the construction engineers. An additional water source must also be provided to supply the tanks installed under the modules if required.

Figure 8. Rooftop with M12 modules

A building can have several terraces, and in Figure 9 we can see how one of the faculties on the USAMV campus can contain up to 650 photovoltaic panels that produce 260 Kwh of energy per hour of sunshine, well above the building's own consumption, the amount additional energy can be delivered to the national grid. Also, more than 3,000 plants in 10-liter containers can be grown under the panels installed on the building.

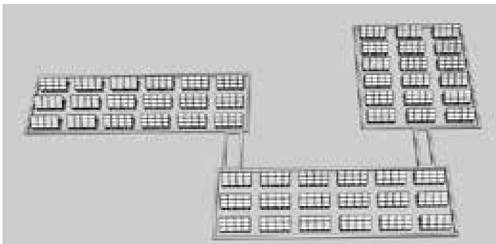

Figure 9. Multiple terraces of a faculty from USAMV Bucharest

Preliminary assessments can be made using the Google Maps application which provides us with valuable information on the orientation of the terraces towards the cardinal points and the existing obstacles on the respective terrace, as can be seen in Figure 10. However, the final solution can be adopted only after visits with all stakeholders and specialists, as unforeseen situations may arise, such as the renovation and consolidation of the building soon. Moreover, such an action can be an opportunity, because a horticultural solution can be taken into account in the structural calculation, which can be beneficial for both energy generation and the environment.

Figure 10. Orientation of the terraces towards the cardinal points and the existing obstacles

Finally, Figure 11 represents a sketch on a close scale of an important part as an area of terraces in the buildings located in the USAMV Bucharest campus, respectively the roofs of the student hostels and the canteen. Together with the terraces of other buildings on campus, we have reached an area of 18,800 square meters, which can be arranged with 26,000 plants and 5,000 photovoltaic panels of 0.4 kWh each, which can produce in a single hour of full sun almost 2 MW of electricity.

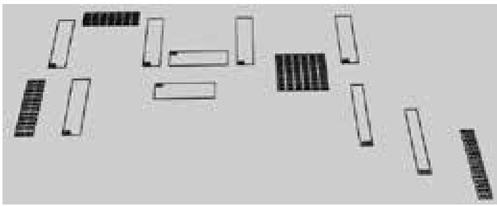

Figure 11. Student hostels and canteen terraces in USAMV Bucharest

CONCLUSIONS

Agrivoltaics is one of the areas of great interest at present, with research being carried out on all continents. It allows a dual use of land both to produce electricity from photovoltaic sources and for agricultural production. In this study, we defined “Hortivoltaics” as a branch of “Agrovoltaics” that refers to small cultivation areas, as well as to the terraces of public or residential buildings that can house under photovoltaic panels plants grown in containers, irrigated as much as possible by rainwater. recovered from the surfaces of photovoltaic panels, but which also have a backup power supply in case of drought. Cultivated plant species are utilitarian but also ornamental, with varying requirements for light requirements, although most of them will be tolerant of shade, heat and water stress. It is necessary to develop prototypes of light photovoltaic modules, but resistant to wind and seismic forces, whose weight resulting from structures and containers to be supported by the terraces of buildings.

The example in this study referred to the terraces identified in the USAMV Bucharest campus using the Google Maps application, but other areas and terraces with a high horticultural potential can also be identified.

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MORPHOLOGICAL AND PHENOLOGICAL VARIABILITY OF SOME VARIETIES OF GLADIOLUS CULTIVATED UNDER CLIMATIC CONDITIONS OF CRAIOVA

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Abstract

The study was carried out to evaluate the variability of some morphological characteristics in six cultivars of gladiolus, 'Blue Frost', 'Green Star', 'Nova Lux', 'Peter Pears', 'Priscilla' and 'Purple Flora', cultivated in the climatic conditions of Craiova city, Romania. The results showed that the minimum number of days from planting to flowering and the maximum plant height, number of leaves per plant and spike length were recorded in 'Green Star' cultivar, which was superior to the other cultivars. 'Nova Lux' performed the best for the number of florets per spike (16.5), and 'Blue Frost' for the diameter of floret (12.26 cm), wich are important quality indicators. Among the cultivars 'Purple 'Flora' and 'Priscilla' had the longest flowering duration. The coefficient of variation in terms of vegetative and flowering parameters, ranged between 7.75% for the number of leaves per plant and 19.89% for the number of florets in inflorescence. The highest coefficient of variation was obtained for the flowering duration (23.73%).

Key words: *cultivar, flowering duration, morphological characteristics.*

INTRODUCTION

Gladiolus (*Gladiolus hybridus* Hort.) is a perennial geophyte plant, belonging to the Iridaceae family, and it is especially valuated for the beauty and elegance of the inflorescences. It is cultivated in many countries both in the field and in greenhouses, depending on the local climatic conditions. It is one of the most important commercial flower crops, with a high economic value, for cut flower and corm production. There are numerous gladiolus cultivars today on the world market, which distinguish by the great diversity of sizes, shapes and colours of flowers, the number of flowers in a spike, the length of floral stems, the resistance to diseases and pests, as well as vase life duration of cut flowers. The majority of cultivars used for cut flower production have long spikes with large florets.

Gladioli present round or flat underground stems (corms), covered with fibrous, brownish tunics. During the vegetation period, 1-2 vigorous flower stems develop from axillary buds on the corms, and are wrapped at the base in a few leaves and have various heights, depending on the variety and corm size. During every growing season, the plants produce one new corm on top of the old corm, which is consumed and disappears until autumn, and numerous cormels develop at the base of the new corm. The leaves are long, narrow, lanceolate or linear, green, with obvious veins and the appearance of a sword. The flowers are arranged in spike-like inflorescences, they are funnel-shaped, with a straight, wavy or fringed edge, and a very wide range of colours in different attractive shades, from white, pink, red, yellow, orange to purple, violet and even green or colour combinations. Choudhary et al. (2011), Patra & Mohanty (2014) showed that spike length, number of florets per spike, floret size, weight and diameter of corm are important quality characteristics and may be considered as selection indices in gladiolus breeding programme and crop improvement. The cultivars that produce a high yield of cut flowers and increased rate of corms production are the most appreciated and looked for by producers and consumers.

Gladiolus can be grown with good results in sandy-loam, rich in nutrients, loose, well drained soils, with constant moisture and pH 6-7, in sunny sites. Ahmad et al. (2013) reported the beneficial effects of humic acid and NPK applications on vegetative growth, yield and commercial quality of cut gladiolus stems.

Baldotto & Baldotto (2013) reported that the plants produced a higher number of florets per spike and a higher number of cormels, with a larger diameter, which may be due to the effects of humic acid application.
The nitrogen, phosphorus, potassium with micronutrients especially boron and zinc had a significant effect on size, weight and number of corms and cormels increasing (Halder et al., 2007; Shaukat et al., 2012).
Environmental factors such as temperature and light intensity control the growth and development of plants, which are closely dependent on climatic conditions (Dole & Wilkins, 2005). Nagar et al. (2017) reported an increase in the vegetative and quality parameters of the plants grown under higher temperature and long day conditions. Zeshan et al. (2016) studied the growth and development of three cultivars of gladiolus under different ecological conditions and concluded that temperature is an important factor for the vegetative growth, the variety-climate interaction being significant. Gladiolus plants are sensitive to water stress, which results in reduced flower yields (Wilfret, 1992).
Gladioli are vegetatively propagated through corms and cormels for commercial production. The corm size is a determining factor in plant growth and the quality of the flowers (Narayan et al., 2013; Azimi, 2020). The date of planting plays an important role in improving the vegetative growth, quality and corm production. The different planting dates have a significant effect on the number of days to corm sprouting and affect floral characteristics and number of florets/spike (Zubair et al., 2006; Akpinar & Bulut, 2011; Saaie et al., 2011; Adil et al., 2013). *In vitro* protocols have been developed for the mass production of gladiolus cormels, using different media and various explants of the plant (Memon et al., 2016).
Several studies have been conducted regarding the influence of plant growth regulators on growth, flowering and gladiolus corm production and it has been reported that the treatments with different growth regulators are effective in the breaking of corm dormancy and promote more number of quality corms and earlier sprouting (Baskaran et al., 2009; Bhujbal et al., 2014), as well as an increase in plant height, flower quality parameters and the chlorophyll content (Chopde et al., 2012b; Sajid et al., 2015).
Gladiolus are mainly used as cut flowers, in bouquets and floral arrangements, but can also be used alone or in combination with other perennial and annual species, in simple or mixed borders, in groups, for the summer decoration of parks and gardens, as well as in pots and containers, in patios or balconies.
The study was conducted to determine the morphological and phenological variability of six cultivars of gladiolus, under the climatic conditions of Craiova city, located in the southwest of Romania.

MATERIALS AND METHODS

The research was carried out in the Floriculture Research Area, Faculty of Horticulture from Craiova, Romania, on six cultivars of gladiolus with a different range of floret colours from light lilac with white ('Blue Frost'), light green ('Green Star'), yellow ('Nova Lux'), orange ('Peter Pears'), white with pink edges ('Priscilla') to dark purple ('Purple Flora'), grown in open field conditions. The biological material consisted of medium sized corms imported from The Netherlands, which were planted in April 2018, as soon as the temperatures began to rise, at a spacing of 30 cm between rows, 15 cm between corms along the row and the depth of 10 cm, in moist, loam-sandy soil. Cultural practices have been applied during the vegetation period.
The climate is temperate-continental with Mediterranean influences in Craiova city and it is characterized by very hot summers, with low rainfall and moderate winters. In 2018, the average annual temperature was 12.3°C and the amount of annual rainfall was 725.1 mm.
The experiment was laid out in randomized complete block design with three replications. Observations and biometric measurements on the main phenological phases and morphological characteristics were made during the study period. The growth parameters (number of days to sprouting, plant height, number of leaves per plant, leaf length) and flowering parameters (number of days from planting to flowering, number of florets per inflorescence, spike length, floret diameter,

duration of flowering) were recorded and analysed statistically using descriptive statistics in Minitab 17 software.

RESULTS AND DISCUSSIONS

The phenological stages of gladiolus plants are divided into four developmental phases: corm dormancy phase, sprouting, vegetative phase and reproductive phase (Schwab et al., 2015). The initial phases of plant growth are affected by local climatic conditions such as temperature and rainfall.

In the present study, the different cultivars have shown various responses with respect to the analysed morphological characteristics.

In Table 1 there are shown the results obtained for the vegetative features. The sprouting percent was 100% in all the studied cultivars and the average number of days required for sprouting of the plants was between 9.13 and 15.86. Among the cultivars, 'Priscilla' had the earliest sprouting (9.13 days), while 'Peter Pears' recorded the maximum number of days from corm planting to sprouting (15.86), followed by 'Purple Flora' with 14.23 days.

Regarding the plant height, a difference was observed among the studied cultivars. The highest value was recorded in the 'Green Star' cultivar (132.51 cm), while the 'Blue Frost' had the lowest value of this parameter (102.33 cm). Many cultivars behave differently even grown under the same environmental conditions. The corms used in this study had about the same size, so the variation observed in plant height of gladiolus cultivars might be due to the environmental conditions and genetic traits. Hossain et al. (2011) reported a wide variation in plant height amongst some genotypes of gladiolus. The height of the flowering stem is one of the important traits for the cut flower trade.

The maximum number of leaves per plant was recorded in 'Green Star' (8.43) and a similar result was reported by Naresh et al. (2015) and Pattanaik et al. (2015), followed by 'Purple Flora' (8.12), while the 'Peter Pears' cultivar had the minimum number of leaves (6.75). In terms of the average leaf length, the analysis of the data shows that, the lowest value was recorded at 'Peter Pears' (52.56 cm), compared to the other cultivars. The highest value of this parameter was recorded at the 'Priscilla' cultivar (69.83 cm), which is in accordance with the results of Kumar & Kulkarni (2009), wich obtained similar values for the leaf length as well as for the number of leaves.

The coefficient of variation (CV) was low for the number of leaves per plant (7.75%), the plant height (9.47%) and medium for the length of leaves (12.00%). The highest coefficient of variation was recorded for the number of days to sprouting, and indicates differences from one cultivar to another. This aspect may be due to the genotypic differences, that may contribute to the various levels of gibberellins and abscisic acid in corms, which is controlling the dormancy period (Kaur & Bajpay, 2019).

Table 1 Vegetative characteristics of gladiolus cultivars

Cultivars	Number of days to sprouting	Plant height (cm)	Number of leaves/plant	Leaf length (cm)
Blue Frost	12.45	102.33	7.33	54.21
Green Star	9.26	132.51	8.43	58.40
Nova Lux	11.93	127.53	7.87	67.62
Peter Pears	15.86	123.42	6.75	52.56
Priscilla	9.13	125.16	7.66	69.83
Purple Flora	14.23	110.78	8.12	66.24
Mean	12.14	120.29	7.69	61.48
SD	2.67	11.39	0.59	7.38
CV%	22.00	9.47	7.75	12.00

In Table 2 are presented statistical data on morphological characteristics of the studied cultivars. Analysing the average values obtained for each cultivar, it was observed that the lowest number of days from corm planting to flowering correspond to 'Green Star' cultivar (78.45) and the highest number of days to flowering was recorded in 'Purple Flora' (101.60). The others cultivars ranged between 80.45-98.12 days taken to first floret open after planting. The planting depth of the corms and the distance between the plants play an important role in the number of days until the opening of basal florets (Niranjan et al., 2018). Variation in the number of days required for first floret opening might be attributed to the genetic constitution of cultivars which respond to the vegetative and reproductive growth and to the other factors such as water, light and nutrition (Thakur et al., 2015).

The spike length, the number of florets per inflorescence and the diameter of florets at full opening are important quality indicators for the decorative and commercial value of gladioli.

The maximum spike length was recorded at 'Green Star' (66.23 cm), and the minimum length was recorded at 'Blue Frost' (47.20 cm), the average value for this parameter being 55.80 cm. Sharma & Gupta (2003) revealed that spike length was significantly improved by increasing size of mother corms and planting spacing.
The number of florets/spike was influenced by cultivar and ranged from 9.60 at 'Purple Flora' to 16.50 at 'Nova Lux'. Kumar et al. (2017) reported that cv. 'Nova Lux' produced the maximum number of florets per spike (15.53). The 'Peter Pears' cultivar produced an average number of florets/spike of 14.33, the finding being in line with the result obtained by Singh et al. (2020). The diameter of floret was measured on the flowering stem and had the maximum value at 'Blue Frost' (12.26 cm), followed by 'Priscilla' (12.14 cm) and the minimum values at 'Green Star' (9.41 cm) and 'Nova Lux' (9.78 cm). Kumar (2015) reported a similar value of the diameter of floret for the 'Nova Lux' cultivar. Naresh et al. (2015) recorded similar values to those obtained in this study, for the number of days taken for basal floret to fully open, the spike length and the floret diameter, at 'Green Star' cultivar. The variation in the number of florets per spike, the flower diameter and spike length may be due to genetic variability among the different cultivars of gladiolus and prevailing environmental conditions during the field experiment (Safeena & Thangam, 2019).
It is important to know the flowering time of the cultivars, for a staggered flowering on a longer period. Duration of flowering in the field also varied depending on cultivar and it was between 9.33 days at 'Green Star' and 17.92 days at 'Purple Flora', with an average value of 13.61 days. The coefficient of variation was medium for the floret diameter (11.06%), the spike length (13.30%) and the number of florets per spike (19.89%). The high coefficient of variation (23.73%) was recorded for the flowering duration.

Table 2. Flowering characteristics of gladiolus cultivars

Cultivars	Number of days to flowering	Duration of flowering (days)	Number of florets/spike	Length of spike (cm)	Diameter of floret (cm)
Blue Frost	98.12	11.42	10.43	47.20	12.26
Green Star	78.45	9.33	13.81	66.23	9.41
Nova Lux	93.67	12.26	16.50	62.67	9.78
Peter Pears	90.18	14.27	14.33	53.45	10.33
Priscilla	80.45	16.48	12.67	55.73	12.14
Purple Flora	101.60	17.92	9.60	49.50	10.82
Mean	90.41	13.61	12.89	55.80	10.79
SD	9.36	3.23	2.56	7.42	1.19
CV%	10.35	23.73	19.89	13.30	11.06

CONCLUSIONS

Regarding the emergence of plants, the results obtained show that it started after a certain number of days from corms planting that is specific to each cultivar, the dormancy period of corms varying from one cultivar to another, depending on the cultivar and environmental conditions. In the area where the experiment on evaluation of different cultivars has been carried out, the climatic conditions correspond to the ecological requirements both for gladioli and for many ornamental species. 'Green Star' was superior to the other cultivars in terms of the plant height, number of leaves and spike length and bloomed the earliest, after 78.45 days from planting. 'Nova Lux' had the maximum number of florets per spike and the highest diameter of floret was observed in 'Blue Frost' cultivar. The longest flowering duration was recorded in the 'Purple Flora' and 'Priscilla' cultivars.
All studied cultivars had vigorous, good quality stems, with over 100 cm height, and can be used both for cut flowers, especially valuated in bouquets and flower arrangements for their elegant spikes with attractive florets of various colours and shades, and in gardens during the summer season.

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REHABILITATION OF THE DENDROLOGICAL PARK IN BUHUSI, BACAU COUNTY

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Abstract

The idea of rehabilitation proposed in the present project presented in this paper aims primarily to capitalize on green spaces in the park located in downtown Buhusi, increase the area of land legally framed as green space, giving up some parasitic elements such as basketball court on the territory park, increased accessibility and the creation of the best conditions to cover the need for relaxation, culture and education of all categories of population without discrimination.
The rehabilitation solution proposes a completely new architectural concept with landscape elements - defining the organic style. The arrangement scenario consists of free, sinuous shapes meant to create a harmonious, balanced rhythm and to strengthen the feeling of space. The interior of the proposed rehabilitation solution is intended to provide a gradual transition between the various functional areas of the park.
The vegetation will consist of small shrubs and conifers arranged in clusters that grow in decorative terraces throughout the year. The arrangement of deciduous and coniferous trees will be made so that the colour offered is diverse in all seasons.

Key words: *composition, dendrological, park, rehabilitation*

INTRODUCTION

The environment can be defined as a system of three elements, the natural environment, the urban environment and the urban living environment, between them there is a close interdependence and interaction that ensures the balance in the environment necessary for human survival and evolution (Nordh et al., 2009). Therefore, the balance of the environment is vital for humanity, the proper functioning of each element being essential (Borsari et al., 2010). As a result, if we consider the improvement of the quality of life of the population in small and medium-sized cities in Romania at national level, statistics indicate that in small and medium-sized cities, compared to large urban centers appear the risk of poverty and social exclusion. Thus, the proportion of the urban population unaffected by housing, employment or human capital development deficiencies decreases with the size of the city, registering the lowest values at the base of the urban network, of 43% in small towns (10,000-20,000 inhabitants) and 29% in very small towns (less than 10,000 inhabitants). Buhuși, according to the local territorial administrative unit, on 1st of July 2015, had a population of 20,871 inhabitants and two localities under administrative subordination: Marginea and Runcu.
Taking into account these aspects, the rehabilitation project presented in this paper aims to simultaneously solve the improvement of social, educational, cultural and recreational services, as well as the improvement of urban public spaces in the central area of Buhusi, with implications for improving the quality of life. The integrated approach to the city's problems will lead to the simultaneous solution of several requirements and needs of the population, between which there are interdependent relationships, thus contributing to the fulfillment of the vision of the city's development (Accati et al., 2010).
The general objective of the project treated in this paper is the rehabilitation/modernization of the Dendrological Park in Buhuși, Bacău County, by arranging recreation spaces for all categories of population, without discrimination and creating facilities at the same time, in order to achieve a quality public

space according to the principles of urban regeneration.

Improving the quality of life in Buhuşi by rehabilitating the green spaces in the Dendrological Park is one of the main components of the rehabilitation project that aims to improve urban public spaces in the central area of the city (A = 6,633,000 sqm).

MATERIALS AND METHODS

The Dendrological Park is located on Mihai Viteazu Street which connects with the Victoria Cinema building to Mihai Viteazu Street and Ion Ionescu de la Brad Street. The space is called Dendrological Park, although it does not meet the necessary conditions for authorization as a dendrological park with an area of less than 1 ha. This name is found in the usage of the citizens of Buhuși and in the public administration field.

Figure 1. Location of the project design

Currently, the land belonging to the Dendrological Park is used improperly, being degraded, having an aged vegetation and not at all in tune with the contemporary needs of the inhabitants. The project presented in this paper proposes the arrangement of a green area consisting of a park and garden for unlimited public access.

Within the arrangement of the space related to the park, it is proposed to build pedestrian alleys made of pavers placed on a sand support layer 3 cm thick, the foundation layer being made of ballast 10 cm thick after compaction. The width of the designed alleys is variable, being between 2 and 5 m, which also serve as occasional access for the necessary interventions. Thus, within the park, the surface of the arranged green spaces is 4393.80 sqm - provided to be planted with trees, shrubs, flowers, etc., with the complete lawning of the remaining surfaces and their automated irrigation.

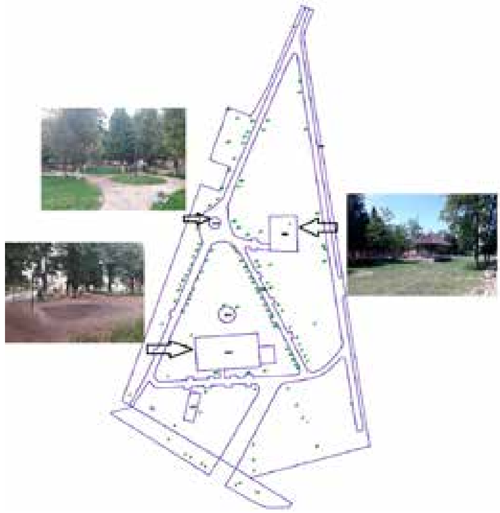

Figure 2. Plan of the existing situation

From a climatic point of view, the site is located in an area with a temperate-continental climate with strong Baltic influences, which gives a rich rainfall regime both in winter and in summer and temperatures 1-2^{0}C lower compared to other regions in the Cracau-Bistrita depression. From the multi-year meteorological observations, it is found that from a thermal point of view, the analyzed area is characterized by average annual temperatures of 8-9^{0}C. The minimum air temperature drops to approx. -20^{0}C in the winter months and reaches maximum values of approx. +32^{0}C in the summer. The warmest month of the year is July (with an average temperature of 18-19^{0}C), and the coldest, January (-3.5 ÷ -20^{0}C).

As public spaces, green spaces contribute to increasing social inclusion, by creating opportunities for people of all ages to interact both through informal social contact and by participating in community events (Tassinari et al., 2010). The dendrological park development project proposes the creation of a setting suitable for bringing to the landscape the areas for various social and cultural events, such as local festivals, civic celebrations or theater, film, etc. Thus, they "help to form the cultural identity of an area, are part of its unique profile and give a sense of place to local communities" (Toscano et al., 2017). Well-maintained green spaces play a significant role in promoting the health of the urban population.

They offer opportunities to encourage a more active lifestyle, such as walking, running, exercising, cycling, etc., including travel between inhabited areas and/or various public facilities (Pivetta et al., 2010).

Some studies show that the main value of green spaces derives from their ability to restore the "well-being" of those who frequent them (Zilemenos & Paraskevopoulou, 2017).

They provide citizens with quiet places to relax and reduce stress, to escape from the built environment and traffic. Green spaces respond mainly to human needs for recreation and leisure. In the case of people without income or time, the park remains the most convenient solution for recreational activities (Loukil et al., 2010). Also, the Dendrological Park we propose to host playgrounds for children, contributing to their physical, mental and social development. Urban green spaces are also of great importance from an aesthetic point of view, as they attenuate the impression of rigidity and aridity of any built environment (Nikologianni et al., 2017).

RESULTS AND DISCUSSIONS

In order to make the arrangement proposal, a detailed study was taken into account on the existing situation in the Dendrological Park, following which it was found that the accesses to the park are undervalued, not being imposing due to degradation and sizing.

The alleys are degraded and there is no route to ensure a circulation with axes of perspective and points of interest.

It is necessary to completely replace the pavilion, as it shows strong signs of degradation, being physically and morally outdated, as well as the restoration of perimeter sidewalks that are currently missing or are cracked and detached from the base of the building and no longer play the main role, to carry rainwater away from the construction. Over time, these lead to subsidence of the foundation, which affects the entire structure.

The fencing is made of unsightly materials (prefabricated concrete panels and wire mesh and reinforced concrete pillars) on all sides of the park. The access gate made of wood is degraded and does not present safety in operation, requiring a rehabilitation, both structurally and in terms of finish.

If we study the definition of a dendrological park - "an area where trees and shrubs of different species are planted, intended to study the conditions of their development, the result of both the natural spread of tree species and human cultivation on the same area, of native species as well as some exotic ones" in the context presented in this park we will find that the name of "dendrological park" is a purely given one by locals (Glaeser, 2010). In reality, the vegetative material is insufficient - the vegetation consists only of trees of size I (*Tlilia tomentosa* and *Thuja* sp.). These trees require crown correction grooming. The fact that the lower layers of vegetation are missing could create air currents that favor the circulation of dust (Glenn et al., 2017). Proper vegetation is also vital to provide chromatic dynamics throughout the growing season.

The surface of the soil is mostly uncovered, favoring the raising of dust, and in some areas *Convolvulus arvensis* and *Polygonum aviculare* have developed, which have suffocated the grasses from the initial turf.

The park does not have adequate lighting, most of the lighting fixtures are malfunctioning and incorrectly arranged in relation to the alleys and related spaces. There is no public drinking fountain in the park, mandatory according to the requirements of the specific regulations.

The urban furniture, consisting of benches with metal structure and wood, is insufficient and incorrectly placed, there is no functional connection between benches and alleys, they are randomly arranged on the green space.

Concrete planters are structurally and morally obsolete, in which random vegetation is currently growing.

The park does not have an irrigation system, it is not watered properly and it is not constantly maintained.

The children's playground is not delimited by the rest of the park and the equipment in it does not ensure a satisfactory degree of safety for the users. The risk of accidents is imminent. At the same time, it is not designed to be of interest to little ones.

The basketball court is also indefinite, incorrectly located and unsanitary, it has a high degree of insecurity. In fact, its presence in the area can be considered unproper because the practice of a sport in the center of the park will inevitably lead to the discomfort of all other users.

Due to the very high degree of damage and the faulty arrangement of all the equipment present in the park, it is necessary to replace them with some that respect the minimum conditions of resistance, stability and functionality imposed by the current normative acts.

In conclusion, the Dendrological Park does not invite and does not present itself as a pleasant space and although it is located in the central area of the city and in the vicinity of two important educational institutions it is unfrequented, being for the most part unpopulated.

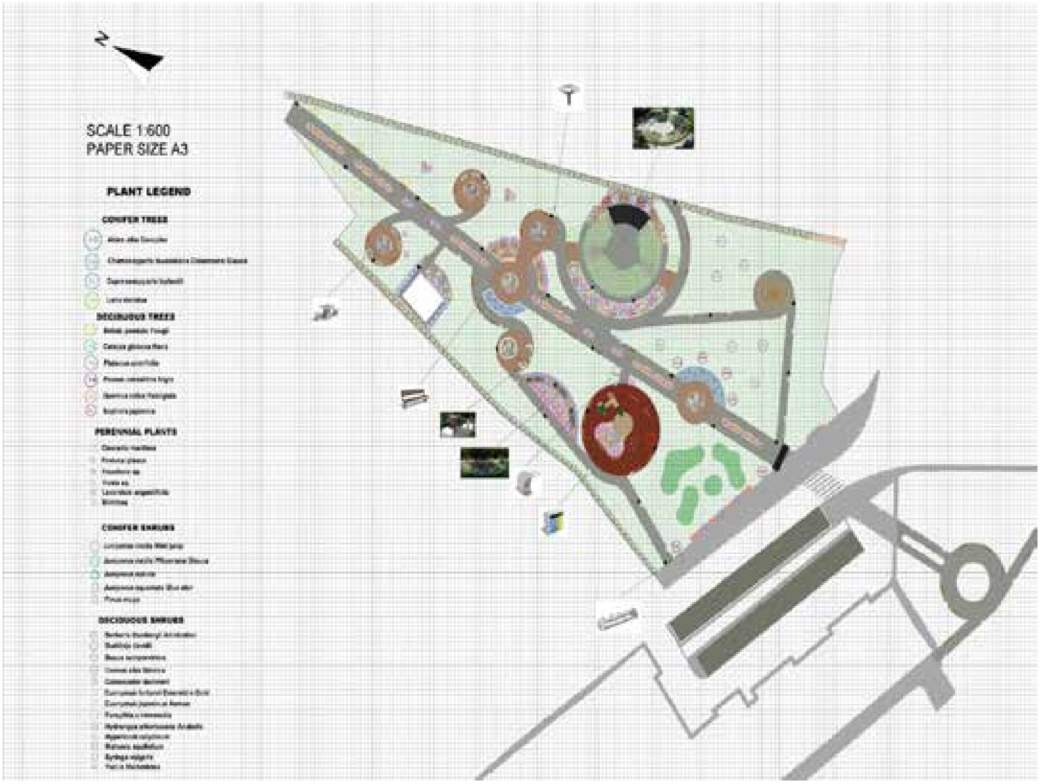

Figure 3. The proposed arrangement plans

The design of the arrangement proposes a different architectural concept, the landscape elements that are part of it defining the mixed style. The script, built of regular shapes that combine with free shapes, creates a harmonious, balanced rhythm and strengthens the feeling of space. The interior path of the proposed green spaces provides a gradual transition between the different functional areas, as can be seen in Figure 3.

In order to help preserve the nature on site, the shrub and arboreal vegetation has been carefully placed in the proposed development to facilitate the application of maintenance and possibly correction work when needed, so as not to represent a danger to those who frequent the area. These spaces, by rehabilitating the green areas, restore their functionality and vitality, reconnecting the connection between man and nature. Thus, by respecting the design principles, the Administrative Territorial Unit (ATU) Buhuşi City, Bacău County will not be aware of the specific forms in the plan, but will benefit of the countless pleasant relationships produced by the projected ambiance.

Figure 4. The main entrance to the Dendrological Park

In order to emphasize the recreational effect of the site and at the same time to create a safe space for promenade in the area at the entrance to the park, it was proposed to install circular elements designed to protect the space from car access but also to relax the gaze through their organic volume, as can be seen in Figure 4.

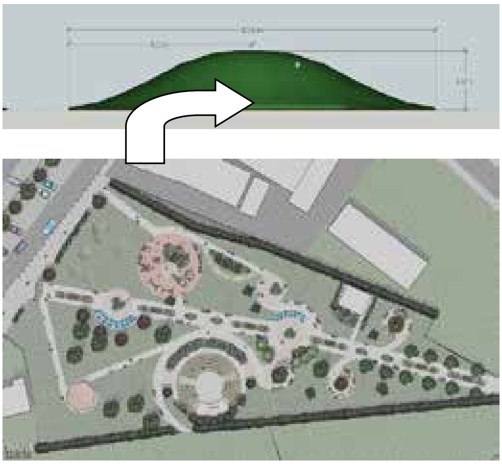

Figure 5. Artificial hills

In order to accentuate the organic forms, artificial hills of different shapes were created, highlighted both during the day and in the evening through the system of placing the vegetal material in the siting places (Figure 5).

The artificial hills could also function as sitting places.

Figure 6. Alveolus located inside the alleys

For aesthetic purposes, closely related to one of the basic human needs, is the need for beauty, namely the aesthetic function. In this context, the aesthetic function of this site was proposed to be capitalized by the plant elements that liven up green spaces.

As a result, in each alveolus located inside the alleys shown in Figure 5, the intent was to create some vegetal compositions that would offer a pleasant image and a discreet scent, to all those who either walk or transit the park.

Figure 7. Composition using *Festuca* and *Lavandula* species

In order to facilitate the easy maintenance of the green areas in the park and for sustainability reasons, the water element was simulated by introducing a transparent vegetation, whose noise produced by the wind blowing through the leaves to simulate the rustling of the water and whose color contrasted with turquoise stones resemble the luster of a lake (Figure 7). Through this intervention on the landscape, it can be concluded that this arrangement sums up all the natural factors: water, air, soil, subsoil, solar energy and existing organisms, which determine the living conditions of man and the development prospects of society.
The place from which the major axis of the composition starts is proposed to be the "gateway" and is the area of connection with the city and its turmoil. The role of this area, which will occupy the entire current location of the central park, is to stop, invite and prepare passers-by for the experience offered by the areas arranged in the perimeter of the dendrological park. This area, due to the positioning between two heavily trafficked streets, remains a transit area and the way of carrying out the arrangement and the proposed endowments mainly respects this fact.
The alleys in the rehabilitation proposal will be made of prefabricated tiles made of antique vibro-pressed concrete (finish obtained by technical procedures for aging concrete slabs), to create an aspect that expresses the passage of time. The pavers will be delimited with borders of the same material.

Figure 8. Composition using *Prunus* and *Abies* species

The purpose of bringing the *Prunus cerasifera* and *Abies concolor* species (Figure 8) into the designed landscape was to define and accentuate the volume of the site, both for the winter and summer decoration. The shapes in this arrangement that can be seen in Figure 8 are defined by lines and are what we see for the first time when we look at the arrangement from a distance.
An important note that was taken into account when the plant plan was systematized at the site level is also related to the physical characteristics of the land in question, namely: location, size, surface and shape of the land, topography, plot, access to utilities, pedological features, local climatic conditions.

Free or built, the value of the land is created by the utility or capacity of the land area to meet the wishes and needs of society, as a result, the introduction of a children's space in the design determines the exponential increase in the value of the site.
Utilizing this type of land contributes to the sanitary function. Also, in the whole arrangement a sense of unity is created by the introduction of an amphitheater (Figure 9), taking into account the fact that this park is close to an educational center.

Figure 9. Amphitheater

The functional urban furniture and the beautifully arranged green spaces as can be seen in Figure 10, create a pleasant and civilized atmosphere in the city. In this space, the points of interest are represented not only by the built elements but also by the diversity of plants and woody vegetation.
Studies have shown that people are able to talk to each other more easily by focusing on their common focus, which is why they have proposed the introduction of eight chess tables with two chairs, and it has also been proposed to create a space for forest-park, thus demolishing all barriers of coercion on socialization in which people regularly perceive themselves as strangers (Gunnarsson & Lorentzon, 2017).

Figure 10. Combining the vegetal elements with the built ones

This way of systematizing the arrangement aimed at the efficient and intense capitalization of the land by creating new points of attractiveness that could contribute considerably in the development of the tourist circuit, but also in bringing an aesthetic value to the whole area.

CONCLUSIONS

The present solution proposes a completely new architectural concept with landscape elements - defining the organic style. The arrangement scenario consists of free, sinuous shapes meant to create a harmonious, balanced rhythm and to strengthen the feeling of space.

By rehabilitating the green areas, the place is given functionality and vitality, to restore the connection between man and nature and the perspective on the space is all the more underlined by the different plant compositions located at the site in order to animate the park, transforming in a local center of interest.

In order to achieve the sustainability of this arrangement, plant species were chosen due to their resistance to pollution and poor quality of the soil, but also because of their decorative properties. They transmit to the landscape the characteristic features of the season according to the phenophase, underlined by diversity and chromaticity. Thus, the harmony and polychromy of the colors from the point of view of the aesthetic orientation can emphasize the the strong points of the rehabilitated landscape.

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COULD CACTUSES ENDURE WINTER IN ROMANIAN CLIMATIC CONDITIONS?

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Abstract

Cacti are one of the most interesting group of plants, because of their unique and special way of life. Due to their impressive beauty of their flowers and spines, are used nowadays as potted plants in different areas beside their native habitat. Moreover, for this reason growers are testing their amelioration to frost endurance. Frost resistant at is a complex physiological trait with a genetic basis. For the present study we have selected ten cacti species as followed: Cylindropuntia imbricata, Cylindropuntia whipplei, Opuntia basilaris, Opuntia fragilis, Opuntia polyacantha, Opuntia rutila, Echinocereus coccineus, Echinocereus reichenbachii var. baileyi, Escobaria leei and Escobaria vivipara var. radiosa on which we have analysed the damages caused by frost and winter injuries. The selected cacti species were subjected to two experimental conditions, first, when plants were covered and second, when remained uncovered. Results indicated that frost hardiness of some species was greatly influenced by the coverage, while at other no differences were observed. In conclusion, the present work strengthens the possibility of using some of the cacti species in the Romanian landscape design.

***Key words**: bonitation score, cacti, frost resistant, coverage.*

INTRODUCTION

Cactaceae are one of the most interesting plant families on earth, several of the species are members of the arid vegetative regions of America (Edwards et al., 2005; Guerrero et al., 2019). They have a special unique way of life, compared to other known plants. Because of their impressive beauty, nowadays the landscapers or the consumers are trying to incorporate them in the landscape architecture. Cacti are one of the first plants that were brought back from the American continent by the Europeans in the 15th century (Howard and Touw, 1981). The spread of many species could be explained by the fact that the horticulturist trades, them and propagate for their unique way of life (Walters et al., 2021).
Cactuses can make some of the most dramatic modifications observed in the plant kingdom (Guerrero et al., 2019), these changes can occur at all phenotypic levels (Gibson & Nobel, 1986), and can be known as succulent syndrome (Edwards & Ogburn, 2012). This syndrome allows the cactuses adaptation to different dry environments, which rely on the water storage in their tissues to avoid dehydration (Guerrero et al., 2019).
Cacti in general are plants of warm climates stretching through the American continent. Frost resistant is characteristic of the plant, being an inherited biological feature that can be very little influenced in a new environment with gradual adaptation (Sakai and Larcher, 2012). To get a frost-resistant variety in a group of frost-sensitive plants millions of years of climatic adaptation are needed. Frost resistant is complex, it is a physiological trait that is genetically based. In plant cells that adapted to frost occur complex phenomena: while preparing for hibernation, cell components regroup and grains of starch crystals are formed in the cell, the chloroplasts form nodules, so they get disorganized. At the same time, a phenomenon that can be seen with the naked eye: the evergreen leaves get yellow and red

and lose their rigidity cacti in their tissues lose moisture to install contraction. Frost resistant means that a plant can survive on certain low temperature (Szabó-Mohácsi, 2007).
The aim of the present study was to test ten species/varieties of cactuses, if they can strive and overwinter the cold conditions in the Romanian landscape, without freezing or getting frost injuries, which can decrease their decorative values. Furthermore, which species/variety of cactus from the selected ones is more resistant to frost and could be used probably as a landscaping design plant in the future.

MATERIALS AND METHODS

The study was carried out in the village of Bozeni, Mureș County between October and March (46°31′48″ N 24°40′26″ E) as an open field experiment.
The average temperature during the experiment was between 0.42 and 9.99°C, and the lowest minimum temperature -21.9°C (Figure 1).

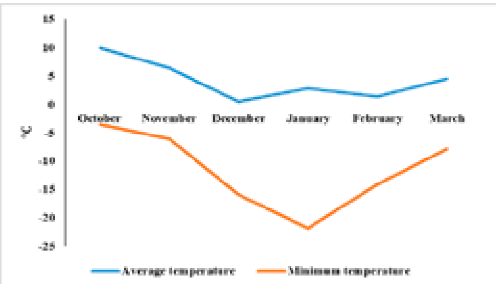

Figure 1. Average and minimum temperature during the open field experiment (October and March)

The lowest average precipitation was recorded in January 43.7 mm, and the highest in October with 93.4 mm quantity (Figure 2).

Figure 2. Average precipitation during the open field experiment (October and March)

We have selected ten cactuses for this experiment, which were purchased from a Hungarian cacti collector, 20 seedlings per species/variety.
Cylindropuntia imbricata (Figure 3): Mature plants can reach a height of 1.5 to 3 m; shoots can branch, they are becoming woody. Every areola has 10-30 pieces of 2-3 cm long, reddish brown spines. The flowers grow at the end of the shoots, they are 4-6 cm long and 8-9 cm wide with bright red colour. They are native to Colorado, Arizona, New Mexico, Kansas, Oklahoma, Texas as well as Northern and Central Mexico (Virág, 2000a).

Figure 3. *Cylindropuntia imbricata*

Cylindropuntia whipplei (Figure 4): Forms a rich shrub and can reach a height of 1.5 m. It grows straw-coloured spines that get a silver colour and are about 8-12 cm in length. It blooms in May with yellow-green flowers with a diameter of 2 to 3.75 cm. Are native to Northern Arizona, Navajo, Sitgreaves National Forest, and New Mexico (Szutorisz, 2004).

Figure 4. *Cylindropuntia whipplei*

Opuntia basilaris (Figure 5): Has a height of 12-20 cm, the shoots are about 12-20 cm, gray-blue, velvety. Areolas are sunken, the tuft of glochids are reddish-brown and fall early. The flower has a diameter of 6 cm, bright red. Are native to Southern Utah, Nevada, Western Arizona and South-eastern part of California (Debreczy, 1976).

Figure 5. *Opuntia basilaris*

Opuntia fragilis (Figure 6): The specie is short, thallophytes, with a height of 5-10 cm. Shoots are round, ovoid, 2-4 cm long, 1.2 to 5 cm long and 1.2 to 2 cm thick. Spines are tipped gray brown. Their length is 1.2 to 1.5 cm, sometimes 2.5 cm. The areola contains 1-6 spines. The flower has a diameter of 4-5 cm, yellow, yellow-green. Are native to Canada - British Columbia: Manitoba, Ontario, USA - Washington, Michigan, Illinois, California and south Texas (Virág, 2000b).

Figure 6. *Opuntia fragilis*

Opuntia polyacantha (Figure 7): Forms a shrub of 7.5 to 15 cm high, the shoots having a length of 5-10 cm and 1 cm thick. Each areola has spines which are stiff, firm and smooth. Spine length is 0.6 to 1.2 cm long at the bottom and 2.5 to 3.8 cm long above. The flowers are 4.5 to 8 cm in diameter, 4.5 to 6 cm long, its colour is yellow, sometimes pink. These cactuses are native to Canada - British Columbia, Alberta, Saskatchewan, USA - Idaho, Dakota, North-eastern Nevada, Utah, New Mexico, North-eastern Kansas, South-western Missouri, Oklahoma, Texas (Virág, 2000).

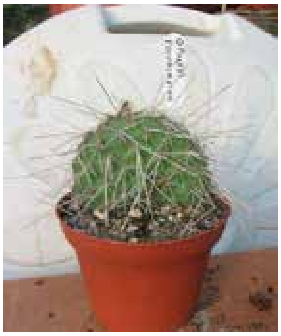

Figure 7. *Opuntia polyacantha*

Opuntia rutile (Figure 8): It is a creeping specie with ovoid stems, 4-10 cm long, cylindrical, 2-5 cm long with some spines. These spines are white or white brown-tipped. Their number alternates between 1-6 pieces on the areola and are 0.5 to 3 cm long. It has a flower with a diameter of 6 cm, yellow to reddish. They are native to Western Colorado, Wyoming (Virág, 2000c).

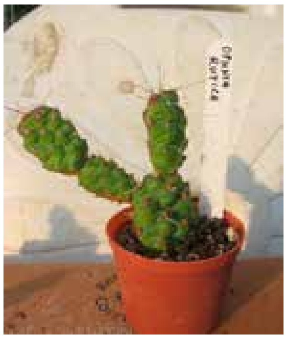

Figure 8. *Opuntia rutile*

Echinocereus coccineus (Figure 9): It is a plant with numerous stems, which has a length of 3.8 to 7.5 cm and 2.5 to 5 cm in diameter and 9-10 ribs. The colour of the spines is gray, white, pink or yellowish brown, sometimes yellow with a length of 2.5 to 6.2 cm. Spines are straight, smooth and rarely rugged. The flowers have the diameter of 2.5 to 3.8 cm and the length of 3-5 cm. Are native to USA - California, Nevada, Utah, Colorado, Arizona, New Mexico, Texas, Mexico - Western Sierra Madre, Durango, San Luis Potosi (Virág, 2000d).

Figure 9. *Echinocereus coccineus*

Echinocereus reichenbachii var. *baileyi* (Figure 10): It has a cylindrical shape with a length of 10-15 cm and the width of 5-7 cm. The number of ribs is about 16. The flowers are near to the top with 6-7 cm in diameter and length with bright red colour. Are native to Oklahoma (Virág, 2001).

Figure 10. *Echinocereus reichenbachii var. baileyi*

Escobaria leei (Figure 11): It is a specie with highly branched stems. The largest being green, cylindrical, with a length of 2.5 to 7.5 cm and 1.2 to 2.5 cm in diameter. The spines are very dense, covering the stems with a number of 6-9/areoles. Their colour is white with pink tip and with the length of 4.5 to 9 cm. The diameter and length of the flowers are 1.2 to 2 cm, brownish-pink. Are native to New Mexico (Virág, 2000).

Figure 11. *Escobaria leei*

Escobaria vivipara var. *radiosa* (Figure 12): Has a round shape, it grows isolated or in small groups with a height and width of 5 cm. Spines are white or brown with 1 to 4.2 cm in length. Flower length and width is 3.5 cm which are near to the top of the plant and their colour is pink. Are native to from Kansas to western Texas (Szutorisz, 2006).

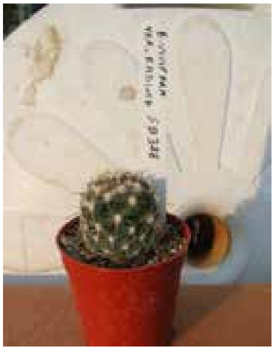

Figure 12. *Escobaria vivipara var. radiosa*

The selected cactuses were planted in the start of October on a 10 sqm alpine garden. The experimental field was divided into two parts one where the uncovered cactuses were planted and the other where the covered ones (10–10 for each species/variety).

In the end of March, we have determined the frost effect on the biological material used in the experiment.

The bonitation scale was determined as followed:

- 1 point if the cactus has not suffered any damage during the winter;
- 2 points if the cactus is 20% damaged;
- 3 points if the cactus is 50% damaged;
- 4 points if the cactus is 80% damaged;

- 5 points if the cactus is 100% damaged (the bud is completely dead).

Data were analysed using Past 4 statistical software (Oslo, Norway). Data were tested for normality of errors and homogeneity of variance.

All data were normally distributed. The significance of the differences between the treatments was tested by applying 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 $p < 0.05$.

RESULTS AND DISCUSSIONS

Considering the frequency of the injuries (Figure 13) caused by the frost, could be determined that the most injuries were observed at the cactuses which were remained uncovered during the winter season.

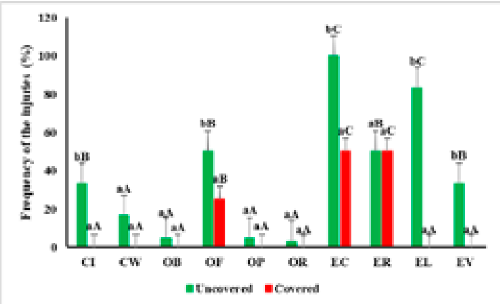

Figure 13. Frequency of the injuries caused by the winter frost on the selected cactuses. Different lowercase letters above the bars indicate significant differences between the uncovered and covered cactuses, and different uppercase letters indicate the significant differences between the species/varieties, according to Tukey test ($p = 0.05$).

However, is important to note that some of the selected plants suffered a higher damage from the frost, even EC (Figure 16a) plants have died. CW (Figure 15b), OB (Figure 15c), OP (Figure 15e), and OR (Figure 15f) reported a higher resistance against the cold days.

At the previously mentioned cactuses the damages were almost undetectable after the frosty days. Regarding the covered cactuses it was observed that, the coverage improved their resistance against the frost. EC and ER (Figure 16b) reported higher damages, even if they were protected.

On the other hand, CI (Figure 15a), CW, OB, OP, OR, EL (Figure 16c), and EV (Figure 16d) did not suffer any frost damage, during our experiment.

When comparing the coverage type on the same plants, could be concluded that at CI, OF (Figure 15d), EC, EL, and EV reported significant changes.

Is important to mention, that the uncovered *Echinocereus coccineus* suffered the most during the winter, in the spring these species all individuals had wilted/died.

In previous research was found that the most common damage to cacti species was dieback and not individual mortality (Alvarez-Yepiz et al., 2018).

In a study was reported that *Opuntia fragilis* survived several harsh winters in southern Finland with no frost damages, even the next year produced flowers (Leppänen, 2018). Guevara et al., (2000) recommends that is important to protect the cacti in the winter season at least for 1–2 years after planting.

From the data can be observed that *Cylindropuntia* and the *Opuntia* species obtained a better bonitation score compared to *Echinocereus* and *Escobaria*.

This could be explained by a species-dependent process. Significant changes were observed when comparing the species one to another. Also, a significancy was detected at EC between the uncovered and covered experimental conditions.

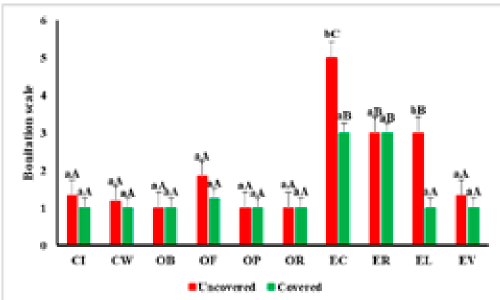

Figure 14. Bonitation scale of the injuries caused by the winter frost on the selected cactuses. Different lowercase letters above the bars indicate significant differences between the uncovered and covered cactuses, and different uppercase letters indicate the significant differences between the species/varieties, according to Tukey test ($p = 0.05$).

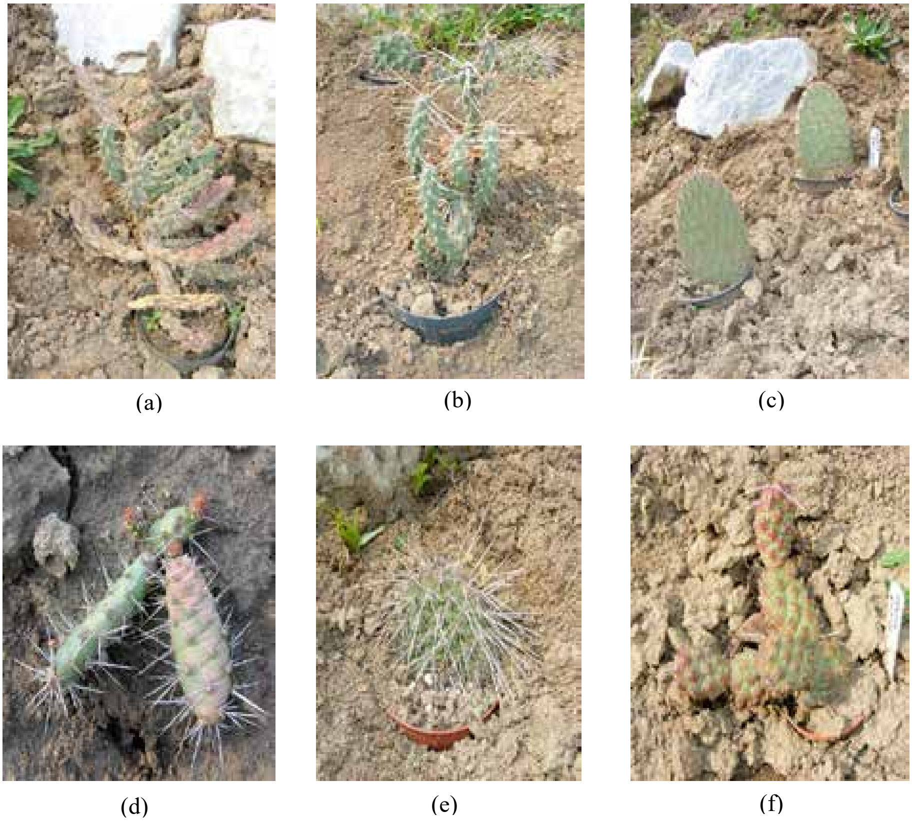

Figure 15. The selected cactuses after the winter frost. (a) - *Cylindropuntia imbricata*; (b) - *Cylindropuntia whipplei*; (c) - *Opuntia basilaris*; (d) - *Opuntia fragilis*; (e) - *Opuntia polyacantha*; (f) - *Opuntia rutila*

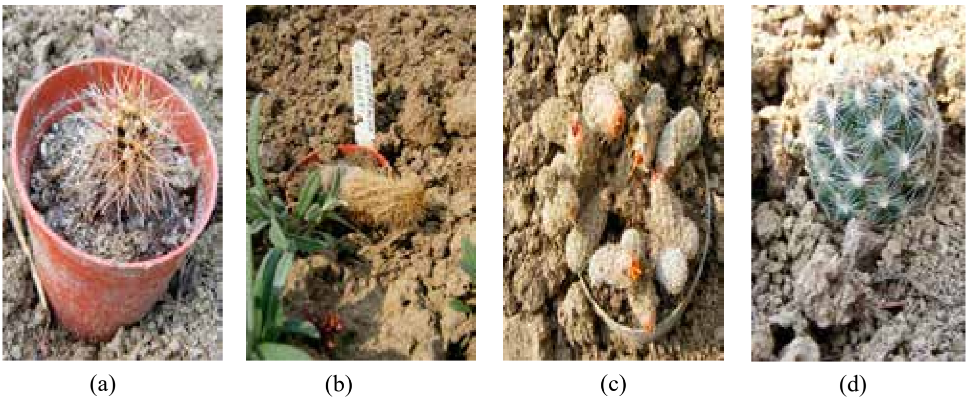

Figure 16. The selected cactuses after the winter frost. (a) - *Echinocereus coccineus*; (b) - *Echinocereus reichenbachii* var. *baileyi*; (c) - Escobaria leei; (d) - *Escobaria vivipara* var. *radiosa*

CONCLUSIONS

In ornamental landscaping day by day an innovation appears, which can be explained by the landscapers' ideas or by the consumer expectation. However, these new innovations not every time are suitable or can be put in practice. The present study provides new experimental data on the comparison of ten cactus species/varieties regarding to frost resistant. According to the results, it can be concluded that some of the selected cactuses in this experiment tolerated in a positive way the winter season, on the other hand some did not. From the results could be concluded that the covered cactuses endure in a much higher way the frost, than the uncovered ones. Furthermore, when comparing the species/varieties between them, can be concluded that EC, ER, and EL are less tolerant to frost than the other selected cactuses, but future experiments need to be conducted. However, due to the high frequency of absolute minimum temperatures in the experimental site, it appears to be necessary to protect/cover the plants in winter.

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PARTIAL RESULTS REGARDING THE DETECTION AND IDENTIFICATION OF PATHOGENS ON DENDROFLORIC PLANTS IN DIFFERENT GREEN SPACES OF CRAIOVA MUNICIPALITY

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Abstract

The purpose of the study in this paper was to detect and identify pathogens in some dendrofloric plants in different green spaces of Craiova. The detection and identification of the attack of different phytopathogens consisted both in periodic visual observations in the experimental area on certain organs of the host plants that showed symptoms of disease, and in performing laboratory tests. Regarding the detection of certain bacterial diseases, in the monitored period, following bacteria were identified: Agrobacterium tumefaciens, Erwinia carotovora, Pseudomonas marginalis, Xanthomonas hyacinthi, Xanthomonas campestris pv. campestris, Xanthomonas hortorum pv. pelargoni. For proper identification, not only macroscopic observations, but also laboratory tests are required to certify the initial identification in the field and, where possible, a pathogenicity test on susceptible plants was performed, obtaining the reproduction of the disease in the test plants.

***Key words*:** *observation, test, isolation, detection, identification.*

INTRODUCTION

From ancient times the man has lived in close contact with nature showing interest in it and especially in dendrofloric plants, the wonders of the world that enrich our lives with the scent, color, shape and health it offers us by producing oxygen, an indispensable gas of life. The most important role in bacteriology was played by Erwin Smith (1854-1927) who laid the foundations of the research method in phytobacteriology, being recognized as the father of this branch of science (Alexandri et al., 1967; Hatman et al., 1989). In Romania, Săvulescu, Rădulescu, Olga Săvulescu, Severin, Preda, Popescu, Pop, Lazăr, Alexandri, Ghimpu and Iliescu laid the foundations of phytobacteriology and had important contributions in this field. As a result of the interaction between bacteria and the host, smaller or larger changes appear in the host plant, so that various symptoms may occur (Rădulescu & Negru, 1967). More than 200 species of phytopathogenic bacteria are known worldwide (Mitrea R., 2006).

The „Fire blight" caused by the pathogen *Erwinia amylovora* was first reported in the United States in 1780 as one of the oldest phytopathological diseases described, and only a century later it was shown that the disease was caused by a bacteria capable of killing destroys considerable crops in just a few weeks (Burte, 1992). It was conducted the first study to investigate *Erwinias'* relationships using several strains of *E. chrysanthemi* and all *E. carotovora* subspecies (Avrova et al., 2002). In a study conducted in 2018 on the assortment of flowering plants cultivated in different green spaces of Craiova Municipality the attack of twenty-seven pathogens was reported on the nineteen host plants (Sălcudean (Ioniță) et al., 2019).

According to (Severin & Iliescu, 2006), the main symptoms caused by bacteria in plants are:

- spots and burns of different shapes and sizes, with or without halo - may occur on all aerial organs of the plant; are produced by bacteria of the genera *Erwinia, Pseudomonas and Xanthomonas*;
- wilting - are due to the invasion of the xylem by bacteria, which subsequently multiplies and then migrates throughout the plant; wilting usually kills part or all of the plant; are produced by some bacteria of the genera *Clavibacter, Curtobacterium, Pseudomonas, Ralstonia* and *Xanthomonas*;

- soft rot - may occur in vegetation or storage, giving off a characteristic odor; are due to bacteria of the genera *Erwinia* and *Burkholderia*;
- hypertrophies - are represented by galls and tumors that can develop on roots, leaves and stems; bacteria of the genera *Agrobacterium* and *Rodococccus* are responsible for such manifestations;
- ulcers - usually appear on the stem and branches of the plant, being represented by necrotic areas, longitudinal cracks of the scab, growths or cavities; bacteria of the genera *Clavibacter, Pseudomonas* and *Xanthomonas* are responsible for the existence of ulcers.

In general, for bacteriosis, the main means of movement and dispersal is considered the propagating material, and trade is an important factor in the spread of pathogens.

Another important aspect is related to the use of their own propagating material, obtained from infected crops and which led to the spread and growth of areas where bacterial diseases have been reported in ornamental plants.

MATERIALS AND METHODS

The researches was carried during the period 2018-2021, the studied material being represented by dendrofloric plants from different green spaces of Craiova Municipality. Field phytosanitary checks show that several pathogens can attack a variety of dendrofloric species, and the manifestations can be similar, even if they are attacked by different pathogens. The biological material was represented by plants and parts of plants with symptoms belonging *Prunus nigra*, *Hyacinthus orientalis, Brassica oleracea var. acephala* and *Pelargonium* sp.

In addition to macroscopic observations, the detection and identification of some of the phytopathogenic agents was performed in the biology laboratory of the high school "Dimitrie Filișanu" - Filiași, where they were planted in pots and grown in laboratory conditions in order to be able to make several observations until the appearance of obvious symptoms of the disease, part of the samples taken from the field. The detection and identification of the attack of different phytopathogens consisted both in periodic visual observations in the experimental area on certain organs of the host plants that showed symptoms of disease, and in performing laboratory tests. The different test methods used were developed according to Janse (2005) and Schaad et al. (2001), as follows:

- **macroscopic observation -** the material suspected of being infected with bacteria was subjected to visual examination; thus, if visible changes were detected **-** rotten areas or areas with spots and necrotic lesions, tumor like growths, additional investigations were performed to highlight various bacterial diseases;
- **isolation method on culture medium -** isolation was made from plant material suspected of being infected with phytopathogenic bacteria (examples: leaves, stems, bulbs etc.); the first step was washing, followed by disinfection (with 70% ethanol) of the infected material; then small portions of the affected areas were taken; sampling was performed at the boundary between the healthy and diseased material, and the pieces of material obtained were placed in extraction bags and crushed with a hammer (Figure 1); sterile water was added until the material was covered and then stirred at room temperature for 30 minutes for the soft plant material (leaves, bulbs) and 1-2 hours for the wood material (tumors); the isolation of the obtained suspensions was made in sterile conditions, in a vertical laminar air flow cabinet by streaking media with o loop; semi-selective culture media (King´B medium) were used for the detection of *Pseudomonas* and non-selective media (NA - Nutrient Agar, YPGA - Yeast Peptone Glugose Agar) for the detection of other bacteria; incubation of inoculated plates was performed at 27°C for at least 5 days; suspicious colonies were subjected to additional tests, such as rapid serological tests, biochemical tests, hypersensitivity tests and/or pathogenicity tests;
- **rapid serological tests -** Fast Lateral Flow test was used, which is very easy to perform and is based on an antigen-antibody reaction; it allowed rapid detection of antigens using specific antibodies. This test was used only to

detect *Xanthomonas hortorum* pv. *pelargonii*, using a kit from Loewe-Germany (Figure 2); a suspicious colony was resuspended in a buffer provided by the manufacturer; the mixture was stirred for a few seconds and then 3-4 drops of it were placed in the well of the test box; the result was read and interpreted after 5-15 minutes at room temperature;

Figure 1. Test preparation for analysis (original)

Figure 2. Fast Lateral Flow Test (original)

- **biochemical tests -** young colonies were needed to perform biochemical tests. They were either resuspended in sterile water to obtain slightly turbid suspensions, or were used as such; the following biochemical tests were performed:

- *test on liquid Beef medium* (meat broth) - was performed by pipetting 500 µL suspicious bacterial suspension in a tube with liquid Beef medium; after inoculation, the tubes were incubated for 3-5 days at an optimum growth temperature of 27°C;
- *oxidative band method* - a suspicious young colony was chosen and placed on an oxidative band; interpretation of the result was performed after 1 minute;
- *hydrogen sulfide* (H_2S) *production* - suspicious colonies were transferred using a sterile loop and inoculated by pricking in a tripton medium; the inoculated tubes were incubated for 3-5 days at 27°C; a strip of paper soaked in lead acetate was then inserted into the tube, securing it with a stopper so that the tripton medium would not be touched; the tubes were further incubated and followed for 1-2 days until a reaction occurred;
- *starch hydrolysis* - a suspicious colony was streaks on a Petri dish with solid soluble starch medium; the plates were incubated at 27°C for 3-5 days, until bacterial growths appeared; Lugol iodine solution was pipetted onto the Petri dish and waited a few minutes for the results to be interpreted;
- *mobility* - young, suspicious colonies were taken with a loop; they were inoculated by stabbing in a tube with motility-specific medium (medium with peptone and sodium chloride); interpretation of the results was performed after 3-5 days of incubation at 27°C;

- **hypersensitivity test** - this test was performed on tobacco plants, *Nicotiana tabacum* in the stage of 3-4 true leaves; the suspicious bacterial colonies were placed in 2 mL of sterile water, obtained a slight turbidity bacterial suspensions; they were inoculated, using a hypodermic needle syringe, into the intracellular space of the leaves of the tobacco test plants, which were then incubated at room temperature and monitored for 1-2 days;

- **pathogenicity test** - the pathogenicity test may be performed on carrot or potato slices, on detached leaves or on test plants susceptible to target bacteria; bacterial suspensions were made from suspicious colonies and inoculated on the material used to establish pathogenicity:

- *vegetal tissue test* - was performed on carrot slices for the detection of *Agrobacterium tumefaciens* and on detached leaves from *Pelargonium* sp. for the detection of *Xanthomonas hortorum pv. pelargonii*; the surface of the plant material was disinfected with 70% ethanol for a few seconds, after which the excess ethanol was removed with a napkin; in the case of using carrots, they were sliced to obtain 0.5 cm thick rounds, which were placed in Petri dishes with 1% agar medium; then were made bacterial suspensions from the suspicious colonies; the plant tissue of the test plant was injured by scratching in the case of carrot slices and by stinging in the case of geranium leaves; between 100-1000 µL of bacterial suspension was pipetted over the injured wounds; the plates thus inoculated were placed in a box with a lid to maintain a moist environment and then incubated at 27°C for several days, until symptoms appeared.

- *vegetal tissue maceration test* - was performed to establish the pectolytic activity of bacteria: *Xanthomonas campestris pv. campestris* on *Brasica oleracea var. acephala* (decorative cabbage), *Erwinia carotovora*, *Pseudomonas marginalis* and *Xanthomonas hyacinthi* on *Hyacinthus orientalis*; this test was performed on potato slices; thus, after the surface of the potato tubers was disinfected with 70% ethanol and the excess alcohol was removed with a napkin, the vegetable tissue was sliced and placed in a Petri dish with 1% agar; bacterial suspensions were made in sterile water from suspicious colonies; then about 1000 µL of bacterial suspension was pipetted over the tissue used; to maintain a moist environment, inoculated Petri dishes were placed in a container with a lid and incubated for 48 hours at 27°C;
- *plant test* - was performed for the artificial reproduction of the disease on sensitive test plants. The test was performed on plants of *Pelargonium sp.* for *Xanthomonas hortorum pv. pelargonii* and plants of *Solanum lycopersicum* 'Money Maker' for *Agrobacterium tumefaciens* and *Xanthomonas hortorum* pv. *pelargonii*; plants were used in the stage of 1-2 true leaves; in the case of confirmation of the bacterium *Agrobacterium tumefaciens*, bacterial suspensions obtained directly from the extract; in the case of reproduction of the disease caused by *Xanthomonas hortorum* pv. *pelargoni* were used bacterial suspensions made by resuspending suspicious young colonies in sterile water; the suspensions were inoculated into the stem and the plants were incubated at 26-28°C until the onset of disease symptoms.

RESULTS AND DISCUSSIONS

Regarding the detection of certain bacterial diseases, following macroscopic observations, in the monitored period, various symptoms produced by bacteria were identified (Table 1).

Table 1. Bacteriosis identified in the experimental area

Host plant	Harmful organism	Disease - Symptoms
Prunus nigra	*Agrobacterium tumefaciens*	**Bacterial cancer** - tumors like cauliflower inflorescences, at the base of the stem (Figure 3)
Hyacinthus orientalis	*Erwinia carotovora*	**The soft rot of the bulbs** - small plants, bulbs soft, macerated, mucilaginous and blackened, with an unpleasant odor, characteristic of rot (Figure 4)
	Pseudomonas marginalis	**Marginal burning of leaves** - stagnation of plant growth and necrotic lesions at the top of the leaves (Figure 5)
	Xanthomonas hyacinthi	**The yellow rot of hyacinth bulbs** - areas of macerated tissue and necrotic areas inside the bulbs; yellowed and browned areas towards the top of the leaves or leaf rot (Figure 6)
Brassica oleracea var. acephala	*Xanthomonas campestris pv. Campestris*	**The black nervation of the cruciferous leaves** - yellowing and internervurian necrosis; vascular ring necrosis (Figure 7)
Pelargonium sp.	*Xanthomonas hortorum pv. Pelargonii*	**Staining of leaves and rot of geranium stalks** - leaves with internervurian necrotic areas, with yellowed edge of parenchima; whole dried leaves (Figure 8)

To confirm the disease caused by ***Agrobacterium tumefaciens***, pathogenicity tests were performed on *Daucus carota* slices and on plants a test of *Solanum lycopersicum* cv. 'Money Maker'. On the carrot slices, after about 3 weeks, whitish tumors appeared near the vascular ring (Figure 9. a).

Also, on the stems of the test plants, after 4-6 weeks after inoculation, tumors of different sizes, light green-whitish color were observed (Figure 9. b).

Erwinia carotovora was detected by the hydrolysis of starch, a test in which clear areas appeared around the bacterial growths, the rest of the medium being stained due to the activity of Lugol's iodine. Also, following the establishment of the pectolytic activity of the suspicious bacteria, it was found that they showed such an activity that led to changes in the substrate (Figure 10.a). The color and consistency of the potato slices changed, leading to the maceration of the vegetal tissue (Figure 10.b)

Figure 3. Tumors of *Agrobacterium tumefaciens* on *Prunus nigra* (original)

Figure 4. Symptoms of *Erwinia carotovora* on *Hyacinthus orientalis* (original)

Figure 5. Symptoms of *Pseudomonas marginalis* on *Hyacinthus orientalis* (original)

Figure 6. Symptoms of *Xanthomonas hyacinthi* on *Hyacinthus orientalis* (original)

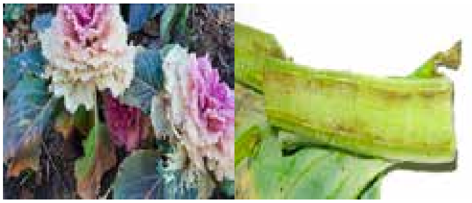

Figure 7. *Xanthomonas campestris* pv. *campestris* on *Brasica oleracea* var. *acephala* (original)

Figure 8. Symptoms of *Xanthomonas hortorum* pv. pelargonii on *Pelargonium* sp. (original)

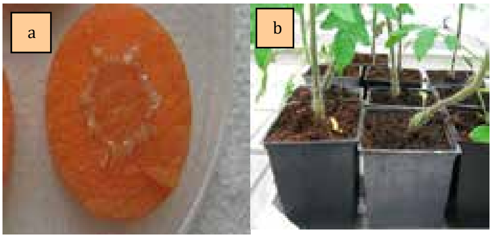

Figure 9. *Agrobacterium tumefaciens* - laboratory tests a - pathogenicity test on carrots; b - pathogenicity test on tomato plants (original)

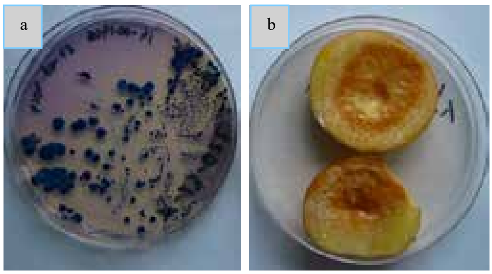

Figure 10. *Erwinia carotovora* - laboratory tests a - hydrolysis of starch; b - vegetal tissue maceration test (original)

Pseudomonas marginalis was isolated on King´B medium, obtaining fluorescent, translucent colonies (Figure 11.a). The first test performed after obtaining suspicious bacterial colonies was the oxidative band test. After the test was completed, a purple coloration was observed (Figure 11.b). The purple color indicates a positive reaction. Another test performed was the vegetal tissue maceration test which was also positive. The vegetable tissue of the potato slices used was macerated and browned (Figure 11.c). Being a bacterium of the genus *Pseudomonas*, the hypersensitivity test of tobacco leaves was also performed. At the injection point of the bacterial suspension appeared a yellow area that extended into the parenchyma of the leaf, followed by necrosis and drying (Figure 11.d).

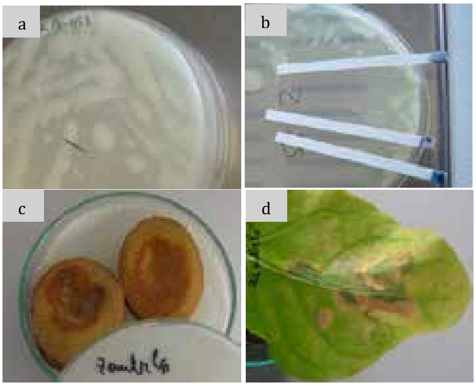

Figure 11. *Pseudomonas marginalis* - laboratory tests
a - isolation on King´B medium; b - oxidase test;
c - vegetal tissues maceration (on potato slices);
d - hypersensitivity test on tobacco leaves (original)

Xanthomonas hyacinthi was identified by the following tests: isolation, biochemical, hypersensitivity and vegetal tissue maceration. On the culture medium the colonies were glossy and light yellow. Four biochemical tests were performed and all corresponded to the biochemical profile of this bacterium.

Thus, the bacteria did not produce hydrogen sulfide (H_2S) (Figure 12.a), hazing the Beef medium (Figure 12.b), were mobile (Figure 12.c) and weakly hydrolyzed the starch (Figure 12.d).

Therefore, the liquid Beef medium was hazing due to the growth of bacteria and the paper soaked in lead acetate did not turn black, so no H_2S was produced.

The test of vegetal tissues maceration leds to the total maceration of the potato slices used, which indicated a very high pectolytic activity (Figure 12.e). The hypersensitivity test on tobacco leaves revealed the appearance of necrotic areas in the area of the parenchyma injected with bacterial suspension (Figure 12.f).

Xanthomonas campestris pv. campestris was identified by isolation on non-selective YPGA (Yest Peptone Glucose Agar) culture medium. After a few days of incubation, small, circular, smooth, yellow, shiny colonies appeared (Figure 13.a, b). The colonies also showed pectolytic activity leading to maceration and blackening of the plant tissue used in the test (Figure 13.c, d).

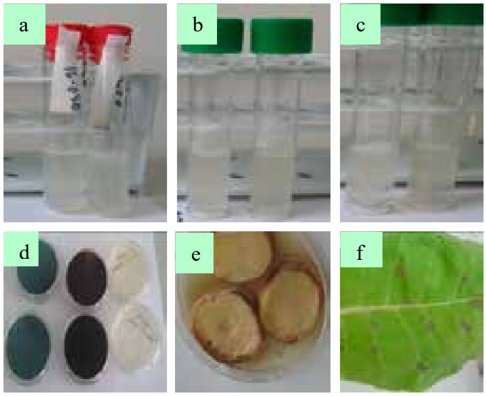

Figure 12. *Xanhomonas hyacinthi* - laboratory tests
a - H_2S production; b - test on liquid beef medium (broth); c - mobility test; d - starch hydrolysis;
e - vegetal tissue maceration test;
f - hypersensitivity test (original)

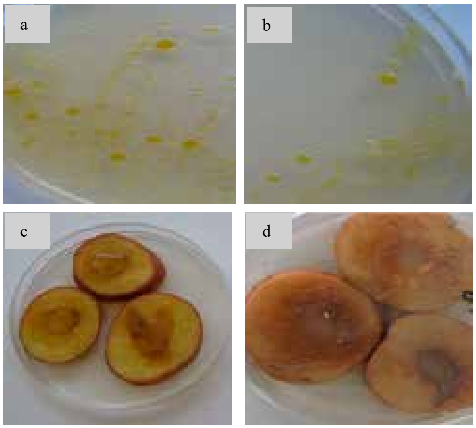

Figure 13. *Xanthomonas campestris pv. campestris* - laboratory tests
a, b - isolation on non-selective culture medium;
c, d - vegetal tissue maceration test (on potato slices) (original)

Xanthomonas hortorum* pv. *pelargonii was identified by the following tests: isolation on

culture media, hypersensitivity, vegetal tissue test and plant pathogenicity. Isolation on culture medium was performed on non-selective media (YPGA - Yeast Peptone Glucose Agar and NA - Nutrient Agar).
After 3 days of incubation, yellow, smooth and translucent colonies appeared. The color of the colonies was more intense on the YPGA medium than on the NA (Figure 14.a, b). Bacterial suspensions were made from the suspected colonies, which were tested serologically, obtaining a positive reaction to the Fast lateral flow test (Figure 14.c).

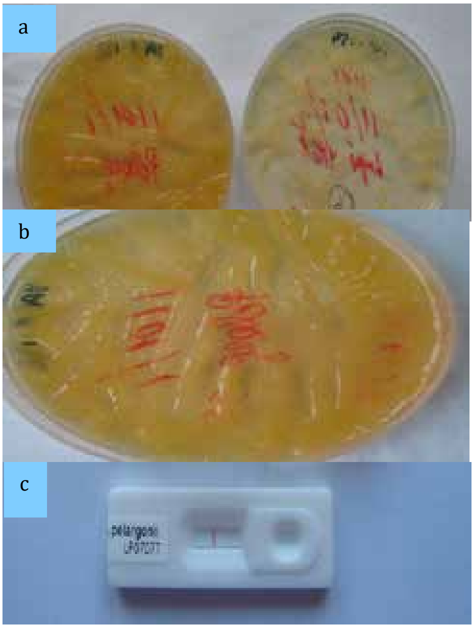

Figure 14. *Xanthomonas hortorum* pv. *pelargonii* - laboratory tests
a, b - isolation test on non-selective culture media;
c - Fast lateral flow test (original)

Also, the suspensions that were inoculated by injection into *Nicotiana tobacco* and *Pelargonium* sp. leaves led after 24-48 h to the collapse of the inoculated parenchyma (Figure 15.a and 15.b).
During the pathogenicity test, after inoculation of the seedlings of *Solanum lycopersicum*, it led to necrotic lesions on the leaves and to the upward rolling of the leaf (Figure 15.c).

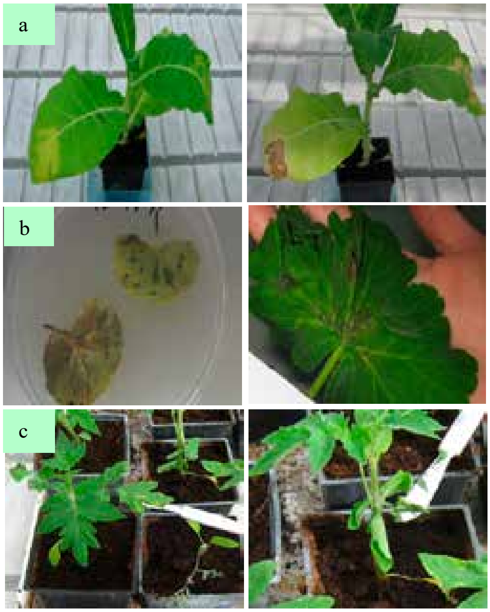

Figure 15. *Xanthomonas hortorum pv. pelargonii* - laboratory tests
a - hypersensitivity test; b - pathogenicity test on leaves detached from *Pelargonium* sp.; c - test on *Solanum lycopersicum* (original)

CONCLUSIONS

The starting point in the detection of diseases caused by bacteria were the macroscopic observations made in the field that showed the existence of symptoms, but without being able to make an exact diagnosis.
For proper identification, not only macroscopic observations but also laboratory tests are required to certify the initial identification in the field.
Regardless of the host plant or the bacterium suspected of producing the present symptoms, the first laboratory test was to isolate the bacteria from the suspicious material on various non-selective or semi-selective culture media.
After isolation, bacterial colonies were used for identification by additional tests (serological, biochemical and hypersensitivity tests) and, where possible, was performed a confirmation test (pathogenicity test on susceptible plants) in order to reproduces the disease in test plants under laboratory conditions.

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EFFECT OF SALINITY STRESS ON SOME PHISIOLOGICAL INDICES OF WHITE CLOVER (*TRIFOLIUM REPENS)*

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Abstract

Salinity is an factor which has a critical influence on seed germination and plant establishment. During the present study the effect of salinity on seeds germination, seedling growth and chlorophyll content of Trifolium repens was studied. Experiments were conducted in laboratory condition. The salinity was induced using three concentration of NaCl (0 mM NaCl, 40 mM and 80 mM). Determinations were recorded in three periods, after (3, 10 and 17 days) and were analysed percentage of germination, MDG and DGS, seedling growth and chlorophyll content. In normal condition Trifolium repens showed highest percentage of germination 79.33%, while in stress salt condition percentage of germination was reduced in proportion to the increase in saline concentration. In salt stress condition we observed that the mean daily germination (MDG) decreased, while daily germination speed (DGS) increased. The effect of the NaCl concentration on the growth in the clover seedlings they showed values between 2.372 cm on the V3 variant and 3.603 cm on the Vo variant. The application of treatments that induced saline stress led to a significant decrease in growth in proportion to the level of differences between treatments. Referring to the effect of the environment on the accumulation of chlorophyll, it was observed that the chlorophyll content decreased under conditions of saline stress (24.15 SPAD). Salt tolerance data may be used to select clover with the highest potential for agronomic production.

Key words: *clover, salt stress, germination, chlorophyll content.*

INTRODUCTION

One of the largest genera of the *Leguminosae* (Fabaceae) family is *Trifolium* with ca. 255 species (Zohary et al., 1987). All species are herbaceous perennials or annuals. Their habitats are temperate and, to a lesser extent, subtropic regions. White clover is very widespread in all ecological areas of our country. *Trifolium repens* L. is grown as a forage crop and is planted in various landscapes for soil conservation. In each year are numerous reports of failed white clover stands. A good understanding of the biology of white clover seed germination in relation to environmental factors is essential for the successful establishment of the crop. The successful establishment of any plant depends on its germination. Various factors such as temperature, water availability, light, salinity etc. can influence seed germination (Al-Ahmadi et al., 2007; Guma et al., 2009; Khan et al., 1984; Khan, 1987; Pérez-García et al., 2007; Ruano et al., 2009). Worldwide, almost 6% of the earth's total surface area is affected by salinity (Zoya Baig et al., 2015). 6.3 million are affected by salinity out of 20.36 million hectare of cultivable land (Qureshi, 1998). Globally, a major stress for plants is increasing soil salinity. In agricultural ecosystem plant production is directly affects by salinity. When plants are exposed to salinity according to Munns (1993), they immediately experience osmotic stress due to low water potential of the substrate. Soil salinity is considered to be one of the major environmental stresses affecting plant growth and productivity, leading to significant productivity losses (Azeem et al., 2011). Water potentials in the leaves of clovers (*Trifolium* sp.), length and dry mass of the stem is reduced by salinity, and are affected the length and conductivity of the root (Orak et al., 2005). For selecting salt tolerance in plants the most viable criteria used are germination and seedling characteristics. The most useable criteria for cultivar selection are germination percentage, germination speed and seedling growth. Screening for seeds with a greater tolerance to salt stress aids in the development of salt tolerant cultivars. A critical point in seedling subsequent and establishment plant health and vigour is seed germination (Azeem et al., 2011). The main colour agent responsible for photosynthesis is chlorophyll. Under adverse circumstances, the chlorophyll level is a good indicator of the photosynthesis function (Xu et al., 2008). Salinity stress can cause severe disruptions in the plant morphology and physiology. The aim of this study was to explore salinity tolerance of *Trifolium repens,* variety Rivendel.

MATERIALS AND METHODS

The experiments were conducted at the Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timişoara. The biological material was represented by *Trifolium repens*, variety Rivendel. Seeds of *Trifolium repens*, with similar sizes were selected for each experiment and germinated under different salinity levels (40mM and 80mM) and version control without salt (0 mM NaCl). We used for germination tests Petri dishes with two layers of filter paper moistened with 6 ml of water or a NaCl solution. Three repetitions were used for each treatment and 100 seeds were placed in each Petri dish. The dishes were incubated in germinators at a constant temperature of 24°C days/20°C night. Germination was recorded at 3, 10 or 17 days intervals. Were considered germinated seeds those which presented emergence of radicle (Bewley et al., 1994). The data were transformed into germination percentages and were calculated means. The seeds were monitoring every day for 17 days, and the following parameters were analysed:

Mean daily germination (MDG) - This is an index of daily germination speed and calculated by:

MDG = FGP/d,

where: FGP: final germination percent; d: test period

Daily germination speed (DGS)

This index is converse of mean daily germination and calculated by:

DGS = 1/MDG (Niste et al., 2014).

The seedling growth was recordered after 7, 14, 21 days. The chlorophyll content were measured after 7, 14, and 21 days, using a clorophylmeter SPAD-502 (Konica Minolta), which measures the absorbance at 650 nm, (Barraclough et al., 2001). To process the experimental data were used analysis of variance and the "t" test [6]. Symbols (*, 0) were used to signify the differences between varieties (Ciulca, 2006).

RESULTS AND DISCUSSIONS

The effect of salinity (V1 - 0 mM NaCl to the control, V2 - 40 mM, V3 - 80 mM, NaCl) upon seeds germination at clover Rivendel variety was observed for 17 days.

Clover Rivendel seeds in laboratory conditions (Figure 1) started to germinate on 3nd day and in 17th day was obtained highest percentage of germination.

In control version (0 mM Nacl) the variety Rivendel registered a percentage of germination of 79.33%.

Compared to the control germination rate was affected by the first concentration of NaCl. At a concentration of 40 mM (V2) germination rate was 75.0% and in V3 (80 mM) was 73.33%.

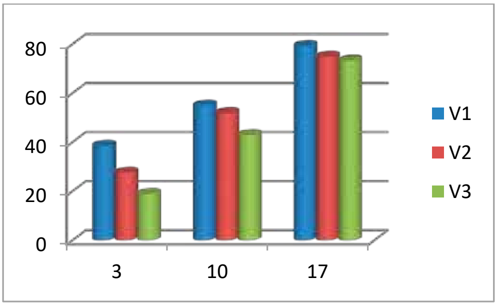

Figure 1. Percent of germination in clover variety Rivendel

On germination percentage it could be noticed that periods had a very significant influence, according to presented data in Table 1.

After a period of (17days) the average values of germination percentage shows an increasing in its value. The results obtained showed statistics assurance.

Table 1. The effect of period in percent of germination

Periods	Average (%)		Relative value (%)	Difference	Significance.
10 days-3 days	51.111	28.444	179.688	22.667	***
17 days-3 days	80.222	28.444	282.031	51.778	***
17 days-10 days	80.222	51.111	156.957	29.111	***
			LSD 5%	LSD 1%	LSD 0.1%
			4.756	6.551	9.019

Regarding the unilateral effect of NaCl concentration on germination percentage at clover seeds (Table 2), it can be observed that above mentioned treatment caused its significant decrease.

Germination percentage varied between 57.66%in variant V1 (0 mM NaCl) and 47.33 % in V3 variant (80 mM NaCl). Decreasing of germination due to salt stress has been reported in another research (Niste et al., 2015; Zoya et al., 2015).

Regarding some indices (Table 3) it can be observed that, MDG (4.31) decreased in stress condition while DGS (0.23) is increased.

Table 2. The effect of medium in percent of germination

Variants	Average		Relative value %	Difference	Signification
V2-V1	54.778	57.667	94.990	-2.889	
V3-V1	47.333	57.667	82.081	-10.333	oo
V3-V2	47.333	54.778	86.410	-7.444	o
			LSD 5%	LSD 1%	LSD 0.1%
			8.192	11.095	14.833

Table 3. Means comparison of seed germination and some indices

Variants	MDG	DGS
V1	4.666	0.21
V2	4.411	0.22
V3	4.313	0.23

The amount of water that the plant uses decreases due to the osmotic stress caused by the increase in the amount of salt in the soil and as a result physiological drought occurs.

Salinity affects seed germination by osmotic effect by preventing or delaying germination, toxicity or ions, causing unviable seed (Cokkizgin, 2012). Germination is one of the most critical periods in the life cycle of the plant according to (Debez, 2004). In plants, during different stages of development level of salinity tolerance is different from germination to mature plant (Khan et.al., 1998; Zoya et al., 2015).

It is also very important for plant establishment in varying geological and environmental regions around the world (Zoya et al., 2015).

Table 4. The effect of period in seedling growth of clover

Periods	Average (%)		Relative value %	Difference	Significance.
14 days - 7 days	3.164	2.239	141.326	0.925	**
21 days - 7 days	3.742	2.239	167.178	1.504	***
21 days - 14 days	3.742	3.164	118.293	0.579	*
			LSD 5%	LSD 1%	LSD 0.1%
			0.527	0.726	0.999

Regarding the effect of the determination period on the growth of the clover seedlings, it can be seen from Table 4 that the seedlings registered an average growth between 2.23 cm and 3.742 cm, 21 days after emergence. The analysis of the obtained results (Table 4) shows that the third determination period ensures a very significant increase in seedling growth superior to the first determination period for the analysed genotype. Also, the second determination period achieves a significantly higher increase in seedling growth compared to the first determination period.

Table 5. The Efect of mediului upon seedling growth of clover

Variants	Average (cm)		Relative value %	Difference	Signification.
V2-V1	3.167873	3.603778	87.90423	-0.4359	-
V3-V1	2.372833	3.603778	65.84294	-1.23094	ooo
V3-V2	2.372833	3.167873	74.90304	-0.79504	oo
			LDS 5%	LDS 1%	LDS 0.1%
			0.490831	0.664766	0.888737

Considering the analysis of the effect of the culture medium on the growth of the seedlings in clover, a decrease of the growth on the medium with a saline concentration of 80mM NaCl is observed, registering an amplitude of variation of 1.231. Regarding the unilateral effect of the NaCl concentration on the growth in the clover seedlings (Table 5) these showed values between 2.372 cm on the V3 variant and 3.603 cm on the Vo variant (0 Bars H_2O). The application of treatments that induced water deficit led to a significant decrease in growth in proportion to the level of differences between treatments. Chlorophyll is one of the major components of chloroplasts, so the chlorophyll content is positively correlated with the rate of photosynthesis. Reduction of chlorophyll content in drought conditions is considered a typical symptom of oxidative stress that may be the result of photooxidation of pigments and degradation of chlorophyll (Anjum et al., 2011). In addition, a reduction in chlorophyll content in water-deficient conditions would be caused by the destruction of the chloroplast membrane, excessive swelling and denaturation of the vesicle lamellae, and the appearance of lipid droplets (Kaiser et al., 1981). A low concentration of photosynthetic pigments can lead to a direct reduction in photosynthetic potential and thus production. From the data in Table 6, it can be seen that the chlorophyll content accumulated in the study material showed values between 30.467 (SPAD) 7 days after stress induction and 29.9 SPAD after 21 days after water stress induction. Considering the analysis of the effects of seedling age on the chlorophyll content, it is observed that it does not have a significant influence.

Table 6. Effect of time on chlorophyll content (SPAD) in clover

Periods	Average (SPAD)		Relative value, %	Difference	Signification
14 days - 7 days	30.144	30.46783	98.9371	-0.32384	
21 days - 7 days	29.9	30.46783	98.13629	-0.56783	
21 days - 14 days	29.9	30.14399	99.19059	-0.24399	
		L DS5%	LDS 1%	LDS 0.1%	
		1.350535	1.860171	2.560921	

Regarding the effect of the environment on the accumulation of chlorophyll, it can be seen that under conditions of saline stress the chlorophyll content decreased (24.15 SPAD) (Table 7).

Table 7. Effect of the medium on chlorophyll content (SPAD)

Variants	Average (SPAD)		Relative value %	Difference	Signification.
V2-V1	30.55429	35.80554	85.33395	-5.25126	oo
V3-V1	24.15199	35.80554	67.45321	-11.6536	ooo
V3-V2	24.15199	30.55429	79.04616	-6.4023	ooo
		LDS 5%	LDS 1%	LDS 0.1%	
		3.248587	4.399785	5.88215	

CONCLUSIONS

Highest percentage of germination was obtained on 17th day. In control version (0 mM Nacl) the variety Rivendel registered a percentage of germination of 79.33%, while in salt conditions V3 (80 mM) germination rate was 73.33%.

The unilateral effect of NaCl concentration on the germination rate of clover seeds led to a significant decrease. Germination percentage varied between 57.66% in variant V1 (0 mM NaCl) and 47.33 % in V3 variant (80 mM NaCl). Mean daily germination (4.31) decreased in stress condition while daily germination speed (0.23) is increased.

The effect of the NaCl concentration on the growth in the clover seedlings they showed values between 2.372 cm on the V3 variant and 3.603 cm on the Vo variant. The application of treatments that induced saline stress led to a significant decrease in growth in proportion to the level of differences between treatments. Regarding the effect of the environment on the accumulation of chlorophyll, it was observed that under conditions of saline stress the chlorophyll content decreased (24.15 SPAD).

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SOME ASPECTS REGARDING THE PROPAGATION BY CUTTINGS OF ORNAMENTAL SPECIES ON DIFFERENT ROOTING SUBSTRATES

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Abstract

Plants, as intelligent organisms, have developed mechanisms for survival, which humans have studied and used to expand the methods of multiplication of various categories of plants. In the field of ornamental plants one of the most common method of propagation is the vegetative method by cuttings. In the present work, the testing of the most commonly used rooting substrates (sand, perlite, peat, peat mixed with perlite 1:1) for the top cuttings of shoots of some indoor ornamental species, decorative by flowers and leaves (Nerium oleander, Pelargonium odoratissimum, Croton variegatus, Hypoestes sanguinolenta, Coleus hybridus) continued. The parameters analysed to evaluate the efficiency of the rooting substrate were some biometric characteristics the average length of the main root and the average length of shoots together with the rooting percentage. For the statistical interpretation of these parameters, the IRP (index of Root and Peak) indicator was developed. The consistency of the IRP indicator was analysed using the Alpha Cronbach coefficient.

Key words: *cuttings, rooting substrates, Alpha Cronbach.*

INTRODUCTION

Propagation of plants by cuttings is based on the ability of these fragments - from the various organs of plants (stems, shoots, leaves, roots) to form at the basal pole, when they are placed in optimal conditions, roots and on apical pole the aerial organs, resulting in new plants, very similar to the mother plants.
According to the literature, the cuttings obtained from the tops of the branches take root faster than the cuttings of the section (or obtained from the lateral branches), due to the higher auxin content (Burzo et al., 2005).
Billions of new plants are produced each year, starting from cuttings harvested from carefully selected mother plants. The quality of young plants is the result of the interaction between the genetic and physiological limits of the plant, the rooting substrate and the ensured environmental conditions (Druege, 2020).
Last but not least, pruning is one of the methods used to ensure the survival of endangered plant species. Thus, in the species *Jasminum parkeri* Dunn (Kashyap et al., 2021), a woody ornamental shrub, not only was it possible to protect the species from population decline but there was even an increase in the adaptive potential of the species, which may be a sign of superior uses as a decorative plant in India, simultaneously with the reintroduction of ex situ cultivated plants into natural populations.
Wild populations of *Hoya imperialis* and *Hoya coronaria* (family Apocynaceae), particularly decorative by flowers, are under special pressure in their areas of origin (Brunei), due to the continuous decline of habitat and unsustainable harvests. Two methods of propagation have been studied for ex situ preservation: by cuttings and by micropropagation. The cuttings were treated with rooting stimulants and placed on a substrate of peat and perlite. The results showed a good multiplication rate, even in the case of unstimulated cuttings. Micropropagation did not work (Mohd Don et al., 2021).
What is very important to note is that continuous innovation in vegetative propagation is one of the drivers of plant breeding programs facilitating the production of high-quality plants with the same genetic characteristics as the mother plant and without diseases or pests (Rufo and Colombo, 2020).
Peat is a type of natural substrate of organic origin, often used in flower production, being found alone or in various combinations, both in

the composition of rooting substrates and those for containerized culture. It should be noted that concerns have now begun to be identified about the possibility of replacing peat due to its non-renewable resource characteristics (Aboksari et al., 2021).Substrates for rooting ornamental cuttings can be classified as "light" substrates, usually simple or mixed, consisting of natural components (peat, sand) and artificial components (perlite) (Davidescu et al., 2001), combined in various proportions depending on the specific requirements of the plants (Manda et al., 2019).

It is good to know that water, simple, can also be used as a rooting "substrate", for certain species in genera such as *Nerium, Pelargonium, Saintpaulia, Hibiscus, Cyperus, Tradescantia, Ficus, Hedera, Scindapsus* (Șelaru, 2006).

A quality substrate is, in most cases, beyond the quality of the biological material available, one of the essential conditions for the success of plant propagation through cuttings. Such a substrate must have good air porosity, be able to retain water and be sterile - free of pathogens.

MATERIALS AND METHODS

This preliminary research was carried out in the greenhouses of the Hortinvest Center of the USAMV Bucharest during 2019-2021.

The studied biological material consisted of cuttings from a number of five ornamental species, decorative by habitus, foliage, flowers, grown in pots in the conditions of our country.

Croton variegatus L. (sin. *Codiaeum variegatum* (L.) Rumph. Ex A. Juss), family Euphorbiaceae, ord. Malpighiales, native to Malaysia, Australia, plant with permanent vegetation, reaches heights of 60-80 cm, decorative by its rich foliage, elliptical leaves elongated, entire or cut symmetrically or asymmetrically, with straight or wavy edge, are leathery and variously colored with spots, dots, streaks of red, yellow, orange, pink, purple that give surprisingly beautiful combinations. The yellow flowers placed in clusters are not of decorative interest (Șelaru, 2006).

Hypoestes sanguinolenta (Van Houtte) Hook. f., family Acanthaceae, ord. Lamiales, species native to Madagascar, herbaceous, with a height of up to 30-40 cm, has continuous vegetation and rapid growth. The leaves, placed opposite, have an oval-elliptical shape with a sharp tip and numerous small pink spots on the green background of the upper face. The reverse of theleaf is purple. The small, purple flowers have no decorative value (Șelaru, 2006).

Nerium oleander L., family Apocynaceae, ord. Gentianales, is native to India, Singapore, Nepal, China, Mediterranean regions. It can bloom from spring to late autumn. As a potted plant, the oleander can reach heights of 1-2 m and has the appearance of a bush shrub. The flexible shoots are trimmed with leathery leaves, very similar to willow or olive leaves. The flowers can be simple or wrapped, grouped in rich bouquets and have a wide range of colors, from white, pink, yellow to red or purple (Șelaru, 2006).

It is a very important species, especially in the Mediterranean area, where there is a high demand, which justifies the efforts of growers to obtain and perpetuate plants with valuable characteristics, vegetative propagation by cuttings is one of the most important methods in this regard, along with of more sophisticated methods such as *in vitro* multiplication (Ochoa et al., 2003; Simion and Anton, 2009; Vila et al., 2010; Aryan and Rani, 2016). But this species is important not only from a decorative point of view, but also as a factor that contributes to the decrease of pollution in the urban environment due to its resistance (Doganlar et al., 2012; Vasquez et al., 2016; Elloumi et al., 2017).

Pelargonium odoratissimum (L.) L'Hér. family Geraniaceae, ord. Geraniales - the apple geranium, or the scented geranium (Thirumalachar, 1944; Toma, 2009), or in Romanian “drușaim” Șelaru, 2006).The species comes from South Africa (Cape of Good Hope), has deeply lobed leaves (7-11 lobes) with serrated edges. The bluish- green tongue has a fine pubescence. The flowers are purple, small, less decorative. The whole plant is strongly fragrant, being used in food as an aromatic plant and in medicine.

There are also opinions about this species, according to which the propagation by stem cuttings does not give the expected results, due to the difficult rooting (Ebrahimzadeh et al., 2021), while studies carried out on other species of the genus confirm the ability of reproduces faithfully from cuttings, with or without the use of rooting stimulants (Georgescu et al., 2012; Toma et al., 2012).

Coleus blumei Benth. (sin. *Plectranthus scutellarioides* (L.) R.Br.), family Lamiaceae ord. Lamiales, although is mainly known as a garden plant (Șelaru, 2007), it is beginning to establish itself in the assortment of indoor plants, due to the diverse coloration of its particularly decorative leaves. Although it can be propagated by seeds, due to genetic instability, pruning is practiced using stem cuttings with apical leaves, treated with rhizogenic substances (Belniaki et al., 2018) when it is necessary to quickly obtain large quantities of uniform plant material for the establishment various forms of decoration (vegetable carpets, mosaics etc.). At the same time, some studies show that the exposure of cuttings to the light of different types (Cho et al., 2022) favors the further development of new plants.

Long shoots were harvested from the existing mother plants in the didactic collection, from which top shoots were made with 3-5 knots each. Cuttings with relatively different lengths resulted since the distance between the nodes on the stem is different, being larger at the base and smaller towards the top.

After sampling, the cuttings were shaped differently, the leaves from the lower node were removed, and the others from the upper nodes were shortened, almost in half, to the species that allow this intervention, to have a smaller leaf area, so a process of less intense perspiration.

In this context, a bifactorial experiment (type 5 x 4) was organized, as follows:

A factor- cuttings of the 5 ornamental species mentioned above, of 30 repetitions each.

B factor- 4 rooting substrates: river sand (V_1), perlite (V_2), peat (V_3), and a mixture of peat and perlite (1:1) (V_4).

Once made, the cuttings were distributed in the properly prepared rooting substrates, in alveolar plates (51 x 33 x 4.4 cm; 70 cells, usable volume about 4 cm^3/root cell). Throughout the rooting, the appropriate environmental factors were ensured: temperature 20-22°C, atmospheric humidity 95-97%, the maintenance of the permanent substrate revived by frequent watering. The rooting time was 4-5 weeks.

Indicators were defined that define the process of rhizogenesis: percentage of rooting, length of main root and length of rooted cuttings.

An indicator has been defined to reflect the three types of measurements: percentage of rooting (**PR**), the average of the root length of the cuttings main (**RL**) and the average length of the rooted shoots (**LS**), for each type of substrate.

The data were normalized using the unity-based normalization method (Myatt and Johnson, 2014) using the formula:

$$X_{new} = \frac{X_{old} - X_{min}}{X_{max} - X_{min}}$$

The comparison of the data sets was made using the IRP indicator (index of Root and Peak):

IRP = 45% x PR + 10% x LR + 45% x LS,

The Alpha-Cronbach coefficient (Cronbach, 1951), used to study the consistency of the IRP indicator (reliability of IRP index) was calculated using GNU pspp 1.4.0. This software was used for ONEWAY ANOVA to compare the averages of the IRP indices for each species (DF=4, $p<0.05$)

RESULTS AND DISCUSSIONS

The process of rhizogenesis was influenced differently depending on the substrates in which the cuttings were rooted.

1. Percentage of rooting recorded very different values, between 50% and 100%, influenced by both the substrate used and the species (Figure 1).

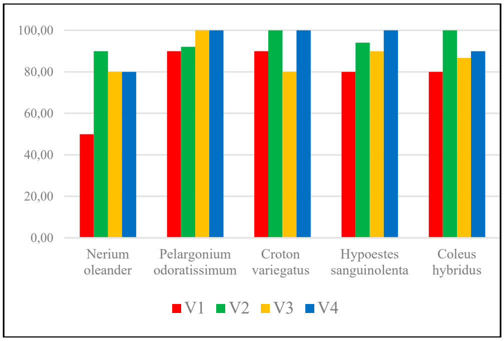

Figure 1. Percentage of rooting on different substrates

In *C. variegatus* cuttings, the rooting percentage was 100% for experimental variants of V_2 and $V_4$are in accordance with the data from literature (Şelaru, 2006), 90% for V_1 cuttings and 80% for V_3 cuttings. The best results were obtained in substrates with perlite due to its granular structure, being easy to cross for roots.
H. sanguinolenta recorded, as can be seen, the highest percentage of rooting on the substrate composed only of peat and perlite (V_4), the percentage value being 100%. In the V_2 cuttings have rooted in the percentage of 94%, take in V_3 90%. The lowest yield was obtained in cuttings rooted in V_1, the percentage of rooting on this substrate being 80%.
In *C. hybridus*, the rooting percentage was different depending on the substrate, so the highest value was obtained in cuttings that had V_2 as their rooting substrate (100%) (confirming the literature data, Nicu and Manda, 2021), then, in descending order, those rooted in the substrate composed of V_4(90 %), 87 % V_3 and V_1 (80 %).
N. oleander: the highest rooting percentage was obtained in the case of the substrate composed only of perlite - V_2 (90%). The lowest yield was obtained in cuttings rooted in the substrate formed only from V_1 (50%). On the other substrates (V_3 and V_4) the rooting percent were 80%.
Concerning *P. odoratissimum* cuttings, from the analysis of the results obtained for peak cuttings, in each substrate, it can be concluded that on the rooting substrate represented by V_4, the best 100% results were recorded. The worst results were obtained in the 90% V_1 substrate.

2. The length of the main roots

The main root length (Figure 2) in *C. variegatus* had values in the range of 7.10 cm in V_4, and 5.1 cm in V_1. Intermediate values were recorded onV_3 (6.90 cm) and V_2 (5.50 cm).

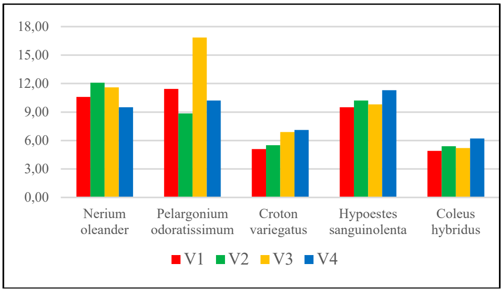

Figure 2.The length of the main root to rooted cuttings on different substrates (cm)

Taking into account the obtained results it can be easily seen that the longest main roots in the rooted cuttings of the species *H. sanguinolenta* were in the case of V_4 (11.30 cm), and the shortest roots were observed in the cuttings rooted in the V_1, 9.50 cm. The length of the roots of the cuttings rooted in V_2 (10.20 cm) and V_3 (9.80 cm) had very close values.
Regarding the biometric parameters studied in the case of the *Coleus hybridus* species, both in the case of the aerial vegetative part (shoots) and in the case of the root system, it was found that their values are close, higher values can be observed in the evolution of the cuttings rooted in V_4, on the second position were placed the cuttings rooted in V_2, and the lowest values were recorded in the cuttings rooted in V_1. The length of the main root recorded values ranging from 6.20 cm (V_4) to 4.90 cm (V_1).
In the case of *N. oleander* species it can be easily seen that the longest roots have grown in plants rooted in V_2 (12.10 cm). The V_4 gave the lowest values, 9.5 cm.
The main root length for the species *P. odoratissimum* was between 21.82 cm (V_3) and 14.84 cm in the case of V_2.

3. The length of the shoots

In the case of the shoot length parameter (Figure 3), in the *C. hybridus* species, the values were close, noting the roots formed in V_4 substrate (19.10 cm), the V_2 ones 18.40 cm, 18.21 cm those from V_3 and the lowest values (17.90 cm) in the case of V_1.

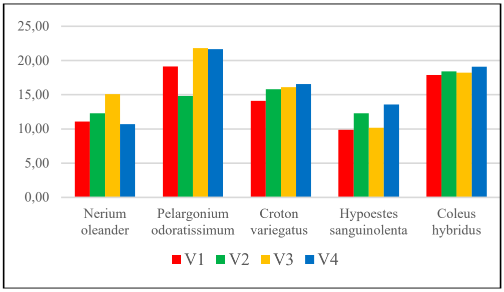

Figure 3. The length of shoots at rooted cuttings on different substrates (cm)

The length of the shoot at *C. variegatus* recorded close values, the highest - 16.56 cm in V_4, the smallest - 14.10 cm in the V_1. Root and shoot have the same decreasing tendency from V_4, V_3, V_2 to V_1.

In the case of the species *H. sanguinolenta* the length of the shoot from V_4 had the highest value - 13.58 cm, then 12.30 cm in V_2, 10.19 cm in V_3 and 9.88 cm in the V_1. All the indicators followed had the lowest values in the cuttings rooted in the V_1, average values in the case of cuttings rooted in V_2 and V_3, and the V_4cuttings gave the best results.
In the species *C. hybridus*, shoots of an average length of 19.10 cm were observed in the case of $V_4$and 17.90 cm in the case of cuttings rooted in V_1.
The species *N. oleander* recorded the following values of the length of the shoot, as can be seen from the graph: maximum 15.10 cm in V_3; minim 10.70 cm in V_4, intermediate values of 12.30 cm in V_2 and 11.10 cm in V_1. *P. odoratissimum* had the longest shoots among the species studied, the size of the shoots being between 21.82 cm in V_3 and 14.84 cm in V_2. However, the experimental results show that cutting is a good method for the multiplication of *P. odoratissimum* plants, inciting some contrary opinions in the literature (Ebrahimzadeh et al., 2021).
By applying the IRP coefficient, it was aimed at identifying the response of each studied species to the substrates used for rooting (Figure 4), resulting in the following:

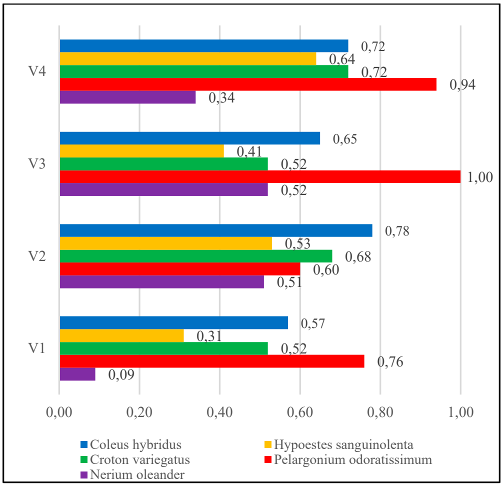

Figure 4. The IRP coefficient on the substrate

River sand (V_1) - the best results were obtained at the rooted cuttings of the species *P. odoratissimum*, at the opposite pole being the rooted cuttings of *N. Oleander* (which confirm the results obtained by Ochoa et al., 2003).
Perlite(V_2) - the best behaviour was recorded in the case of *C. hybridus* rooted cuttings and the worst result was obtained in *H. sanguinolenta*.
Peat (V_3): the greatest influence it had in the cuttings of *P. odoratissimum*, and the lowest on the cuttings of the species *H. sanguinolenta*.
The mixture of peat + perlite (V_4) was the most favourable for the cuttings of *P. odoratissimum* and the least favourable for the cuttings of *N. oleander*.
Subsequently, the averages of the IRP indicators for each species were calculated and the averages were compared with the help of ONEWAY ANOVA, obtaining significant differences (Figure 5).
Following the data processing with the help of ONEWAY ANOVA, it can be seen that, in the case of the substrates studied, the best results were obtained when rooting the *P. odoratissimum* cuttings, and the worst results were recorded in the case of *N. oleander* cuttings, which may lead to the idea that for this species other types of rooting substrates should be tested.

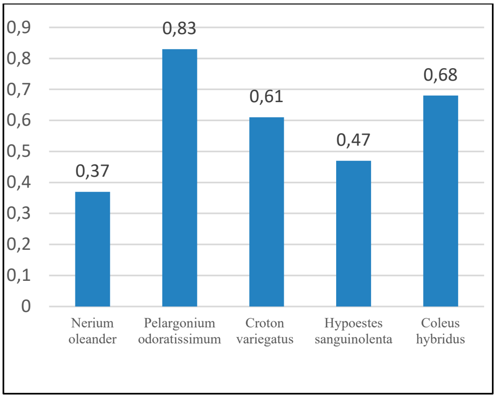

Figure 5. The mean of the IRP coefficient

CONCLUSIONS

The use of the IRP coefficient allowed the customized identification of the efficiency of the rooting substrates for each species. Thus, at *N. oleander* - the best results were obtained on V_3 (but almost the same in V_2) and the weakest on the V_1; at *P. odoratissimum* - the best behaviour was in V_3 and the weakest in V_2; at

C. variegatus - it was best for V_4 and the weakest for V_1 and V_3; at *H. sanguinolenta* - the best result was in V_4 and the weakest in V_1; at *C. hybridus* - the maximum was recorded at the V_2 and the minimum at the V_1.

Such coefficients, of the type used in this paper (IRP), which quantify, from a mathematical point of view, the evolution of some biometric parameters, in this case, those that characterize the process of rooting of cuttings, can be useful tools, within the reach of researchers, to highlight the interaction between the elements that intervene in this type of processes.

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BIBLICAL GARDENS AT CHEIA MONASTERY DOMAINS

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Abstract

The paper deals with the research and valorisation of the relationship between sacred spaces and forest landscapes through the insertion of biblically-themed gardens into a 20 ha monastic domain, surrounded by the forests of Cheia mountain resort, located in central Romania. The site is crossed by a natural water course with two ponds and comprises a series of mixed tree groups and groves, increasing the landscape diversity of Cheia Orthodox Monastery area. The first stage of the project consisted in a complex research of the site from a cultural, historical, functional, ecological and visual point of view. Following the site assessment, a landscape redevelopment strategy has been proposed through minimal interventions. It is intended to introduce biblical thematic gardens focused on the conservation of the dendrological vegetation, on the valorisation of the local ambiances through visual and functional integration of the site in the context of the mountain forest landscape and Cheia resort. The proposed vegetation presents a distinct symbolic value in each thematic area that confers a particular visual identity and a specific spiritual role. The conclusions reveal the high potential of the site from visual, environmental and touristic point of view. The opportunity to increase the attractiveness of the monastic domain is highlighted by inserting multiple uses, such as educational, religious, cultural, recreational etc.

Key words*: Bibllical gardens, Cheia Monastery, Landscape design, Landscape analysis.*

INTRODUCTION

The site is located in a natural setting with a high landscape value and presents a tourism potential which is currently insufficiently exploited. At present, it is necessary to create a suitable area for the spiritual preparation of the monks and pilgrims, since the monastery domain is largely unused. Its integration in the context of the Cheia resort and the tourist circuits in the area by promoting the spiritual identity can significantly contribute to increasing the tourist attraction on the local level (Bândiu, 2009). Given the increasing acceleration of traffic problems and the anthropic pressure on the Prahova Valley, the entire tourist area has the potential to develop in the next decade as an alternative for tourists from Bucharest and beyond.

Măneciu commune is located at a distance of 47 km from Ploiești and 18 km from Vălenii de Munte, being the last commune of Prahova county from the border with Brașov county. Cheia resort, part of Măneciu commune is located on DN1A road at the border of Brașov and Prahova counties, at an average altitude of 870 m. The annual average temperature is 6^0C and the annual precipitation regime is 750-800 mm. The studied site occupies about 20 ha - an important area of the territory of the locality. The village of Cheia, was formed gradually from the second half of the nineteenth century. (Ivan, 2010; Niculescu, 1977)

MATERIALS AND METHODS

The study was based on several applied research methods - used to perform field analyses on the Cheia Monastery Domains, Măneciu commune, Cheia village, Prahova county. The following research methods were used for the analyses: - Field visits - for analysis of vegetation, environments, functions, movements; - Field photos - for all analyses; - Site observations - for all analyses; - Consultation of bibliographic resources - for researching the physical environment, analysing functions, analysing traffic, researching tourist resources; - Consultation of websites - for physical environment research, traffic analysis, tourist resources analysis - Monks' queries - for all analyses;

Following the applied research, the information collected for each analysis was processed using a SWOT analysis. Subsequently, the synthesis of the analyses and a diagnosis with the main dysfunctions and strengths of the site was prepared, based on which the vision, mission and strategy were developed (Holden and Liversedge, 2014; LaGro, 2007; McHarg, 1967).

RESULTS AND DISCUSSIONS

Circulation. There are two accesses to the monastery courtyard, on DN1A (made of gravel) and an asphalt road inside the Cheia resort, formerly marked by a double alignment of *Picea abies*. In the courtyard of the monastery the composition of the alleys is geometric. The outside domain of the monastery court is restricted, fenced and access can only be done with the permission of the monks. Currently, there is no perimeter alley that ensures accessibility throughout the area. The main access path is an alley that crosses the area. The watercourse is a barrier to accessibility on the site from several points, with only one concrete bridge in the middle of the site.

Land use. In the domain there are spaces with different functions. The monastery we can see today, dating from 1835 and kept in good condition, is surrounded by rectangular shaped cells. In the immediate vicinity of the monastic complex there are areas where the monastery supports itself by animal farming, greenhouses with vegetables, orchard and two pools with trout.

Vegetation and landscape value. Following the analysis of vegetation, two major categories of vegetation were identified, spontaneous and planted. The spontaneous vegetation occupies the largest area of the site and includes areas with tree and shrub vegetation, lawn that dominates the site with herbaceous plants and marsh and river vegetation near the watercourse and lakes. The planted vegetation is present especially near the monastery.

Depending on the water surface, vegetation and relief, several types of landscape have been identified. The zones were named according to the volume, textures, colors and openness. Each area was evaluated with grades from 1 to 5 depending on the physical values, among which - relief, water and vegetation; perceptual values - depth, amplitude, volumetric aspects, accents, color, textures and balance value, depending on which a global value has been established (Table 1). The analysis was designed based on combining landscape assessment methods proposed by landscape architects Ian McHarg, James LaGro and Florin Teodosiu (Boc, 2012).

Also, for each area the following criteria were evaluated: the ratio between spontaneous and planted vegetation, the share of tree, shrub, herbaceous, dominant species, invasive species and those in a degraded state (Table 2).

Zone 1 has been called the *Water Flow*, and the state it transmits is "Between static and dynamic". It includes a stream and an artificial lake. In Zone 1, the spontaneous vegetation, the swamp vegetation (*Typha angustifolia*) and the river vegetation predominate, where *Salix caprea* has an invading character.

Zone 2 was called the *Obscure Forest*, characterized by a high diversity of tree species. It comprises vegetation predominantly planted with different species of deciduous and resinous trees (*Abies* sp., *Picea abies, Pinus silvestris, Fagus* sp., *Acer pseoudoplatanus, Larix* sp.). The vegetation is characterized by a diversified texture, chromaticity and volumetry.

Zone 3 is called the *Tree of Life* and expresses the idea of centrality. It is a meadow area, where the dominant element is a *Fagus sylvatica* tree.

Zone 4 called the *Hidden Mirror* arouses the curiosity of discovery. It is an area with natural lake, being the largest water mirror on the surface of the domain, surrounded by spontaneous vegetation with an invasive character, predominantly deciduous (*Fagus* sp., *Betula* sp., *Salix caprea, Rosa canina*).

Zone 5 is called the *Test Hill*, which awakens states of uncertainty. It is the highest point on the site, it contains a *Fagus sylvatica* grove, and offers wide perspectives to the entire surface of the domain.

Zone 6 is called the *Illuminated Forest* and creates a feeling of tranquility. This area is discovered in a deciduous forest, predominantly composed of *Fagus sylvatica*, located at the edge of the site.

Table 1. Landscape value assessment

LANDSCAPE VALUES	ZONE 1	ZONE 2	ZONE 3	ZONE 4	ZONE 5	ZONE 6	ZONE 7	ZONE 8	ZONE 9	ZONE 10	ZONE 11
PHYSICAL VALUES	3,25	3,33	2,66	4,33	4,5	2,66	2,33	2,5	1,33	3,66	3
topography	3	2	2	4	4	1	1	1	1	2	1
water	5	3	2	5	-	2	-	3	-	-	3
vegetation	3	5	4	4	5	5	2	2	1	4	5
built elements	2	-	-	-	-	-	4	2	2	5	-
PERCEPTUAL VALUES	3,83	3,66	3,5	4	3,5	3	3,16	1,33	1,16	4	2,83
landscape depth	4	2	5	3	3	2	5	2	1	3	4
amplitude	4	2	5	4	3	2	5	2	2	3	1
volumetry	3	5	2	5	5	5	1	1	1	3	5
accents	4	5	4	4	4	2	4	1	1	5	3
colors	4	4	3	4	3	3	2	1	1	5	2
textures	4	4	2	4	3	4	2	1	1	5	2
BALANCE VALUE											
unity vs diversity	4	5	3	5	4	4	3	2	1	5	4
GLOBAL VALUES	3,69	4	3,05	4,4	4,16	3,22	2,83	1,94	1,16	4,22	3,27

LEGEND	
Very low value	1
Low value	2
Average value	3
High value	4
Very high value	5

Albei Boc, Vladimir, 2012. *Strategii de branding pentru valorificarea peisajului în mun. Brăila**

*Analysis based on the association of proposed methodol by McHarg, James LaGro and Florin Teodosiu

ZONA 4

ZONE 2

ZONE 5

ZONE 1

ZONE10

Table 2. Vegetation assessment

VEGETATION TYPES	ZONE 1	ZONE 2	ZONE 3	ZONE 4	ZONE 5	ZONE 6	ZONE 7	ZONE 8	ZONE 9	ZONE 10	ZONE 11	TOTAL
1 SPONTANEOUS VEGET.	80-100%	20-40%	80-100%	80-100%	80-100%	80-100%	80-100%	40-60%	40-60%	40-60%	<20%	60-80%
1.1 Arborescent	<20%	20-40%	<20%	80-100%	80-100%	80-100%	<20%	<20%	<20%	<20%	<20%	20-40%
Abies alba	-	-	-	<20%	-	-	-	-	-	-	-	<20%
Picea abies	-	-	-	<20%	-	-	-	-	-	-	-	<20%
Alnus glutinosa	-	-	-	20-40%	-	-	<20%	-	-	-	-	<20%
Fagus silvatica	<20%	40-60%*	<20%*	40-60%**	80-100%	80-100%	<20%	-	-	-	-	20-40%
Betula pendula	<20%	-	-	20-40%	-	-	-	-	-	-	-	<20%
Salix caprea	20-40%*	20-40%*	-	40-60%**	-	-	-	-	-	-	-	<20%
1.2 Shrubs	<20%	<20%	<20%	20-40%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%
Cornus sanguinea	-	-	-	20-40%	-	-	-	-	-	-	-	<20%
Rosa canina	<20%	<20%	-	20-40%	<20%	-	-	-	-	-	-	<20%
1.3 Herbaceous	20-40%	<20%	80-100%	20-40%	20-40%	20-40%	80-100%	<20%	<20%	20-40%	<20%	60-80%
Hypochoeris radicata	<20%	-	-	<20%	<20%	<20%	20-40%	<20%	<20%	40-60%	20-40%	<20%
Trifolium sp.	<20%	20-40%	-	<20%	-	<20%	20-40%	<20%	<20%	60-80%	40-60%	20-40%
Astrantia major	-	20-40%	-	-	-	-	-	-	-	-	-	<20%
Centaurea phrygia	-	-	-	20-40%	-	-	20-40%	-	-	-	-	<20%
Holcus lanatus	20-40%	-	40-60%	<20%	<20%	<20%	40-60%	-	-	<20%	-	20-40%
1.4 Palustrine	40-60%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%
Typha angustifolia	40-60%	-	-	-	-	-	-	-	-	-	-	<20%
2 PLANTED VEGETATION	<20%	60-80%	<20%	<20%	<20%	<20%	<20%	40-60%	40-60%	40-60%	80-100%	20-40%
2.1 Arborescent	<20%	60-80%	<20%	<20%	<20%	<20%	<20%	40-60%	40-60%	40-60%	80-100%	20-40%
Abies concolor	-	-	-	-	-	-	-	-	-	-	<20%	<20%
Picea abies	-	40-60%	-	-	-	-	-	40-60%	20-40%	20-40%	80-100%	<20%
Pinus silvestris	-	<20%	-	-	-	-	-	60-80%	<20%	-	-	<20%
Larix deciduas	-	40-60%	-	-	-	-	-	-	-	-	-	<20%
Thuja occidentalis	-	-	-	-	-	-	-	-	-	60-80%	-	<20%
Tilia tomentosa	-	-	-	-	-	-	-	-	-	<20%	<20%	<20%
Acer pseudoplatanus	-	<20%	<20%	<20%	-	-	-	-	-	-	-	<20%
Fruit trees	40-60%	-	-	-	-	-	-	20-40%	<20%	-	-	<20%
2.2 Shrubs	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	40-60%	<20%	<20%
Juniperus sabina	-	-	-	-	-	-	-	-	-	20-40%	-	<20%
Berberis thunbergi	-	-	-	-	-	-	-	-	-	20-40%	-	<20%
Syringa vulgaris	-	-	-	-	-	-	-	-	-	<20%	-	<20%
Chaenomeles sp	-	-	-	-	-	-	-	-	-	<20%	-	<20%
2.3 Herbaceous	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	20-40%	<20%	<20%
Hosta sp	-	-	-	-	-	-	-	-	-	20-40%	-	<20%

LEGEND	
Very low coverage	<20%
Low coverage	20-40%
Average coverage	40-60%
High coverage	60-80%
Very high coverage	80-100%

*Invasive species

*Vegetation in poor condition

Zone 8 and 9 are production areas and arouse a sense of chaos. Here, there are fish, vegetables, fruit trees and animal husbandry activities.

Zone 10 is called the *Spiritual Court* and transmits a state of peace, otherwise specific to a holy place. It comprises the monastery

complex and the inner courtyard which contains a planted vegetation composed of deciduous and coniferous shrubs and perennial species.
Zone 11 is called the *Forest Tunnel*, which puts you in the position to choose between two directions. It represents the main entrance, marked by a double alignment of *Picea abies* in a precarious state.
The zones with the highest landscape value are those located near the watercourse (zones 1 and 4), the areas with rich tree vegetation (zones 2 and 5) and the courtyard of the Cheia monastery.

Synthesis. The presence of a natural environment with insufficiently enhanced landscape diversity resulted. From the point of view of the neighborhoods, it was noticed a picturesque natural framework, several vantage points to the monastery complex and the mountain massifs. On the ecological level, the vegetation on the site is in good condition and is integrated into the local natural landscape. Functionally, several areas within the site are difficult to access and there is a physical barrier between the site and the resort. The production zones are unsightly (Figure 1).

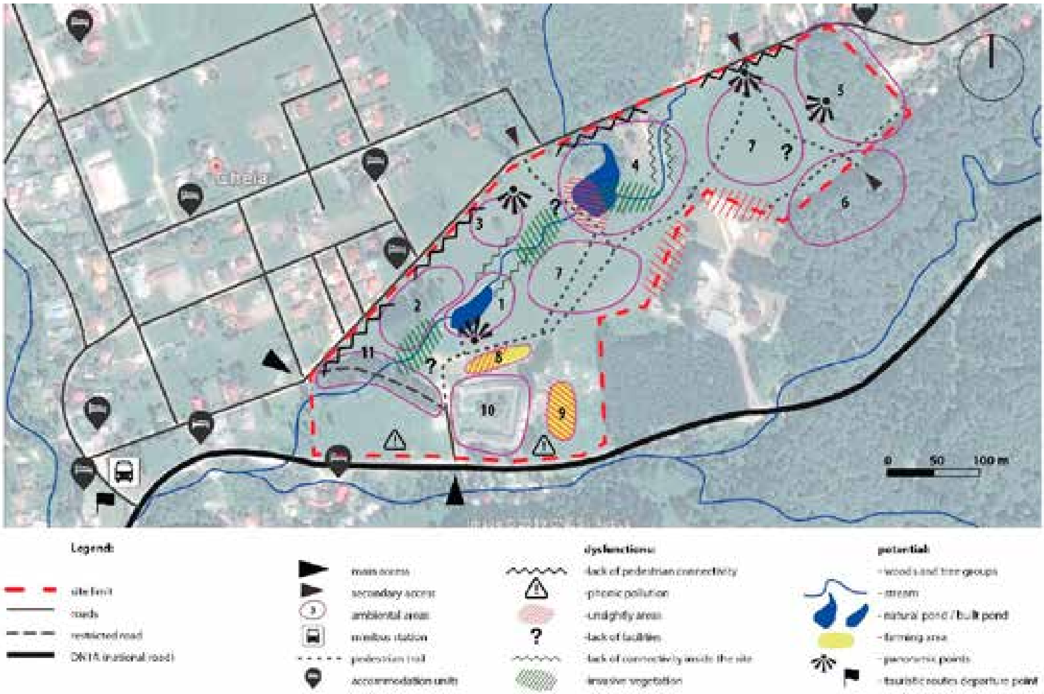

Figure 1. Synthesis plan

Strategy. Based on the diagnosis, the proposed vision targets the landscape enhancement and functional integration of the site as an element of tourist attraction of the Cheia resort (Figure 2).
The mission includes the following objectives:
- context: connecting the site with the resort and enhancing the natural environment;
- ecological: the conservation of valuable vegetation in zones 2, 5, 6, 11 and its completion with vegetal compositions in zones 1, 3, 4, 7 that integrate in the local context;
- functional: creating a pedestrian traffic network that interconnects existing and proposed access points and points of interest; introducing functions and facilities that enhance the specificity of the place;
- aesthetic: enhancing the types of landscapes by creating a concept that highlights the connection between the natural environment and the sacred space (Bândiu, 2011);

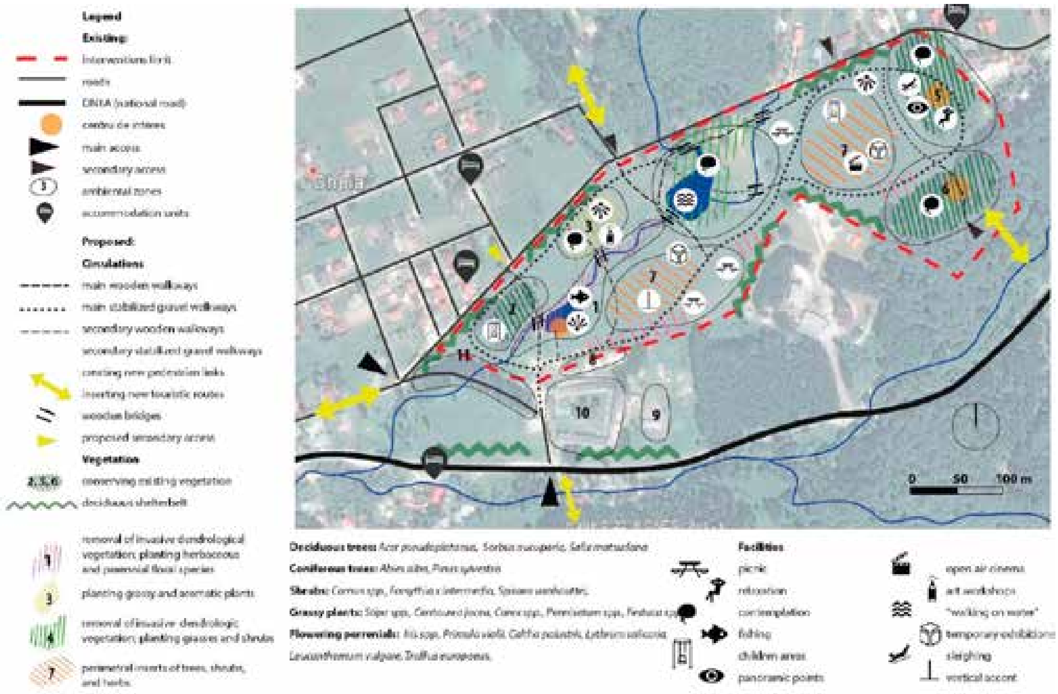

Figure 2. Strategy plan

Concept. Starting from the landscape zones identified on the site, the concept involves the creation of thematic areas through minimal interventions. Thus, seven bible-themed gardens are proposed, each marking an important event in the Old and New Testaments (Bândiu, 2011; Douglas, 1995). The proposed concept responds both to the needs of the monks community and to the need to promote the locality of Cheia, as a tourist resort of national interest, considering that there are very few parks and gardens with a biblical theme in the world.

The landscape design solution for the Domains of the Cheia Monastery proposes the creation of a free style composition, through which the proposed points of interest are interconnected and linked to the six access points to the site.

The vegetal composition preserves the existing structure, along with a series of minimum and medium insertions of groups of trees and shrubs, but also of sub-shrub and herbaceous vegetation. It is proposed to introduce new specimens from the species existing on the site, but also a selection of new species suitable with the local climate (Iliescu, 2008). Completing the existing species with the proposed ones offers a rich seasonal variation throughout the year.

From the point of view of the proposed functions, the space is characterized by a relatively large diversity without having a strong visual impact. Functions and facilities are proposed with the role of relaxation, walking, contemplation, artistic creation, playgrounds, exhibition spaces, but also areas with utilitarian role.

CONCLUSIONS

The proposed solution respects the principles of ecological, economic and social sustainable development. At the ecological level, it has a minimal impact on the environment, most of the interventions built being minimal, and the proposed species are integrated into the local ecosystem. From an economic point of view, the investment is designed to involve relatively low costs, made possible by the use of predominantly local materials and plants in small quantities. At the social level, the site is addressed to both monks and pilgrims, as well as to the general public through the proposed functional and environmental diversity.

The implementation of the biblically themed concept can lead to the emergence of a unique type of park in the Orthodox space that can become an important tourist attraction center in the region (Figures 3 and 4)

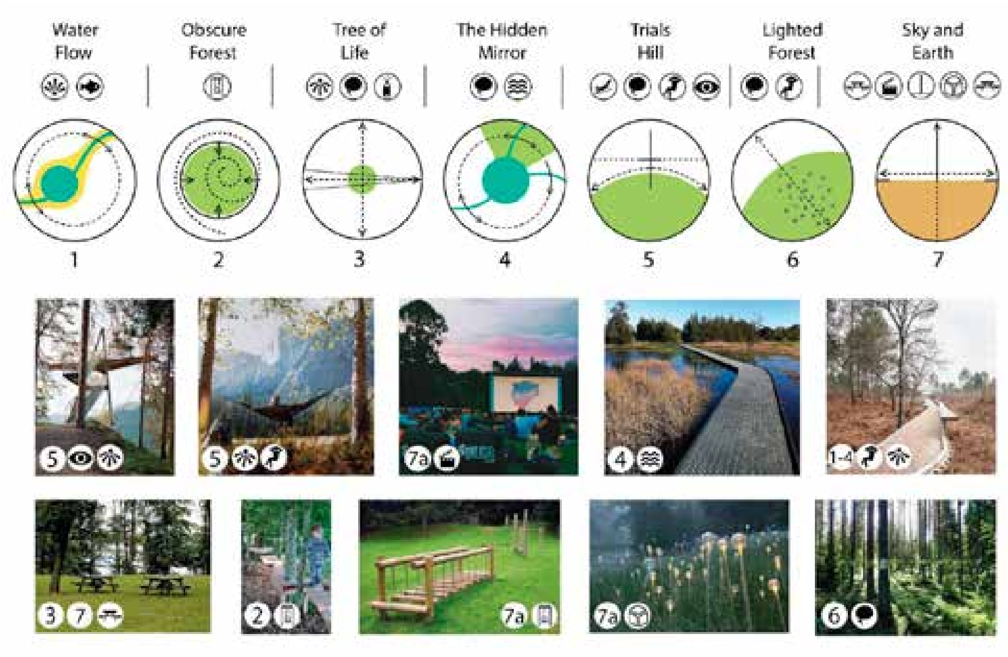

Figure 3. Concept

Figure 4. Landscape design proposal

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USING FRUIT GROWING SPECIES FOR GREEN ROOFS

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Abstract

Green roofs are a way of landscape development that combines aesthetics with ecological functions of these types of facilities. The sustainability of this approach lies in combining economic, aesthetic and especially functional, making them the primary aspects anchored in determining the choice of the most suitable crop systems. Romania is an European country where culture tree species with ornamental value is well represented by a great diversity of species and varieties that are favorable climatic conditions for growth and flowering, while ensuring décor staggered throughout the year. A difference from the landscaping from the ground is that the arrangements of roofs and terraces rarely work with plants decorative through flowers, such as the use of tree species is more than appropriate in this case. Extending the concept of Green Roof has advantages in terms of encouraging environment, educational system and the community life, fostering solidarity population to achieve a framework for proper management of long-term spaces. Implementing the concept of green roofs in public areas can be very successful, both in the short and long term, primarily by reducing pollution and improving the aesthetics of such spaces furnished. Diversification research in this area and finding new techniques for obtaining more efficient and rapid production of a higher quality is one of the current priorities of food safety programs.

Key words: *green roof, sustainability, substrate, strawberry, optimization.*

INTRODUCTION

The issue regarding the arrangement of green roofs is relatively recently addressed both in Romania and internationally. The originality of the study basis refers to the use of fruit species for this type of arrangement.

In recent decades, architects, builders and urban planners around the world have begun to use green roofs not for aesthetic reasons - a rather secondary concern - but for their practicality and ability to mitigate the environmental extremes specific to conventional roofs.

Despite the delays due to the novelty of the theme, many communities have enthusiastically embraced the implementation of these types of roofs in public spaces (Haggas C., 2006).

Following the observations and research, it was concluded that the implementation of the concept of green roofs in public spaces can be a real success, both in the short and long term, primarily by reducing pollution and improving the aesthetics of such spaces.

The promotion of the concept of "green roof", in correlation with the already existing model of "roof garden", wants to promote new production ideas not yet exploited at full capacity so far in our country.

This implies the use of these innovative concepts, thus starting a continuous process of communication, information and counseling, worth mentioning.

With the growth of the world's population, the demand for agricultural products, which constitute the human food base, has increased and will continue to increase. It is known that in agriculture, in general and fruit growing, in particular, the cultivar, through its qualities clearly superior to the species, is the main means of production.

Diversifying research in this area and finding new, more efficient and faster techniques for obtaining a higher quality production is one of the current priorities of food safety programs.

Extending the concept of green roof has advantages in terms of encouraging environment, educational system and the community life, fostering solidarity population to achieve a framework for proper management of long-term spaces. Some of the directions investigated worldwide arising from the paper "*Quantifying the effect of slope on extensive green roof storm water retention*" by Kristin L. published in 2007 in the journal, *Ecological Engineering*, on the benefits of green roofs are:

increased water retention, reducing pollution, low noise, increased thermal insulation, sealing effective protection against ultraviolet rays action.
The presentation of other experiences with similar result, belonging to the urban communities of Vancouver and Winnipeg - Canada, Bahia de Caraquez - Ecuador, Aomori, Osaka - Japan, Nairobi - Kenya, Beijing - China, Dhakka - Bangladesh, illustrated these concerns, proposing specific solutions assumed responsibly, in accordance with the principles of sustainable urban development (Wong, N.H., Cheong, D.K.W., Yan, H., Soh, H., Ong, C.L., Sia, A., 2003; Varras, G., Vozikis, K.-TH., Myriounis, C., Tsirogiannis , I.L., Kitta, E., 2015).

MATERIALS AND METHODS

The present work is an way to make people, communities and general public aware about the advantages of green roofs cultivation in terms of environmental sustainability, development and management.
To exemplify this concept, we chose a 50 sqm roof, located in a new residential complex, on a 4-storey building in the Tatarasi neighborhood, Iasi (Figure 1).

Figure 1. Location of the project design

Exclusive advantages of a green roof: increased rainwater retention, reduction of pollution, dust particles, noise attenuation, increased thermal insulation, effective protection of waterproofing against the action of ultraviolet rays, protection of waterproofing against day/night and summer/winter temperature variations, improving the quality of life, integrating the building into the natural environment, increasing the value of the building and others (Bibbiani, C., Campiotti, A., Giagnacovo, G., Incrocci, L., Pardossi, A., Latini, A., Schettini, E., Vox, G., 2018; Olate, E., Gómez , M.F., Musalem, M., Sepúlveda , C. and Ferrer J.M., 2013).
Secondary advantages of using fruit tree species for green roofs: ensures flavor, freshness and color to site, offers a decorative spring look through flower and color, attracting pollinators and offering delicious and fragrant fruits. The surplus fruit can be used by processing in different forms. In addition, fruit growing practices can be a relaxing activity influencing the well being on all levels (Lille, T., Karp, K., Varnik, R., 2003).
Therefore, when choosing the varieties intended for this type of arrangement, several factors are taken into account, such as: varieties adapted to the climatic conditions of the area in which they are to be planted, resistant to diseases and pests, with a shallow root system and heights as low as possible, which form few and short stolons.
Being a shallow rooted annual crop, strawberry can be fitted anywhere in urban horticulture development with little but frequent irrigation facility.

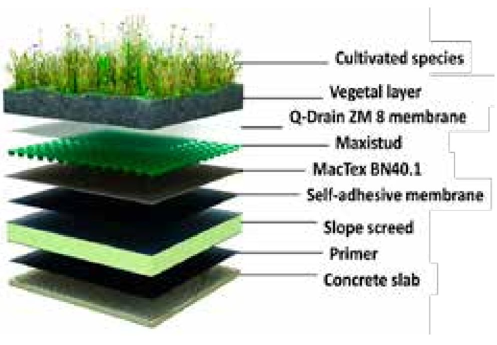

Figure 2. Layers placed in the gutters on the roof

Through its objectives, this project will not only contribute to maintaining the sustainability of the environment by maintaining the ecological balance of the area, but will also use the natural resources of climate and soil without disturbing the ecological balance (Pramanick, K.K., Kishore, D.K., Watpade, S., Sharma, Y.P., 2017).
For container mounting (Pașcu Roxana, Zlati Cristina and Bernardis R., 2017) we have used innovative materials meant to protect both the insulation of the roof they were mounted on and the cultivated plants. For good container insulation, we used a special membrane, called MacTex BN40.1 200gr, and in order to retain rainwater in the containers we used Maxistud**,** which is a membrane with tronconic protuberations of HDPE, of high thickness (20 mm) and exceptional mechanic characteristics, that can retain up to 6l of water on 1 m^2. Over this membrane, for the distribution of the substrate weight and in order to insure good water drainage, we used a Q-Drain ZM 8 membrane (Figures 2 and 3).
As planting material there will be used refrigerated stolons.

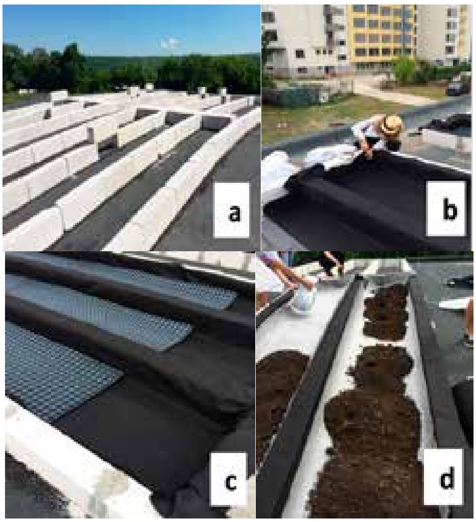

Figure 3. Containers mounting: a-BCA briks, b-MacTex BN40.1, c-Maxistud, d-Q-Drain ZM 8

Strawberry varieties description:
‘**Albion**’ is a Californian variety, high yielding, robust plants grow conical shaped berries of good flavour and excellent firmness, ideal for eating fresh. They produce multiple crops and are usually available throughout the summer months (from May to September).
‘**San Andreas**’ strawberry is a moderate day-neutral with a production pattern very similar to Albion. Plant vigour for ‘San Andreas’ is somewhat higher than for ‘Albion’ early in the season, but berry size throughout the fruiting season is similar to ‘Albion’. The fruit colour for ‘San Andreas’ is slightly lighter than for ‘Albion’, and it has similar post harvest characteristics. The flavour of ‘San Andreas’ is very good and it also shows good disease resistance.

RESULTS AND DISCUSSIONS

The opportunity of the study is achieved by increasing green areas in urban landscape in terms of continuous demographic growth. Landscape architecture, being directly related to ensuring the ecological balance of the environment, has as main objective the preservation and development of landscapes and their associated values for the benefit of current and future generations.

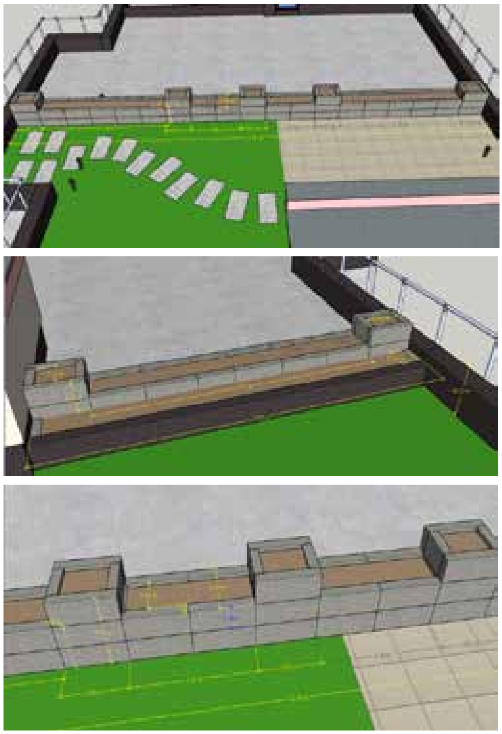
Figure 4. Space systematization for green roof project design

The sustainability of this approach lies in combining economic, aesthetic and functional aspects, especially of such areas, making them the primary aspects anchored in determining the choice of the most suitable crop systems (Figure 4).

In Romania, the ornamental culture (Cojocariu Mirela, Elena Liliana Chelariu, Chiruţă C., 2022) but also fruit trees with ornamental value is well represented by a great diversity of species and varieties that find favorable climatic conditions for growth and prosperity, while ensuring decor spread throughout the year (Torchyk, U.I., 2010). For strawberry, there are developed many culture methods that have dual role: ornamental and economically.

Many communities have embraced enthusiastically implementing these types of roof in public areas (Pradhan, S., Mitra (Sarkar), M., 2017; Rowe, D.B., 2018). Following observations and researches have concluded that implementation of the concept of roof greenery in public spaces can be very successful, both in the short and long term, primarily by reducing pollution and improving the aesthetics of space designed.

Promoting the concept of "green roof" in conjunction with the already existing model of "garden on the roof", aims to promote new ideas untapped production at full capacity so far in our country. This involves the use of these innovative concepts, such as an ongoing process of communication, information and advice noteworthy.

The scientific research addressed in this paper focused on some strawberry varieties, with the purpose of including them on green rooftop culture as a new way of cultivation (Figure 5).

Figure 5. Design proposal for landscaping the green roof garden with emphasize on strawberry

Considering the importance of green spaces, the proposed solution presents an important contribution both to the development of knowledge in the field, including novelty, originality and complexity and integration of strawberry culture in urban design (Zlati Cristina, Pașcu Roxana, 2021).

We have included in this project (Table 1) species that are also suitable for this kind of roof design and an evaluation of potential production that can be obtained. As we can observe in Table 2, the production can cover the necessity of building inhabitants. Also, the production overplus can be sold at a premium price as early or late crop.

Table 1. List of proposed vegetation, species and quantity required

Species used for roof composition	Quantity (pieces)
Abies concolor	1
Acer palmatum ‘Fire Glow’	2
Berberis thumbergii ‘Atropurpurea’	2
Buxus faulkner ‘Ball’	2
Cupressus leylandii ‘Green’	6
Cupressus arizonica ‘Fastigiata’	6
Euonymus japonicus ‘Aureomarginato’	3
Lavandula angustifolia	4
Ligustrum ovalifolium ‘Aureum’	3
Photinia x *fraseri* ‘Red Robin’	15
Rosa ‘The Fairy’	4
Yucca filamentosa ‘Golden’	3
Fragaria grandiflora ‘Albion’	35
Fragaria grandiflora ‘San Andreas’	35

Table 2. Strawberry varieties production estimation

Variety	Production per plant (grams)	Nr. of plants	Total Production (Kg)
‘Albion’	800	35	28
‘San Andreas’	700	35	24.5

With world population growth has increased and will further increase the demand for agricultural products which are the basis of human food. It is known that in agriculture in general and fruit growing, in particular, cultivar, by its superior qualities, is the main means of production (Kuchi, V.S., Kabir, J., 2017). Diversification of the research in this area and finding new techniques for obtaining more efficient and rapid production of a higher quality is one of the current priorities of food safety programs.

The productivity of strawberry varieties could be up to 900 grams per plant in full ripening season.

Detailed images and zoning of the design project could be observed in (Figure 6).

Figure 6. Details of the design proposal

CONCLUSIONS

The present topic is a way to raise the awarness upon the advantages of green roofs for sustainable environmental development and management.

It intertwines concerns about caring for the environment, sustainable urban development and quality of life.

Our study and the literature reviews has been proven that greening plays a very important role in building an environmentally friendly and heathy society.

Green roof as a landscape solution would serve to replace ecological functions lost in the development of the land.

Using fruit tree species for these type of landscapes will bring major benefits to environment in terms of healthy and economic aspects of life.

In the same time, the project results will increase the adoption of sustainable soil management practices in commercial strawberry small farms.

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TRICHODERMA SPP. – MECHANISMS OF ACTION IN THE CONTROL OF STORAGE PATHOGENS - REVIEW

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Abstract

Aim: The purpose of the paper is to revise the multiple methods of biological control of Trichoderma spp. The need to reduce the use of fungicides in phytosanitary control and makes it necessary to develop technologies that allow easy, economical and effective ways to obtain products from endogenous microorganisms with sufficient quality and quantity to their application in the crops areas. In addition to the industrial importance of the genus, certain Trichoderma species have the ability to antagonise plant pathogens. Trichoderma interacts with other microorganisms, but mainly with pathogenic fungi. These interactions include hyperparasitism, competition, and antibiosis. Hyperparasitism is connected with the direct contact of an antagonist with a pathogen and is composed of such stages as: pathogen recognition, attack, gradual penetration of the pathogen cells and death. Contamination of crops with phytopathogenic genera such as Fusarium, Aspergillus, Alternaria, and Penicillium usually results in mycotoxins in the stored crops and are designated as the most devastating species for small grain cereals.

Key words*:* biological control, phytopathogens, *Trichoderma spp.*

INTRODUCTION

Fungi in the genus *Trichoderma* have been known since at least the 1920s for their ability to act as biocontrol agents against plant pathogens. Phytopathogenic microorganisms and insects have been coexisting with plants since the very beginning of agricultural evolution. For about 70 years, *Trichoderma* spp. have been known to be able to attack other fungi, to produce antibiotics that affect other microbes, and to act as biocontrol microbes (Weindling, 1936; Weindling, 1934). Some landmarks along the way include the discoveries that these fungi frequently increase plant growth and productivity (Lindsey, 1967) either in the presence (Chang, 1986) or absence of other microorganisms and that they can induce disease suppression in soils (Chet, 1981). Further, strains differ remarkably in their abilities to colonize roots (to be rhizosphere competent) and the most effective strains will colonize roots and provide benefits for at least the life of annual crops (Harman, 2000). In addition, the complex mechanisms of mycoparasitism, which include directed growth of *Trichoderma* toward target fungi, attachment and coiling of *Trichoderma* on target fungi, and the production of a range of antifungal extracellular enzymes, were elucidated. These fungi were found to produce a wide range of other extracellular enzymes and some of these were implicated in the biological control of plant diseases (Harman, 2006).

Trichoderma spp. can be efficiently used as spores (especially, conidia), which are more tolerant to adverse environmental conditions during product formulation and field use, in contrast to their mycelial and chlamydospore forms as microbial propagules (Amsellem, 1999). Conidia and mycelia can be produced in either solid-state or liquid fermentation. In general, liquid fermentation is more suitable method over solid-state fermentation for large scale production, still special techniques are required for abundant conidia production (Mausam, 2007). *Trichoderma* fungi are well known for their antagonism against several soil-phytopathogens, involving fungi, invertebrates, and bacteria.

RESULTS AND DISCUSSIONS

Biocontrol genes of *Trichoderma*

The genome sequencing of Trichoderma species has provided exclusive data for phylogenetic and bioinformatic analyses toward understanding the roles of these opportunists in agroecosystems and forced the development of systems biological approaches, initiated and enhanced whole genome expression analysis (Singh et al., 2018). Many genomic studies reveal that Trichoderma spp. contains many valuable genes with great variety of expression patterns, which allows these fungi to use as biocontrol agents, plant growth promotional activities and their environmental adaptation (drought, salt, heavy metal tolerance etc.) At present the most important genome sequences of seven species are: *Trichoderma harzianum*, *Trichoderma asperellum*, *Trichoderma reesei*, *Trichoderma virens*, *Trichoderma atroviride*, *Trichoderma longibrachiatum* and *Trichoderma citrinoviride* (Sharma et al., 2011).

Plant-Trichoderma interaction

The release of various compounds into the rhizosphere by plant roots generates a carbon rich environment and also contributes towards establishing a dynamic molecular communication between roots and soil-inhabiting microbes (Bais et al., 2006). One of the main carbohydrates secreted by plant roots is sucrose, which has been detected in high concentrations near root tips (Kraffczyk et al., 1984; Jaeger et al., 1999; Mahmood et al., 2002). Studies have revealed that this secreted disaccharide plays important roles in the uptake of minerals and in the association of plants with microbial communities (Kraffczyk et al., 1984; Jaeger et al., 1999; Mahmood et al., 2002; Baudoin et al., 2003). Plants, generat sucrose that have several fundamental roles involved in the control of various plant developmental processes. Sucrose coordinates the carbohydrate distribution network in the whole plant and is involved in carbohydrate-mediated signalling pathways. The degradation of sucrose inside the plant cells also yields important sources of carbon and energy for microbes during plant–microbe associations (Dennis and Blakeley, 2000; Koch, 2004). Many fungal species express extracellular invertases that influence sink activity in host tissues and are likely to attract greater amounts of carbohydrates to the infection site (Doidy et al., 2012; Vargas and Kenerley, 2012). Similarly to mycorrhizal fungi, *Trichoderma* spp. can also use monosaccharides produced in the plant root; however, a distinctive feature between *Trichoderma* spp. and mycorrhizal fungi is that most *Trichoderma* spp. are also able to use sucrose as a substrate. In contrast to mycorrhizal fungi, these species of *Trichoderma* gained the ability to use the sucrose released into the rhizosphere with no dependence on the plant metabolism (Vargas et al., 2013). In the presence of plant roots or when growing saprophytically in the presence of sucrose, *T. virens* expresses a highly active intracellular invertase (Vargas et al., 2009). Photosynthesis and carbohydrate metabolism establish the basis for the control of plant growth and productivity. Among carbohydrates, sucrose is the main photosynthetic product that is transported from the source to the sink tissues (Dennis and Blakeley, 2000). The basis of the results reported, sucrose may play two important functions in *T. virens:* (i) as a signal molecule to control plant root colonization; and (ii) as a source of carbon and energy in the rhizosphere.

The endophytic *Trichoderma*

The mechanisms by which *Trichoderma* contributes to disease management are divergent and isolate/species dependent (Harman et al., 2004). Antimicrobial activities, such as mycoparasitism, antibiosis and induced resistance, along with factors contributing to plant growth promotion, such as improved photosynthesis efficiency, nutrient uptake, production of antioxidants and solubilization of nutrients, are some of the most studied of these biological attributes (Howell, 2003; Harman et al., 2004; Vinale et al., 2008; Hermosa et al., 2012). For mycoparasitism to occur, Trichoderma must at least be in the proximity of the target organism to take advantage of metabolites released through enzymatic/toxin activities. In the best described interactions, Trichoderma comes in direct contact with the target organism's cell walls. In some interactions, *Trichoderma* coils around its target, forms appresoria-like structures and directly penetrates (Rocha-Ramirez et al., 2002; Howell, 2003; Harman et al., 2004). The ability of *Trichoderma* to parasitize other hyphae-forming microbes can be both *Trichoderma* specific and target microbe specific (Bailey et al., 2006). During penetration, *Trichoderma* produces many different hydrolytic enzymes capable of degrading the targeted hyphae cell wall. Production of these enzymes can be a direct response to signals coming from the target hyphae (Vinale et al., 2008; Druzhinina et al., 2011). *Trichoderma* species produce a vast array of secondary metabolites, many of which are selectively toxic against microbes rather than to plants, resulting in the process that is called antibiosis (Howell, 1998). Notable examples include gliotoxin and its related metabolites. Gliotoxin has limited toxicity to plants compared with its inhibitory activity against microbes such as *Phytophthora cactorum* (Smith et al., 1990), *Botrytis cinerea* (Di Pietro et al., 1993), *Rhizontonia solani* (Howell et al.,1993) and *Pythium* spp. (Chet et al., 1997). In comparison, viridiol shows poor selectivity, having significant toxicity towards plants (Jones et al., 1988). It is here that endophytic associations, at least at some level, have been implicated in contributing to plant disease and stress management. The best characterized systems indicate that when *Trichoderma* colonizes plant root systems, it penetrates the roots, going a few cells in, where it induces both local and systemic defence reactions that limit further colonization (Harman et al., 2004). Root colonization, which as described includes penetration, may be essential for induced resistance and plant growth promotion (Hermosa et al., 2012). In terms of protection in the field, induced resistance provides protection against disease that is spatially and temporally separated from the application of *Trichoderma* (Harman et al., 2004). *Trichoderma* has been shown to promote growth in a range of crop plants through increased root development, increased secondary root formation (Samolski et al., 2012) and increased shoot size (Hermosa et al., 2012). *T. harzianum* T22 increased maize root growth for the entire cropping season while increasing efficiency of nitrogen use and elemental uptake (Harman et al., 2004). An increase in micronutrient concentrations was implicated in the increased growth of cucumber colonized with *T. harzianum* (later on identified as *T. asperellum* and subsequently as *Trichoderma asperelloides*) T-203 (Yedidia et al., 2001). As mentioned above, root colonization includes the formation of penetration structures (Viterbo and Chet, 2006), expression of enzymes (Yedidia et al., 1999, 2000; Viterbo et al., 2004; Brotman et al., 2008; Moran-Diez et al., 2009), and penetration between cells to a depth of a few cells into the root cortex where plant recognition and defence in the form of enzymes and callose deposition stop growth of the fungus (Yedidia et al., 1999; Harman et al., 2004).

Promotion of plant growth and induction of systemic defence

Plant growth and development are greatly affected by environmental stresses such as drought, salinity, nutrient deficiency, and adverse temperatures. Pathogens can also have a severe impact on plant health, decreasing agricultural production. For the past 50 years, the major challenge of providing sufficient food for the increasing human population has been facilitated by the application of high inputs of chemical fertilizers containing nitrogen (N), phosphorus (P) and potassium (K), which, together with advances in crop and agricultural techniques focusing on shoot biomass and seed yield, has resulted in increasing productivity (Gonzaxszlez et al., 2009; Xing and Zhang, 2010). Current production methods based on high amounts of nutrients are not only costly but also lead to several environmental and health problems (Conway and Pretty, 1988). Additionally, in crops such as wheat and maize, intensive arable cultivation is no longer sustainable because it often leads to soil degradation. Several fungi are known to proliferate in the rhizosphere, the part of the soil that receives the influence of plant roots, or even penetrate plant tissues without causing disease. These include endo- and ecto-mycorrhizas, binucleate *Rhizoctonia spp., Piriformospora indica* and *Trichoderma spp.* (Waller et al., 2005; Shoresh et al., 2010; Harman et al., 2011). Some of these organisms were initially appreciated because of their biocontrol properties antagonizing root pathogens and protecting plants from diseases, but recent studies have demonstrated that they may possess additional attributes for application in agriculture. Many complex interactions between plants

and microorganisms occur at the rhizosphere, the soil zone in close contact with roots. The root system performs the essential activities of providing water, nutrients and physical support to the plant (Contreras-Cornejo et al., 2013). The primary root originates in the embryo and produces many lateral roots during vegetative growth, and each of these will produce more lateral roots. A further adaptation to take in water and nutrients is performed by root hairs. These are long tubular-shaped outgrowths from root epidermal cells. Root hairs play an important role in the uptake of sparingly soluble nutrients that have low diffusion in the soil, such as phosphate. Because they have a small radius, root hairs explore a larger volume of soil per unit of surface area than thicker lateral roots. Root hairs also play a role in modulating the properties and composition of the rhizosphere because they exude high quantities of organic compounds, including organic acids, aminoacids, sugars, proteins, mucilage, phenolics and secondary metabolites (Contreras-Cornejo et al., 2011). Root exudates perform diverse functions in the rhizosphere including mineral weathering, mobilization of nutrients, metal detoxification and growth inhibition of pathogenic bacteria, invertebrate herbivores, or neighbouring plants (Badri and Vivanco, 2009). Some compounds such as organic acids can act as chemotactic signals to attract symbiotic fungi and bacteria (Rudrappa et al., 2008), whereas sugar plays a fundamental role in interactions with mycorrhizal fungi and *Trichoderma* (Vargas et al., 2009, 2011). Microorganisms and plants emit signalling molecules for communication. Plants are able to recognize microbe-derived compounds and adjust their defence and growth responses according to the type of microorganism encountered. This molecular dialogue will determine the final outcome of the relationship, ranging from pathogenesis to symbiosis, usually through highly coordinated cellular processes (Ortiz-Castro et al., 2009). Plant-growth-promoting rhizobacteria (PGPR) are natural rhizosphere inhabiting bacteria that belong to diverse genera such as *Pseudomonas* and *Bacillus* species (Soleimani et al., 2005). The general effect of PGPR is an increased growth and productivity of plants. Their contribution can be exerted through different mechanisms including modulation of root system architecture and increased biomass production through the release of phytohormones such as auxins or cytokinins (Lugtenberg et al., 2002; Lopez-Bucio et al., 2007; Ortiz-Castro et al., 2009). Besides, several fungi such as mycorrhizas, *Trichoderma spp.* can interact with plants in many beneficial ways, some of which resemble those of PGPR. *Trichoderma spp.* are free-living fungi that are common in soil and root ecosystems. They have been widely studied for their capacity to produce antibiotics, parasitize other fungi and compete with deleterious plant microorganisms (Harman et al., 2004). Until recently, these traits were considered to be the basis for how *Trichoderma* exert beneficial effects on plant growth and development. It is clear, however, that certain strains also have substantial direct influence on plant development and crop productivity (Harman, 2006, 2011). It was reported that cucumber seedlings grown in soil amended with *Trichoderma harzianum* propagules sustain a 30% increase in seedling emergence 8 days after sowing. Three weeks later, these plants exhibited a 95% and 75% increase in root area and cumulative root length, respectively, and substantial increases in dry weight (80%), shoot length (45%) and leaf area (80%) were registered (Yedidia et al., 2001).

The use of high quantities of chemical fertilizers in agriculture causes pollution of soils and water bodies. Thus, a major goal of biotechnology is to develop novel strategies to optimize fertilizer use. With this aim, Molla et al. (2012) tested the ability of *Trichoderma spp.* to increase growth of tomato plants when supplied together with fertilizer. It was found that supplementation of fertilizer with Trichoderma enhanced plant production by 50% compared with a standard dose of NPK macronutrients, minimizing the use of fertilizers and their potential negative effects in the environment. A recent application in the field came from manipulation by genetic means of the *T. harzianum,* which encodes a cysteine-rich cell-wall protein (Samolski et al., 2012). It is generally believed that plants activate defence responses upon pathogen or insect attack. This means that plants save energy under enemy-free conditions and could invest photosynthetically fixed carbon in growth and reproduction. Interestingly, some types of soil can suppress the symptoms of plant diseases. Research has shown that the observed increased resistance in these plants is the result of the presence of rhizosphere microorganisms, including bacterial and fungal species, which exert their protective effect by directly inhibiting the growth of pathogens or by means of the activation of a part of the plant's immune system (Pieterse et al., 2009).

***Trichoderma*: mechanisms for controlling pathogens**

Trichoderma spp. are the most widely studied mycoparasitic fungi. However, their mycoparasitism is difficult to demonstrate *in situ* until very recently due to technical difficulties in making *in situ* microscopic observations (*e.g.*, fluorescence imaging and differential staining), such as at the soil–root interface. Moreover, techniques involving antibodies, such as combined baiting-ELISA (enzyme linked immunosorbant assay) techniques to detect *Trichoderma* spp. in composts, would certainly increase our understanding of the mycoparasitic interaction of these fungi (Thornton, 2002).

The biocontrol mechanisms attributed to *Trichoderma* spp. are: competition for nutrients, parasitism, antibiosis, secretion of enzymes, and the production of inhibitor compounds (Guédez et al., 2009; Zimand et al., 1996; González-Estrada et al., 2019). This biocontrol agent attacks and penetrates fungal cells, causing an alteration with the consequent degradation of the cell wall, causing retraction of the plasma membrane and disorganization of the cytoplasm (Tronsmo et al., 1977). These mechanisms are favored by the ability of *Trichoderma* to colonize the rhizosphere of plants. Competition is defined as the unequal behavior of two or more organisms before the same requirement (substrate, nutrients), if the use of this substrate by one of the organisms reduces the amount or space available to others. This type of antago- nism is favored by the characteristics of the biological control agent as ecological plasticity, growth rate primarily as chlamydospores (Hjeljord et al., 1998) speed of development, and external factors such as soil type, pH, temperature, and humidity (Ahmad, 1987). Nutrient competition can occur for nitrogen nonstructural carbohydrates (sugars and polysaccharides such as starch, cellulose, chitin, laminarin, and pectin, among others) and microelements (González-Estrada et al., 2019). Mycoparasitism is defined as an antagonistic symbiosis between organisms, generally involving extracellular enzymes such as chitinases, cellulases, and which correspond to the composition and structure of the cell walls of parasitized fungi (Lorito et al., 1990). Degradation of the cell walls of the host is observed in the late stages of the parasitic process (Carsolio et al., 1999), which leads to almost total phytopathogen weakening. *Trichoderma* can excrete metabolites like cellulases, glucanases, lipases, prote- ases, and chitinases in order to facilitate the insertion of hyphae for nutrient uptake of the pathogen, ending with the loss of cytoplasmic contents of the host cell (Demain et al., 2008). Antibiosis is the inhibition of pathogen development by metabolized products and small toxic molecules, volatile and lytic enzymes, which operate structural polymers, such as chitin and β-1-3-glucans of the cell wall in most pathogenic fungi, producing an adverse effect on development and differentiation (Goldman at al., 1994). Given the above, it is said that the greater the amount of metabolic products, the antagonistic power increases; additionally, some authors mention that this mechanism is not the principle, due to the risk of emergence of the antibiotic-resistant pathogens (Goldman at al., 1994). The production of enzymes such as chitinase and/or glucanases produced by the fungus of *Trichoderma* is involved in the control of pathogenic fungi. These hydrolytic enzymes can degrade the cell wall polysaccharides (chitin and β glucans) affecting its stability and integrity (Howell et al., 2003; González-Estrada et al., 2019).

For example, *Trichoderma* has been applied as postharvest biocontrol agent in different crops such as strawberries, tomatoes, apples and pears. Existing different species of *Trichoderma* with high antagonistic capacity are *T. asperellum*, *T. viride*, and *T. harzianum.* Several authors have investigated different species of *Trichoderma* with the objective to find the most effective biocontrol agent for each crop and pathogen. At present, *Trichoderma* is produced at industrial level as active component of biological products “biopesticides”; other ingredients that conform the biopesticides are the edible polymers, which can form coating for easy adhesion to the fruit and give to the product protection and stability dur- ing its shelf life. The application of biopesticides is widely used in agriculture and can be applied by immersion or spraying during the industrialization of agricultural products (Marín et al., 2017). The incorporation of *T. harzianum* into edible coatings as biopesticide produced higher inhibition of the pathogens *Botrytis cinerea* and *Penicillium expansum* compared to the application as simple conidial suspension of the antagonist on the fruits (Batta et al., 2015). The same author previously reported the same effect on other fruits such as pears, grapes, apples, strawberries and peaches [Batta et al., 2004, 2007; González-Estrada et al., 2019). Yasmeen et al. (2017), demonstrated that shoot and root length of maize significantly declined with the increase in salinity concentration in the soil. However, seed of maize treated with *Trichoderma harzianum* (has shown substantial increase in plant mM NaCl treatments as compared to those plants that were not treated with *Trichoderma.* Anothert study (Zhang et al., 2019) also showed that *Trichoderma* species played a critical role in host plant metabolic processes in response to NaCl stress. The use of *T. longibrachiatum* TL-6 strain increased proline content in wheat seedlings under NaCl stress, which helped maintain the cell osmo regulation (Rasool et al., 2013) and energy storage (Aggarwal et al., 2012).

Shoresh and Harman observed that tomato plants inoculated with *T. harzianum* expressed genes that encode antioxidant enzymes (Shoresh and Harman, 2008). Mastouri et al. (2010) proved that under conditions of osmotic saline stress or extreme temperatures, tomato seeds treated with *T. harzianum* T22 germinated earlier and more evenly than did non-inoculated seeds. Similarly, soil application of *T. viride* along with farm yield manyre (FYM) and NPK increased the plant growth parameters, grain yield, and biomass of wheat crop (Mahato et al. ,2018). Hajieghrari (2010) found that maize crop treatment with T. *hamatum* T614 isolate increased the leaf area, shoot weight, and fresh roots of seedling compared to the non-inoculated seedling. Similarly, bulb treatment with *T. harzianum* enhanced the plant growth of tuberose and resulted in increased flower production and quality (Nosir, 2016). The application of *Trichoderma* strains TH1 and T4 increased root weight, shoot weight, and dry root weight against Pythium. In addition, lateral root development and nodule formation were also found to be enhanced. Application of *Trichoderma* strains TH1, T4, and T12 significantly increased the soil fungal population and significantly reduced the cell-wall- degrading enzyme activities of *Pythium* (Naseby et al., 2000). A typical interaction of *Tricoderma* with *Fusarium* involves attraction, attachment, coiling, and lysis by hydrolytic enzymes or secondary metabolites (Mukherjee et al., 2012).

Many previous studies have established a beneficial effect of *Trichoderma* on several pathogens that affect this crop, especially the most representative, *Fusarium oxysporum, Phytophthora capsici*, and *Rhizoctonia solani*, so its application is common as part of integrated management programs of these diseases. It was observed that the greatest effect on root length was *T. asperellum* with the lowest concentration of conidia (1.0×10^6 conidia/ml), which draws attention to the issue of doses that are recommended for field applications (Bal and Altintas, 2006).

CONCLUSIONS

Trichoderma species establish complex and dynamic interactions with inhabitants of the rhizosphere, such as plant roots and microbes.

The metabolic activity of the fungal cells mainly depends on the uptake and degradation of different carbohydrate sources. Because of the metabolic complexity of carbon metabolism in fungal cells, intricate regulatory pathways control the carbon flux from the rhizosphere to the fungal cells that is vital for root colonization and the fungal life cycle.

Peptaibols, linear oligopeptides produced by *Trichoderma spp*., inhibit beta-glucan synthase which prevents the pathogen from reconstructing its cell wall. Culture filtrates of a *T. harzianum* isolate changed the colony color of *A. flavus* and had a clear effect on the growth. *T. viride* showed a potent antagonisms of *F. verticillioides* in an in vitro assay which was proven by the suppression of radial extension of the fungus by 46% after 6 days and by 90% after 14 days. Like biocontrol agent is a safer and the cheapest method to control these severe diseases and to facilitate better plant growth, yield, and production. The pathogenic fungi *Fusarium spp*. generally cause wilt nearly in all the crop plants from various groups and cause a severe threat to food demand. Among the various biocontrol agents, *Trichoderma spp*. are considered to be the most beneficial, and they assure increased level of safety with minimum environmental impacts.

Trichoderma spp. are well-known fungi that enhance plant growth by nutrient solubilization in the soil, growth hormones production in plants, and defense against pathogens. Due to these characteristics, *Trichoderma* protect the plants against various biotic as well as abiotic stresses. To defend against pathogenic fungi, various mycotoxins have been reported to be produced in this fungus. In the interaction of *Trichoderma* with *Fusarium* a mechanism involves in a series from attraction to cell lysis via attachment and coiling of hyphae through different hydrolytic enzymes and second- ary metabolites.

Trichoderma-based bioinoculants are increasingly used in agriculture, with several hundred formulations available as registered products worldwide. Several strategies have been applied to identify the genes and signals involved in the interactions of *Trichoderma* with plants. Biological control is applicable and many novel methods are being discovered, mostly based on microbiological research and the application of microorganisms that can suppress fungal growth and detoxify mycotoxins. *Trichoderm*a reduce the damage and suppress the fungal growth, it is common to add antifungal substances during growth in the field or storage.

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IMPROVING AND DIVERSIFYING THE CHICKPEA GERMPLASM COLLECTION FOR SUSTAINABLE USE IN BREEDING AND AGRI FOOD CHAIN

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Abstract

Food legumes are crucial for key agriculture-related challenges, such as agrobiodiversity conservation, sustainable agriculture, food security and human health. Comparing to other grain legumes species, chickpea is considered one of the most nutritious for human consumption, having no antinutritional factors except the raffinose-type oligosaccharides, but proven health benefits. One of the major objectives of breeding programs is development of high-yielding, early-maturing cultivars suitable to short cropping season. The current study was developed in frame of national project in ADER program 2019 – 2022 and had as aim the improvement and diversification of chickpea germplasm collection to ensure the optimal use of genetic resources, as a precondition to increase the crop sustainability. The narrow genetic base of cultivated chickpea is one of the major obstacles to sustaining and improving its productivity and renders the crop vulnerable to new biotic and abiotic stresses. In-situ conservation is based on empirical principles and on-farm conservation activities are marginally developed. Seed collections are assembled and are maintained on accession basis, where each accession usually comprises a mixture (population) with an unknown composition of genotypes. This study monitored neutral and functional diversity both at the genetic and phenotypic levels, which means characterizing it, investigating its interactions with the environment in order to define useful functional variation in given environments, establishing a link between variation of phenotypes and ―biomarkers to predict potentiality of accessions for specific breeding goals. Plant genetic resources comprising landraces, obsolete varieties and crop wild relatives were investigated. Performance has been achieved by applying new approaches for germplasm characterization and evaluation like development of core sets, mini-core sets, reference sets and trait-specific subsets, etc. Better utilization and conservation of these accessions raises substantial challenges due to the genetic changes that can be occurring during seed multiplication.

Key words: *biodiversity, Cicer, nutritional profile, quality traits.*

INTRODUCTION

Legume crops as bean, lentil, lupin, mung bean, chickpea, have suffered from the lack of genomic resources for genetic and genomic analysis – they have literally been ‘orphans’ from the genomics revolution (Varshney et al., 2009).

The success of chickpea improvement program largely depends on the wealth of the genetic resources (Ramanappa et al., 2013). Despite its high morphological variability, genetic variation is low (Udupa et al., 1993).

Knowledge about the amount, kind and magnitude of variability in the germplasm and genetic relationships among breeding materials could be an invaluable tool in crop improvement strategies (Joshi and Dhawan, 1966; Kumar and Arora, 1992).

Yadav et al. (2004) observed a negative correlation between yield and seed size under water-limiting environment (Gowda er al., 2011). Developing large-seeded high-yielding kabuli cultivars is an important breeding objective in most chickpea improvement programs. In chickpea, the large-seeded kabuli types produce a lower yield than the small-seeded kabuli cultivars.

Analyzing the available data, we observed there are large gaps between potential and actual yields of chickpea independent of the location of cultivation. New crop rotation and intercropping schemes are tested and encouraged for implementation as key components of the strategy to deal with the biotic stresses that affect chickpea cultivation. In countries with smallholder production, extension work is needed to inform farmers about modern agronomic practices, particularly to enable them to deal with pests and diseases.

The profitability of chickpea is lower than that of cereals like wheat and barley, because of which cultivators with access to irrigation prefer to grow these latter crops (Rawal et al., 2019). In any case chickpea production, needs lower manpower and external inputs than cereals (Sharasia, 2017). Another challenge faced in chickpea production is considerable post-harvest losses because of poor storage infrastructure (Rawal et al., 2019). Average yields of chickpea are nearly 780 kg ha^{-1}, although farmers can harvest more than 2.500 kg ha^{-1}. The crop potential is nearly 5.000 kg ha^{-1} (Sarmah et al., 2012).

According to the FAOSTAT (2017) chickpea is largely cultivated in worldwide in 55 countries. India is listed as the most important producer with 9 million of tons (mt). Among EU countries, are the major European producers, according to the same sources are: Spain, Italy, Bulgaria and Greece. Europe countries specially Spain, Italy, Portugal, Germany, and France import substantial quantities of chickpea from the global market. The analyses of consumption patterns in Europe indicated a rapid increase.

Apart from technological challenges, there are various economic and policy-level issues that need to be dealt with for further expansion of chickpea production.

In the recent years, there has been clear recognition of the role of food legumes in the transition to a plant-based diet, along with the importance of their wider use in the fight against climate change and to promote food security and human health (Bellucci et al., 2021). There are funds allocated in different programs and projects to support and stimulate the conservation for valuable use of legume species. Massive collections are developed by structures that involves phenotypic, genotypic, and agronomic integration to facilitate the access and the use of resources in breeding and along agri food chain.

This work presents the structure of worldwide chickpea collection and investigation results of several traits collected to be integrated and analyzed as potentially useful resources in breeding works. The final aim is to provide new genetic materials, that will benefit growers, seed industry, able to be largely exploited in different cultivation systems under current pressure of climate change. The result of the study will facilitate the exploit of genetic variation of *Cicer arietinum* for enhanced productivity.

MATERIALS AND METHODS

The study is developed in frame of ADER 2020 national research project that deals with the urgent need to provide climate-resilient cultivars technics and methods addressed competitive production systems.

The structure of the paper includes two parts: the first part presents aspects related current situation of chickpea collections as searched in open access catalogs/ data bases of EURISCO and GENESYS, using as filter *Cicer* genus with emphasis on *artiethinum* species.

The second part presents the preliminary results of field investigation aimed to evaluate and to detect the most suitable chickpea resources in terms phenological characterization and yield performance in climatic conditions of Northeast part of Romania, as presented in Figures 1a-1d.

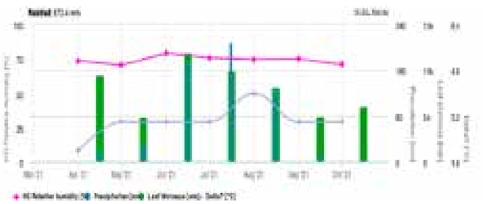

Figure 1a. Relative humidity (%), precipitation (mm), leaf wetness

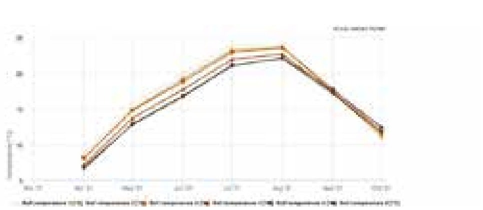

Figure 1b. Soil temperature at different deep levels

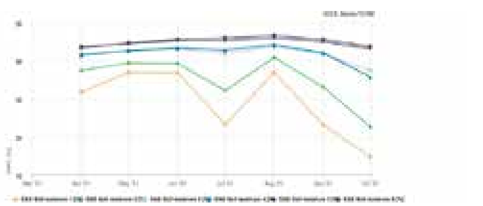

Figure 1c. Soil moisture at different deep levels

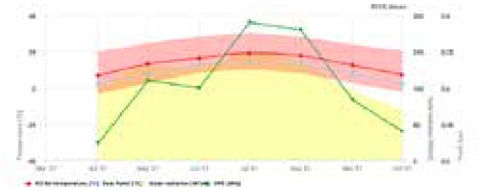

Figure 1d. Air temperature and soil radiation

A collection of eight accessions with different biological status as presented in Figure 2 was established by seed exchanges based on SMTA and collection missions of material from small farms.

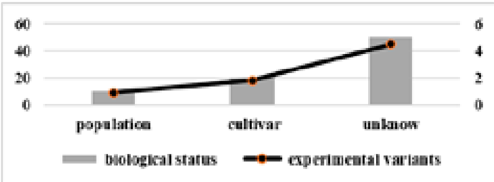

Figure 2. Biological status of investigated material

The heterogeneous biological material studied was collected and maintained as populations During the investigations Single Seed Descent (SSD) lines, meaning seeds derived from a single plant and maintained as pure lines by selfing cycles were developed.

All this material is part reported in EURISCO.

Phenological investigations were conducted by a specific protocol developed using as a reference *Chickpea descriptor* elaborated by Biodiversity International; International Center for Agricultural Research in the Dry Areas (ICARDA); International Crops Research Institute for the Semi-Arid Tropics (ICRISAT); and the Indian Agricultural Research Institute (IARI).

The investigated traits were grouped in three main categories:

- *phenological observations* (sowing date, harvest date, degree of pigmentation and hairiness of the plant, number of days from sowing to early flowering (10%), number of days from sowing to flowering 50%, flowering time 90%, pod ripening)
- *features that impress productivity* (percentage of germinated seeds, number of plants with pods in experimental plot), number of seeds per pod, number of pods per plant, number of seeds per plant, seed mass per plant, appreciation of dehiscence, MMB, resistance and tolerance to pathogen attack)
- *plant architecture* (growth rate of the plant, pod length (mm), plant height (cm), number of leaflets on the leaf, leaf type, canopy height (cm), canopy width (cm), flower color, number of primary basal shoots number of secondary basal shoots number of primary apical shoots).

RESULTS AND DISCUSSIONS

Improving and diversifying the plant germplasm collection for sustainable use in breeding and agri food chain, by creation of Intelligent Collections has been proposed for different legumes species as common bean (Cortinovis et al., 2021), lentil (Guerra-García et al., 2021), and lupin (Kroc et al., 2021) and chickpea (Rocchetti, 2022). The basic principle includes phenotypic characterization, focused on traits considered important for crop adaptation to the different environments (the time to flowering, podding and maturity). Phenology of the crop has an immense influence on productivity and stability. Berger et al. (2006) confirmed the importance of high harvest index (HI) and drought escape in chickpea under natural drought stress through early flowering and maturity. Therefore, most breeding programs aim at developing early-maturing cultivars whose maturity period matches with the available crop duration (Upadhyaya et al., 2010).

According to www.eurisco.ipk-gatersleben.de the European Search Catalogue for Plant Genetic Resources (EURISCO) is a web-based tool that provides information about a significant volume of accessions (more than 2 million accessions of crop plants and their wild relatives), preserved *ex situ.* The net includes about 400 institutes, subject of a network of National Inventories of 43 member countries. The major role of collaborating institutions is supported by a consolidated effort for the preservation of large genetic agrobiological diversity. This online catalog functions based on data automatically received from the National Inventories through country National Focal Points (NFPs). It is in fact a European network of *ex situ* National Inventories (NIs) in purpose to make the European plant genetic

resources data available everywhere in the world.
Based on EURSICO National inventory report taxonomy (ipk-gatersleben.de) a total number of 12192 *Cicer arietinum* accessions, and 110 different taxa were detected (Figure 3). Romania has uploaded in EURISCO 127 accessions. The material used for investigations by SCDL Bacau in this work is subject of this collection (Figure 4).

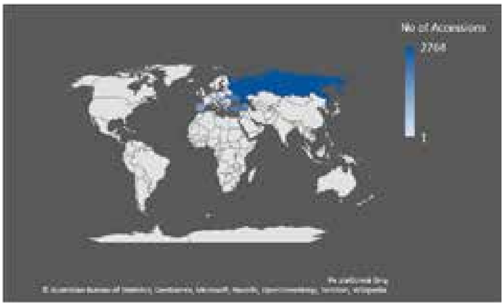

Figure 3. Chickpea accessions availabilities according to EURISCO catalog

Figure 4. Presence of investigated material in this study in EURISCO catalog

Another important tool is represented by Genesys, a database which allows users to explore the world's crop diversity conserved in gene banks through a single website. Genesys brings together some of the world's largest databases of individual gene banks and plant genetic resource networks. These networks often incorporate numerous institutions and many more individual networks – for example, the almost two million accessions provided by ECP/GR through the European Plant Genetic Resources Search Catalogue (EURISCO) come from hundreds of research centers, gene banks and institutions across Europe. Likewise, the CGIAR genebanks are close collaborators and update almost 800.000 passport records every year.
In addition, many individual genebanks, research institutions and smaller networks publish their collection information on the portal to share with the world.
Based on available GENESYS data (https://www.genesys-pgr.org) 66171 *Cicer* accessions are available, 64018 being *Cicer arietinum* accessions.
The largest chickpea collections are maintained at ICARDA and ICRISAT, CGIAR centers with unique accesses estimated at more than 15.000 and 20.000. Collection of wildlife and derived introgression lines is maintained at UC Davis in California. Other worldwide collections of chickpea germplasm Office of National Plant Genetic Resources (NBPGR), India and Gene Banks in Australia, USA, Iran and other countries. The European Chickpea Database was set up in 1996, following the ECPGR meeting in Copenhagen, Denmark, being maintained by the National Institute for Agricultural and Veterinary Research (INIAV). This database includes passport data of cultivated PGR, breeding material and CWR maintained in germplasm collections in European research institutes and gene banks, in line with FAO / IPGRI list according to the Multi-Crop Passport Descriptors. Exchanges and requests for PGR are made based on SMTA directly to the holding institutes. Globally, the need to collect more wildlife has been recognized.
Study of gaps identified in the world collections were:
• the lack of local chickpea varieties in the unexplored areas as Hindu Kush-Himalayas, West and North China, Ethiopia, Uzbekistan, Armenia and Georgia that has been noted for greater diversity;
• the need to collect and investigate CWR which are exploitable sources for tolerance to the pressure of biotic and abiotic factors.
Among the wild species of chickpeas, *Cicer reticulatum* and *Cicer echinospermum*. Chickpea evolved from a unique ancestor, *Cicer reticulatum*.
Diversity is exceedingly low in the modern gene pool, owing to successive human-induced bottlenecks (https://www.pulsesincrease.eu).

Related Cicer species *C. bijugum, C. chorassanicum, C. cuneatum, C. pinnatifidum, C. judaicum, C. yamashitae* and *C. anatolicum*, are annual, apart from *Cicer anatolicum* which is a perennial species (Figure 5).
These wild species have been used to transfer genes for resistance to biotic and abiotic stress. *Cicer bijugum* is reported mainly in western, southern, and southeastern Turkey, northern Iraq, and northeastern Iran, while *C. vistatum* is found in Ethiopia and Pakistan;
• gene transfer from wildlife is not very easy, therefore, studies on characterization, evaluation of various desired characteristics are needed;
• chickpea collections are not fully exploited due to the lack of evaluation data associated with the collected material.

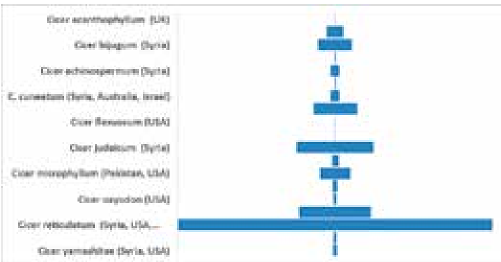

Figure 5. *Cicer* sp. - accessions distribution in different countries

Gene bank collections would become more useful if the collected genetic material will be associated with detailed characterization as well as evaluation data. Chickpeas are suitable for cultivation in extensive agro-climatic conditions that differ depending on the photoperiod, temperature, and precipitation.
A comprehensive screening of chickpea collections revealed a high degree of diversity. Breeders can find and select from global collections genetic resources according to the specifics of their interest. Some web sources able to provide info and plant genetic material are:

- (http://www.genesys-pgr.org),
- (http://www.icrisat.org/),
- (http://www.ars-grin.gov/),
- (http://www.ecpgr.cgiar.org/),
- (http://www.gene.affrc.go.jp/).

The characterization and maintenance of chickpea genetic resources and their exploitation in pre-breeding has major contribution to development of more sustainable agri-food chain**.**
The collection consisted in eight chickpea resources was investigated in open field conditions for two years in different experimental variants, including use of seedings and direct sowing in conventional versus organic, in soil and in pots. Different periods for experiments development were tested.
Characteristics of investigated materials are summarized in Table 1 and Figure 6.

Table 1. Characteristics of biological material

V	Hair ness	Pig menta tion	Growth habit *	No leaflets	Seeds color **	Testa texture ***
Na1	low	green	5	11	10	3
Na2	low	green	5	13	10	3-5
Na3	low	green	4	13	10	3-5
Na4	low	green	5	11	10	3
Na5	low	violet	4	13	2	7
Na6	low	violet	4	11	1	3
Na7	low	green	2	13	16	3-5
Na8	low	green	2	13	16	3
*	Growth habit	see figure 6				
**	Seed color	beige=10, brown=2, black=1, yellow beige=16				
***	Testa texture	rough=3, smooth=5, tuberculated=7				

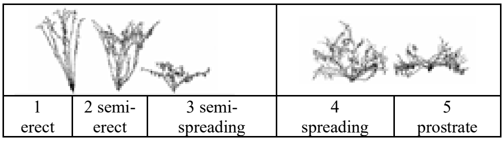

Figure 6. Growth habit of investigated material

During the vegetation period, phenological observations and biometric measurements, individual plant harvesting (SSD). Planting was carried out on land modelled on furrows with a crown width of 140 cm, the distance between rows 70 cm, and 20 cm between plants in a row. The comparative chickpea culture included variants displayed in four replicates in random blocks.
The selection and use of high-quality seeds is a first step in inducing a rapid germination process, able to imprint quality and competitiveness of crops. Inoculation, fertilization, and pest control will be of limited value if the seeds sown do not produce a healthy, vigorous seedling.
Sowing pure, high-quality seeds has contributed to: increasing the tolerance of seedlings to disease; promoting the rapid and uniform establishment of the experimental field;

increased tolerance to abiotic stress at the beginning of the season, such as unfavorable temperature and humidity; rapid development of roots; uniform maturity, higher yields and superior seed quality (purity, germination and vigor and disease-transmitted disease levels).

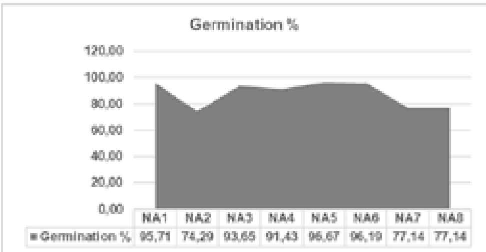

Figure 7. Germination rate at each variant

Three variants recorded values between 74.29 and 77.14%, and five variants germinated over 91%. (Figure 7). Variant Na 5 with a germination of 96.67% was noted as having the highest germination level and the average germination was 87.78% (Table 2).

Table 2. Chickpea germination

	Germination (%)
Min	74,29
Max	96,67
Average	87,78
Standard deviation	9,18
Coeff of variation %	0,10

Regarding the dynamics of the growth and development processes, we found that the first flowers appeared after an interval of 44-68 days from seed emergence.

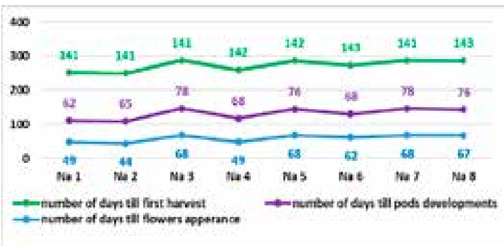

Figure 8. Dynamic of flowering and pods set

The first pods development was observed starting 62 to 68 days after seed emergence. If in the case of the two indicators there were differences between the experimental variants, the first harvests were performed approximately uniformly, after 141 - 143 days from seeds emergence (Figure 8).

The rate of pods maturation (considered at 50% matured by changing color) was between 50 and 65 days after the appearance of the pods. Na 3 recorded the fastest maturation and Na 1 the slowest, in the climatic conditions of 2021.

For a maturation of at least 90%, the experimental variants required an average interval of 78 days from the appearance of the first pods (Figure 9).

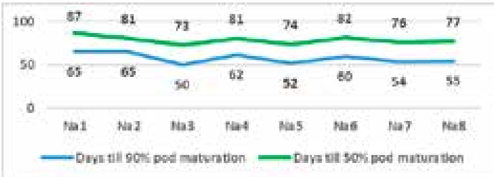

Figure 9. Pod maturation rhythm

We found that Na 3 required 73 days for 90% maturation. Na 1 was noted for its longest interval, 87 days, till 90% maturation. In conclusion, the ripening rate of the pods had a similar dynamic, constant for the two investigated indicators: maturation 50% and minimum 90%.

Table 3. Morphological traits to assess plant architecture

Variant	Plant height (cm)	Canopy height (cm)	Canopy width (cm)	First pod Insertion (cm)
Na1	68,00	31,50	82,50	20,50
Na2	61,50	42,50	72,00	21,00
Na3	82,00	48,50	79,00	39,50
Na4	54,50	40,00	69,50	11,00
Na5	84,50	48,00	77,00	34,50
Na6	57,50	34,00	81,00	15,00
Na7	70,00	34,00	61,00	26,00
Na8	74,00	50,00	79,50	26,50
Min	54,50	31,50	61,00	11,00
Max	84,50	50,00	82,50	39,50
Average	69,00	41,06	75,19	24,25
Standard dev	10,81	7,06	7,50	9,72
Coeff of variation %	0,16	0,17	0,10	0,40

The plants height varied between 54.5 cm and 84.5 cm. Variant Na 5 recorded the highest plants, and those with the lowest port were the plants of variant Na 4. This character, cumulated with the first pod insertion height is of particular importance when establishing harvesting methods (Table 3). At the same time, the height of the plants also influences the amount of biomass provided.

In general, all variants had pods with one and very rarely two seeds. Thus, the average number of seeds in the pod varied between 1 and 1.5. Variant Na 2 had the lowest average number of pods per plant, 44.67. Variant Na 3 recorded the highest number of pods per plant (Table 4).

Table 4. Yield components for each variant

Variant	Number of pods per plant	Pod length (mm)	Number of seeds in pod	MMB (g)
Na1	164,33	20,55	1,10	295
Na2	44,67	20,33	1,10	296
Na3	165,33	20,85	1,00	285
Na4	108,33	22,22	1,11	275
Na5	93,25	20,00	1,50	305
Na6	130,67	16,92	1,15	265
Na7	108,75	18,06	1,18	310
Na8	129,58	20,76	1,15	295
Min	**44,67**	**16,92**	**1,00**	**265**
Max	**165,33**	**22,22**	**1,50**	**310**
Average	**118,11**	**19,96**	**1,16**	**290,75**
Standard deviation	**40,91**	**1,76**	**0,16**	**15,41**
Coeff of variation %	**0,35**	**0,09**	**0,14**	**0,05**

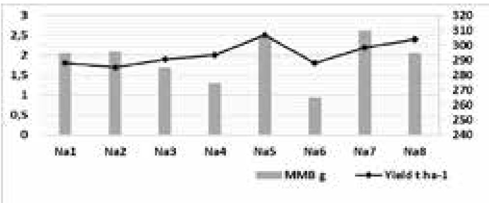

Figure 10. Variation of yield and MMB at investigated accessions

Regarding breeding for a higher yield, chickpeas have an indeterminate character, it is photo-sensitive as well as heat-sensitive, being characterized by a poor management of photosynthesized substances, resulting in low yields. Figure 10 shows the fact that Variants Na 5 and Na 7 were noted for their productivity, and MMB values.

Seed harvesting was done individually from potential plants, identified during the vegetation. Single Seed Descendance is a simple, convenient, inexpensive, and time-saving method, ensuring the possibility of a large number of crosses that can be evaluated by this method, because it requires less space and manpower in each generation. This method preserves considerable variability. The material can be improved quickly. This strategy of developing and using SSD purified accessions has been applied in several projects (e.g., INCREASE, BRESOV); The general conclusion is that SSD lines offer the possibility to associate phenotypes to a unique genotype, thus promoting genetic resources conservation and their use in pre-breeding programs. (Bellucci et al., 2021) (https://www.pulsesincrease.eu/).

CONCLUSIONS

The existing of freely available, national databases is a real support for searching information related the conservation of different species and accessions worldwide.

A special attention is recommended when filter insertion is used in databases, to include synonyms and correct writing of species name.

In terms of chickpea investigation in Northeast part of Romania our preliminary results showed a superior productivity of variant Na 5. Variant Na 3 was highlighted by its earliness.

All variants are retained for the study in competition cultures.

Variants were noted and harvested separately according to performance indices for later use in breeding. Seed harvesting was done individually from potential plants, identified during the vegetation. Single Seed Descendance seems to be a useful tool, simple, convenient, inexpensive and time-saving method, ensuring the possibility of a large number of crosses that can be evaluated by this method. The benefits of the method: it requires less space and manpower in each generation, preserves considerable variability.

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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF *CARTHAMUS TINCTORIUS* EXTRACTS AGAINST NOSOCOMIAL MICROORGANISMS

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Abstract

Drug-resistant pathogens are the main cause of health-associated infections. They continue to significant infections, increase mortality of hospitalized patients and also have a negative financial impact. Taking into consideration those factors, there is a great need to find and develop new compounds with low toxicity, specific activity, and high bioavailability to combat them. In this way, have increased the attention on medicinal plants and their bioactive compound with antimicrobial properties. This review evaluates the antimicrobial activities of different Carthamus tinctorius (safflower) extracts from seeds, leaves, and flowers against nosocomial pathogens. The study concludes that safflower different extracts might be used against multidrug-resistant microbes that cause nosocomial and community-acquired infections.

***Key words**: antimicrobial activity, nosocomial pathogens, phenolic compounds, safflower extracts.*

INTRODUCTION

In the European Union, nosocomial infections affect approximately 3 million people each year, of which about 50,000 cases are fatal (data from European Centre for Disease Prevention and Control) (Miklasińska - Majdanik et al., 2018). The most frequently multidrug resistant (MDR) pathogens which are the main cause of nosocomial infections among hospitalized patients are: *Acinetobacter baumannii, Escherichia coli (E. coli)*, *Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa* (*P. aeruginosa*), *Pseudomonas* spp, *Enterococcus faecalis* (*E. faecalis*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Candida albicans* (*C. albicans*) (Chen et al., 2022; Iyer et al., 2022; Urusova et al., 2022; Dhasarathan et al., 2021; Luo et al., 2021; Mo et al., 2019; Othman et al., 2019; Périer et al., 2020; Wang et al., 2019; Moreno et al., 2013). The extensive use of synthetic antimicrobial agents can cause concern due to their development of microbial resistance and environmental pollution potential. Considering this issue, it is important to develop new antimicrobial agents able to prevent microbe's adhesion and proliferation on materials surfaces and reduce their negative effects (Ivanov et al., 2022; Khameneh et al., 2021; Mittal et al., 2019). The growing incidences of MDR pathogens have increased the attention on several medicinal plants and their active principles for antimicrobial properties (Moreno et al., 2013). According to Khameneh et al., 2021, phytochemicals like alkaloids, tannins, carbohydrates, glycosides, terpenoids, flavonoids, steroids, and coumarins have particular clinical value because their bioactivity does not lead to resistance. Safflower (*Carthamus tinctorius* L.) which belongs to the Asteraceae family, contains many bioactive compounds for which it has biological activities, involving antidiabetic, anticancer, antioxidant, anti-ageing, anticoagulant, hepatoprotective and anti-inflammatory, analgesic and antibacterial activities (Sura et al., 2020). According to Górniak et al, 2019, mechanisms of this activity still require more studies.

PHENOLIC COMPOUNDS FROM SAFFLOWER WITH ANTIMICROBIAL ACTIVITY

Safflower has been identified to contain more than 200 compounds including flavonoids, alkaloids, lignans, alkane diols, riboflavin, steroids and quinochalcone C-glycosides (Sun et al., 2020; Hussain et al., 2021; Ozkan et al., 2022).

The pathogens have developed several mechanisms of antibiotic resistance, such as overexpression of the efflux pumps, destroying the antibacterial agents, structural modification of porins, and modification of antibiotics. Therefore, inhibition of them is an integral part of combating antibiotic resistance (Khameneh et al., 2021). The phenolic compound can modify the permeability of cell membranes, make changes in various intracellular functions induced by hydrogen bonding with the enzymes or by the modification of the cell wall rigidity with integrity losses due to different interactions with the cell. This may induce irreversible damage to the cytoplasmic membrane and coagulation of the cell content that can even lead to the inhibition of intracellular enzymes. All of these mechanisms are possible due to the lipophilic character of phenolic compounds. Polyphenols also can suppress biofilm formation and exert a synergistic effect with antibiotics (Bouarab - Chibane, 2019; Othman et al., 2019; Miklasińska - Majdanik et al., 2018).

Polyphenols can also produce cell membrane damage of bacteria, indicated by the release of intracellular K+, with greater effects observed with gram-negative bacteria strains than with gram-positive bacteria strains (Liu et al., 2021). The interaction of flavonoids with lipid bilayers of bacterial cell membranes is made through two mechanisms: partition of the more non-polar compounds in the hydrophobic interior of the membrane, while the second includes the formation of hydrogen bonds between the polar head groups of lipids and the more hydrophilic flavonoids at the membrane interface (Górniak et al., 2019). Flavonoids also may act by inhibiting both energy metabolism and DNA synthesis thus affecting protein and RNA syntheses, in Gram-positive bacteria can modify intracellular pH, also can interfere with the energy (ATP) generating system. (Bouarab-Chibane et al., 2019; Xie et al., 2017). Long aliphatic chain substitution in flavonoids leads to an increase in their hydrophobicity and facilitates interactions with the bacterial cytoplasmic membrane, in this way resulting in an increase in antibacterial activity of these compounds. The presence of phenolic hydroxyl groups, which have a high affinity for proteins, and microbial enzyme-inhibition, also may enhance the antibacterial effects of flavonoids. Hydroxylation of flavonoids improves antibacterial activity even against Methicillin-resistant *Staphylococcus aureus* (MRSA) strains (Othman et al., 2019; Miklasińska-Majdanik et al., 2018). The most important phenolic compounds from *C. tinctorius* and their antimicrobial mechanism of action are shown in Table 1. Daphnoretin, a flavonoid compound found in *C. tinctorius*, had a potent binding affinity with TLR4 and TLR8. These findings could be used as a basis for further investigations for daphnoretin as the possible inhibitors for hyper-inflammatory conditions in COVID-19 by targeting Toll-like receptors (TLRs) (Hansur et al., 2021). Terpenes exert antimicrobial activities against both the antibiotic-susceptible and antibiotic-resistant bacteria, having the ability to promote cell rupture and inhibition of protein and DNA synthesis, disruption in microbe multiplication and development, interfering with their physiological and metabolic activities. Like flavonoids, terpenoids make their effects by disruption of microbial membranes (Masyita et al., 2022). Besides terpenes, another compound found in safflower is trans-trans-3,11-tridecadiene5,7,9-triyne-1,2-diol, which has antifungal properties (Attia et al., 2021).

In safflower, were identified also anti-nutritional factors (ANFs) which are present in the form of tannins, luteolin, acacetin, and serotonin derivatives. ANF compounds can be used as antibacterial, anti-inflammatory, antioxidant and anticoagulant agents for various health and pharmaceutical applications (Singhal et al., 2018).

Table 1. The most important phenolic compounds from *C. tinctorius* and their antimicrobial mechanism

Nr crt.	Phenolic compound	Antimicrobial mechanism of action	References
1	Ferulic acid	-Decrease the hydrophobicity of *P. aeruginosa*. -Propylferulate can be used for the use of efflux pump inhibition of *Staphylococcus aureus* IS-58 strains.	Liu et al., 2020; Pinheiro et al, 2022
2	Catechins	-Cause alteration in the membrane permeability and membrane damage. -Interfere with the biosynthesis of the bacterial cell wall by binding with the peptidoglycan layer. -Neutralize bacterial toxic factors from *Vibrio cholerae*, *S. aureus*, *Vibrio vulnificus*, *Bacillus anthracis*, and *Clostridium botulinum*. -In the combination with reuterin, exhibits synergistic antimicrobial activity against *S. mutans* by repressing growth, biofilm formation, water-insoluble glucan production, and virulence genes expression. -Damage to bacterial cells through binding to their surfaces and the bactericidal action of hydrogen peroxide generated through catechin autooxidation.	Miklasińska-Majdanik et al., 2018; Górniak et al, 2019; Zhang et al, 2021
3	Quercetin	-Decrease in proton-motive force in *S. aureus.* -Cell membrane disruption (membrane rigidification), DNA intercalation, DNA gyrase inhibition, Type III secretion inactivation, dehydratase inhibition (HpFabZ), protein kinase inhibition in *E. coli*, *Helicobacter pylori*. -Inhibit the neurotoxin from *C. botulinum* -Hyaluronic acid lyase (Hyal B) inhibitors in *Streptococcus agalactiae*. The inhibitory effect of the flavonoids increased with the number of hydroxyl groups present in the flavonoid structure. -Antibiofilm agent against *Salmonella spp.* biofilm formation, as well as biofilm caused by other bacteria. -Quercetin in combination with polymeric nanoparticles produces an alteration in membrane permeability, adsorption, and adhesion of nanoparticles with fusion with the cell membrane.	Rempe et al 2017; Miklasińska-Majdanik et al., 2018; Górniak et al, 2019; Kim et al, 2022; Kumar et al., 2016
4	Morin	-Cause destabilization of the membrane structure by disordering and disorientation of the membrane lipids and induced leakage from the vesicle. -Inhibit sortase—the enzyme presents in the cytoplasmic membrane of Gram-positive bacteria, which is responsible for anchoring protein virulence factors to the cell wall peptidoglycan. Morin presence makes that *S. aureus* to have a reduced affinity to fibrinogen Binds to FtsA close to ATP binding site affecting the ATPase activity of FtsA and inhibiting its polymerization. -Inhibition of FtsA polymerization affects the cell division of *Vibrio cholerae* altering its morphology to an elongated one. -Promoting bacterial aggregation, leakage of the cell membrane, intervention in the biofilm growth, and the down-regulation of the PBP2a-mediated resistant mechanism. inhibit *S. aureus*' virulence. -Inhibit the initial biofilm formation of *Listeria monocytogenes* strains.	Miklasińska-Majdanik et 2018; Nag et al., 2021; Rajput et al., 2021; Sivaranjani et al.2021
5	Apigenin	-Cause destabilization of the membrane structure by disordering and disorientation of the membrane lipids and induced leakage from the vesicle. -Dehydratase inhibition (HpFabZ) and protein kinase inhibition. -Adherence and fusion of liposomal apigenin with the bacteria produce membrane perturbation through reactive oxygen species generation. -DNA cleavage assay showed that API inhibited DNA gyrase harbouring the quinolone resistance mutation gyrA (Ser84Leu) of *S. aureus*.	Rempe et al 2017; Banerjee et al 2015; Miklasińska-Majdanik et 2018.
6	Naringenin	-Have antibacterial activity against MRSA, reducing the fluidity in hydrophilic and hydrophobic regions of both inner and outer cellular membrane. -Change the membrane fluidity and fatty acid composition. -Inhibit *S. mutans* growth and biofilm formation, increased *S. mutans* surface hydrophobicity, reduced bacterial aggregation, and downregulated the mRNA expression of gtfB, gtfC, comD, comE, and luxS.	Miklasińska-Majdanik et 2018, Wang et al., 2018;Yue et al., 2018
7	Rutin (quercetin-3-O-rhamnoglucoside)	-Decrease the bilayer thickness, and disrupt the lipid monolayer structure. -Decreased biofilm production. Down-regulated the expression of luxS gene and wabG gene in strong biofilm producing *K. pneumoniae*.	Miklasińska-Majdanik et al., 2018; Wang et al., 2021
8	Kaempferol and kaempferide-3-O-glucoside	-Activity against amoxicillin-resistant *E. coli*, but also the ability to reverse the resistance via inhibition of peptidoglycan and ribosome synthesis. -Against *L. monocytogenes*, could cause cell death through cell membrane damage and decrease the expression of the virulence factors. -Inhibit DNA gyrase from *E. coli*. Inhibit the neurotoxin from *C. botulinum.* -Damage to the *E. coli* ATCC 25922 membrane.interaction with the polar head-group of the membrane followed by penetration into the hydrophobic regions.	Miklasińska-Majdanik et 2018,.Liu et al., 2021; Górniak et al, 2019

ANTIMICROBIAL ACTIVITY OF *CARTHAMUS TINCTORIUS*

Evaluation of antimicrobial activity of safflower leaves

In the composition of leaf essential oil, was identified caryophyllene oxide as a chemotype. Other representative compounds were found such as methyl eugenol (16,79%), α-pinene (8,03%) and cinnamyl acetate (7,29%). Eugenol is a very important compound useful in the pharmaceutical field because of its antibacterial, antifungal, anti-inflammatory and antioxidant properties and its insecticide effect (Bhuiyan et al., 2010).

In the leaves during growth and processing, were also identified eight flavonoids, apigenin-6-C-β-D-glucopyranosyl-8-C-β-D-glucopyranoside (AGG), quercetin 7-O-β-Dglucopyranoside (QG), luteolin 7-O-β-D-glucopyranoside (LG), quercetin 7-O-(6”-O-acetyl)-β-D-glucopyranoside (QAG), luteolin 7-O-(6”-O-acetyl)-β-D-glucopyranoside (LAG), quercetin (Q), luteolin (L) and acacetin 7-O-β-D-glucuronide (AG).

Steamed and roasted safflower leaves are a rich source of flavonoids and may be a good source of bioactive components with antibacterial activity (Lee et al., 2006). Moneim et al. (2017) investigated the antibacterial activity of water and methanol extracts from the leaves of some medicinal plants and their synergistic effect with antibiotics.

They used various antibiotic-resistant bacterial species, including: *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumonia), Acinetobacter baumannii (A. baumannii), Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus)* and *Salmonella* spp.

The results showed that methanol extracts from most of the plants tested inhibited the growth of the bacteria tested, however, the largest inhibition was by Senna, followed by Harmal and safflower.

The synergism between plants extract and antibiotics resulted in inhibition of the tested organisms (Moneim et al., 2017). Omidpanah et al. (2019) and Salem et al. (2014a), demonstrated that safflower methanolic and aqueous leaves and flower extracts have an effect on *Aspergillus flavus*, *Aspergillus carbonarus*, *Sclerotinia sclerotiorum*, *Penicillium digitatum* on the fruits.

Evaluation of antimicrobial activity of safflower flowers

Safflower florets contain yellow and red quinochalcone natural dyes such as safflower yellow A, safflower yellow B, safflomin C, precarthamin, and carthamin. These chalcones are the main constituents of glycosylated flavonoids in safflower which were not detected in other natural products.

The antimicrobial activity of quinochalcones, precarthamin and carthamin, was demonstrated by Salem et al (2014 b), against three Gram-positive bacteria (*E. coli, S. aureus,* and *B. cereus*), one Gram-negative bacteria (*P. aeruginosa*), and one yeast strain (*C. albicans*). The potency of the chalcones against *C. albicans* depended on their ability to interact with sulphydryl groups. Extracts of *Carthamus tinctorius* L. flowers harvested at the last stage of flowering, were shown to have significant antimicrobial effects against a fungal strain (*Candida albicans*) and certain bacterial strains (*E. coli*, *S. aureus*, *P. aeruginosa*, and *Bacillus cereus*). The most intense activity was observed against *E. coli* (Attia et al., 2021).

Flowers contain also polysaccharides that had immunomodulating activities. The immune system being the most effective weapon for preventing the invasion of the pathogen (Yao et al., 2018). Oil extracts from flowers have activity on *E. coli* and *S. aureus* (Sabah et al., 2015).

The antibacterial activity of hydro - alcoholic extracts from 3 genotypes of dried safflower flower was demonstrated by Ozkan et al., 2021 against Gram-positive bacteria (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus*) and Gram-negative bacteria *(Escherichia coli, Salmonella typhimurium*). The antibacterial activity of the safflower genotype extracts was determined by using the agar diffusion method. In addition, safflower can be used as antioxidants and vitamin stabilizers in some food types (Adamska and Biernacka, 2021).

Evaluation of antimicrobial activity of safflower seeds

The edible seed oil extracted by cold pressing it is an extraction method that does not use any chemical products during the extraction process and ensures the preservation of oil components (Khémiri et al., 2020). Phenolics, polysaccharides, flavonoids, alkaloids, lignans (tracheloside), steroids, carboxylic acids, quinochalcone C-glycosides and quinone-containing chalcones have been the chemical groups isolated from safflower seeds (Singhal et al, 2019; Mani et al., 2020). Safflower seeds contain 38–48% oil, 15–22% proteins, and 11–22% fibres (Mani et al., 2020). Safflower oil contains 70% polyunsaturated linoleic acid and 10% monounsaturated oleic acid. Seven antioxidative serotonin derivatives were isolated from the oil of safflower (Mani et al., 2020). *C. tinctorius* L. seed oil display high antioxidant and antimicrobial effects (Attia et al. 2021).

Khémiri et al., 2020 show the antimicrobial activity of safflower seed oil on 2 gram-negative bacterial strains (*E. coli* and *Enterobacter cloacae*), 2 gram-positive bacterial strains (*S. aureus* and *Streptococcus agalactiae*), 3 yeast species strains (*Candida albicans*, *Candida parapsilosis*, and *Candida sake*), and 3 fungi species (*Aspergillus niger*, *Penicillium digitatum*, and *Fusarium oxysporum*) and Padole et al. (2021) on *E. coli*. Their reports showed that the oil extracted from safflower seeds under cold pressing exhibited high antioxidant activities and notable antimicrobial effects against human skin opportunistic pathogenic bacteria, yeast, and fungi, which commonly are involved in altering the healing of skin wounds.

Safflower oil nanoemulsion and cumin essential oil combined with oxygen absorber packaging delayed the growth of mesophilic and psychrotrophic bacteria, *Enterobacteriaceae*, and lactic acid bacteria during the storage period of refrigerated lamb loins (Hasani-Javanmardi et al., 2021).

The **seed coat** has effect on *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumonia*, and *Salmonella typhi* as gram-negative bacteria and *S. aureus* (Tayebeh et al., 2021).

On the vegetables Son et al., 2017 revealed the antimicrobial activity of safflower **seed meal** extract on *Listeria monocytogenes* on fresh lettuce.

Yavuzer et al., 2021 and Kuley et al., 2019, showed that ethanolic safflower and bitter melon extracts have activity in controlling the growth of fish spoilage bacteria (*Acinetobacter lwoffii*, *Pseudomonas oryzihabitans*, *Enterobacter cloacae*, *Shigella spp*., *Morganella psychrotolerans* and *Photobacterium phosphoreum*) and food-borne pathogens (*S. aureus*, *Klebsiella pneumoniae* and *Salmonella paratyphi* A). As a result, safflower and bitter melon extracts could be used as antimicrobial agents to inhibit bacterial growth in food.

Aqueous extract from **waste** (mixture of stem and leaf obtained after the seed collection process) has antibacterial activity on *S. aureus* (Gram positive) and *Pseudomonas fluorescens* (Gram negative) (Rodríguez-Félix et al., 2021).

Aqueous extract from air dried powder plant has antibacterial activity on: *Proteus vulgaris*; *S. aureus*, *Erwinia carotovora*, *Bacillus subtilis*, *Klebsiella pneumonia*, and *C. albicans* (Ibrahim et al., 2019) and on *E. coli*, *Klebsiella pneumoniae* (*K. pneumonia*e), *Acinetobacter baumannii*, *P. aeruginosa*, *S. aureus* and *Salmonella* spp. (Moneim et al., 2018)

In the literature, there are more studies about safflower antibacterial action on human pathogenic bacteria and fungi action, including Gram-positive *B. cereus*, *L. monocytogenes*, *S. aureus* and Gram-negative bacteria *E. coli*, *P. aeruginosa*, *Salmonella enterica* (Bouarab-Chibane et al., 2019; Thornfeldt, 2018; Sulieman et al., 2017; Karimkhani et al., 2016; Bessada et al., 2015; Salem et al., 2014).

CONCLUSIONS

This review reveals the importance of various extracts of *Carthamus tinctorius* (safflower) from seeds, leaves and flowers in the control of nosocomial pathogens. The bioactive components of *Carthamus tinctorius* L. may be considered a good alternative to natural therapeutic treatments. The health-promoting applicability of safflower increases with the progressing knowledge of its chemical composition. Subsequent investigation of the plant extracts to isolate and recognize the active ingredients is highly recommended.

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INFLUENCE OF THE TYPE OF THE SPRAYING ON SOME TECHNOLOGICAL AND ECONOMIC INDICATORS IN PESTICIDE TREATMENT OF VINEYARDS

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Abstract:

The aim of the present study is to compare some technological and economic parameters in the treatment of vines with pesticides using: axial and tangential fan sprayers. The following indicators are the degree of coverage of the leaf mass, the degree of penetration of the pesticide into the canopy of the vines and the price of the sprayers. From the experiments, calculations and analyzes conducted so far, it can be concluded that both sprayers meet to a very good extent the technological requirements after mandatory treatment of each vine row on both sides. The degree of coverage of the leaf mass with drops is about 65% for axial and about 78% for tangential sprayers. The difference is due to the more concentrated and directed jet (from air and solution) in the tangential sprayer. This allows for good mixing and passing through the leaf mass. Regarding the other technological indicator - penetration rate - the results are approximately the same. Given the small difference in prices, this is a reason to recommend working with a tangential sprayer when treating vineyards with pesticides.

***Key words**: axial and tangential fan sprayers, degree of coverage, degree of penetration.*

INTRODUCTION

The main part of the fan spray system is the fan. Often in the upper and lower part of its outlet hinge deflector plates are hinged, which direct the created air flow in a certain direction. Pipelines are attached on both sides of the fan. At their upper end they are closed. The nozzles are mounted to them at a certain interval. Depending on the design of the fan outlet, two types of spray systems are observed:
- classic - In this case, the air jet created by an axial fan hits a reflector, turns 90º and spreads evenly 360º on the plane of the reflector. The movement of the air jet, together with the working upwards sprayed by the nozzles, is limited by deflectors, which when spraying vines, low-stemmed orchards and shrubs are placed in a horizontal position, and when spraying tall stems are inclined to a vertical position depending on the size of the crown. and the height of the trees.
To improve the quality of work and reduce the loss of working fluid in the air (Godyn A., 2008) as a variant of the classical two-fan systems are made with vertical arrangement of the two fans. Dual fan sprayers have not yet been well studied. Only prototypes and prototypes have been created without entering mass production and use in fruit growing practice. From the authors' research it can be concluded that these machines and spraying systems are efficient and with good quality of work at the height of the orchards up to 4 m.
- cantilever ("tower" type) - The difference with the classic system is that here the fan is covered by a metal casing, with two narrow slotted holes on the side (up to 10 cm wide). The height of these holes varies from 100 to 250 mm depending on the height of the treated plants. The other difference is that the pipes are straight and not arched as in the classic system. Due to the small area of the outlets, the air flow, respectively the working fluid carried with it, moves at a higher speed, has greater kinetic energy and penetrating force. Cantilever sprinklers have the added advantage of directing the airflow at the top of the tower downwards, which reduces the amount of solution released irreversibly into the atmosphere (Fox R. et al., 2008).
In addition to fan sprinkler systems, pneumatic and frame twin systems are used in the treatment of perennials.

- pneumatic spray systems. They are known as "spider", "octopus" and others. In them, the crushing of the working fluid and the transfer of the droplets is carried out by an air flow with a speed of 80-100 $m.s^{-1}$. Monodisperse dispersion is achieved, with an average droplet diameter between 50-80 μm. The flow is created by a centrifugal fan or compressor and is characterized by high speed. It is supplied to the sprinklers via special air ducts, which are very often flexible and allow the individual positioning of the spray nozzles (diffusers) in height and in the sides. This in turn leads to better directing of the working fluid to the treated object. Sprinklers during operation are at a short distance from the plants - from 0.5 to 2 m. Although with a small mass, the droplets move at high speed (that of air flow), due to which they have a great penetrating force and easily enter the habitat of plants.

- frame (tunnel) twin system - (Panneton B. et al., 2005; Świechowski W. et al., 2004) The main difference between conventional frame systems and this is that in addition to the frame with nozzles located above it, a sleeve is mounted in which high pressure air is supplied. Holes are made just above the nozzles in the sleeve, through which the compressed air flows at high speed. It absorbs the sprayed working fluid, further breaks it into smaller drops and transports it to the crown and inside it on the treated fruit trees. A disadvantage of this system is the loss of working fluid on the ground, which in some cases reaches 18% (Molari G. et al., 2005), which necessitates the creation of improved structures with traps of excess fluid for more complete its use (Wenneker M., J. van de Zande, 2008).

The authors (Doruchowski G. et al., 2002; Świechowski W. et al., 2004) observed the operation of three orchard sprayers for the amount of working fluid supplied, the losses and the biological efficiency depending on the spraying system. Sprayers with classical, column (cantilever) and pneumatic spraying systems have been studied. During the research 3 layers were observed in the crown of the treated trees: layer 1 - outer, closest to the sprayer; layer 2 - central; layer 3 - outer, furthest from the sprayer. In the case of the sprayer with a classic sprayer system, there is a clear tendency to reduce the volume of air in the individual layers, as the distance from the sprayer increases. There is no such clear trend for the other two sprayers. There are no differences in the delivered average amount of working fluid in the individual spray systems. However, the pneumatic sprayer delivers more inside the crop crown and less at the tips than the conventional sprayer. This excellent crown penetration in the pneumatic sprayer is combined with 50% lower air volume, which is a prerequisite for lower energy consumption. The loss of working fluid in the soil is not significantly different with individual sprayers, while the loss of air in a conventional sprayer is several times greater than that obtained when using sprayers with column or pneumatic systems.

Derksen R. et al., 2004, observed two sprayers - a classical and a column sprayer system. They found significant differences in the average amounts of working fluid for the two sprayers. Statistical estimates also show that the conventional sprayer delivers significantly larger amounts in the upper and middle parts of the crown. The column sprayer delivers a significantly larger amount in the upper than in the middle. In general, however, the differences in the amount of working fluid deposited in height are smaller for the column sprayer compared to the conventional sprayer

MATERIALS AND METHODS

The aim of the present study is to compare some technological and economic parameters in the treatment of vines with pesticides using: axial and tangential fan sprayers.

The following indicators are the degree of coverage of the leaf mass, the degree of penetration of the pesticide into the canopy of the vines and the price of the sprayers.

The experiments were performed in a vineyard with a row spacing of 3 m, a row distance of 1.2 m, a row length of 200 m, a spray rate of 450 $l.ha^{-1}$ and a movement speed of 6.8 $km.h^{-1}$. The leaf mass was located in a belt with a height of 1.05 m to 1.83 m.

Two mounted, fan sprayers were used: axial AGP 440 and tangential AGP 440U with a tank of 450 l each.

To determine the monitored technological indicators along the row, in 5 randomly selected places (in the middle of the belt), water registration papers (Figure 1) are attached on the front and back sides of selected sheets. During the research 3 layers were observed in the vine: layer 1 - outer (positions 1 and 2), closest to the sprayer; layer 2 - central (positions 3 and 4); layer 3 - outer (positions 5 and 6), furthest from the sprayer. The first working stroke is performed with the sprayer, after which the area covered with droplets on the 6 water registration papers is counted with a planimeter at each of the 5 points in a row. The sprayer passes on the other side of the sprayed row and the second stroke is performed. Again, the area covered with droplets is reported. This operation is performed for each of the two observed sprayers.

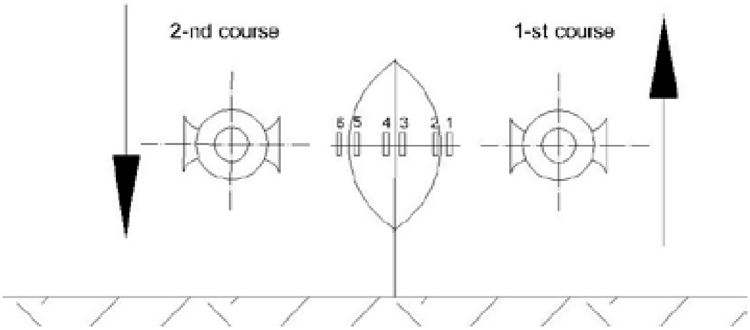

Figure 1. Scheme of the experiment

In the first stroke, position 1 is closest to the sprayer and position 6 is furthest away. In the second stroke it is exactly the opposite.

The degree of coverage is determined according to formula 1:

(1) $$M_S = \frac{S_1}{S}$$

S - area of the water registration paper, cm^2; S_1 - area covered with drops, cm^2.

The degree of penetration provides information about the possibility of uniform saturation of the entire leaf mass of the vine with a solution of the preparation. Determined by formula 2:

(2) $$M_n = \frac{M_{S1}}{M_{S5}}$$

M_{S1} - degree of coverage in item 1 (Figure 1), M_{S5} - degree of coverage in item 5. The closer to 1 the value obtained, the better the solution penetrates the leaf mass.

In terms of economic indicators, most of them are derived from the price of the sprayer. For this reason, in the present work, its price is indicated as an economic indicator.

RESULTS AND DISCUSSIONS

Water-registered papers measuring 7 x 5 cm were used in the experiments. The results obtained are reflected in the following tables.

Table 1 Area covered with drops after operation with axial fan sprayer, cm^2

Position of water registration paper	first work move, points of row					second work move, points of row				
	1	2	3	4	5	1	2	3	4	5
1	16,8	16,1	17,2	16,5	15,6	6,2	4,3	6	6,1	5
2	12	10,2	10,9	12	11,3	12,1	13	12,2	12,4	11,5
3	14	13,4	13,4	14	13	9	9,5	7,8	9	9,3
4	9	9,5	7,8	8,8	9,5	14,1	12,5	13,6	14,2	13,2
5	12	12	12,3	12,3	11,6	12	10,1	11	11,9	11,5
6	6,3	4,2	5,6	6	4,9	16,7	16,2	16,9	16,4	15,7

By moving the sprayer away from the observed layers of the leaf mass, a reduction of the area covered with drops is reported both on the front (positions 1, 3, 5) and on the reverse side (positions 2, 4, 6). The total area covered with drops after passing the sprayer on both sides of the row is obtained by summing the results for each position of each stroke (Table 2). There is a leveling of the area with delayed drops on both the front and back of the leaf.

Table 2 Total area covered with drops after operation with axial fan sprayer, cm^2

Position of water registration paper	Point of row					Mean
	1	2	3	4	5	
1	23	20,4	23,2	22,6	20,6	21,96
2	24,1	23,2	23,1	24,4	22,8	23,52
3	23	22,9	21,2	23	22,3	22,48
4	23,1	22	21,4	23	22,7	22,44
5	24	22,1	23,1	24,2	23,1	23,3
6	23	20,6	22,5	22,4	20,6	21,82

The results of the operation of the tangential fan sprayer are presented in Tables 3 and 4. They are similar to the operation of the axial sprayer, with the difference that the area covered with drops is larger.

Table 3 Area covered with drops after working with a tangential fan sprayer, cm2

Position of water registration paper	first work movee, points of row					second work move, points of row				
	1	2	3	4	5	1	2	3	4	5
1	17,3	17,4	17,6	17,2	17,6	10,7	10,3	10,2	10,6	10,9
2	13	11,5	11,8	12,8	12,7	13,9	13,8	14,3	14,7	15,1
3	16	15,9	16,1	15,7	15,8	13	10,9	12,4	11,7	10,7
4	12,5	11	10,8	11,3	10,7	16,1	15,8	16,2	16	15,7
5	14,7	14,7	15,6	14,9	15,2	13	11,7	12	12,6	12,8
6	10,8	10,2	9,5	10,5	10,6	17,5	17,1	17,6	17,3	17,6

Table 4 Total area covered with drops after operation with tangential fan sprayer, cm^2

Position of water registration paper	Point of row					Mean
	1	2	3	4	5	
1	28	27,7	27,8	27,8	28,5	27,96
2	26,9	25,3	26,1	27,5	27,8	26,72
3	29	26,8	28,5	27,4	26,5	27,64
4	28,6	26,8	27	27,3	26,4	27,22
5	27,7	26,4	27,6	27,5	28	27,44
6	27,9	27,3	27,1	27,8	28,2	27,66

Degree of coverage

According to formula 1, the degree of coverage on the front and back of the sheet is determined after the first stroke and after the final treatment of the row for the two sprayers used (Table 5).

Table 5. Degree of coverage, %

Position of water registration paper	Axial fan sprayer		Tangential fan sprayer	
	After firs work move	After final processing	After firs work move	After final processing
1	**47**	**62,7**	**49,8**	**79,9**
2	35	67,2	35,3	76,3
3	**42,2**	**64,2**	**45,4**	**79**
4	28,3	64,1	32,2	77,8
5	**37,8**	**66,6**	**42,9**	**78,4**
6	16,9	62,3	29,5	79

- The results on the front of the sheet are indicated in bold

It can be seen that after 1 move:

With the axial fan sprayer, the degree of coverage decreases with distance of the observed position from the spray system from 47% for position 1 to 37.8 for position 5 from the front of the leaf and from 35 to 16.9% for the back of the leaf. There is a significant difference in the degree of coverage of the front and back of the sheet. This is due to the large

scattering of the air jet and the reduction of its penetrating force.
When working with the tangential fan sprayer, the same picture is observed for the degree of coverage of the front and back of the sheet. Due to the small outlet, the air stream is more concentrated, penetrates with greater force into the leaf mass and mixes it well. This allows for better coverage on both the front and back of the sheet

After the second (final processing) move:
In both sprayers there is an equalization in the degree of coverage of both the front and back of the sheet. The degree of coverage when working with the tangential sprayer is about 10% higher. This is due to the more concentrated direction of the air flow.

Degree of penetration

It is determined using formula 2. The results of the calculations are shown in Table 6.

Table 6 Degree of penetration

Axial fan sprayer		Tangential fan sprayer	
After first move	After final processing	After first move	After final processing
0,79	**0,94**	**0,86**	**0,98**

Both sprayers have a good degree of penetration both in the first stroke and in the final treatment of the row. Due to the above reasons for the specifics of the air flow and this indicator, the results are better with tangential sprayers. What has been said so far confirms the work of Derksen R. et al., 2004 regarding pesticide treatment of perennials and in particular orchards.
Price. There is a small difference in the prices of the two sprayers. For the axial fan sprayer AGP 440 it is BGN 4332, and for the tangential AGP 440U - BGN 5112 (Traktor Invest, 2022).

CONCLUSIONS

From the experiments, calculations and analyzes conducted so far, it can be concluded that both sprayers meet to a very good extent the technological requirements after mandatory treatment of each vine row on both sides. The degree of coverage of the leaf mass with drops is about 65% for axial and about 78% for tangential sprayers. The difference is due to the more concentrated and directed jet (from air and solution) in the tangential sprayer. This allows for good mixing and passing through the leaf mass. Regarding the other technological indicator - penetration rate - the results are approximately the same. Given the small difference in prices, this is a reason to recommend working with a tangential sprayer when treating vineyards with pesticides.

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WHAT COLOUR SUITS YOU BEST? A GENERAL OVERVIEW OF METHODS FOR FUNGAL ORGANISMS STAINING AS POST-VITAL MICROSCOPICAL PREPARATIONS USED IN PLANT PROTECTION

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Abstract

Staining fungi or anatomical parts of them (spores, conidiae, conidiophores, mycelia etc.) is still one of the most used study method in phyto- or zoo- or anatomopathology. In order to fix, dehydrate and clarify them, lactophenol remains the best reagent. Even if various modern techniques are used today, some of the nowadays neglected „classical ones" can sometimes provide an alternate investigation method which can led to interesting morphological and systematic data, or, at least reveal in more specific ways microscopical structural details.

Key words: *Fungi staining, dyes, spore staining, mycelia staining.*

INTRODUCTION

In plant (as in animal or human) pathology, staining of microbial and fungal tissues, isolated from the host or inside it are a valuable method of investigation, we can say a fundamental one. From the discovery of the lactophenol, its use in association with various stains (from which the water-soluble aniline blue, still called „Cotton blue" by the mycologists) represented the standard method of primarily investigation in micology and the starting point for various modifications, which help us to understand how do microscopical fungi infect their hosts, how do they develop, reproduce and can spread the disease to another plant (or animal) host.
Because in many cases, the microscopical fungi are +/- transparent, their refraction indices having values near the ones of glass or water (around 1,00, the coloured ones being not taken into account here) they need in order to contrast them to be stained.

MATERIALS AND METHODS

The presented methods have been validated in the laboratory by staining a conidial suspension and mycelia of *Beauveria bassiana* and *Fusarium* sp. mycelia, both cultivated on potato-dextrose-agar medium (PDA). The stains used, their Colour Index number, class and synonims are presented in the Table 1 (Gabe, 1970, Llewellyn, B.D., 2019).
The preparation methods for the mountants and iodine reagents (Table 2), simple stains (Table 3) and double stains (Table 4) as well as the bibliographical indications the results and (where it was the case) photographs (Figures 1-5) of the stained fungi are also presented.
The inspection of the preparations and the doccumentaion was performed by using a NOVEX-B series microscope, with a total magnification of 1000x and captions were acquired via a MicroQ S series camera with ToupView V 3.2 software.
The mycelia were recolted with the aid of a lanceolate needle previously flame-sterilised.
Saline solution spreading of the material: A small amount of biological material was suspended in a drop (2-3 mm diameter) of Ringer-Locke solution on a alcohol-degreased microscopical slide previously flame-sterilised (the heating of the slide increases the surface's hydrophobicity helping the formation of small, almost spherical liquid droplets, thus enhancing the homogenous distribution of the biological material between the slide and the coverslip).

A alcohol-degreased coverslip is placed with care (in order to not include air bubbles) onto the droplet containing the biological material.
A small pice of blotting paper is placed over the coverslip, after a little pressure being exerced by gently tapping onto the coverslip with a match. The Ringer-Locke solution excess is absorbed by the blotting paper. Care should be exerced in order to not smash excessively the biological material. Staining was performed by the following method: A drop of the staining solution is placed at one of the coverslip margins. A piece of blotting paper is placed at the opposite margin of the coverslip, which will absorb the Ringer-Locke solution, permitting the access of the staining solution by capilarity under the coverslip.
Dye solution spreading of the material: A small amount of biological material is suspended in the same conditions as above, but in a staining solution.
Heating the preparation after adding the staining solution isn't always necessary, but can be helpful when dealing with mycelia which don't „catch" the stain at room temperature. In any case, care must be exerced in order to avoid the boiling of the solution, which can led to the formation of small air bubbles between the slide and the coverslip. The best methods are: i) „step" onto a small, blue burner flame some two to five times with the preparation (coverslip side up) for one to two seconds. The preparation must be warm, not hot (check by touching with the preparation the back of the hand – the slide must be supportably warm; and ii) heat the preparation on a hot plate setted at about 50 degrees Celsius for 5 to 10 seconds, then check the temperature as previously. The entire procedure must not be repeated more than two times, and the preparations must be examined as soon as possible, but not later than 5 to 10 minutes.
The margins of the coverslip and its' superior surface are gently wiped with blotting paper and the preparation can be examined. The doccumentation will take place as soon as possible, because the aqueous solutions can evaporate in less than half an hour and some of the preparations transferred in lactophenol did not reatin the stain in a satisfactory manner, because the dye dissolves in the lactophenol.

Table 1. Name, Colour Index recommended name and number, chemical category and synonims of the stains quoted in the text.

Name	Colour Index recommended name and number	Category	Synonims
Aniline Blue, water soluble (Mixture of Methyl Blue * and Water Blue **	* Acid blue 22 (42.755) ** Acid blue 93 (42.780)	Acid, Triarylmethane	*China Blue, Cotton Blue, Methyl Blue ** Water Blue, Aniline Blue, acid
Sudan III	Solvent Red 23 (26.100)	Azo, Lysochrome	Sudan Red, Sudan Red BK
Eosin B	Acid Red 91 (45.400)	Acid, Fluorone, Xanthene	Eosin Red, aqueous, Eosine bluish, Imperial Red
Toluidine Blue	(52.040)	Basic, Thiazine	Toluidine Blue
Trypan Blue	Direct Blue 14 (23.850)	Acid, Azo, Vital	Niagara Blue 3B
Phloxine B	Acid Red 92 (45.410)	Acid, Fluorone	Phloxine
Acid Fuchsin	Acid fuchsin (42.685)	Acid, Triarylmethane	Acid Magenta, Acid Rosein, Acid Rubin, Acid Violet 19, Rubin S
Janus Green B	Diazine green (11.045)	Basic, Phenazine, Azo, Vital	IDEM!
Congo Red	Direct Red 28 (22.120)	Acid, azo	Direct red, Cotton red
Chrysoidin	Basic Orange 2 (11.270)	Acid, azo	Chrysoidin G, Chrysoidin Y

Table 2. The preparation methods for the mounting media and the iodine-based reagents

Reagent	Quantity	Function	Compounding procedure
Amann's lactophenol (Amann, 1896; Langeron, 1949; Langeron & Vanbreuseghem, 1952; Constantinescu, 1974)			
Phenol	10 g	Clarifying and fixing agent	The liquids must be weighed, not measured. When the mixutre is homogenous, add the phenol. Freshly prepared is transparent, but with time it becomes yellow than deep brown.
Lactic acid	10 g	Fixing agent and solvent	
Glycerol	20 g	Clarifier and solvent	
Distilled water	10 g	Solvent	
Results	Claryfies and permanentises the microscopical preparations. In time it can get brown due to the oxydation, leading to a possible permanent darkening of the preparation, making it impossible to be examined.		
Amann's chloral-lactophenol (Langeron & Vanbreuseghem, 1952)			
Phenol	10 g	Clarifying and fixing agent	As the previous solution
Chloral hydrate	10 g	Clarifying and fixing agent	
Lactic acid	10 g	Fixing agent and solvent	
Results	Claryfiing agent more effective than the „classical" lactophenol (n = 1..49), but also softens and inflates the material more.		
Berlese mounting medium modified acc. to M.A. Ionescu (Ionescu, 1937; Toma & Anghel, 1976)			
Chloral hydrate	40 g	Clarifying agent	See below the „Observations" rubric
Gum acacia (Gum arabic)	10 g	Density enhancer	
Glucose syrup	5 g	Density enhancer and solvent	
Glacial acetic acid	5 g	Acidifying agent and solvent	
Anhydrous glycerol	5 g	Solvent	
Observations	Weigh all the ingredients. Dissolve the gum acacia (15 g) in 20 g of water at room temperature for one day with occasional stirring. After all the impurities sedimented, filter through glass wool. Dissolve the glucose (15 to 20 g) in the minimum amount of water (about 15 mL) with stirring and heating on water bath, in order to obtain a honey-like consistency. The chloral hydrate is put in a flask, adding the solutions of gum acacia, glucose, the acetic acid and the glycerol. The flask is heated on a water bath until the mixture liquefies. The solution is decanted the next day, in order to permit the impurities eventually present to sediment. The solution must be kept in a dark glass-stopped bottle.		
Results	Claryfies and permanentises the microscopical preparations. The degree of clarification is higher compared to the lactophenol. Also, the medium does not darkens due to oxydation.		
Lugol's iodine solution			
Potassium iodide	2 g	Increases the iodine solubility	Dissolve the potassium iodide the necessary quantity of water (approx. 10 mL), then add the iodine and disolve it. Add the rest of the water and homogenise.
Iodine	1 g	Reagent for the amyloid	
Distilled water	100 mL	Solvent	
Results	The amyloid components of the fungi stains blue, the non-amyloid stains dark yellow to brown.		
Melzer's iodine reagent (Melzer, 1924)			
Chloral hydrate	100 g	Clarifying and fixing agent	Prepare the iodine-iodide solution as previously indicated (Lugol's solution). Add the chloral hydrate and homogenise. Use as such.
Potassium iodide	5 g	Increases the iodine solubility	
Iodine	1,5 g	Reagent for the amyloid	
Distilled water	100 mL	Solvent	
Results	Same results as the Lugol, but the material is clarified due to the chloral hydrate.		

Table 3. Preparation of the simple stains solutions

Reagent	Quantity	Function	Compounding procedure
Sudan Red – Lactopenol (saturated solution) (Langeron & Vanbreuseghem, 1952, Constantinescu, 1974)			
Sudan III	q.s.	Lipophyle stain	Dissolve the stain in the lactophenol in small portions, until a parcel remains undisolved. Filter and use as such.
Lactophenol	100 mL	Solvent, clarifying and fixing agent	
Results	The lipohyle parts of the fungi stains reddish-orange against the almost clear background.		
Observations	Even if 0,1 g of Sudan III are used, the dye dissolves poorly, resulting a supersaturated solution which precipitates when in contact with serum or distilled water. Better results are obtained when mycelia is placed directly in the stain than when placed in saline which is replaced by the stain.		
Acetic Cotton Blue (Langeron & Vanbreuseghem, 1952)			
Cotton Blue C4B	0,5 g	Stain	Dissolve the stain in the distilled water. Add the acetic acid and homogenise. Use as such.
Glacial Acetic acid	3 mL	Acidifier	
Distilled water	100 mL	Solvent	
Results	The fungi stains blue against the transparent ground. This aqueous acidified solution is comparable in almost all aspects with the lactophenol one, with the exception that it doesn't clarify the examined material.		
Toluidine blue O (Sangeetha & Thangaduray, 2013)			
Toluidine blue O	0,1 g	Stain	Staining solution (see Observaqtions)
Distilled water	3 g	Solvent	
37% Hydrochloric acid	0,1 mL	Acidifier	
Absolute ethanol	7 mL	Solvent	
Glacial acetic acid	4,5 mL	Solvent	Sulfatiomn reagent (See Observations)
98% Sulphuric acid	1,5 mL	Sulfation reagnet	
Results	The fungi stains reddish-blue against the pale blue ground.		
Observations	Staining solution: Dissolve the stain in the water, add the acid, homogenise, ad the etahnol and homogenise. Sulfation reagent: Pour dropwise carefully the sulphuric acid in the acetic acid, mix well and let cool. Add the sulfation reagent to the wet preparation: 10 mins. Replace with water (4-5 times). Add the stain solution: 3 mins. Rinse sucessively with 95% Ethanol: 10 sec., Absolute ethanol: 10 sec., Xylene: 10 sec., mount and examine		
Trypan blue (Constantinescu, 1974, Schunert et al., 1987)			
Trypan blue	0,1 g	Stain	Dissolve the stain in the buffer solution, check the pH and homogenise. Use as such.
Acetic acid acid – sodium acetate buffer solution acc. to Walpole, pH = 6,4	9,9 mL	Solvent and buffer component	
Results	The fungi stains greyish-blue against the transparent ground.		
Observations	According to Schubert et al., (1987), the stain can be dissolved at 0,05% in lactophenol. The aqueous buffered solution did not worked accordingly, the lactophenolic one sucessfully stained the fungi.		
Eosin (Langeron & Vanbreuseghem, 1952)			
Eosin	1 g	Stain	Dissolve the stain in the solvent. Use as such.
Distilled water	100 mL	Solvent	
Result	The fungi stains vivid pink against the transparent ground.		
Observation	This is a personal modification of the Phloxine staining method, as the two dyes have very similar structures (also indicated by their C.I. numbers). Also, Langeron & Vanbreuseghem, 19XX claim that the stain to be dissolved in a phenol solution.		
Lactophenolic Rubin S (Langeron & Vanbreuseghem, 1952; Constantinescu, 1974)			
Rubin S	0,1 g	Stain	Dissolve the stain in the solvent. Use as such.
Lactophenol	99,9 g	Solvent and clarifying agent	
Results	The fungi stains vivid reddish-violet against the transparent ground.		
Mayer's Mucicarmine (Gabe, 1968; Sangeetha & Thangaduray, 2013)			
Mucicarmine	0,30 g	Stain	Dissolve the satin in the alcohol. Dilute 1:4 (v/v) with distilled water before use.
Ethanol 50%	20 mL	Solvent	
Results	The mycelia stains violet		

Observations	The fungi species (or at least the strains used in this work) stained poorly with this solution, even when the preparation was heated.		
Janus Green (Constantinescu, 1974)			
Janus green	0,005 g	Stain	Mix the two buffer solutions, homogenise and check the pH. Dissolve the dye in the solution and mix well. Use as such.
0,2 M Acetic acid	25,5 mL	Buffer solution acc. to Walpole, pH = 4,6	
0,2 M Sodium acetate	24,5 mL		
Result	The fungi stains green-bluish against the transparent ground.		
Congo Red (Langeron & Vanbreuseghem, 1952; Constantinescu, 1974)			
Congo Red	Stain	5 g	Mix the two components.
Distilled water	Solvent	100 mL	
Results	The fungi stains red against the pale ground.		
China ink (Langeron & Vanbreuseghem, 1952; Rapilly, 1968; Constantinescu, 1974; Sangeetha & Thangaduray, 2013)			
China ink	1 mL	Contrast substance	Mix the two components.
Distilled water	5 to 10 mL	Solvent	
Results	As this is not a stain *per se*, this solution will provide a black background against which the colourless fungi can be observed.		
Observations	The China ink can be used as such (without dilution). Better results are obtained when mycelia is placed directly in the China ink than when placed in saline which is replaced by the China ink.		
Nigrosin, water soluble (Langeron & Vanbreuseghem, 1952; Constantinescu, 1974)			
Nigrosin	5 g	Contrast stain	Mix the two components.
Distilled water	100 mL	Solvent	
Results	Incolor fungi on a black ground.		
Dosch's Chrysoidine (Langeron & Vanbreuseghem, 1952)			
Chrysoidine	2 g	Stain	Dissolve the stain in water. Use as such.
Distilled water	100 mL	Solvent	
Results	The fungi stains bright orange against the transparent ground.		
Observations	Use only 0,1 g of stain. Homogenise and use as such.		

Table 4. Preparation of the double stains solutions

Reagent	Quantity	Function	Compounding procedure
Cotton Blue C4B - Sudan Red – Lactophenol (Langeron & Vanbreuseghem, 1952)			
Saturated Sudan Red solution in lactophenol	100 mL	Staining, clarifying and fixing agent	Prepare the saturated Sudan Red solution in lactophenol as indicated below (Sudan red in lactophenol). Add the Cotton blue and homogenise.
Cotton Blue C4B	0,5 g	Stain	
Results	The hyphae stains blue, some parts of them stains light orange due to the presence of lipophyle structures.		
Observations	Even if 0,1 g of Sudan III are used, the dye dissolves poorly, resulting a supersaturated solution which precipitates when in contact with serum or distilled water. Better results are obtained when mycelia is placed directly in the stain than when placed in saline which is replaced by the stain.		
Picro-Nigrosin – lactophenol (Langeron & Vanbreuseghem, 1952; Constantinescu, 1974)			
Nigrosin	0,2 g	Stain	Dissolve the nigrosin in the picric acid saturated solution. Use this solution instead of water to prepare the lactophenol: The liquids (minus the stain solution) must be weighed, not measured. When the mixutre is homogenous, add the phenol.
Picric acid, saturated aqueous solution	10 mL	Stain and solvent	
Phenol	10 g	Clarifying and fixing agent	
Lactic acid	10 g	Clarifying and fixing agent	
Glycerol	20 g	Clarifier and solvent	
Results	The fungi stains yellowish – light green with darker parts agains the pale ground.		
Observations	Better results are obtained when mycelia is placed directly in the stain than when placed in saline which is replaced by the stain.		

RESULTS AND DISCUSSIONS

The vital or post-vital staining methods and colour reactions used were compiled and adapted from various authors (see bibliography). Most of the methods are merely "citations of other citations", as in some cases is almost impossible to find the original source (even if the author's name is known, some of the periodicals in which the paper was published are not available, not even in electronic format, also being quite difficult to find a physical copy. Thus, four major works have been used, two classical ones (Langeron, 1949, Langeron & Vanbreuseghem, 1952), a modern one (Constantinescu, 1974) and a recent one (Sangeetha & Thangadurai, 2013).

In the investigated literature are quoted more techniques, which at the time they have been developed and played an important role in mycology. Amann (1896) prepared and used for the first time a mixture which later becomed one of the most precious aid in fixing, clarifying, observing and mounting fungi – the "lactophenol" ("the mycological reagent by excellenece", as Langeron (Langeron, 1949, Langeron & Vanbreuseghem,1952) classified it).

The original recipe suffered some modifications in order to increase its' refractive index, originally n = 1,44 ("hydrated lactophenol") to 1,48 ("anhydrous lactophenol"). So, by adding chloral hydrate, the refractive index raises up to n = 1,49 or even more as in the case of the Berlese's medium modified acc. to Ionescu (1937), quoted by Toma & Anghel (1976) which provides a permanent mounting medium which claryfies the material more effective than the lactophenol. Due to the fact that the phenol itself auto-oxydises in time in contact with the atmospheric oxygene, its' colour changes gradually from translucent to cherry-red, due to a multitude of oxydation products with complex formulae, thus the lactophenol also brunishes with time, becoming yellow then dark brown, process which is accelerated in the slide preparations, even if the coverslip borders have been sealed.

Various staining methods, more or less specific, from which ones have been specially developed (e.g. lactophenolic acid aniline blue, which the mycologists use under a older chemical synonim – Cotton blue) (Şerbănescu-Jitariu et al., 1983) or adapted from the botanic or zoological histology protocols (e.g. eosin) have been used in order to evidentiate specific morphological and anatomical structures pertaining to the fungi, both free or inside their plant or animal hosts.

Some of them lack the precise preparation or clearly defined structure as required by chemistry, as in the case of some stains which are used in saturated or even supra-saturated solutions. In some cases, the stain concentration in the specific solvent – in this case the lactophenol - need to be raised in order to obviate in some cases the low permeability of the fungal cell wall.

But, in some cases, empirical formulations have been used as such (apparently succesfull), as in the case of a sulfanylamido- conjugated stain (sulphanylamidochrysoidine), initially developped as a chemoterapeutic agent (Rubiazol™, "Prontosil soluble") by I.G. Farbeindustrie and later used by Dosch to stain fungal structures. At the time, probably the indicated concentration (2%) of the sulphanilamido-chrysoidine worked, but the actual water solubility of the stain itself (chrysoidine) is way lower. In this work, a sample of the stain dissolved at 0,1 to 0,5%, thus, the original concentration was re-calculated, a final 0,5% aqueous chrysoidine solution giving far better results. But, when used at the prescribed 2% concentration, even if as such, (so not even in contact with the lactophenol) it began to crystallise under the coverslip, making the preparation quite embarassing to examine due to a multitude of small dye crystals floating in the solution.

The same happened when using Janus green (as a vital stain) and partially with aqueous eosin. Some other stains quoted in the literature (e.g. lactophenol-saturated Sudan III solution) didn't worked simply because the stain didn't disolved completely, even at 0,1%, resulting a solution of the stain which contained small particles of undisolved dye. From four different batches tested, none of them dissolved at concentrations higher than 0,025 to 0,04 %. The conservation of the stain by the examined structures in lactophenol was poor, the dye dissolving in the mounting medium, with the exception of some of the staining solutions in which the solvent was the lactophenol itself, thus making almost impossible to make permanent preparations – the almost immediate doccumenting (microphotography) being necessary.

The China ink, which when used (according to one of the quoted references) diluted 1:1,5 with saline or distilled water formed a solution, which even if appeared as black, at the microscope was light grey. The China ink was used undiluted, with good results. Instead of using a saturated solution of Cotton blue in lactophenol, a 0,5% solution gave same (even better) results.

Same problem apply to othe staining solutions, which at the time they were developped little or not at all was known about the best concentration to be used or to the method of progressive staining. In all those cases, the anatomy of the investigated fungi and the impediment of the host's +/- lignified tissues (with some exceptions) the use of concentrated solutions was necessary. Afterwards, by routine, those concentrated solutions were used for staining even thin-walled fungi. The author does not doubt in any moment the level of expertise of the researchers who in a brilliant manner discovered a multitude of fungal species and unveiled large areas of their unknown universe. He can only simply give some advices concerning the concentrations of the staining solutions, which, in his opinion, can be adjusted so as their staining properties remain the same, without dispensing quite significative amounts of dyes which only role is to assure a "saturated" solution prepared "according to the literature".

The dyes (with some exceptions, as in the case of the stabylised diazonium salts or of some compound dyes, as is the mucicarmine), due to their complex structure, are stable and conserve their staining properties over long periods of time. This fact is important, as the named substances can be sucessfully conserved and used (it's important to test their staining properties prior to use, though) over long periods of time. Also, by using more diluted solutions (progressive staining) or preparing smaller volumes it can improve: i) the laboratories' working costs (by avoiding useless spills of over-saturated solutions), ii) the environment protection, by minimising the amount of stains used and therefore the quantity of waste solutions resulted which need proper collection and withdrawal, and iii) the worker's health, as more of the stains and other chemicals used (e.g. phenol) are, if not carcinogenic or mutagenic, at least irritant or simply harmful.

Some of the staining and mounting methods are not currently used today, some of them are not widely known, but in some cases they can led to interesting results (e.g. the modification of the Berlese's chloral-lactophenol mounting medium modified acc. to Ionescu) which provides a permanent mounting medium which claryfies the material more effective than the lactophenol. In some cases, the lactophenolic solutions of stains gave good results in the author's hands, but the aqueous solutions of the same ones used for the staining, when replaced with lactophenol under the coverslip didn't conserved the coloration, because the dye used simply dissolved in the mounting medium.

The use of the China ink diluted 1:3 v/v with saline serum or distilled water (or water-soluble nigrosin) in order to obtain a black fundal on which the fungi would appear transparent didn't worked, but the undiluted China ink worked accordingly.

Some of the methods which were developed for the study of the fungal organisms inside their hosts could not be tested simply because they have been developed for working on sections fixed on slides. Even if some of the spores or hyphae affixing methods on slides have been elaborated, the proportion of the organisms sucessfully retained thorough all the stining process is quite low. When using a 0,1% w/w collodion solution (nitrocellulose dissolved in a 1:1 v/v absolute ethanol-dry diethyl ether) film in order to obtain a transparent membrane to hold in place the fungi as in the animal histology, the affixing were good, but the structure conservation was affected.

It may be possible that the quoted methods would work with another fungi species, or at least with other strains or cultivation methods of the two species used hereby by the author. The colleagues which kindly provided to the author the cultivated fungi have done their best to obtain cultures comparable in any aspect to the ones they use.

As for the stains used in this study, their tinctorial properties are fully conform with the literature and the results obtained by using them are perfectly comparable with the expected ones, at least when they were used in the histological study of other organisms by the author.

CONCLUSIONS

The 0,5% lactophenolic Cotton blue, the 0,1% lactophenolic Rubin S (Figure 1), the 0,05% lactophenolic Trypan blue (Figures 2, 3 and 4), the lactophenolic Picro-Nigrosin (Figure 5), the 0,5% aqueous Eosin (used instead of Phloxine, due to their very similar structures) (Figure 6), the 0,5% aqueous Chrysoidine (Figure 7), and the buffered Janus green solution stained well (Figure 8).

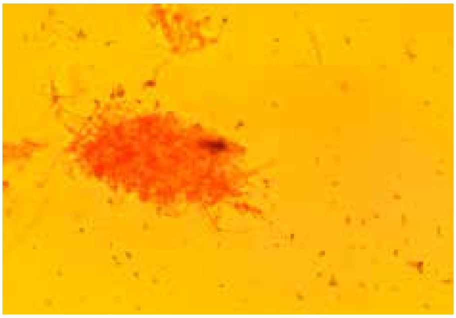

Figure 1. *Fusarium* sp. – 0,1% lactophenolic Rubin S – 200x

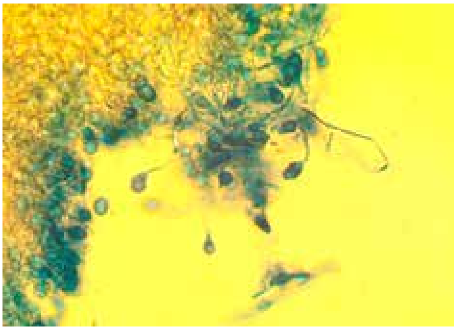

Figure 2. *Fusarium* sp. – 0,05% lactophenolic Trypan blue – 400x

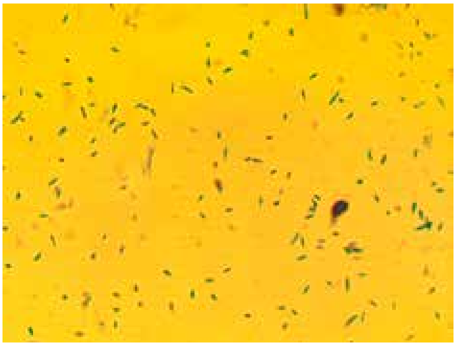

Figure 3. *Fusarium* sp. – 0,05% lactophenolic Trypan blue – 1000x

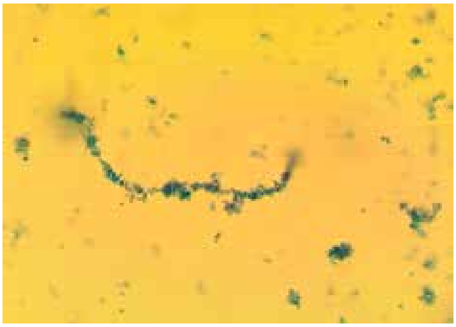

Figure 4. *Beauveria bassiana* – 0,05% lactophenolic Trypan blue – 1000x

Figure 5. *Fusarium* sp. – 0,1% lactophenolic Picro-nigrosin – 400x

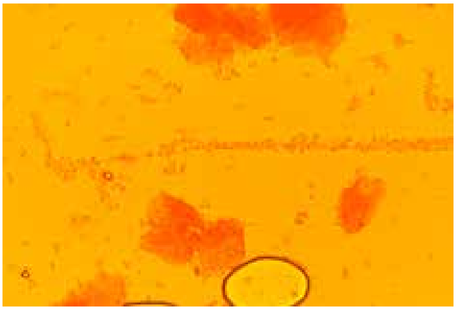

Figure 6. *Beauveria bassiani* – 0,5% aqueous Eosin – 400x

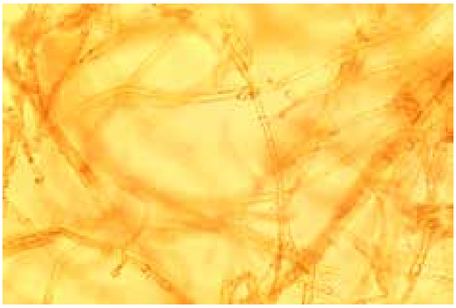

Figure 7. *Fusarium* sp. – 0,5% aqueous Chrysoidine – 400x

Figure 8. *Beauveria bassiani* – 0,1% aqueous buffered Janus green – 400x

The buffered Trypan blue solution didn't worked accordingly, the tissue stained lightly. The Mayer's Mucicarmine solution did'n worked at all. The Sudan III-lactophenol solution, used on saline-suspended hyphae precipitated the stain in small crystals. When hyphae were placed in the same solution, they stained light yellowish, but the solution also crystalised. The Cotton blue – Sudan III in lactophenol stained deep blue the hyphae (at least at the margin of the examined sample), only a small central portion becoming faint yellow.

The structures didn't retained any stain when mounted in lactophenol (even if they were examined one day later), with the exception of Cotton blue and Trypan blue (100% colour intensity conserved), Eosin red (75 to 100%) and Rubin S (roughly 50%).

The staining methods (from which the cotton blue-lactophenol appears to be the preferred one nowadays) currently in use are well-established and well known. But, what can appear in this work as „personal preferences" can be of interest in studying a more broad array of fungi that the two species that the author had at hand.

By using some of the techniques detailed here, it would be possible to gain some interesting structural and morphological criteria of identification, or at least some well evidentiated taxonomic characteristics which could be helpful in our comprehension of the representants of such a diverse phylum as Mycota is.

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COMPARATIVE LEAF AND FLOWER MORPHO-ANATOMICAL STUDY OF WILD AND CULTIVATED GOJIBERRY (*LYCIUM BARBARUM* L.) IN ROMANIA

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Abstract

Goji berry (Lycium barbarum L.) is widely used as food and medicine in Asian countries and recently had a dramatic gain in popularity on American and European continents. Due to their complex composition and recommendations in traditional Chinese Medicine, goji berry is also one of the most studied species in the recent years. The species grow wild in Romania, being appreciated for its bush density for hedges and fences. Initially, imported L. barbarum varieties were used by goji berry growers for commercial plantations, while in the last year five new varieties were homologated. The morpho-anatomical structure of leaves and flowers of the wild and cultivated goji berry from the Bucharest area was compared, to determine important traits that could be relevant for goji breeders but also for taxonomists. Morphological differences were found regarding the leaves shape, position, and leaves width. The wild L. barbarum has cuticle-covered leaves, highly developed vascular bundles and vascular bundle sheaths were present in the mesophyll. The palisade cells appeared to be very large. These characteristics of the leaf's anatomy are also relevant in the context of biotic stressors, as eriophyid mites, that are one of the most important pests of goji berry shrubs.

Key words: *wild and cultivated Lycium barbarum, leaf anatomy, mesophyll, vascular bundle.*

INTRODUCTION

Goji is the generic name for different plant species from the genus *Lycium*, belonging to the Solanaceae family. It represents one of the most popular products of traditional Chinese medicine that is also used outside of China (Wetters et al., 2018). The genus *Lycium* includes more than 80 distinct species, distributed in temperate or sub-tropical regions (Levin & Miller, 2005).

The exact origin of *L. barbarum* is not known, but its natural habitat is between Southeast Europe and Southwest Asia. While the wild *L. barbarum* is also known as fences sea buckthorn, the "goji" name can be considered a trading name, given initially by the North American ethnobotanist Bradley Dobos, being the species with the most significant commercial value (Tabără, 2020a, 2020b). Moreover, given that *L. barbarum* L. is a naturalized species in most countries of the world, this has hindered the process of differentiation between the species and especially between the subspecies of this shrub. *L. barbarum* is also found in Romania, being observed in the Dobrogea area, including the Danube Delta (Doroftei, 2009), in the region of Moldova, in Oltenia, where it was described as an adventitious plant with a pronounced invasive character (Răduţoiu & Stan, 2013) and in the city of Timișoara (Coste & Arsene, 2003).

The invasive potential of this species was also observed in 34 localities in Oltenia (Răduţoiu & Băloniu, 2021). Also, *L. barbarum* is found in the spontaneous flora of the Republic of Moldova, being an allochthonous and naturalized plant that was introduced from China for ornamental purposes and penetrated into natural ecosystems, acquiring a potentially invasive character (Palancean, 2015).

While originally grown as a health food in Asia, *L. barbarum* and its berry are now known worldwide for its health benefits, including a high fiber, potassium, magnesium, iron, vitamin E, vitamin C, carotenoids, and beta-carotene content (Niro et al., 2017). *L. barbarum* plant has been consumed for over 2500 years with no toxicity being reported (Donno et al., 2015). The fruit, leaf, root bark of many species of the genus *Lycium* have long been used as local foods and/or medicines. Recently, *Lycium* fruits, have become increasingly popular in the western world because of their nutritional properties (Amagase and Farnsworth, 2011; Mencinicopschi, 2013a, 2013b; Asănică et al., 2016; Qian, et al., 2017), they are even advertised as "superfood" in Europe and North America (Chang & So, 2015). Phytochemical studies indicate that the richness in numerous constitutions of different classes, such as polysaccharides, carotenoids, flavonoids, alkaloids, amides, terpenoids, endows *Lycium* species with a variety of biological activities (Yao et al., 2011; Qian, et al., 2017;). Plant-based products are important sources of both food and medicine. Whether a plant is used as food or medicine depends on a wide range of factors but is not necessarily due to its pharmacological or nutritional properties (Leonti, 2011; Jennings, et al., 2015). *L. barbarum* has special medicinal properties, so in recent years this plant has gained more and more attention from consumers due to its antioxidant properties and for improving vision (Shen et al., 2012), anticancer and immunomodulatory effects (Gan et al., 2004; Tang et al., 2012), antioxidant activity (Henning et al, 2014; Benchennouf et al., 2017; Shi et al. 2017), hepatoprotective activity (Ahn, et al., 2014), hypoglycemic properties (Guowen et al. 2010), neuroprotective effects (Ho et al., 2010). Especially polysaccharides, zeaxanthin dipalmitate, vitamins, betaine, and mixed extracts were reported to be responsible for anti-aging, improving eyesight, and anti-fatigue effects (Wu and Guo, 2015; Yao et al., 2017).

A protective property of goji extracts on retina cells has been proved in the early stage of the degeneration of the retina. It is proposed that absorbing the light zeaxanthin and luteolin present in goji fruit displays an inhibitory effect on neuron apoptosis (Ni et al., 2013), antioxidant and antimicrobial activities (Mocan et al., 2014).

In the present paper, we compared for the first time the morphology of the leaf and the flower and the anatomical structure of the leaf lamina of the wild and cultivated species. These studies highlight morpho-anatomical characteristics of the species. As the leaves are the plant organs with the highest plasticity and receptivity to changes in the environment, we focused on the leaf anatomy.

MATERIALS AND METHODS

The wild goji leaves and flowers were gathered form the spontaneous flora, from the Morii Lake shore (Ilfov county) and the cultivated goji leaves and flowers were taken from the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania. From both sites, samples were collected during the flowering period, from August to October 2021. A collector number was given, and the specimens were dried according to standard herbarium methods. The specimens are kept in the herbarium of USAMV Bucharest. The leaves were sectioned by hand using razor blades to make semipermanent and permanent slides for microscopic studies. The sections were cleansed with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine (Georgescu et al., 2015). Analyses and observations of the cross-sections were performed at the Research Center for Studies of Food Quality and Agricultural Products, at USAMV Bucharest. Photos were taken and measurements were made using the Leica DM1000 LED microscope, equipped with a Leica DFC295 video camera and with a Leica S8 APO Stereomicroscope, as well as a Sony photo camera. Photos were taken using the light microscope at different magnifications.

RESULTS AND DISSCUSIONS

Leaves morphology

Studies related to leaf morphology and micromorphology were made by Săvulescu et al., 2019: Luchian et al, 2019, 2020, 2021; Toma et al., 2021; Vârban et al., 2021. The leaves of the studied shrubs were solitary or arranged in bundles 5-16 (rosettes) especially in the wild *L. barbarum* (Figure 1). The solitary leaves that had an alternating disposition predominated on the new shoots that emerged in the second half of the growing season. The wild goji had the leaves grouped in bundles of 5-16 leaves each, on the branches of previous years or at the base of the yearly shoots. The shape of the leaves was obovate (Figure 2), elliptical in wild goji and mainly lanceolate or elliptical and leathery in the cultivated goji (Figure 3). The wild plants had leaves with a blade of 5-9 cm long, 1.5-2.5 cm wide and the petiole 0.5-1.5 cm long, while the leaves of cultivated plants were 2.5-5.5 cm long, 0.4-2 cm wide and a petiole 0.3-0.4 cm long.

Figure 1. Obovate leaves arranged in bundles in the wild *Lycium barbarum*

Figure 2. Obovate leaves and flower of wild *Lycium barbarum*

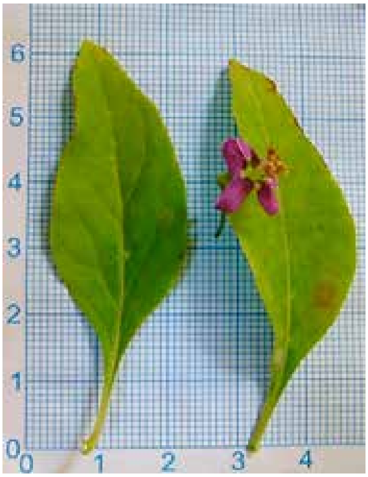

Figure 3. Lanceolate leaves of cultivated *Lycium barbarum*

Flower morphology

Studies related to flower morphology were made by Anghelescu et al., 2021. On the analyzed goji shrubs, all the developmental stages of flowers and fruits were found simultaneously. The flowers of the shrubs appear in the nodes area, being solitary or in inflorescences of 3-5 flowers each. The flowers have a calyx with 2-3-4 sepals, pubescent (the calyx predominates with 2 sepals, sometimes a sepal is bent at the top), the corolla is rotated, having 5-6-7 petals (predominantly corolla flowers with 5 petals) of one-color, from lilac to an intense purple (Figures 4-7). The petals of wild goji have a corolla tube of 0.9 cm, and the lacini are 0.5-0.8 cm and 0.4-0.5 cm wide, the diameter of the open flower can reach 1.9-2 cm. In wild and cultivated goji, the five anthers open longitudinally, from the top to the base (Figures 8-9). In the wild species the anthers have filaments 1-1.4 cm long, and in the cultivated one the length of the filaments is 1-1.6 cm long. Towards the end of their developmental cycle, the flowers gradually lose their color and become beige.

Figure 4. Hairy calyx, with two sepals on wild *Lycium barbarum*

Figure 5. Two-lobed calyx on wild *Lycium barbarum*

Figure 6. Wild *Lycium barbarum* corolla with 5-6 petals

Figure 7. Corolla tube of a wild *Lycium barbarum* flower

Figure 8. Flower of cultivated *Lycium barbarum*

The corolla tube has hairs at the top, towards the lacins, both in the wild and cultivated *L. barbarum*. Also, the stamens filaments have hairs towards the base, in the form of a tuft, in both cultivated and wild goji (Figure 10). All goji flowers have darker stripes on the petals. The petals of wild goji have multicellular hairs, rarely arranged on the edge of the petal, and in the cultivated species multicellular hairs are observed, abundant (Figures 11-12).

Figure 9. Stamen's detail of anthers, with longitudinal opening

Figure 10. Hairs in the upper part of the corolla tube and at the base of the stem filaments on wild *L. barbarum*

Figure 11. Rare hairs on a wild *Lycium barbarum* petal

Leaves anatomy

Research on the anatomy of the genus Lycium has been done by Jobert et al., 1984; Norverto, 2000; Selvi et al., 2009; Tabără, 2020a; Konarska, 2018; Amanova & Duschanova, 2021.

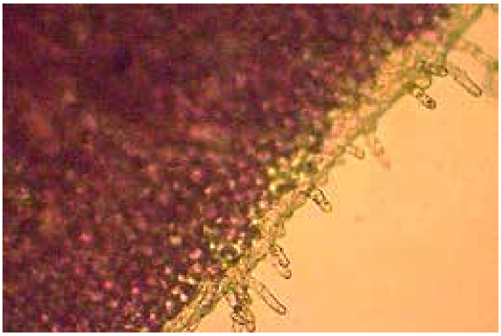

Figure 12. Dense hairs on a cultivates petal of *Lycium barbarum*

The anatomy of the leaves was studied in sections, on fresh or fixed preparations. On the transverse sections taken from the middle part of the leaf the following were observed: the epidermis is composed of a single layer of cells, and the cells are rectangular. The cells of the upper epidermis are larger than the lower epidermis ones, the walls of the cells are corrugated, and the epidermal cells are covered with a cuticle. Stomata are presented on both surfaces (amphistomatic leaves) and the stomata are more on the abaxial surface than on the adaxial surface. Stomata is generally present on both surfaces of the leaves, these findings also reported Metcalfe and Chalk, 1979.

Both epidermises are covered by the cuticle. In the wild goji, the upper epidermis was 11.3-11.5 µm, the cuticle 5.5-6 µm, and the lower epidermis 8.3-8.5 µm and the cuticle 5 µm. In cultivated goji, the upper epidermis was 15 µm and a cuticle of 5-5.9 µm, the lower epidermis was 6-8 µm and the cuticle of 4.8-5 µm.

The leaves of wild and cultivated goji possessed bifacial and amphistomatic structure with well-developed adaxial and abaxial epidermis (Figures 13-16). Upper epidermis of wild goji present elongated trichomes (Figure 17). The mesophile is arranged 1-2 layers of palisade on wild goji and 2-3 layers of palisade in cultivated goji (Figures 17-19). The shape of the palisade parenchyma in cross section is cylindrical. Over abaxial epidermis spongy parenchyma is disposed consisting of 2–4 layers circular or ovoidal cells. Adaxial phloem is absent from the smaller lateral veins. Middle vein on leaf is surrounded by parenchymatic cells, vascular bundles are bicollateral and usually cells and upper and lower parts are accompanied by collenchyma (Figure 20). The thickness of wild goji leaf was between 150-172 µm, and in cultivated goji the leaf thickness was 450-470 µm.

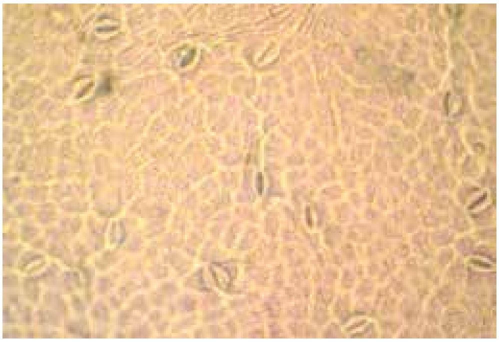

Figure 13. Upper epidermis in cultivated *Lycium barbarum*

Figure 14. Lower epidermis in cultivated *Lycium barbarum*

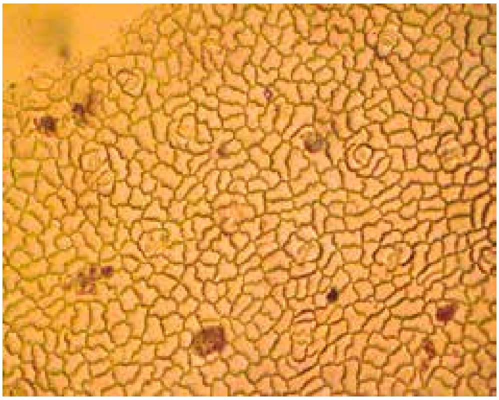

Figure 15. Upper epidermis in wild *Lycium barbarum*

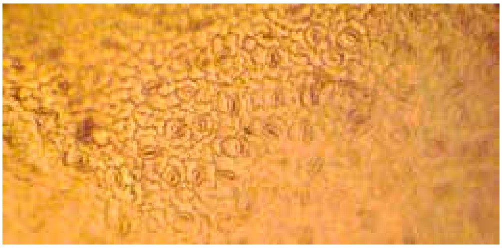

Figure 16. Lower epidermis on wild *Lycium barbarum*

Figure 17. Leaf-upper epidermis with trichomes, on wild *Lycium barbarum* leaf

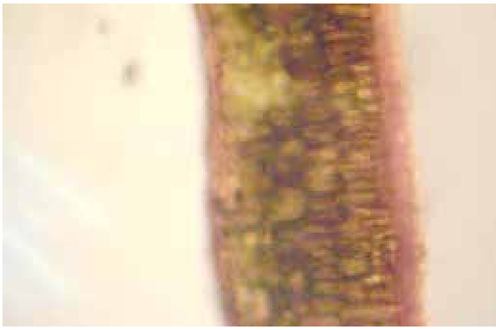

Figure 18. Cross section of a Lycium barbarum leaf - wild

Figure 19. Leaf anatomy on cutivated *Lycium barbarum*

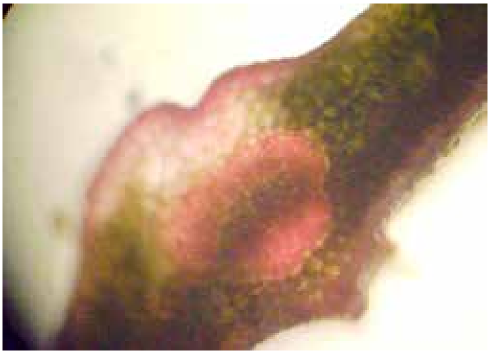

Figure 20. Midrib - bicollateral vascular bundle on wild *Lycium barbarum*

CONCLUSIONS

The anatomical studies carried out on the Lycium genus are just at the beginning, more aspects requiring in depth studies.

The micromorphological and anatomical characteristics are of great interest and significance to the discussion of the taxonomy of the species.

The petals of wild goji have multicellular hairs, rarely arranged on the edge of the petal, and in the cultivated species multicellular hairs are observed, abundant.

On the sampled wild goji, the upper epidermis is thinner than in cultivated goji and display elongated trichomes. The mesophile is arranged 1-2 layers of palisade on wild goji and 2-3 layers of palisade in cultivated goji.

The thickness of wild goji leaf was between 150-172 µm, and in cultivated goji the leaf thickness was 450-470 µm. These aspects will be further analyzed on multiple samples, collected from different places, to exclude the influence on climatic factor on the leaves.

The anatomical studies we performed on leaf cross-sections as well as on leaf surface sections demonstrate for the first time the anatomy of the vegetative organs of specimens growing wild in Romania. The microscopic observations made on leaves show structural-anatomical characters, with role in species adaptability and will be further analyzed on samples of *L. barbarum* collected from different places, to see the influence of climatic factors on the anatomical characteristics of leaves.

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RHIZOSPHERE EFFECT OF HORTICULTURAL PLANTS LETTUCE (*LACTUCA SATIVA* L.) AND TOMATO (*SOLANUM LYCOPERSICUM* L.) ON *NEMATOPHAGOUS* SPECIES FROM FUNGAL COENOSES

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Abstract

The presence of nematophagous fungi in microbial communities from horticultural plants grown in greenhouses is considered beneficial because they are possible biological control agents of plant-parasitic nematodes. Previous studies performed to elucidate the rhizosphere effect on nematode-trapping fungi are scarce and therefore is important to investigate the ecology of nematophagous fungi in the rhizosphere of different plants. The aim of this paper is to present the results of research carried out in greenhouse conditions to compare the rhizosphere effect of two horticultural plants, represented by lettuce (Lactuca sativa L.*) and tomato (Solanum lycopersicum* L.*) on naturally occurring nematophagous fungi with special focus on nematode-trapping fungi and endoparasitic fungi, as well as ecological aspects in fungal community structure and functions. Nematophagous fungi from lettuce rhizosphere belong to nematode-trapping species (Arthrobotrys oligospora, Dactylaria candida, Monacrosporium cionopagum) and endoparasitic species (Harposporium anquillulae,). Excepting Dactylaria candida, the same nematophagous species were identified in the rhizosphere of tomato plants. In both plant rhizospheres, different adhesive or non-adhesive hyphal structures to capture nematodes and non-adhesive infection conidia detected were photographed and presented.*

Key words*: nematode-trapping fungi, rhizosphere effect, microbial communities, endoparasitic fungi, biological control agents.*

INTRODUCTION

Rhizosphere is defined as the zone in soil which is influenced by the physical, chemical and biological processes of plant roots (Jeffery et al., 2010).
The composition of plant exudates depends on cultivar and is influenced by growth stage or plant exposure to stress.
Plants' exudates, also determine microbial species richness and their abundance in the rhizosphere (Singh et al., 2007; Verma et al., 2018; Alawyie & Babalola, 2019).
Plants are able to fix the carbon in the photosynthates, partly being transported into the root and excreted from root tissues in rhizosphere. Various metabolites secreted by the roots such as organic acids (aliphatic and aromatic acids - lactic, malic, oxalic, pyruvic, succinic, and amino acids), amides, carbohydrates (glucose, xylose, fructose), play chemotactic role. They are also plants growth promoting bioactive factors (Babalola, 2010).
Literature reported the antagonistic capacity of various fungi from rhizosphere of wheat (Matarese et al., 2012) or tomato against specific plant myco-pathogens (Mogle & Mane, 2010; Sharma & Singh, 2014; Jaiswal et al., 2017).
Nematophagous fungi, especially from rhizosphere of horticultural plants grown in greenhouses, play a beneficial role in biological control of plant-parasitic nematodes (Chen et al., 2020). Nematophagous fungi can be divided into four categories: nematode-trapping fungi, endoparasitic fungi, fungi which parasitise eggs and females, and toxin-producing fungi (Dackman et al., 1992).
Research has been carried out in greenhouse conditions to compare the rhizosphere effect of two horticultural plants, represented by lettuce (*Lactuca sativa* L.) and tomato (*Solanum lycopersicum* L.) on naturally occurring nematophagous fungi with special focus on nematode-trapping fungi and endoparasitic fungi, as well as ecological aspects in fungal community structure and functions.

MATERIALS AND METHODS

Rhizosphere samples (consisting in roots with closely adhering soil) were collected (Persmark & Jansson, 1997) by gently shaking for removing the most of the soil from the roots of 18 weeks old tomato plants (*Solanum lycopersicum* L.) cultivar Cindrel and lettuce (*Lactuca sativa* L.) cultivar Lolobionda cultivated in experiment under controlled conditions.
Research has been conducted in greenhouse of HORTINVEST-Research Center for Studies of Food Quality and Agricultural Products, from University of Agronomic Sciences and Veterinary Medicine – Bucharest, Faculty of Horticulture, during summer 2020.
Microbiological analyses were performed by soil dilution method on specific solid culture media for fungi (PDA and water-agar).
After 7 days incubation at 25ºC in the dark, colonies were counted and results expressed as colony forming units (c.f.u. x 10^{-3}), each reported to gram of dry soil (Matei, 2011).
Taxonomic identification was carried out using morphologic criteria, according to Domsch & Gams (1970) and Watanabe (2002) determinative manuals for fungi. Nematophagous fungi were identified using specific keys (Cooke & Godfrey, 1964; Schenck et al., 1977; De Hoog & Oorschot, 1985; Liu et al., 1992; Philip, 2001; Meyer & Carta, 2005; Kendrick, 2020; Zhang et al., 2021).
All morphological characteristics were measured under a MC 5.A optic microscope and photographs of different adhesive or non-adhesive hyphal structures to capture nematodes and non-adhesive infection conidia were taken by attached ToupCam.
The total number of species in community (S) was recorded for lettuce and tomato rhizosphere.
The ratio between the number of species in community and microbial effectives expressed species richness index (SR_2).
Comparative composition analysis in harvested, culturable fungal species from lettuce and tomato rhizosphere was performed to interpret the relationships between the two sets of species identified (Gentleman & Ihaka, 1996) by using a Venny 2.1 program to create Venn diagrams.
Similarity index (SI) between lettuce and tomato rhizosphere communities of fungi was calculated (Tiwari et al., 1994).
The Shannon index (*H'*), evenness (ε) were used to evaluate the fungal diversity (Mohan & Ardelean, 1993).
The index of Brillouin (1956) and Simpson (D) index (Stugren, 1982) were also used to calculate the diversity and “equitability” component of fungal species diversity.
The constancy, referring to the presence of the species in samples, was expressed by the value of frequency (F%) and species were grouped in: accidental species (F value by 25%), accessory species (F = 25-50%), constant species (F = 50-75%), euconstant species (F = 75-100%) (Mohan & Ardelean, 1993).
All assays were carried out in triplicate and results represent the average mean of 3 replicates.

RESULTS AND DISCUSSIONS

The results of microbiological analyses evidenced that composition of fungal cenosis and the relative abundance values of the species were influenced by rhizosphere environment, with higher number of species and lower abundance values in lettuce rhizosphere (Figure 1) and lower number of species with higher abundances in tomato rhizosphere (Figure 2).

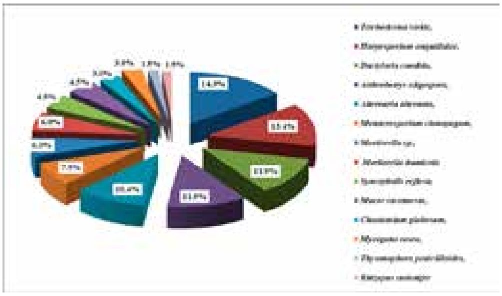

Figure 1. Percent mean relative abundance of fungal microflora composition in rhizosphere of lettuce

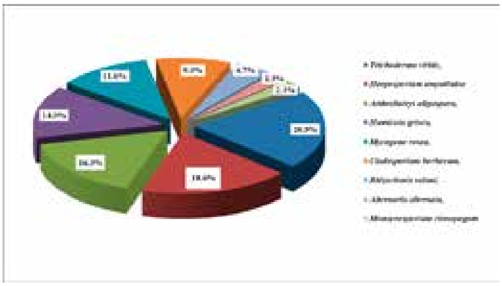

Figure 2. Percent mean relative abundance of fungal microflora composition in rhizosphere of tomato

There were clear differences in the relative abundances of the nematophagous fungal species between the crop plants. The data analysis showed that there was higher relative abundance of *Harposporium anguillulae* in rhizosphere of tomato plants (18.6%) than in rhizosphere of lettuce (14.6%). The nematode-trapping species *Arthrobotrys oligospora* was also found with higher abundance in rhizosphere of tomato (16.3%) than in rhizosphere of lettuce (11.9%, the same relative abundance as for differential species *Dactylaria candida*). Conversely, *Monacrosporium cionopagum* was most abundant in rhizosphere of lettuce (7.5%) as compared with rhizosphere of tomato (2.3%).
Examination of Petri plates by optic microscopy revealed morphological characteristics and specific fungal structures (Figure 3) developed by nematophagous species on water agar medium.

Figure 3. Conidia and adhesive reticulate traps of *Arthrobotrys oligospora* - tomato rhizosphere (300x)

Images ilustrate aspects of the specific morphology of conidia with apical cell twice larger than the basal cell (Figure 4) and three dimensional adhesive nets formed by *Arthrobotrys oligospora* to capture nematodes (Figure 5). Nordbring-Hertz & Stålhammar-Carlemalm (1978) found a lectin in the fungal net matching with a carbohydrate in the cuticle of the nematode. Tunlid & Jansson (1991) described the proteases involved in the infection and immobilization of nematodes by the nematophagous fungus *Arthrobotrys oligospora.*

Figure 4. *Arthrobotrys oligospora* conidia with apical cell twice larger than the basal cell - lettuce rhizosphere (600x)

Figure 5. Digestion of nematode trapped in adhesive reticulate net by predaceous fungus *Arthrobotrys oligospora* - lettuce rhizosphere (300x)

The endoparasites belonging to genus *Harposporium* are obligate parasites, nutritionally dependent exclusively on nematodes, which they infect with non-adhesive spores, respectively .
The nematode-trapping fungi (*Arthrobotrys oligospora, Dactylaria candida, Monacrosporium cionopagum)* may also feed saprophytically in soil and are less dependent on their ability to consume nematodes. In order to capture nematodes, they develop different traps: adhesive hyphae, three-dimensional networks, as previously presented for *Arthrobotrys oligospora,* adhesive branches to *Monacrosporium cionopagum*, or non-constricting rings and two branches joined to form a loop for *Dactylaria candida*) (Figure 6).

Figure 6. *Dactylaria candida* - nematode-trapping devices - lettuce rhizosphere (300x)

The fungi in both groups completely destroy the nematode host by enzymatic lysis followed by consumption of the body content (Figure 7).

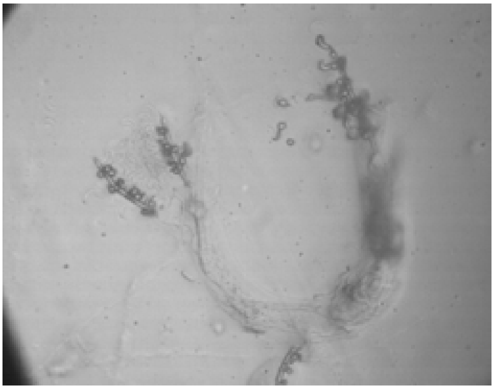

Figure 7. *Harposporium anquillulae* fungal structures emerged from digested body of the nematode (600x)

The image illustrates the conidiophores of *Harposporium anquillulae* emerged outside the destructed content of nematode body and sickle-shaped infecting conidia spread on the culture medium. After their ingestion by another nematode, the new infection appears even from the pharynx where the conidia stick.
The hyphal system developed inside the host dissolute its content and after this, penetrates the cuticle and develops its own structures outside, to continue the cycle of infections.
Further analysis of the other fungal components of rhizospheres revealed that, in both fungal communities, the most abundant species was antagonistic fungus *Trichoderma viride* (Figure 8) but with higher relative abundance value recorded for rhizosphere of tomato plants (20.9%) than for rhizosphere of lettuce (14.9%).

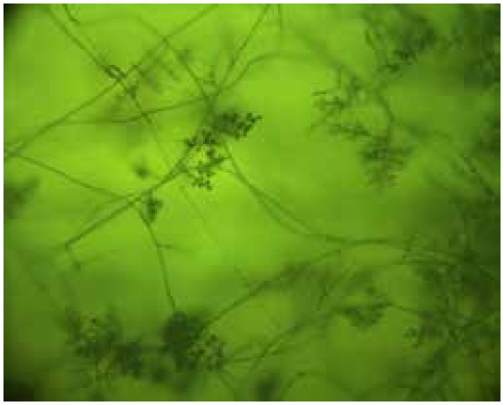

Figure 8. *Trichoderma viride* from the tomato rhizosphere(150x)

Recent studies (Yan et al., 2021) reported the role of *Trichoderma* spp. in improving plant defences against plant pathogenic nematodes by increasing the synthesis of secondary metabolites and defences-related enzyme activity in plant.
Apart of the relative abundance of species, their frequency in samples was helpful for establishing their constancy and the status of a certain species in the structure of myco-cenosis from rhizosphere of lettuce or tomato plants.
In Table 1 are also presented the values of the most utilized indices of characterization of biodiversity and evenness in microbial communities.
Thus, the values of Shannon index (H') and evenness (ε) were higher in rhizosphere of lettuce plants as compared with those from rhizosphere of tomato plants. The value of this diversity index increases with the increased number of fungal species (14 species in rhizosphere of lettuce as compared to 9 species in rhizosphere of tomato) and is also higher when the species are evenly distributed (ε=0.749 in rhizosphere of lettuce as compared to ε=0.708 in rhizosphere of tomato), as confirmed by other results from literature (Morris et al., 2014).
The values of index of Brillouin and D index of Simpson calculated confirmed the higher diversity and "equitability" component of fungal species diversity in rhizosphere of lettuce than in rhizosphere of tomato plants.

Thus, according to index of Simpson that takes values between 0 and 1, depending on the number of species and their proportion of representation, the diversity index value D=0.901 in myco-cenosis from lettuce rhizosphere is higher than the value D=0.850 in myco-cenosis from tomato rhizosphere, and the values of "equitability" (Brillouin) E=0.735, respectively E=0.698, too.

Table 1. Taxonomic composition, species status and biodiversity indices of fungal rhizosphere communities of lettuce and tomato, in greenhouse experiment

Lettuce rhizosphere (Fungal species constancy)	**Tomato rhizosphere** (Fungal species constancy)
[1]*Trichoderma viride,* [1]*Harposporium anquillulae,* [2]*Dactylaria candida,* [2]*Arthrobotrys oligospora,* [2]*Alternaria alternata* [3]*Mortierella humicola,* [3]*Mortierella* sp., [3]*Monacrosporium cionopagum,* [3]*Syncephalis reflexa,* [3]*Mucor racemosus,* [3]*Chaetomium globosum,* [3]*Mycogone rosea,* [3]*Thysanophora penicillioides,* [3]*Rhizopus stolonifer*	[1]*Trichoderma viride,* [1]*Harposporium anquillulae,* [2]*Arthrobotrys oligospora,* [2]*Humicola grisea,* [2]*Mycogone rosea,* [3]*Cladosporium herbarum,* [3]*Rhizoctonia solani,* [3]*Alternaria alternata,* [3]*Monacrosporium cionopagum*
S=14 SR_2=0.208 Shannon H'=2.441 H'_{max}=2.639 ε=0.749 Brillouin H=0.933 B=62.531 H_{max}=1.269 E=0.735 Simpson Index D=0.901	S=9 SR_2=0.209 Shannon H'=1.999 H'_{max}=2.197 ε=0.708 Brillouin H=0.751 B=32.296 H_{max}=1.085 E=0.698 Simpson Index D=0.850

[1]*euconstant species (F=75-100%)*
[2]*constant species (F=50-75%)*
[3]*accessory species (F=25-50%)*
[4]*accidentally species (F=by 25%)*

Comparative analysis of fungal microbiomes composition from lettuce and tomato rhizosphere revealed that the exudates from plant roots influenced the composition of rhizosphere myco-cenoses, with only 6 shared (common) species (SI=52.17%), half of them being represented by nematophagous species *Arthrobotrys oligospora, Harposporium anquillulae* and *Monacrosporium cionopagum)*.

Other 3 shared species were represented by antagonistic and cellulolytic *Trichoderma viride*, as dominant species in both rhizospheres, *Alternaria alternata*, potential pathogen for lettuce and tomato plants and *Mycogone rosea*. The last species is rarely isolated from soil, being more often reported as parasite on Basidiomycetes (Baron, 1968).

Humicola grisea, Cladosporium herbarum, and *Rhizoctonia solani* were differential species, isolated only from tomato rhizosphere. Differential species, isolated only from lettuce rhizosphere were nematode-trapping fungus *Dactylaria candida* and other 7 species from genera *Mortierella, Syncephalis, Mucor, Chaetomium, Thysanophora* and *Rhizopus.* Venn diagram (Figure 9) represents the number and proportion of shared (common) and differential species.

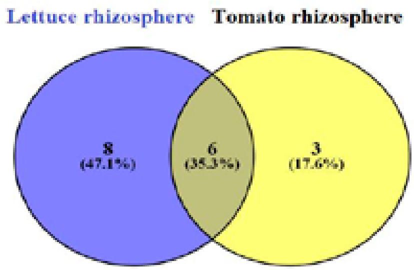

Figure 9. Venn diagram of unique and shared number and proportion of fungal species from lettuce and tomato rhizospheres

Microbial communities were dominated by antagonistic fungi *Trichoderma viride* and endoparasitic *Harposporium anquillulae*, present in all samples (frequency F=100%) as euconstant species from both rhizospheres. accompanied by species with role in organic matter decomposition, plant growth promoting, improvement of soil structure, humification (*Humicola grisea, Cladosporium herbarum*), and less frequent phytopathogenic species (*Rhizoctonia solani)*.

Our previous research evidenced the presence of *Harposporium anguillulae* as constant species in rhizosphere of soybean plants, cultivar PR91M10, grown in greenhouse (Matei & Matei, 2010).

Results from the present research are in concordance with other data from literature evidencing the importance of root–soil–microbe interactions in rhizosphere for crop production (Verma et al., 2011; Zhang et al., 2017).

Preece & Penuelas (2016) studied the role of rhizodeposition under drought and underlined the beneficial effect for biodiversity of soil microbial communities and ecosystem resilience.

Other research revealed the rhizosphere effect of three agricultural plants (barley, pea and white mustard) on naturally occurring nematophagous fungi and nematodes in both field and pot experiments and discussed various types of traps found, underlining that nematophagous species forming adhesive networks have a high saprophytic ability and a complex relationship with nematodes (Persmark & Jansson, 1997). The greatest number of species of nematophagous fungi (average of 2.4 species recovered from 0.1 g material) was detected in pea rhizosphere, as compared with white mustard and barley rhizospheres and root-free soil (less than 1.7 species).

The most common species in both soil and rhizosphere was *Arthrobotrys oligospora*. Liang et al. (2017) reported that the Woronin body in *Arthrobotrys oligospora* is essential for formation of fungal traps and the efficiency of pathogenesis process.

In the present experiment, four nematophagous species were identified in fungal communities of lettuce and tomato rhizospheres, with capacity to develop various typical infection structures ranging from adhesive nets, branches, non-constricting rings and ingested conidia.

The genetic reservoir of nematophagous fungal species could be utilised for reducing the populations of nematodes that detrimentally impact a wide variety of economically important horticultural plant species, especially in greenhouse conditions.

Further experiments with isolates of these fungi will need to be followed up to test them as inoculum added to soil in nematode population suppression assays.

CONCLUSIONS

Nematophagous fungi identiffied in lettuce rhizosphere belong to nematode-trapping species *(Arthrobotrys oligospora, Dactylaria candida, Monacrosporium cionopagum)* and endoparasitic species *(Harposporium anquillulae).*

Nematophagous fungi from tomato rhizosphere belong to nematode-trapping species *(Arthrobotrys oligospora, Monacrosporium cionopagum)* and endoparasitic species *(Harposporium anquillulae)*.

Different adhesive or non-adhesive hyphal structures to capture nematodes and non-adhesive infection conidia were detected in rhizospheres of both plants.

There were differences in the relative abundances of the nematophagous fungal species between the crop plants.

The fungal community from rhizosphere of lettuce plants presented a higher biodiversity and homogeneity as compared to rhizosphere of tomato plants.

Plants' exudates influenced the composition of rhizosphere myco-coenoses, with only 52.17% shared species, half of them being represented by nematophagous species *Arthrobotrys oligospora, Harposporium anquillulae* and *Monacrosporium cionopagum)*.

Microbial communities were dominated by antagonistic fungi *Trichoderma viride* and endoparasitic *Harposporium anquillulae*, (euconstant species), accompanied by constant and accessory species with role in organic matter decomposition, plant growth promoting, improvement of soil structure, humification and, less frequent, by potential phytopathogenic species.

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TECHNOLOGY DESIGN FOR *SIDERITIS SCARDICA* AS A NEW CULTURE IN ROMANIA

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Abstract

The current ascending international trends to enclose in production and food sector the aromatic and medicinal plants is similar in Romania too. Due to a steadily loss of biodiversity, many endemic species, especially in the areas of origin, are endangered, among them being the species Sideritis scardica. In Romania, after 1996 it was the subject of acclimatization and breeding research. At the same time, in order to popularize and expand the species in culture, the focus of the research was directed towards the elaboration of the specific growing technology in accordance with the pedo-climatic conditions of our country. This work summarizes the research undertaken at PGRB Buzau in the period 2019-2021 on the optimal culture technology at Sideritis scardica highlighting the fact that the best results were obtained at the establishment of the culture by seedling, obtained on the experimental variant represented by 80% peat and 20% dolomite (limestone). The optimal scheme for planting in the field was of 70 cm between rows and 50 cm between plants/row and the maximum biomass production was obtained in the 3rd year of cultivation, respectively 179 kg green mass/100sqm. Studies have shown that Sideritis scardica can be successfully cultivated in Romania with a real potential for valorization: medicinal, aromatic honey, ornamental and last but not least for the revaluation of arid hollows in mountainous areas.

***Key words**: genotype, breeding, acclimatization, Mursalski, seedling.*

INTRODUCTION

The species *Sideritis scardica* Griseb. known as an endemic plant in certain regions of the Balkan Peninsula originates in the Rhodope Mountains, at altitudes between 1000 and 2200 m.a.s.l. on sunny and steep slopes, being appreciated as one of the most valuable aromatic and medicinal plants worldwide (Shtereva et al., 2015). In Bulgaria, the important role of Sideritis scardica as traditional remedies tea and its conservation status has required its cultivation as market production (Kostadinova et al., 2008).

In Romania, it was taken into study after 2000, by the researcher Costel Vînătoru, using a variety from the mountains of Bulgaria highly appreciated for its multiple qualities: food, medicinal, aromatic, ornamental (Vînătoru, 2019). The researches undertaken were completed with obtaining the first variety of *Sideritis scardica*, patented and registered in the Official Catalogue of Cultivated Plants in Romania, under the name of Domnesc.

In the following period the research was continued at PGRB Buzau, with the aim of enriching the germplasm base and obtaining new genotypes with distinct phenotypic expression. At the same time, a special emphasis was put on the development of specific cultivation technology for the soil and climatic conditions of our country with the aim of achieving technological transfer to producers. Specialists recommend field crop as the most efficient and which favours the increase of resistance and adaptability to agro-ecological conditions (Evstatieva & Alipieva, 2011). The main objective of the present paper is to present the research undertaken in the period 2019 - 2021 on the development of specific cultivation technology.

MATERIALS AND METHODS

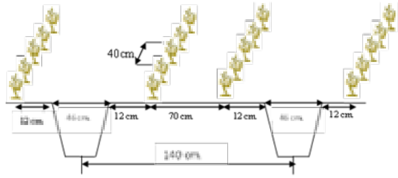
The research works were carried out between 2019 and 2021, at PGRB Buzau, located in the south-eastern part of Romania, in the continental climate zone. In order to establish the cultivation technology, three experimental substrate variants, containing different mixtures, were chosen for seedling production: V1 with 100% simple ground peat, partially decomposed, with microelements, pH 6, with wetting agent and fertilizer 1 g/l, extrafine structure; V2 a mixture of 80% peat and 20% sand and V3 a mixture of 80% peat and 20% dolomite (limestone).
Figure 1 shows pictures of the three substrate preparation methods.

a) b) c)
Figure 1. Three substrate variants:
a) V1 100% peat; b) V2 80% peat+20% sand;
c) V3 80% peat+20% limestone

The genetic material used in this theme comes from the germplasm collection held by PGRB, in this species, namely the Domnesc variety. Three variants were used for the establishment of the field crop: V1 by seedling, V2 by cuttings and V3 by direct sowing, as shown in Figure 2.

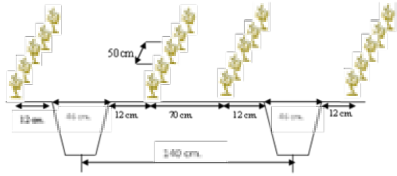
a) b) c)
Figure 2. Material used to establish the culture of *Sideritis scardica*:
a) V1 seedling; b) V2 cuttings; c) V3 seeds

Land preparation was done in autumn, by clearing the land of plant debris followed by deep autumn ploughing at a depth of 30 cm, and in spring the soil was tilled with a disc harrow, equipped with a levelling frame, after which the land was shaped using the L445 tractor in aggregate with MMS 2.8 with a width of 1400, 94cm at the crown, a 46cm ridge and 70cm between rows.
Regarding the schemes for establishing the culture, three variants with different densities were used as can be seen in Figure 3, images a), b), c):
V1 - 70 cm between rows and 40 cm between plants /row (Figure 3a);



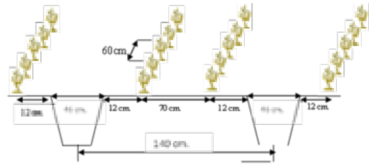
a)

V2: 70 cm between rows and 50 cm between plants / row (figure 3 b);



b)

V3: 70cm between rows and 60 cm between plants / row (Figure 3 c):



c)

d)

Figure 3. Crop establishment schemes with different plant/row densities:
a) V1 at 40cm; b) V2 at 50cm; c) V3 at 60cm;
d) third year of *Sideritis* crop

In the first year, in 2019, the crops were established by the three methods: V1 by seedlings, V2 by cuttings and V3 by direct sowing. Sowing was carried out, for all three variants, in the first decade of March, in heated protected areas (greenhouse), when the average daily temperature was 15-16°C. The three pre-prepared substrates: 100% peat, 80% peat + 20% sand, 80% peat + 20% dolomite, were used to fill the 33 x 53 cm and 70 cells/tray cellular pallets.
Schematically, the three methods are shown in Table 1.

Table 1. Schematic representation of culture establishment methods and distances

Method name	**Method of establishing culture**	**Distance between rows**	**Distance between plants per row**		
			V1	**V2**	**V3**
V1	seedling	70cm	40cm	50cm	60cm
V2	cuttings	70cm	40cm	50cm	60cm
V3	directly sown	70cm	40cm	50cm	60cm

The three substrate variants called V1, V2 and V3 and configured differently, 100% peat for V1, 80% peat and 20% sand for V2 and 80% peat and 20% dolomite for the V3 variant can be found in the table 2 below:

Table 2. Configuration of the three substrate variants

Crt. No.	Substrate type	Peat	Sand	Limestone
1.	V1	100%		
2.	V2	80	20%	
3.	V3	80%		20%

The maintenance works were the usual ones, namely: filling the gaps after planting, weed control by two manual and three mechanical hoeing and supplying the crop with water on time, in critical phenophases using a watering norm of 200-250 m^3/ha.
Irrigation works require special attention due to pubescence leaves because can adhere to the soil surface and rot easily.
If the plant is grown on unmulched land or without rocks, the rain can lead to sticking the hairs of the leaves with the soil, a phenomenon that depreciates the foliar mass and can even lead to compromising the crop. In the picture below you can see two plants, one partially and totally destroyed due to excess water:

a) b)
Figure 4. Two plants of *Sideritis scardica* destroyed due to excess water: a) partially; b) totally

In case of hoeing is very important not to bring soil close to the stem.
During the vegetation period, biometric and qualitative measurements were carried out; the values from the measurements were processed by statistical calculation using SPSS software, performing analysis of variance by ANOVA test followed by DUNCAN test with 95% confidence interval and p-value < 0.05%. Temperatures and precipitation were also monitored during the study period, 2019-2021 and processed to obtain monthly average values and plotted.

RESULTS AND DISCUSSIONS

The natural growing conditions for *Sideritis scardica* do not target high demands, the species prefers rocky soils with sandy-clay composition, pH between 6.9-8 and a low nutrient content. The Buzaului area, where the experiment was carried out, is a lowland area, characterized by a continental climate, with hot and dry summers and low rainfall. The soil is luto-sandy alluvial with good drainage. Charts 1 and 2 represent the main climatic factors with monthly average values; measurements were carried out in the interval 2019-2021, temperature being recorded and expressed in degrees Celsius and precipitation in mm. In chart 1, regarding the evolution of precipitation, a slight upward trend is observed, 2019 being the year with the lowest monthly average of 34.58mm and 2021 the year with a maximum of 49.58mm.

Chart 1. Evolution of average monthly precipitation in the period 2019-2021

Chart 2, on temperature evolution, shows a downward trend, from a monthly average of 12.92°C in 2019 to a monthly average of 11.25°C in 2021. It is worth mentioning that in the June-September flowering period, the monthly averages were identical in 2019 and 2020, i.e. 22.5°C, and in 2021 a monthly average of 20.4°C was recorded in the same period (June-September).

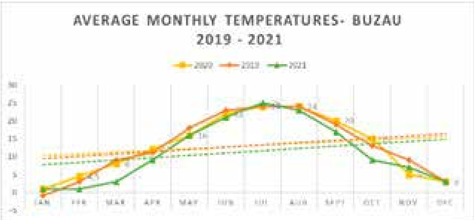


Chart 2. Evolution of monthly average temperatures in the period 2019 – 2021

Throughout the growing season, from sowing, crop establishment to harvest, phenological, biometric and laboratory observations were carried out during all years of study. In terms of seedling production it was found that the best substrate variant is V3, composed of 80% peat and 20% dolomite (limestone). Tables 3 and 4 show phenological observations and biometric measurements of the seedlings obtained from the three substrate variants at the time of planting, i.e. at about 60 days after sowing. Thus, the height of the seedling had a minimum value of 2.41cm in the substrate V1, with 100% peat, and a maximum value of 3.53cm in substrate V3, containing 80% peat and 20% limestone (table 3). Similarly the range of the coefficient of variation is narrow, between 4.08% and 4.62%, with differences in the height of the plants within normal limits. In terms of the number of leaves of the seedlings at the time of planting the calculated average was 5.66 for the 100%turf variant, V1 and equal to 7.66 for the other two variants, V2 and V3. The coefficient of variation (CV) ranged from 9.72% in the case of V2 to 13.15% in the case of V1. The standard deviations (SD) for PH (plant height) were 0.1 for V1, 0.1 for V2 and 0.2 for V3; for NL (number of leaves) the standard deviations (SD) obtained were: 1 for V1, 0.7 for V2 and 0.7 for V3 (Table 3).

Table 3. Seedling growth dynamics of *Sideritis sc.* as a function of substrate: plant height and number of leaves

Substrat type	Plant height (cm)		Number of leaves	
	Mean±SD	**CV%**	**Mean±SD**	**CV%**
V1	2.41±0.1^{c}	4.84%	5.66±1^{b}	13.15%
V2	2.96±0.1^{b}	4.08%	7.66±0.7^{a}	9.72%
V3	3.53±0.2^{a}	4.62%	7.66±0.7^{a}	11.11%

*Letters represent Duncan test results with 95% confidence interval and $p < 0.05\%$.

Regarding leaf length, the lowest average was recorded for substrate V1, 2.18 cm and the maximum was 3.61cm for substrate V3, with a composition of 80% sand and 20% limestone (Table 4).

Table 4. Seedling growth dynamics of *Sideritis sc.* as a function of substrate: length and width leaf

Substrat type	Leaf length (cm)		Leaf width (cm)	
	Mean±SD	**CV%**	**Mean±SD**	**CV%**
V1	2.18±0.08^{c}	3.45%	0.91±0.15^{b}	16.06%
V2	3.35±0.13^{b}	3.76%	1.2±0.09^{a}	7.45%
V3	3.61±0.11^{a}	2.95%	1.3±0.08^{a}	6.12%

*Letters represent Duncan test results with 95% confidence interval and $p < 0.05\%$.

The coefficient of variation (CV) ranged from 2.95% to 3.76%.
Measurements taken to determine leaf width had minimum mean values in V1, namely 0.91cm and 1.3 in the substrate of V3.
The coefficient of variation (CV) ranged from 6.12% to 16.06% (Table 4).
Regarding leaf size the standard deviations (SD) varied as follows: for length the standard deviations were: 0.08 for V1, from observations made on the seedlings at the time of planting: plant height, number of leaves, leaf dimensions (length and width), 0.13 for V2 and 0.11 for V3; for width standard deviations were calculated: 0.15 for V1, 0.09 for V2 and 0.08 for V3 (Table 4).
For a better understanding of the results obtained, the three substrate variants are represented in different colours and segmented by type of quantitave characteristics (Chart 3).

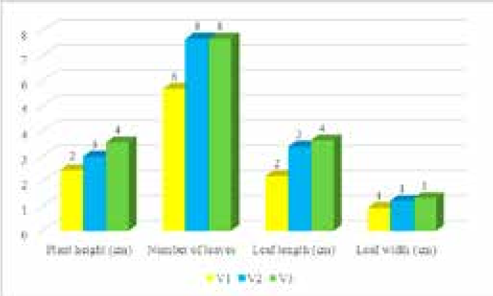


Chart 3. Graphical representation of quantitative characteristics according to substrate type

Regarding the establishment of the crop in the field using seedling, cuttings and seed, it was found that V1, respectively the seedling method is the best and safest way to establish the crop of this species in Romania. One important recommendation is that seedling should not be inserted deeper into the soil than the level of nutrient cube (Figure 5).

Figure 5. Seedling in a nutrient cube

The culture was established on all three expression variants around 1st of May. The percentage of survive in V1 was over 95%, in V2 42% and in V3 by direct sowing only 14% of the seeds germinated. In case of irrigation, excess moisture should be avoided, as the plant may wither.
It was also found that in this variant, V3, the plants had a great competitive opposition with weeds, which required additional hand weeding and transplanting work in the remaining gaps due to uneven sprouting. It should be specified that, although a carefully selected seed with a germination percentage of over 92% was used, the crop had chaotic densities within it, most probably due to the small size of the seed: length 2.23 mm, width 1.45mm (MMB = 1.308 g).
Regarding the establishment of crops using different densities between plants per row, it was found that V2 is the optimal variant of establishing the crop in terms of plant density per row ensuring a higher yield than the other variants, while it also makes good use of the land and can easily perform maintenance work.
The measurements were carried out at the time of harvesting the flower stems. The values obtained were the basis for calculating mean values (M), standard deviations (SD) and coefficients of variation (SD) using statistical formulas. In terms of the number of stems, the values fall within normal growth and development trends. In 2019 (table 5) were recorded at the V1 variant, an average value of 32 rods, 38 rods at V2 and 34 rods at V3. Regarding vegetative mass in 2019 the mean for V1 was 137.99g, for V2 was 184.82g and 157.16g for V3. Coefficient of variations (CV) ranged from minimum average value 1.63% at V2 to a maximum average value 3.27% at V3.

Table 5. Mean values of quantitative characteristics of the vegetative mass of a *Sideritis sc.* bush depending on the type of substrate used - 2019

Year	2019					
Crop variant	Rod number		Rod weight (g)		Vegetative mass (g)	
	Mean ±SD	CV%	Mean ±SD	CV%	Mean ±SD	CV%
V1	32± 0.70^{c}	2.19%	4.31± 0.01^{c}	0.23%	137.99± 3.53^{c}	2.56%
V2	38± 0.70^{a}	1.84%	4.86± 0.02^{a}	0.41%	184.82± 3.01^{a}	1.63%
V3	34±1^{b}	2.94%	4.62± 0.01^{b}	0.22%	157.16± 5.14^{b}	3.27%

In 2020 (Table 6), the values recorded were higher, namely: an average value of rod number was 130.4 at V1, 147.2 rod number at V2 and 140.1 average value of rod number for V3.

Table 6. Mean values of quantitative characteristics of the vegetative mass of a *Sideritis sc.* Bush depending on the type of substrate used - 2020

Year	2020					
Crop variant	Rod number		Rod weight (g)		Vegetative mass (g)	
	Mean ±SD	CV%	Mean ±SD	CV%	Mean ±SD	CV%
V1	130.4± 0.89^{c}	0.68%	5.75± 0.03^{b}	0.52%	750.06± 7.77^{c}	1.04%
V2	147.2± 0.83^{a}	0.56%	5.86± 0.03^{a}	0.51%	862.9± 8.85^{a}	1.03%
V3	140± 1.00^{b}	0.71%	5.75± 0.06^{b}	0.52%	805.86± 12.23^{b}	1.52%

The main quantitative characteristic monitored, was the average value of the vegetative mass of a *Sideritis sc*. bush and the determinations carried out recorded in 2020 an average weight from minimum value 750.06g from V1 to 862.9g maximum value at V2.

In 2021 (Table 7), the values recorded were the highest, namely: an average value of rod number was 179.8 at V1, 211.2 rod number at V2 and 188.2 average value of rod number for V3. The vegetative mass of a *Sideritis sc*. bush and the determinations carried out recorded in 2021 presented an average weight from minimum value 1166.16g from V1 to 1418.35g maximum value at V2.

Table 7. Mean values of quantitative characteristics of the vegetative mass of a *Sideritis sc.* bush depending on the type of substrate used - 2021

Year	2021					
Crop variant	Rod number		Rod weight (g)		Vegetative mass (g)	
	Mean ±SD	CV %	Mean ±SD	CV %	Mean ±SD	CV%
V1	179.8± 0.83[c]	0.46 %	6.486± 0.17[b]	2.62%	1166.16± 30.30[c]	2.60%
V2	211.2± 0.83[a]	0.39 %	6.716± 0.13[a]	1.94%	1418.35± 24.66[a]	1.74%
V3	188.2± 1.09[b]	0.58 %	6.418± 0.07[b]	1.09%	1207.81± 9.66[b]	0.80%

It should be mentioned that *Sideritis scardica* is a valuable honey plant, which blooms in stages between May 15 and June 15. If the inflorescences are harvested early in May, in favorable weather conditions (late frosts), a second flowering occurs starting with September, which lasts until the arrival of frost.

Figure 6. *Sideritis sc.* first year crop

In the image above is a sequence from a culture of *Sideritis sc.* established by the V1 method, shaped land, first year of crop, weak development with reduced number of rods (Figure 6).

Figure 7. *Sideritis sc*.: the second year crop

In the second year after the establishment of the crop the plant shows both vertical and horizontal growth, increasing the number of stems and therefore the vegetative mass (Figure 7).

Figure 8. *Sideritis sc*.: the third year crop

In the third year the plant reaches a vegetative maximum (Figure 8).

The graph below (Chart 4) shows the influence of the composition of the substrate used for the establishment of the crop on the main quantitative characteristic, i.e. the vegetative mass of a *Sideritis sc.* bush in the third year after the establishment of the crop, when the phenotypic characteristics are at their maximum.

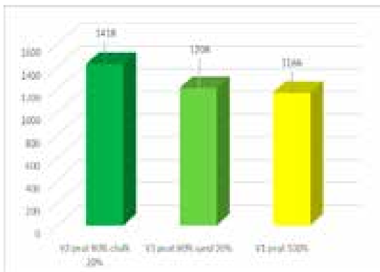


Chart 4. Influence of V2 substrate on the vegetative mass of *Sideritis sc.*

As far as green mass vs. dry mass is concerned, it was found that there are no significant differences. The dry mass decreases by skip up to 38% of the green mass; the percentage is variable depending on the time of harvesting: the more the crop is at an advanced stage of ripening, the lower the percentage of dehydration; the explanation is that the green plant has a low water content (Figure 9).

Figure 9. Green plant vs. dried plant of *Sideritis sc.*

It has been found that *Sideritis sc.* is highly appreciated as a honey plant, providing pollen and nectar for a long period of time, starting from the end of June, second year, until frost.
It is worth mentioning that the flower has a very pleasant lemon-yellow colour and the aroma is a mix of menthol and citrus. The specific and particular aroma of mountain tea (*Sideritis scardica* Griseb.) is perhaps the most important reason for its widespread use by peoples.
In the picture below (Figure 10), *Sideritica sc.,* flowers:

Figure 10. *Sideritica sc.* flowers and bees

After harvesting, the flowering stems are dried in specially designed areas, away from sunlight and draughts; the stems are tied in bunches that weigh about 400g in green (Figure 11):

Figure 11. Drying and storage of *Sideritis sc.*

CONCLUSIONS

It has been found that the *Sideritis* species can be grown successfully in Romania, except in very hot, arid areas. Regarding the best method of establishing the crop is by seedling using a nutrient mixture of peat and dolomite (limestone). The optimum planting distance in the field is recommended to be 70 cm between rows and 50 cm between plants/row. The maximum production potential of the *Sideritis scardica* crop is achieved in the third year after establishment. The study did not detect any diseases and/or pests that would cause significant damage to the crop, which is why the crop can be grown successfully under organic conditions in the soil and climate conditions of Romania.

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MINERAL NUTRITIONAL VALUE OF PRODUCTS CONTAINING ARONIA FRUITS AND JUICES: A REVIEW

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Abstract

The "superberry" Aronia melanocarpa has attracted a lot of attention due to its high content of phenolic phyto-chemicals. However, little attention has been paid to its mineral content. The main objective of this paper is to review the mineral content of black chokeberry fruits, products and by-products, focusing on the health importance of mineral intake from food, against supplements. A lot of food databases and scientific publications demonstrated that comparing to fresh apples, fresh black chokeberry fruits contain high concentrations of minerals such as potassium (290.3 mg/100g) - which contributes to human health by regulating protein synthesis and the metabolization of carbohydrates - and copper (0.211 mg/100 g), zinc (0.147 mg/100 g), selenium (0.028 mg/100 g) - which act as antioxidants. The fresh Aronia melanocarpa berries also contain manganese, which is an essential micronutrient for plant growth and development. Nevertheless, excessive manganese accumulation in human's body may lead to adverse effects like insomnia, memory loss, irreversible nerve damage. Few results have been found for manganese concentrations in black chokeberries (0.0829 mg/100 g dry weight) and in aronia juices (reported to vary between 0.298 ± 0.003 mg/100 g and 1.177 ± 0.005 mg/100 g). Therefore, extensive research conducted in this area is needed in order to identify and quantify the mineral concentrations of aronia fresh berries and products and to contribute to an advance knowledge of mineral content as a key role for healthy natural products. Additionally, the variability of the mineral's concentrations in the aronia fruits should be taken into consideration, as they are in direct relation with crop variety, soil characteristics, climate changes, fertilization and harvesting time.

Key words*: Aronia, juice, minerals, micronutrients, macronutrients.*

INTRODUCTION

Aronia melanocarpa is one of four accepted species of the genus *Aronia* Medik, in the *Rosaceae* family, also known as black chokeberry (Mahoney et al., 2019). It is a 2-3 m shrub (Jurendić & Ščetar, 2021) native to the Great Lakes region and the North-eastern U.S., with a southerly extension into the higher elevations of the Appalachian Mountains (Scott Peterson, n.d.). It was brought to Russia at the beginning of the 20th century from where it spread to Eastern Europe (Ochmian et al., 2012). The other three *Aronia* Medik accepted species are: *Aronia arbutifolia* (red chokeberry), *Aronia prunifolia* (purple chokeberry) and *Aronia mitschurinii* (Mahoney et al., 2019).
There are many cultivars documented in literature, most commonly researched being "Nero" (Czechia), "Rubina" (crossing from Russian and Finnish plants), "Viking" (Finland), "Kurkumäcki" (Finland), "Hugin" (Sweden), "Fertödi" (Hungary) and "Aron" (Denmark) (Kulling & Rawel, 2008) which are usually harvested between August and September (Jurendić & Ščetar, 2021).
Aronia melanocarpa has a very complex chemical composition, which can vary depending on many factors such as: soil composition, fertilisation, climate conditions, berry maturity, harvest method and storage conditions (Tolić et al., 2017).
Fertilisation has a large impact on berries production and quality. Following fertilisation, increased plant growth and berries yield were reported, alongside with a decrease in total acidity and pigment concentration (Kulling & Rawel, 2008).

Polyphenol content

Aronia has attracted a lot of attention due to its high content of polyphenols (proanthocyanidins, anthocyanins, flavonoids and phenolic acids), research showing that these compounds can have a meaningful beneficial impact on health through their antioxidative, anti-inflammatory, antiviral, anticancer, antiatherosclerotic, hypotensive, antiplatelet, and antidiabetic properties (Borowska & Brzóska, 2016).

Aronia melanocarpa was studied primarily for its phenolic constituents, aronia fruits being particularly rich in anthocyanins, flavanols, proanthocyanins and phenolic acids (King & Bolling, 2020a). A study conducted by Denev et al. (2018) shows that the total polyphenol content of aronia fruits varies between 1022mg/100g fresh weight and 1795 mg/100 g fresh weight from which 284-686 mg/100 g fresh weight are anthocyanins.

In a study conducted by Skupień & Oszmiański (2007), *Aronia melanocarpa* plants were fertilised with three types of fertilisers: manganese, commercial fertiliser (nitrogen, potassium and silicon) and a combination of both. The polyphenolic content decreased for all fruits from all treated plants compared to the control group (Skupień & Oszmiański, 2007).

The phenolic content of aronia products and other food products enriched with aronia were extensively studied. Aronia dried fruits have a high polyphenolic content, 40.15g GAE/kg; fresh aronia juice has 6.88±0.23g GAE/kg (Catană et al., 2017). High concentrations of polyphenols have been identified in other products as well: 6.9-12 g GAE/kg jam, 6.7 g GAE/kg compote, 2.6 g GAE/kg syrup (Kapci et al., 2013), 0.86 g GAE/l fruit tea (decoction), 0.89 g GAE/l fruit tea (infusion) (Šavikin et al., 2014), 0.88 g/l wine (Wilkowska et al., 2017), 0.429-2.339 g/l liqueur (Olas et al., 2008).

Yogurt with 2-3% added aronia juice increased its phenol content up to 54.05 mg GAE/g dry weight, showing a higher antioxidant activity (Nguyen & Hwang, 2016). Beer infused with aronia berries shows a higher polyphenol concentration and also a positive effect on sugar utilisation during fermentation (Jahn et al., 2020).

Aronia pomace total phenolic concentration was reported to be of 63.1g GAE/kg (Kapci et al., 2013). Adding Aronia pomace powder to replace 25% of starch for making ready-to-eat extruded cereals will increase the phenolic content of these products (Schmid et al., 2022). Adding either aronia juice or berries to different food products or beverages can increase their antioxidant activity.

Fibre content

After extracting the juice, the pomace fibre content is up to 57.8-71.5 g/100 g dry weight, out of which 43.8-61.7 g/100 g dry weight is insoluble fibre (Schmid et al., 2020). These include: cellulose (34g/100g dry weight), hemicellulose (32 g/100 g dry weight), lignin (22.7 g/100 g dry weight) and pectin (7.52 g/100 g dry weight) (Schmid et al., 2020). A study conducted by Schmid et al. (2021) shows that aronia pomace powder can be used to substitute starch in breakfast cereals and snacks and it's a valuable source of dietary fibre and polyphenols.

Aronia berries contain 5.62 mg/100 g fresh weight of dietary fibre (Kulling & Rawel, 2008). Aronia fibres are a good sorbent for cadmium and led, facilitating the removal of these two heavy metals from the human body (Borycka, 2012).

Total sugar content

The reported total sugar in aronia berries varies between 6.21 g/100 g and 20.92 g/100 g (Ochmian et al., 2012; Skupień & Oszmiański, 2007; Naukowe & Kwiaciarstwa, 2007; Mayer-Miebach et al., 2012; Sidor et al., 2019). Fertilisation also has an impact on the total sugar content. The fruits from aronia plants fertilised with commercial fertilizer showed a decrease in total sugar by 1.6 g/100 g and those from plants treated with manganese fertiliser combined with commercial fertilizer showed a 1.10 g/100 g decrease in total sugars. In fruits from plants treated only with manganese fertiliser sucrose was not detected (Skupień & Oszmiański, 2007).

Total sugars in aronia juice were reported to be 11.0-14.3 g/100 ml by Mayer-Miebach et al. (2012) and 12.0-19.6 g/100 ml by Handeland et al. (2014). After juice extraction, a part of the sugars remain in the pomace, 84 g/kg, the dominant component being sorbitol (Jurendić & Ščetar, 2021). The extraction methods used during juice manufacturing process such as additional pomace extraction after first pressing, will influence the saccharide composition of the fruit pomace (Sójka et al., 2013).

Aronia in the form of juice cand be better tolerated by consumers, even if the astringent taste of the berries is still present. In a study conducted by Duffy et al. (2016), aronia fruits were harvested from August to September once a week for 7 consecutive weeks, and the juice obtained from each harvest was tested for sweetness, sourness, saltiness, bitterness, astringency, and liking/disliking. Additionally, the effects of added sucrose and ethyl butyrate were assessed (Duffy et al., 2016). Juices obtained from fruits harvested in the first three weeks had the lowest sweetness and greater sourness and astringency, compared to juices obtained from berries harvested in the last three weeks, which had greater sweetness (Duffy et al., 2016). Adding 5% sucrose or a mix of ethyl butyrate and 3% sucrose increased the juice sweetness and changed consumers' opinions changing from dislike to like (Duffy et al., 2016). Taking this into consideration, in addition to the complex mineral content, aronia juice can be considered a potential ingredient in different beverages.

Fat content

Very few studies have reported the total fat content of aronia fruits. One paper reported a total fat content in aronia berries of 0.14 g/ 100 g fresh weight (Kulling & Rawel, 2008). Aronia seed oil had the highest content of linoleic acid, over 70%, and α-tocopherol, 166.0-1104.8 mg/kg (Milala et al., 2018).

Aronia juice contains less than 0.1 g/100 g of fat (Sidor & Gramza-Michałowska, 2019). After juice extraction, the dried pomace contains 5.15% of fat (Pieszka et al., 2015) of which 90.49% are polyunsaturated fatty acids and 9.51% are saturated fatty acids (King & Bolling, 2020b).

Protein content

Generally aronia fruits do not contain high amount of protein. Lancrajan (2012) found 0.7 g/100 g fresh weight of protein in aronia berries. A very small amount of protein was reported in aronia juice - 0.2 g/100 g (Sidor et al., 2019). After juice extraction, the pomace contains 10.77% of protein (Pieszka et al., 2015). The seedless part of the pomace has a lower protein content than the seed fraction (Sójka et al., 2013).

Organic acids content

Organic acids are constituents of ripe fruits and are responsible for their sourness and flavour (Famiani et al., 2015). In aronia berries organic acids are represented mainly by quinic acid - 404.4 mg/100 g fresh weight, malic acid - 328.1 mg/100 g fresh weight and ascorbic acid - 65.2 mg/100 g fresh weight (Denev et al., 2018).

The concentration of organic acids extracted from the aronia berries to the juice varies between 12.27 g/l and 21.87 g/l (Sosnowska et al., 2016). During juice fermentation with *L. paracasei*, malic acid is transformed in lactic acid which enhances the preservation of food in time, making aronia juice a potential substrate for the production of functional beverages of enhanced nutritional value (Bontsidis et al., 2021).

Vitamin content

Aronia melanocarpa fruits are high in vitamin C, which is an antioxidant necessary for the human body to prevent scurvy, gum disease and to boost immunity (Lupascu & Sîrbu, 2020). Fresh fruits contain 31.85 mg/100g Vitamin C (Catană et al., 2017), but it has also been reported to be 13.7 mg/100 g fresh weight (Kulling & Rawel, 2008), 2.3 mg/100 g fresh weight (Andrzejewska et al., 2015) and 7.2 mg/100 g (Skrede et al., 2012). Frozen berries have a 9.6% lower amount of Vitamin C compared to fresh berries (Catană et al., 2017). Other vitamins have been also reported in aronia berries: Vitamin B_1 (0.017- 0.019 mg/100 g), Vitamin B_2 (0.016-0.027 mg/100 g), Vitamin B_6 (0.024-0.029 mg/100 g), Vitamin A (0.77 mg/100 g), carotenoids (total carotenoids 48.6 mg/100 g) (Sidor et al., 2019).

In a study conducted by Catană et al. (2017), Vitamin C was quantified in different aronia products: aronia compote - 7.96 mg/100 g, aronia jam - 7.25 mg/100 g, aronia fresh juice - 98.75 mg/100 g, dried aronia fruits - 15.11 mg/100 g. Aronia fresh juice also contains Vitamins B1 (25-90 μg/100 ml), B2 (25-110 μg/100 ml), B6 (30-85 μg/100 ml), pantothenic acid (50-80 μg/100 ml) and niacin (100-550 μg/100 ml) (Kulling & Rawel, 2008), and the total carotenoid content is up to 97.8 μg/l (Oprea et al., 2014).

Mineral content

Research also shows a complex mineral content in aronia fruits, most abundant being calcium, potassium, iron, molybdenum, manganese, phosphorus and iodine (Pavlovic et al., 2015). High amounts of potassium and zinc were found in aronia juice (Kulling & Rawel, 2008). Heavy metal content will depend on harvest site and vegetation period (Ognik et al., 2006).

There are not that many studies that quantify the mineral content in aronia, focusing on aronia fruits, very few also mentioning the mineral content in aronia products (juice, filter bags for infusion and pomace) (Cindrić et al., 2017; Jurendić & Ščetar, 2021; King & Bolling, 2020a; Kulling & Rawel, 2008; Sidor et al., 2019; Pieszka et al., 2015; Pavlovic et al., 2015).

The aim of this paper is to review the mineral content of black chokeberry fruits, products and by-products, focusing on the health importance of mineral intake from food, against supplements, taking into consideration the variability of the minerals' concentrations in the aronia fruits as influenced by cultivar/variety, soil characteristics, climate changes, fertilization and harvesting time.

MATERIALS AND METHODS

Search strategy

Publications were identified through ScienceDirect Freedom Collection, Elsevier, Scopus, Elsevier, SpringerLink Journals, Springer, Mendeley Reference Manager, Google Scholar. Other international databases were used: Office of Dietary Supplements part of the Nation Institutes of Health, the United States Department of Agriculture, the United Stated Environmental Protection Agency.

The search strategy was comprised on two key elements:

- Aronia mineral content - searching with different keywords and permutations of the keywords: "Aronia melanocarpa", "black chokeberry", "aronia review", "aroinia juice", "aronia tea", "aronia pomace powder", "aronia minerals", "aronia mineral profile";
- Identification of the daily recommended intake for macro and micro-nutrients and of maximum accepted levels of heavy metals.

Inclusion criteria and selection process

Studies were filtered between 2005 and 2022. The following keywords were used:

- "aronia" – 1296 results;
- "Aronia melanocarpa" – 848 results;
- "black chokeberry" – 455 results;
- "aronia minerals" – 45 results;
- "aronia review" – 42 results;
- "aronia meta-analysis" – 4 results;
- "aronia juice" – 286 results;
- "aronia tea" – 50 results;
- "aronia pomace powder" – 7 results;
- "aronia fertilisation" – 9 results.

Articles chosen had to include direct measurements of mineral concentrations in aronia berries and products or a literature review on aronia and/or aronia products. From all found articles *only three* of them reported direct measurements on minerals concentrations from aronia berries, *one article* reported direct measurements on minerals from aronia juice and tea, *two articles* reported direct measurements on minerals from aronia pomace and *four articles* were literature reviews that included mineral content in either aronia berries or aronia products.

RESULTS AND DISCUSSIONS

1. Mineral content in aronia fruits

There are very few authors that have studied the mineral composition in *Aronia melanocarpa* fruits (Šnebergrová et al., 2014; Pavlovic et al., 2015; Cindrić et al., 2017; Lancrajan, 2012). Results are shown in Table 1.

Aronia berries are not too popular within consumers due to their bitter and astringent taste. A study on consumer preference and willingness to pay for a new type of berry in the US market shows that US consumers prefer to purchase and consume sweet berries (blueberries, strawberries, raspberries and blackberries), refusing to buy and consume aronia fruits due to their taste.

Positive health information on aronia fruits might persuade customers to purchase this new berry, but because of the bitter taste they might have buyer's remorse and refuse to consume it in the future (Hoke et al., 2017). Provided this information, even if the mineral content of aronia fruits has a lot of potential for the human's health, new products containing aronia need to be developed and tested for consumer acceptance.

Table 1. Mineral concentration in *Aronia melanocarpa* berries

Mineral	Concentration mg/kg	Reference
Na	12.5[a]; 16.8[b]	Pavlovic et al., 2015
	4.27	Cindrić et al., 2017
	26	Lancrajan, 2012
K	2707[a]; 4977[b]	Pavlovic et al., 2015
	6790	Cindrić et al., 2017
	2180	Lancrajan, 2012
Ca	601[a]; 1167[b]	Pavlovic et al., 2015
	1212	Cindrić et al., 2017
	3220	Lancrajan, 2012
	min 119; max 552[c]	Šnebergrová et al., 2014
Mg	164[a]; 578[b]	Pavlovic et al., 2015
	669	Cindrić et al., 2017
	168	Lancrajan, 2012
	min 83.3; max 314.2[c]	Šnebergrová et al., 2014
P	239[a]; 956[b]	Pavlovic et al., 2015
Zn	4.09[a]; 8.40[b]	Pavlovic et al., 2015
	0.55	Cindrić et al., 2017
Fe	9.4[a]; 14.2[b]	Pavlovic et al., 2015
	1.32	Cindrić et al., 2017
	9.3	Lancrajan, 2012
Se	0.21[a]; 0.28[b]	Pavlovic et al., 2015
Cu	0.82[a]; 2.11[b]	Pavlovic et al., 2015
	1.58	Cindrić et al., 2017
Mo	0.021[a]; 0.016[b]	Pavlovic et al., 2015
	0.039	Cindrić et al., 2017
Mn	5.49[a]; 17.89[b]	Pavlovic et al., 2015
	0.829	Cindrić et al., 2017
Ni	0.143[a]; 0.741[b]	Pavlovic et al., 2015
	0.38	Cindrić et al., 2017
V	0.40[a]; 1.58[b]	Pavlovic et al., 2015
Si	2.37[a]; 6.37[b]	Pavlovic et al., 2015
Cr	0.49[a]; 0.53[b]	Pavlovic et al., 2015
	0.029	Cindrić et al., 2017
Li	6.75	Pavlovic et al., 2015
	0.012	Cindrić et al., 2017
Sr	1.57[a]; 7.05[b]	Pavlovic et al., 2015
	1.66	Cindrić et al., 2017
Al	2.88[a]; 4.40[b]	Pavlovic et al., 2015
	158	Cindrić et al., 2017
Sn	0.62[a]; 0.72[b]	Pavlovic et al., 2015

[a] This value is the lowest concentration found in one of the two samples analyzed by Pavlovic et al. (2015) who analyzed two samples from different plants without specifying if they are different varieties or different cultivars.
[b] This value is the concentration found in the second sample analyzed by Pavlovic et al. (2015) who analyzed two samples from different plants without specifying if they are different varieties or different cultivars.
[c] Šnebergrová et al. (2014), reported maximum and minimum Ca and Mg concentrations in berries from nine locations in the Czech Republic and one sample from Poland without giving explicit values for the ten samples.

The cited authors in Tables 1 and 2 did not mention if the aronia berries are locally grown or imported. The only information provided is that the berries were purchased in local markets as follows: Cindrić et al. (2017) – Croatia, Pavlovic et al. (2015) – Serbia, Lancranjan (2012) did not report anything about the provenience of the aronia berries of which he used an alcoholic extract. In Table 1, the cited values from Šnebergrová et al. (2014), are maximum and minimum Ca and Mg concentrations in berries from nine locations in the Czech Republic and one sample from Poland, the authors not giving explicit values for the ten samples.

Table 2. Mineral concentration in *Aronia melanocarpa* berries

Mineral	Concentration mg/kg	Reference
As	0.20[a]; 0.36[b]	Pavlovic et al., 2015
Cd	0.016[a]; 0.041[b]	Pavlovic et al., 2015
	0.055	Cindrić et al., 2017
Ba	1.48[a]; 6.66[b]	Pavlovic et al., 2015
Pb	0.048[a]; 0.091[b]	Pavlovic et al., 2015
	0.041	Cindrić et al., 2017
Sb	0.29	Pavlovic et al., 2015
Co	0.019[a]; 0.043[b]	Pavlovic et al., 2015
	0.019	Cindrić et al., 2017
B	2.88[a]; 14.22[b]	Pavlovic et al., 2015

[a] This value is the lowest concentration found in one of the two samples analyzed by Pavlovic et al. (2015) who analyzed two samples from different plants without specifying if they are different varieties or different cultivars.
[b] This value is the concentration found in the second sample analyzed by Pavlovic et al. (2015) who analyzed two samples from different plants without specifying if they are different varieties or different cultivars.

As it has been shown in Tables 1 and 2, there are large differences between the reported values by different authors. These differences might come from different analytical methods used (Table 3) or from analysing different varieties/cultivars of aronia, or due to different harvest times.

All fruits for which mineral concentrations values were reported were not sampled according to sampling methods for trees and shrubs, and also have not been sampled according to sampling methods from containers or silos (Wulfsohn D, 2010).

Table 3. Analytical methods used for determining the mineral concentrations in aronia fruits and products

Author	Fruit/ product	Analytical method
Pavlovic et al., 2015	aronia berries and aronia juice	iCAP 6000 (Thermo Scinentific, Cambridge, UK); operating conditions: flush pump rate 100 rpm, analysis pump rate 50 rpm, RF power 1150 W, nebulizer gas flow rate 0.7 L min−1, coolant gas flow rate 12 L min−1, auxiliary gas flow rate 0.5 L min−1, sample uptake delay 30s.
Cindrić et al., 2017	aronia berries	ICP-AES spectrometer from Teledyne Leeman (Hudson, NH, USA); operating conditions: flush pump rate 1.0 ml/min, RF-Generator 40 MHz “free-running”, Argon flow Coolant:18 L/min, Auxiliary: 0.8 L/min, Nebulizer: 1 L/min, Peristaltic pump 1.0 mL min−1, Sample uptake delay 30s
Lancrajan, 2012	aronia berries	Not specified
Šnebergrová et al., 2014	aronia berries	Cations (potassium, magnesium and calcium) were determined by isotachophoresis according to Kvasnicka et al. (1993)
Pieszka et al., 2015	aronia pomace	ICP-MS; operating conditions: not specified; method details: not specified
Sójka et al., 2013 cited by Jurendić & Ščetar, 2021	aronia pomace	Not specified

2. Mineral content in aronia products

2.1. Mineral content in aronia juice

Mineral content in aronia commercial juice was quantified by Pavlovic et al. (2015) from 4 different commercially available aronia juices from Serbia, results being shown in Tables 4, 5 and 6. There is a huge variability in the results, information on other juice ingredients and preparation (raw vs. pasteurised) are missing.

Results were compared with the Dietary Reference Intake for children between 4-8 years old, adolescents between 14-18 years old and adults 19+ years old (*Dietary Reference Intakes (DRIs): NCBI Bookshelf*, n.d.), with maximum accepted levels of heavy metals in drinking water in the US (*National Primary Drinking Water Regulations | US EPA*, n.d.).

Based on Pavlovic et al. (2015) findings, aronia juice has a huge potential in providing a fair amount of calcium, magnesium and phosphorus. A 200g portion of aronia juice ensures up to 24.5% DRI (Daily Reference Intake) of Ca, 28.04% DRI of Mg and 29.62% DRI of P for adults. Up to 90.61% DRI of Mg for children 4-8y can be provided with the same portion of aronia juice. Magnesium excess is not a concern for healthy individuals if it’s provided from food intake (*Magnesium - Health Professional Fact Sheet*, n.d.).

Aronia juice has a high concentration of chromium, 200g of juice ensuring 3 to 10 times more chromium than the DRI. There’s no limit established for Cr since there are no adverse effects linked to high intakes in healthy humans (*Chromium - Health Professional Fact Sheet*, n.d.). Trivalent chromium has been shown to reduce insulin resistance (Hua et al., 2012), but there’s no study on the bioavailability of this mineral after aronia juice consumption. Although hexavalent Cr is harmful it was not found in food samples (Vacchina et al., 2015). The absence of hexavalent chromium in foods may be due to its instability in presence of antioxidants (Vacchina et al., 2015).

Copper is an essential mineral present in aronia juice; Pavlovic et al. (2015) reported between 15% of DRI up to 100.22% of DRI for adults. Copper in food and dietary supplements (total of 2.5-3mg/day) shows good results in terms of slowing down bone mineral loss and reducing resorption markers, confirming the effectiveness of copper supplementation on bone metabolism (Rondanelli et al., 2021). Excess copper can result in liver damage and gastrointestinal symptoms (*Copper - Health Professional Fact Sheet*, n.d.). For individuals with liver pathology, caution is advised in the consumption of chokeberry juice due to its possible high Cu concentration (*Copper - Health Professional Fact Sheet*, n.d.).

Pavlovic et al. (2015) also shows a huge amount of selenium in aronia juice, 2-10 times more than the DRI in a 200g portion. Selenium plays an important role in maintaining the homeostasis of the human body, but excess selenium (more than 5mg/d) can cause hair loss, skin and nail lesions, anemia and may increase the risk of type 2 diabetes (Kieliszek, 2019).

A particular concern is manganese. As per Pavlovic et al. (2015), 200 g aronia juice ensures between 25-100% of DRI for adolescents and adults and up to 156% of DRI for children.

Manganese may be used as a fertiliser to lower the sugar content of aronia fruits (Skupień & Oszmiański, 2007) being one of the 17 essential elements for plant growth and reproduction (Alejandro et al., 2020).

Even if manganese treatments will lower significantly the sugar content in aronia fruits, the accumulation of this mineral in the fruits and products and in the human body after consumption need to be taken into consideration as it may lead to adverse effects like insomnia, memory loss, irreversible nerve damage (*Manganese - Consumer,* n.d.).

Compared to the maximum accepted levels of heavy metals in water (*National Primary Drinking Water Regulations | US EPA*, n.d.), a 200g portion of aronia juice contains double the amount of cadmium accepted in water, and almost double the amount of led. This information needs to be corelated with the pollution of the soils and types of treatments used on the aronia plants.

Table 4. Mineral concentrations of *Aronia melanocarpa* commercial juice compared to Dietary Reference Intakes

Aronia juice (Pavlovic et al., 2015)[a]				**Dietary Reference Intakes (DRI) (Dietary Reference Intakes (DRIs): NCBI Bookshelf, n.d.) in mg/day**			**% of DRI for 200g portion of aronia juice**		
Mineral		**mg/kg**	**mg/200g portion**	**Children 4-8y**	**Adolescents 14-18y**	**Adults 19+**	**Children 4-8y**	**Adolescents 14-18y**	**Adults 19+**
Ca	Min	138	27.60	1000	1300	1000	2.76	2.12	2.76
	Max	1225	245				24.5	18.84	24.5
Cr	Min	0.55	0.11	0.015	0.035	0.03	733.33	314.29	366.66
	Max	0.74	0.148				986.66	422.86	493.33
Cu	Min	0.68	0.136	0.44	0.89	0.9	30.9	15.28	15.11
	Max	4.51	0.902				205	101.34	100.22
Fe	Min	0.72	0.144	10	11	8	1.44	1.30	1.80
	Max	1.73	0.346				3.46	3.14	4.32
K	Min	848	169.6	2300	3000	3400	7.37	5.65	4.98
	Max	3204	640.8				27.86	21.36	18.84
Mg	Min	209	41.8	130	410	420	32.15	10.19	9.95
	Max	589	117.8				90.61	28.73	28.04
Mn	Min	2.98	0.596	1.50	2.20	2.30	39.73	27.09	25.91
	Max	11.77	2.354				156.93	107	102.34
Mo	Min	0.05	0.01	0.022	0.043	0.045	45.45	23.25	22.22
	Max	0.064	0.0128				58.18	29.76	28.44
Na	Min	19.60	3.92	1000	1500	1500	0.39	0.26	0.26
	Max	56.30	11.26				1.12	0.75	0.75
P	Min	568	113.60	500	1250	700	22.72	9.08	16.22
	Max	1037	207.40				41.48	16.59	29.62
Se	Min	0.72	0.144	0.03	0.055	0.055	480	261.81	261.81
	Max	1.73	0.346				1153.33	629.09	629.09
Zn	Min	0.89	0.178	5	11	11	3.56	1.61	1.61
	Max	3.45	0.69				13.80	6.27	6.27

[a] Pavlovic et al. (2015) analysed 4 different aronia juices. The Min and Max values are the lowers and the highest values from all four samples.

Table 5. Heavy metal content in *Aronia melanocarpa* juice compared to Maximum Accepted Levels in drinking water by EPA (National Primary Drinking Water Regulations | US EPA, n.d.)

Aronia juice (Pavlovic et al., 2015)[a]				**Maximum accepted level in drinking water (EPA)**
Mineral		**mg/kg**	**mg/200g portion**	**mg/kg**
As	Min	0.37	0.074	0.01
	Max	0.79	0.158	
Cd	Min	0.05	0.01	0.005
	Max	0.064	0.0128	
Ba	Min	0.77	0.154	2
	Max	2.06	0.412	
Pb	Min	0.061	0.0122	0.015
	Max	0.143	0.0286	

[a] Pavlovic et al. (2015) analysed 4 different aronia juices. The Min and Max values are the lowers and the highest values from all four samples.

The minerals in Table 6 may be quantified in aronia products however their concentrations in foods are not regulated in official regulations and provisions.

Table 6. Other minerals in *Aronia melanocarpa* juice

Aronia juice (Pavlovic et al., 2015)[a]			
Mineral		mg/kg	mg/200g portion
Sb	Min	0.13	0.026
	Max	0.54	0.108
Co	Min	0.01	0.002
	Max	0.092	0.0184
B	Min	1.44	0.288
	Max	9.32	1.864
Ni	Min	0.13	0.026
	Max	0.86	0.172
V	Min	0.47	0.094
	Max	1.43	0.286
Si	Min	3.3	0.66
	Max	7.4	1.48
Li	Min	0.016	0.0032
	Max	0.072	0.0144
Sr	Min	0.34	0.068
	Max	3.67	0.734
Al	Min	1.64	0.328
	Max	9.7	1.94
Sn	Min	0.86	0.172
	Max	1.09	0.218

[a] Pavlovic et al. (2015) analysed 4 different aronia juices. The Min and Max values are the lowers and the highest values from all four samples.

2.2. Mineral content in aronia pomace

After juice extraction, the pomace will still be rich in minerals as shown by Pieszka et al. (2015) and Sójka et al. (2013) cited by Jurendić & Ščetar, 2021 (Table 7).

Aronia pomace powder has been studied mainly for the fibre and phenolic content and currently it is used as substitute for cocoa powder in biscuits (Molnar et al., 2020), as a substitute for starch in ready-to-eat extruded cereals (Schmid et al., 2022) and as a colouring foodstuff (Nemetz et al., 2021). Mineral analysis and bioavailability were not presented in these studies.

Table 7. *Aronia melanocarpa* pomace mineral concentrations

Mineral	Concen-tration mg/kg	Reference
Na	37[a]	Pieszka et al., 2015
	50-90[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021
K	2780[a]	Pieszka et al., 2015
	1810-3080[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021
Ca	2750[a]	Pieszka et al., 2015
	2190-4080[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021
Mg	880[a]	Pieszka et al., 2015
	370-2500[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021
P	2390[a]	Pieszka et al., 2015
	2390[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021
Zn	15.7[a]	Pieszka et al., 2015
	06-37[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021
Fe	197[a]	Pieszka et al., 2015
	75-80.6[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021
Cu	1.95[a]	Pieszka et al., 2015
	5-12[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021
Mn	31.5[a]	Pieszka et al., 2015
	32[b]	Sójka et al., 2013 cited by Jurendić & Ščetar, 2021

[a] Pieszka et al., 2015 quantified the minerals in dried pomace.
[b] Sójka et al., 2013 cited by Jurendić & Ščetar, 2021 did not mention if the values are an interval or minum and maximum, nor the number of samples analysed.

CONCLUSIONS

High concentrations of Se, Mn and Cr were reported by Pavlovic et al. (2015) in aronia commercially available juice showing that a 200 mg serving of juice may provide several times the amount of DRI for all age groups. A serving of 200mg of juice will provide up to 11 times the DRI for selenium for 4-8 years old children and up to 6 times for adults 19+ years old. For chromium the same serving will provide between more than 4 times the DRI for adolescents 14-18 years old and adults 19+ years old and up to more than 9 times the DRI for 4-8 years old children.

Based on the reported data, the mineral content of aronia berries shows a large variability. For potassium it was found to be up to 3 orders of magnitude, varying from 2707mg/kg (Pavlovic et al., 2015) to 6790mg/kg (Cindrić et al., 2017b).

Fertilisation will have a strong impact on saccharides concentrations in berries however no reports were found on the influence of fertilisation on mineral concentrations in berries.

Based on the current knowledge, *Aronia melanocarpa* has a large potential to be a good dietary source of essential minerals such as K, Ca, Mg, P, Se, Cu, Cr, Mn, containing highly significant concentrations of polyphenols and a low content in sugar which can be controlled through fertilisation.

It is not adequate to compare the reported values for aronia berries and juices for mineral concentrations due to the fact that the analysed juices are commercially available and not extracted from the berries for which minerals have been reported. Moreover, there is no information on the varieties and harvest conditions of aronia berries used for the commercially available juices. Therefore, research is needed to identify the relationship between concentrations of minerals in aronia berries and juices and to identify the most relevant cultivars/varieties of aronia to produce juices with high mineral content.

There is an acute lack of information on the mineral content of aronia fruits and food products. Using the Mendeley Elsevier searching engine, a total of three article on minerals in aronia products (juice, pomace) were found and a total of 4 articles on minerals in aronia berries in over 1296 article published from 2005.

Extensive research is needed in order to identify and quantify the mineral concentrations of aronia fresh berries and products and to contribute to an advance knowledge of mineral content as a key role for healthy natural products. Additionally, the variability of the mineral's concentrations in the aronia fruits should be studied in relation with crop variety, soil characteristics, climate changes, fertilization and harvesting time.

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IN VITRO RESEARCH STUDY ON THE ANTIMICROBIAL ACTIVITY OF SEA BUCKTHORN, BLACK CUMIN AND GRAPE SEED ESSENTIAL OILS AGAINST SELECTED FOOD SPOILAGE FUNGI

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Abstract

Natural antimicrobial agents such as essential oils obtained from plants can control the growth of different food spoilage microorganisms, thus prolonging the shelf-life and quality of food products. As essential oils are not toxic and already used as flavoring agents, they can be used as substituents for chemical preservatives used to control microbial growth. This study aimed to evaluate the antimicrobial activity by determining the minimum inhibitory concentrations (MIC) of sea buckthorn, black cumin, and grape seed essential oils over four different mould strains usually occurring in the spoilage of fruit products. The fungi used in the experimental research were Penicillium expansum, Fusarium oxysporum, Botrytis cinerea, and Aspergillus flavus. To establish the minimum inhibitory concentration of the essential oils, the agar disc diffusion technique was used. The results showed that all essential oils inhibited the growth of Penicillium expansum and Aspergillus flavus strains at a minimum volume of essential oil of 30 µL.

Key words: *antimicrobial activity, essential oils, fungi, food spoilage.*

INTRODUCTION

Nowadays, food safety and the extension of food products shelf-life are vital factors that are closely studied and analyzed by both researchers and the food industry. Although modern techniques, such as modified atmosphere packaging (Stan et al., 2021), have been emerging over the past few years, food borne diseases are still a significant concern worldwide (Burt, 2004). Therefore, there is a stringent motivation to develop novel methods and techniques that could reduce or eliminate food borne pathogens in all types of food products (Liu et al., 2009; Tenea, 2021). Food products are in general perishable, and protection against spoilage is required during the critical phases of preparation, packaging, storage, and distribution, where the chance to be contaminated or cross-contaminated is high. As consumers have become more concerned about the food they consume and the processing methods used for conserving it, researchers all over the world are looking to replace the traditional methods used for conservation, which may depreciate the nutritional value and sensorial proprieties of the food products, with novel processing techniques (Wendy et al., 2014). Synthetic preservatives have been used to maintain the shelf-life and nutritional quality of large varieties of agri-food products for decades. The main disadvantage of using artificial preservatives is that they may have a negative outcome on the health of the final consumers. The food industry is trying to replace them with non-synthetic, non-toxic, and natural preservatives. Novel and innovative techniques such as essential oils (EOs) and spice extracts are used to extend the shelf-life of different food products (Reis et al., 2022). Essential oils are natural volatile compounds that possess antimicrobial activity, obtained from the plant's secondary metabolism, the most important ones being monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, esters, ethers, aldehydes, ketones, phenols) (Khodaei et al., 2021). To extract them from plants, steam distillation is the most used technique, but there are several other techniques in use, such as expression, fermentation, or extraction. In recent years there have been a large number of research studies that demonstrate the antimicrobial (Michel et al., 2012; Mangalagiri et al., 2021; Lisboa et al., 2022) and antioxidant (Baydar et al., 2007; Mohamed et al., 2016; Rodrigues et al., 2020) activity of EOs. Because of these proprieties, EOs have been used in the food and pharmaceutical industry. EOs and their active components show promising activities against many food-borne pathogens and spoilage microorganisms (Mangalagiri et al., 2021). When applied into a food matrix, a higher concentration of EOs is needed to exert similar antimicrobial activity as those obtained in *vitro* assays. EOs and their active components are novel and innovative techniques used to extend the shelf-life of food products (Tao et al., 2021). The antimicrobial properties of EOs have been reported in several studies (Ermumcu, 2022). The EOs antimicrobial activity results from the complex interaction between the different compounds (phenols, aldehydes, ketones, alcohols, esters, ethers, or hydrocarbons) found in EOs. In some cases, the bioactivities of EOs are closely related to the activity of the main components of the oils.

Black cumin (*Nigella sativa* L.) seeds essential oils have been used in medicinal applications for a long time because of their anti-inflammatory (Woo et al., 2012) and antimicrobial properties (Chaieb et al., 2011). Over 100 different chemical constituents, including all the essential fatty acids (linolenic and linoleic acid), terpenoids, aliphatic alcohols and unsaturated hydroxy ketones) are found in black cumin seeds (Ramadan & Moersel, 2002a). Other beneficial compounds found in the black cumin oils are carvone, an unsaturated ketone, terpene or d-limonene, a-pinene, and p-cymene (Ramadan, 2007). Yasni et al. (2009) studied the antimicrobial activity of black cumin (*Nigella sativa* L.) extracts on the growth of several pathogenic bacteria (*Salmonella typhimurium*, *Bacillus cereus,* and *Staphylococcus aureus*). The results showed that black cumin oil managed to inhibit the development of the pathogenic bacteria at different concentrations. Black cumin oil was incorporated into edible films with chitosan and alginate as matrixes, and the antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was assessed. The results showed that the films containing black cumin oil inhibited the growth of the bacteria in chicken meat samples (Takma & Korel, 2019).

Grapeseed oil is a by-product obtained from the winemaking process, which has great importance in the food industry and the medical sector, due to its therapeutic proprieties (Rosa da Mata, 2022; Maier et al., 2009). Grape seeds contain large amounts of vitamin E and proteins (Lachman et al., 2015). The oil obtained from processing the grape seeds has a significant amount of unsaturated fatty acids (oleic and linoleic acids) (Baydar & Akkurt, 2001; Lachman et al., 2015) and several bioactive components, such as tocopherols, phytosterols, tocotrienols, flavonoids, and phenolic acids (Bail et al., 2008). In a study realized by Mohamed et al. (2016), it analysed the antioxidant activity and bioactive compounds in different grape seed oils samples extracted by supercritical CO_2 and organic solvent. The results showed that this extraction technique represents an efficient green solvent for high-quality oil extraction. Rodrigues et al. (2020) conducted an experiment to assess the antimicrobial activity of a chitosan and gelatine film incorporated with phenolic extracts of grape seed and jaboticaba peel. The antimicrobial test showed that all samples inhibited microorganism growth, extending the shelf-life of the food sample analyzed.

Sea-buckthorn leaves (*Hippophae rhamnoides* L.) are a good source of biologically active substances and nutrients and a rich source of phenolic compounds, fatty acids, and vitamins A, C, E. Some of the most important compounds found in sea-buckthorn are carotenoids, sterols, flavonoids, lipids, tannins, and ascorbic acid, which possess biological activities (Jaroszewska & Biel, 2017). Sea-buckthorn oil is known to have the antioxidant capacity (Tkacz et al., 2020) and antimicrobial activity (Upadhyay et al., 2011), thus making it a valuable component for the agri-food industry. Jeong et al. (2010) studied the antimicrobial and antioxidant activities of sea-buckthorn extracts. The results showed that the EOs extracted from roots and stems had antimicrobial activity compared to other antimicrobial agents.

MATERIALS AND METHODS

Materials

In the present study were used three types of essential oils from Sea-buckthorn (*Hippophae rhamnoides* L.), Black cumin (*Nigella sativa* L.) and Grape seeds (*Vitis vinifera* L.), purchased from Dacia Plant and Hofigal. The four fungi strains used in the experiments (*P. expansum*, *F. oxysporum*, *B. cinerea* and *A. flavus*) were obtained from the collection of the Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest. The fungal strains were inoculated in Potato Dextrose Agar (PDA) medium and incubated at 25°C for a period of seven days.

Methods

The disc diffusion test method was used to investigate the antifungal activity of the three essential oils. PDA culture medium was sterilized at 121°C for 20 minutes and after cooling it was poured into the Petri dishes. After solidification, a quantity of 100 µL suspension (10^6 UFC/mL) of each fungal strain were spread in each plate with the Drigalsky wand. The inoculated Petri Dishes were left to rest for 30 minutes to incorporate the microorganism into it. Four discs of Whatman paper (Φ = 6 mm) were placed on the prepared medium, on which the different quantities of essential oil (10 µL, 20 µL, 30 µL, 40 µL, 50 µL and 60 µL) were added. The control samples consisted of Petri Dishes inoculated with the selected fungi strain, but without any essential oil on the Whatman disks. For each sample 2 repetitions were performed. The dishes were sealed with parafilm to prevent the evaporation of essential oils and incubated for 7 days at 25°C. Evaluation of antimicrobial activity was performed by measuring the diameter of the inhibition zone (including the diameter of the Whatman disc). There are many and different definitions in the literature for the minimum inhibitory concentration (MIC) (Hammer et al., 1999, Demo et al., 2005, Mihai et al., 2015, Vasile et al., 2017), and as a result of the review of these, and in relation to the specific condition in our study, the MIC was defined as the minimum amount of essential oil for which the inhibition halo diameter was at a minimum of 1 cm.

Statistical analysis

For each sample, three repetitions were performed. The obtained data was statistically analysed by using Microsoft Excel 2016. In all tests, it was considered the significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

The antifungal activity of sea-buckthorn, black cumin and grape seeds was determined against four fungi strains with high occurrence in fresh fruits (*P. expansum*, *F. oxysporum*, *B. cinerea* and *A. flavus*). The evaluation of the antifungal activity was performed by measuring the diameter of the inhibition zone and establishing the minimal inhibitory concentration. The results showed that the diameter of the inhibition zone and antifungal capacity are dependent of the quantity of EO used in the experiments. In order to establish the minimum amount of essential oils necessary to inhibit the development of the tested fungi strains, a quantitative screening was performed with 10 µL to 60 µL of each antimicrobial agent used in these experiments.

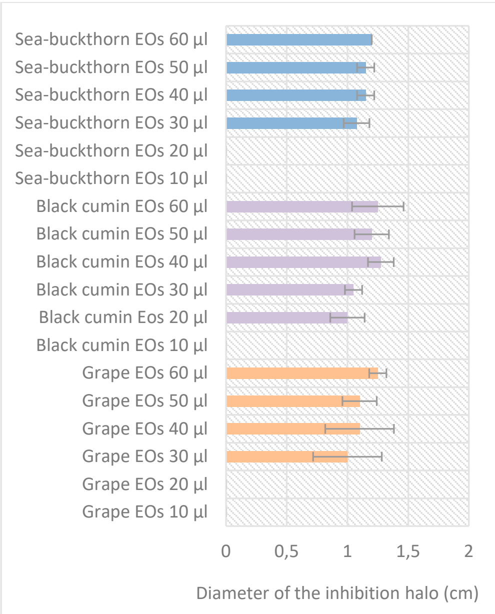

Figure 1. Graphical representation of the inhibition zone (diameter of the inhibition halo) of the essential oils tested on *Aspergillus flavus*

The results showed that in the case of *A. flavus* strain (Figure 1), grape seed and sea-buckthorn EOs presented antifungal activity from 30 µL, the diameter of the inhibition halo being around 1 cm. Black cumin essential oil presented antifungal activity at 20 µL, the diameter of the inhibition halo being 1 cm. The antifungal activity of the three EOs tested was almost the same when the dose was increased from 30 µL to 60 µL, thus the diameter of the inhibition halo for all three EOs ranged from 1 cm at 30 µL to 1.25 at 60 µL.
The tested EOs presented similar antifungal activity results in the case of *P. expansum* strain (Figure 2) as in *A. flavus* strain. Grape seed and sea-buckthorn EOs presented antifungal activity from 30 µL, the diameter of the inhibition halo being around 1 cm. In the case of black cumin essential oil, an inhibitory effect could be seen at 20 µL, the diameter of the inhibition halo being 1 cm.

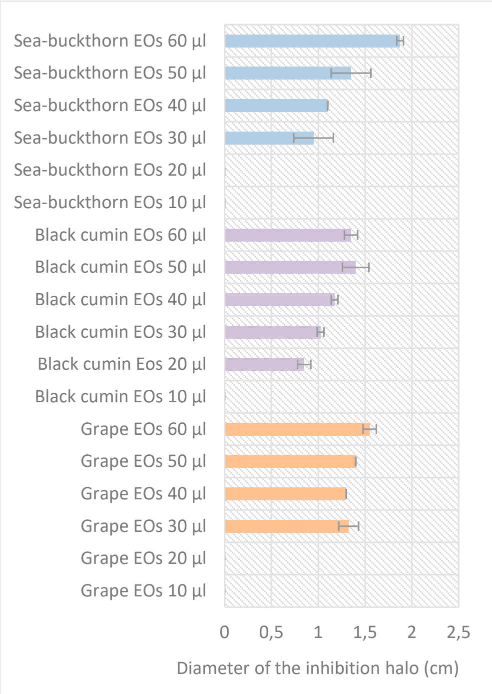

Figure 2. Graphical representation of the inhibition zone (diameter of the inhibition halo) of the essential oils tested on *Penicillium expansum*

The best results regarding the inhibitory effect were obtained at a concentration of 60 µL for the sea-buckthorn essential oil, the diameter of inhibition halo for *P. expansum* being 1.85 cm. At quantities ranging between 40 µL to 60 µL, black cumin and grape seed EOs presented similar inhibitory proprieties, the diameter of the inhibition halo measuring between 1.25 cm at 40 µL to 1.5 cm at 60 µL.
In the case of *F. oxysporum* and *Botrytis cinerea*, sea-buckthorn, black cumin and grape seeds EOs did not show antifungal activity, at any of the tested concentrations.
The minimum inhibitory concentration of sea-buckthorn, black cumin and grape seeds EOs were determined in order to assess their antimicrobial activity. Black cumin essential oil demonstrated lower MIC (20 µL) for both *A. flavus* and *P. expansum* compared with grape seed and sea-buckthorn EOs (Table 1). Grape seed and sea-buckthorn EOs had a MIC of 30 µl for *A. flavus* and *P. expansum*. For *F. oxysporum* and *B. cinerea* all three EOs tested did not show any antimicrobial activity even at concentrations of 60 µL.

Table 1. Minimum inhibitory concentration (MIC) of essential oils tested on *Aspergillus flavus*, *Penicillium expansum*, *Fusarium oxysporum* and *Botrytis cinerea*

Fungal strain	**Grape seed EO**	**Black cumin EO**	**Sea-buckthorn EO**
	MIC (µL)		
Aspergillus flavus	30	20	30
Penicillium expansum	30	20	30
Fusarium oxysporum	NA	NA	NA
Botrytis cinerea	NA	NA	NA

NA - no activity

CONCLUSIONS

In the present study, the antifungal activity of three EOs (sea-buckthorn, black cumin and grape seeds) was determined against four fungi strains with high occurrence in fresh fruits (*P. expansum*, *F. oxysporum*, *B. cinerea* and *A. flavus*). The results showed that all three EOs inhibited the growth of *A. flavus* and *P. expansum* strains at concentrations of 30 µL, the diameter of the inhibition halo being 1 cm. As for *F. oxysporum* and *B. cinerea* strains, none of the EOs tested had any growth inhibitory effect. Sea-buckthorn EO presented the best inhibitory effect over the *A. flavus* and *P. expansum* strains at 60 µL, the diameter of inhibition halo being at 1.85 cm. It can be concluded that the three EOs tested can inhibit the growth of *A. flavus* and *P. expansum* strain. It can be concluded that the three essential oils tested, sea-buckthorn, black cumin and grape seed, presented antimicrobial activity over the four fungi strains tested at concentrations over 30 µL. In order to establish the level of essential oil needed to inhibit the fungal growth in food matrix, further researches need to be made.

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MINIMALLY PROCESSING AND PRESERVATION METHODS FOR SHELF-LIFE PROLONGING OF DIFFERENT TYPES OF FRUITS

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Abstract

Minimally processed fruits are characterized by a short storage period, due to their high sensitivity to microbial and physico-chemical alteration due to the increased respiration rate and more ethylene production, which stimulates the overripening and the injury of the fruit tissue. Moreover, microbial alteration may present a food safety risk for the end consumers, fruits mostly being consumed in raw state. In addition, consumers have become more critical regarding the use of synthetic food additives utilized to increase the shelf life or to improve some sensorial characteristics of the fruits. Controlled temperature and hygiene of the whole supply chain offers the necessary conditions to maintain the quality of the products and to stop the alteration and cross contamination with pathogenic microorganisms. Because fresh fruits shelf life very much depends on certain temperatures and relative humidity parameters, to avoid spoilage, they must be handled properly during all the stages prior to their commercial points. The storage time of the majority of fruits is determined by changes in their sensorial characteristics and, therefore, in order to extend the shelf life and maintaining good quality, farmers and processors must keep the them at the optimum storage conditions that could be specific to each specie and variety.

Key words: *fruits, preservation, quality, shelf-life.*

INTRODUCTION

Fruits are known to be an essential part of the dietary human needs, as they are a major source of vitamins, minerals, carbohydrates and fibres, thus their consumption has been linked with several health benefits (Ma et al., 2017; Yousuf et al., 2018). The shelf life of a food product can be defined as the period in which it can be consumed without being harmful to the human health and its physical, chemical, microbiological and organoleptic qualities remain unchanged. Therefore, in order to extend the shelf life of various types of fruits, various preservation methods, regulations and rules have been established and must be applied by both producers and retailers (Yousuf, 2018). There are three major categories of preservation techniques used nowadays in order to extend the shelf-life of fruits: bio-preservation, physical-based and chemical-based preservation technologies (Ma et al., 2017). In order to maintain or extend the shelf-life of food products through physical-based technologies, temperature, pressure, gas composition and humidity are strictly monitored and modified taken into consideration the food product that is being packaged (Krasaekoopt & Bhandari, 2010). Bio-preservation techniques include the use of natural antimicrobial agents, such as plant extracts, and chemical-based solutions use synthetic additives in order to extend the shelf-life of fruit products (Liu et al, 2020). In recent years, there has been a development for innovative preservation technologies, such as pulsed electromagnetic field (Cao et al., 2019), cold atmospheric plasma, ultrasound treatment, modified atmosphere packaging or edible coatings (Giannoglou et al, 2021; Yildiz et al, 2021). In this review study, the most perishable types of fruits will be discussed and the actual preservation methods for shelf-life prolonging will be outlined for each type of fruit.

1. PRESERVATION METHODS AND SHELF-LIFE FOR APPLES

Apples are being consumed all over the world, all year round, thus making them one of the most economically important fruit in the world (FAO, 2017). The most common technique used to extend the shelf-life of apples is preserving them in cold storage, however over time, some quality losses may appear (Zhang et al., 2021). One of the key factors for maintaining their nutritional and organoleptic qualities during the storage period is determining a correlation between harvest maturity and the temperature of storage (Juhnevica-Radenkova et al., 2014; Zhang et al., 2021). da Rocha Neto et al., (2019) studied the effect of a double-bottom antimicrobial packaging used to extend the shelf-life of apple samples, by inhibiting the growth of *Penicillium expansum*. The results showed that the inoculated apple samples that were stored in the developed packaging material containing palmarosa essential oil had 1/3 less fungal growth, as well as less than 50% ethylene and CO_2 production. Ultrahigh hydrostatic pressure (HPP) apple juice samples were studied by Juarez-Enriquez et al., (2015) in order to observe the treatment influence over the shelf-life of the treated samples. Several parameters such as, ascorbic acid content, pH, titratable acidity, total soluble solids, polyphenol oxidase and pectin methylesterase were determined in the treated and control samples. The results indicated that the HPP treatment preserved the physicochemical parameters, as well as extended the samples shelf-life.

2. PRESERVATION METHODS AND SHELF-LIFE FOR GRAPES

Grapes (*Vitis vinifera* L.) is a worldwide spread fruit crop, with uses in many industries that possess a low physiological activity (Youssef et al., 2020). Because the table grape demand is increasing early, novel processing and preservation technologies are emerging very fast. Because of the high water content and nutrient composition, grapes are an ideal growth medium for fungi, especially *Botrytis cinerea* (Hernandez-Montiel et al., 2018). The main method for inhibiting the growth of fungi is applying chemical fungicides, however this technique is not sustainable, nor environmental friendly (Khalil Bagy et al., 2021). Shahkoomahally et al., (2021) studied the effect of modified atmosphere packaging (MAP) on extending the shelf-life of Muscadine grape (*Vitis rotundifolia* Michx). Grape samples were stored at 4°C and 95% relative humidity for a period of 42 days, in either air or MAP with different percentage of O_2 and CO_2 (6% O2 + 10% CO2), or (4% O_2 + 30% CO_2). The results showed that the samples packaged in MAP presented an improved preservation of compositional quality and delayed softening.

3. PRESERVATION METHODS AND SHELF-LIFE FOR BERRIES

The most perishable fruits on the market are berries, because of their high water content and short shelf-life. The berries market is growing from year to year, hence implying a great research effort in this field so that the producers can offer a longer shelf life and a higher quality for these types of fruits. However, the processing of berries can lead to several changes in the fruits: biochemical, the loss of texture and nutritional value and microbial contamination (Katsaros et al., 2015).

Berries are cultivated on different types of soil, through different technical and agricultural technologies. Some actions during cultivation and harvesting may favour the development of different types of microorganisms that can contaminate the fruits (Toivonen & Brummell, 2008; Paulsen et al., 2021). Due to the action of microorganism, it is estimated that about 30 % of berries stored at improper conditions deteriorate and therefore cannot be sold by the producers. Yearly, the harvest of berries records significant oscillations, as a result of the natural climatic conditions and inappropriate management. Such negative phenomena are accentuated by the high degree of perishability of many species and varieties of the berries (Yildiz et al., 2021). The development of microorganisms on berries is slow, but nevertheless, the microorganisms penetrare into the internat tissues of the fruit, becoming difficult to visually observe during the early stages. Some of the microorganisms, especially micromycetes of some species belonging to *Penicillium* and *Aspergillius* genus, are able to produce secondary metabolites of various compositions, which are toxic to the human health (Drusch & Aumann, 2005). It has recently been found that various alphatoxins, ochratoxins, fumigaciones, rugulosins, patulin, emodin, zearalenone, trichothecene, and other toxic compounds produces by certain species of micromycetes are particularly dangerous to human health (Mandappa et al., 2017).

Blueberries

Blueberries (*Vaccinium corymbosum)* are highly appreciated for their nutritional and medicinal proprieties (Defilippi et al., 2017). The marketing of this high value fruit crop is affected by a number of fungal and bacterial microorganisms (Liato et al., 2017). The shelf-life of blueberries can be maintained through a temperature controlled supply chain, where temperature monitoring and management are vital. In the food industry, deterioration occurs mainly in fresh products due to their short lifespan and perishability. Fresh food requires adequate temperature control through the technology chain. Unlike other products, blueberries need a strictly controlled temperature during the storage and transport processes. Proper and careful management of the temperature along the harvesting and marketing chain is essential for maintaining the quality of blueberries (Paniagua et al., 2014).

Blueberries can be stored for 2 to 4 months at a minimum recommended temperature of 2°C and a maximum recommended temperature of 4 to 5°C. The optimum relative humidity (RH) level is 90-95 %. After harvesting, blueberries are firm and have a thick layer of protective wax, so great care must be taken during the harvesting process, to insure they do not suffer any physical damage (Perkins-Veazie et al., 2008). However, they have a great variability in their shelf life, even in refrigerated conditions.

An earlier harvesting of the blueberries extends their shelf life. This variability in storage life can be reduced by good management of the temperature in the storage rooms, but also by the use of appropriate fungicides. The cold storage temperature is up to 3.3°C, and, at this temperature, the degradation of the berries is delayed, but nevertheless, long term storage is done at lower temperatures, around -1°C to 0.6°C (Nunes et al., 2004).

The dynamic of blueberry respiration plays a major role and affects the shelf life of the fruits because the respiration provides all the energy needed for the biochemical processes (Palanimuthu et al., 2007; Chen et al., 2021). Reducing the respiration rate by changing the storage conditions by lowering the temperature or changing the composition of the gases can significantly increase the shelf-life of berries (Bell et al., 2021**)**. However, there are a series of factors, such as the type of fruit, variety, the degree of maturity, storage time after harvesting, that have also been reported as potential factors affecting blueberry fruits respiration rate (Fonseca et al., 2002). Recently, several studies have investigated the use of high field voltage (HVEF) in drying fresh fruit to improve shelf life (Kao et al., 2019; Lotfi et al., 2022). A number of studies have reported an increase in drying rates for certain fruits and vegetables, for example, potatoes (*Solanum tuberosum)*, radishes (*Raphanus sativus* L.), spinach (*Spinacia oleracea* L), paddy rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) using single of multiple HVEF systems. However, the mechanism by which respiration is controlled by HVEF is not yet well defined and further studies are needed to describe the mechanism and its long-term effects on food (Palanimuthu et al., 2007). As this method of treatment does not consume significant amount of energy, it can be used to improve the shelf life of blueberries, without affecting the quality of the fruit (Kuriya et al., 2020)

Raspberries

Raspberries (*Rubus idaeus),* have a short shelf-life, and the main way of maintaining it is through the maintaining the temperature between 0-2°C and the relative humidity between 90-98% (Huynh et al., 2019).

Fresh raspberries are in high demand in Europe and in other parts of the world. The main constituent of raspberries is water (approximately 87%). Pectin is present in 0.1-

1.0%, but this percentage decreases with the ripening of raspberries due to the process of hydrolysis. The main sugars that are present in the fruits are glucose, fructose and, in small amounts, sucrose. They make up the major soluble component of raspberry juice. A ripe raspberry fruit contains between 5 and 6% sugar. Raspberries also contain small amounts of citric acid and malic acid (Bowen-Forbes et al., 2010). The amount of acid increases in the fruit at the beginning of the fruit growing period, and then decreases once the fruit begins to ripen. The balance between sugars and acids is important for raspberries to taste good and to be accepted by consumers. A fruit with a low sugar content will have a high acid ratio and therefore, a sour taste. The usual pH of ripe raspberries is 3.0 – 3.5; the ratio of sugars and acids (w/w) is about 1.0. Fruits grown in hot, dry summers (daytime temperatures close to 25°C) are sweeter, less acidic, have a stronger flavour, and more strongly coloured. Excessive heat (temperatures above 30°C) will reduce the fruit flavour, and wet weather will reduce the sugar content (de Souza et al., 2014).

Raspberries have one of the highest respiration rates of all berries. This aspect, along with their thin skin makes them very perishable compared to other berries. The optimal stage of maturity for raspberries occurs when berries turn completely red (Gonzalez et al., 2003).

The conditions under which the breathing process can be slowed down are storage at low temperatures, combined with high levels of carbon dioxide and small amount of oxygen in the storage room. The process of respiration is slowed down by high humidity. For raspberries, it is very important to maintain an atmospheric humidity of 90-90%, simultaneously with the low temperature in order to prevent the water loss from the fruit. Special cooling units designed to maintain a high humidity for raspberries are required. At 25°C and 30% relative humidity, fruit loses water 35 faster than at 0°C and 90 % relative humidity (Ozcelik et al., 2020).

Substances that have the ability to act as antimicrobial agents naturally prolong the shelf life of berries have been sought. One such natural compound is chitosan, obtained from the partial deacetylation of chitin, which is a polysaccharide (Kim et al., 2011). Chitosan is one of the most promising coatings for fresh produce due to its excellent coating, broad antimicrobial activity and compatibility with other substances (Kaya et al., 2016). The shelf life can be conditioned either by controlling the agents (growth of microbial populations, enzymatic activities, concentration of reactive compounds) or by monitoring their effects, such as changes in pH, aroma, texture, nutritional value, and the presence of specific compounds, mainly in the early stages. Another way to extend the shelf life is to cover foods with sodium alginate, pectin, and gelatine. They have been shown to be effective, not only in the way they delay water loss (Vimala et al., 2011; Joshi et al., 2021). There is an interest in the use of natural antimicrobials, as they have fewer side effects and have a better biodegradability compared to other available food preservatives (Campos et al., 2011; Kalemba & Kunicka, 2003). These compounds can be extracted from plants or essential oils. Only a few studies have been published on the efficacy of these compounds when incorporated into edible layers applied on fresh fruits (Campos et al., 2011; Rojas et al., 2007).

Raybaudi-Massilia et al., 2008, investigated the use of edible alginate based coatings in which they incorporated malic acid and essential oils of cinnamon, palmarosa and lemongrass on fresh cut melon (*Cucumis melo).* The coating containing 0.3% palmarosa oil appeared to be a promising preservation alternative, as it had received a good acceptation by panellists, maintained the food quality parameters and inhibited the growth of the native microflora and reduced the population of *Salmonella enteritidis.*

A new method of extending the shelf life of berries is to submerge them into various solutions contained polyelectrolytes charged with an opposite electric charge. Antimicrobial coatings with this technique have proved to be successful in treating papaya and pineapple fruits. However, further testing of the coating composition is still necessary due to problems with texture and flavour (Mantilla et al., 2013).

Strawberries

Strawberries (*Fragaria x ananassa)* are well known for their flavour and nutritional value. The quality of strawberries is defined by the intrinsic properties: appearance (color, size and shape), firmness, taste and high antioxidant content of fruits (Di Vittori et al., 2018). Strawberries have one of the most complex aromas of the berries and consists of about 350 volatile compounds (Schwab et al., 2008).

Strawberries are a source of compounds beneficial to human health, such as phenolic compounds, of which anthocyanins are the most abundant. The phenolic content of strawberries is highly variable, mainly due to differences in cultivation, growing conditions, maturity (Tian et al., 2017; Di Vittori et al., 2018).

The fruit skin of the strawberries is extremely thin and the flesh is very soft, therefore, they have a short shelf life of around 7 days (Ayala-Zavala & et al., 2004).

Studies indicate that the optimum storage temperature for strawberries is 0°C to 2°C, a temperature that does not cause the freezing of the tissue and minimizes the alteration of the fruits (Ayala-Zavala & et al., 2004; Aamer et al., 2021). Besides lowering the metabolic processes, storing the fruits at a low temperature also inhibits the growth of molds. The optimum humidity for their storage in order to prevent water loss and wrinkling is 90 to 95% (Di Vittori et al., 2018).

Ikegaya et al. (2020) studied the effect of the storage temperature in the presence or absence of film packaging on two varieties of Japanese strawberries. Storing the fruits at 0°C suppressed the spoilage and the reduction in sugars and organic acids compared to storage at 3°C. However, without the film packaging, storage at both 0°C and 3°C decreased the fresh weight, which results in a loss of quantity. The reduction of weight was lowered to <5% after 28 days by packing the fruits in film packaging. Modified Atmosphere Packaging (MAP) is a post-harvest technology used to extend the shelf life of fresh fruits and vegetables. In this technique, high concentrations of CO_2 and low concentrations of O_2 are often used to slow down the respiration process of the respiration process of the packaged product (Church et al., 1995; Sivertsvik et al., 2002).

Blackcurrant

Blakccurrants (*Ribes nigrum* and *Ribes rubrum)* are perennial plants that are part of the genus *Ribes*, along with gooseberries (*Ribes uva-crispa)*. Their fruits are covered with a protective layer of wax that protects the them form physical damage that can occur both during the growing season and during harvesting. The taste is sweet when the fruits are ripened, with variable acidity (Bakowska-Barczak et al., 2011).

Blackcurrants contain vitamin B, P, E, A, phosphorus, iron and potassium. Vitamin C is particularly valuable in these fruits. To ensure the daily recommended dose of vitamin C, it is enough to consume 20 berries of blackcurrant (Rubinskiene et al., 2005). In red currant (*Rubus rubrum),* the level of vitamin C is slightly lower compared to blackcurrants, but instead, red currants have a higher content of iron, potassium and vitamin P. Blackcurrant and redcurrant normalize the activity of the cardiovascular system and strengthen the blood vessels. Red currants helps a lot in detoxifying and lowering blood cholesterol (Barre, 2001).

Blackcurrants fruits need to be handled with great care, in order to not damage the natural protective coating and it is necessary to maintain the optimal temperature during transport. The boxes in which the currants are harvested should not be left exposed in the sun, as the temperature of the fruit can rise above the air temperature in less than one hour.

Research has shown that cooling currants with forced air at a temperature of 0°C within 2 hours showed a lower degree of degradation (37-46%), after storage for 10 days at -0.5°C, compared with the currants that have been cooled to 1.5°C for 48 hours (Mäkilä et al., 2017). Rapid heat dissipation with forced cold air is referred to as the pre-cooling or pressure cooling stage. After currants are cooled, they can be left in the cold room until they are packaged for their sale in stores. As with other small fruits, currants should be stored in high humidity (95 % at – 0.5°C. Harvested with great care, pre-cooled and stored at 0°C, the currants can reach a shelf life of about 14 days (Osokina et al., 2021).

Blackberries

Blackberries (*Rubus* spp.) are juicy, sweet berries and are considered a summer delicacy. Blackberries are very low in calories. 100 g of fresh blackberries contains only 43 calories.

However, they are rich in soluble and insoluble fiber (100 grams of fruit contain 5.3 of fiber or 14% of the daily recommended dose). Xylitol is low calorie sugar substitute in blackberries. It is absorbed into the bloodstream at a slower rate than glucose, helping stabilize blood sugar levels (Jiao et al., 2005).

Blackberries contain high amounts of phenolic flavonoids, such as anthocyanins, ellagic acid, tannins, gallic acid, cyanidin, pelargonidin, catechins, kaempferol and salicylic acid. Scientific research has shown that these antioxidant compounds may benefit human health and fight cancer, aging and neurological diseases (Hassimotto & Lajolo, 2011). Fresh blackberries are an excellent source of vitamin C, which is a strong natural antioxidant. Eating fruits that are rich in vitamin C helps to develop resistance against infectious agents, against inflammation and helps cleanse free radicals in the human body.

Blackberries can be stored for 2 to 5 days at – 0.6 to 0°C, with a relative humidity of 90 to 95%. Red blackberries gradually darken in colour and turn blue if they are stored for more than 8 days. It should be noted that when stored at 0°C, the colour does not change prominently as when storing blackberries at 4°C (Horvitz et al., 2017). Blackberries must be handled and transported with special care to avoid damage. It is recommended that 15-20% CO_2 should be added into the trucks during the transportation, in order to delay the softening and the degradation effect. Sometimes dry ice is used to supply carbon dioxide.

Blackberries are very perishable, but if they are to be eaten on the same day, they can be stored safely at room temperature (24 to 25°C). The berries are best stored at 1 to 3°C in the refrigerator (Soliva-Fortuny, 2010).

Gooseberries

Gooseberries (*Ribes uva-crispa)* are very perishable and their shelf life is quite short. It can be increased by rapid cooling after harvest. Fruits should be refrigerated at about 1°C within 2 hours of harvesting. Cooling requires a refrigerator with adequate cooling capacity and a fan system so that the cold air can circulate through the pallets of berries (Barney & Hummer, 2004).

Cranberries

Cranberries (*Vaccinium macrocarpon)* are small, red berries that contain significant amounts of vitamins that are beneficial to the human body (Jepson et al., 2012). These are closely related to blueberries (*Vaccinium corymbosum)*, belonging together in the same genus. Fruit ripening begins in mid-summer and the second harvest ripens in late September. Because cranberries are closely related to blueberries, they share a similar nutritional content; they are a good source of vitamin C, contain anthocyanins, about 100 mg per 3.5 kg of fresh fruits (Côté et al., 2010). Anthocyanin, a type of flavonoid, is a powerful antioxidant and it present in cranberries. Research suggests that these antioxidants help reduce the risk of heart disease and the risk of cancer. Anthocyanins help prevent the oxidation of cholesterol in the blood (Hancock et al, 2008).

Cranberries contain high levels of benzoic acid, which naturally offers a longer shelf life. The harvested and cleaned berries can be stored for 3 to 5 weeks before the delivery. Refrigeration at 0 °C will keep the cranberries at an optimum quality (Forney, 2003).

In order to lower the respiration rate, Palanimuthu et al., 2007 used high voltage electric fields (HVEF) of 2, 5 or 8 kV cm^{-1} for 30, 60 or 120 minutes on cranberry fruits. After treatment, the berries were stored at ambient conditions (23°C and 65% RH) for three weeks. After two or three weeks storage, the HVEF treated fruits showed significant lower respiration rates.

CONCLUSIONS

Storage in optimal conditions after harvesting fruits is very important because, after harvesting, the metabolism continues. The shelf life differs from the expiration date. The shelf life is related to the quality of the food products, while the expiration date refers to the safety of the food. The shelf life can be influenced by many factors: light and heat exposure, transmission of gases (including moisture), mechanical stress and contamination with microorganism.

Shelf life is a very important factor for human health. Bacteria and microorganisms are everywhere, and the food that is not properly stored can become contaminated with various types of microorganism that may produce secondary metabolites that are toxic to consumers, especially that fruits are usually eaten raw. It is important that each type of fruit is handled and stored according to their optimal parameters in each stage prior to their selling in stores, to ensure the final consumer not only benefits of unspoiled fruits, but also that the fruits maintain their organoleptic characteristics and their nutritional value.

The most used technique to extend the shelf life of fruits is cold temperature preservation, with controlled relative humidity and CO_2 levels, but new research suggests that other methods, such as the use of antimicrobial additives, pulsed electromagnetic plasma, ultrasound treatment, modified atmosphere packing or edible coating are also efficient in maintaining the nutritional values and organoleptic characteristics and inhibiting the growth of microorganisms of fruits.

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EFFICIENT RECYCLING OF FRUIT TREE WASTES THROUGH CONTROLLED CULTIVATION OF EDIBLE MUSHROOMS

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Abstract

The aim of this work was to establish the best biotechnology for efficient recycling of fruit tree wastes by using them as growing sources for mushroom species. In vitro experiments were carried out in controlled conditions for the optimal cultivation of Pleurotus ostreatus and P. eryngii to get their carpophores as food and medicinal biomass. There were set up three variants of substrates consisting of lignocellulosic wastes belonging to apricot, pear and walnut trees. After inoculation with pure cultures of mentioned species, the substrates were placed into growth chambers at constant temperature of 23 °C, during the incubation between 15-30 days. The physical and chemical parameters during the period of carpophore formation and development were set up and maintained at optimal levels, depending on each mushroom species. After a period of 30 up to 45 days, the registered results revealed a faster development of carpophores and a better productivity of P. ostreatus in comparison with P. eryngii, but the controlled cultivation of both mushroom species turned out to be an efficient recycling biotechnology to get value-added products.

Key words: *carpophores, in vitro cultivation, lignocellulose wastes, Pleurotus eryngii, Pleurotus ostreatus.*

INTRODUCTION

The woody wastes produced every year in any orchard, during the fruit tree pruning, represent in total a huge amount of redundant materials that need to be recycled through their using as main substrates for edible mushroom cultivation.

All these huge amounts of lignocellulosic materials that are coming out from the fruit trees are composed of dried trunks, branches, leaves and even fruit seeds.

However, the most part of these woody wastes are not used at all being stored in improper places or left on the fields leading to the environmental pollution (Zhang, 2008).

Sometimes, these wastes are used almost exclusively as heating sources in their rough shape or as briquettes or pellets (Soetaert and Vandamme, 2006).

The innovative procedure for recycling the fruit tree wastes through the controlled growing of mushrooms is based on the know-how of both submerged cultivation and solid-state development of such mushroom species and was carried out in specific conditions by keeping the strict correlation between both physical and chemical parameters as well as the biological factors represented by selected fungal species (Petre and Petre, 2016).

In this respect, the aim of this work was to solve this problem by recycling all fruit tree wastes through the controlled cultivation of mushroom species to get their edible carpophores, providing simultaneously the environment protection inside the orchards.

MATERIALS AND METHODS

Selected mushroom species

Following the main purpose of this research work, two mushroom species belonging to Basidiomycetes group, namely *Pleurotus ostreatus* and *P. eryngii*, from the mushroom culture collection of University of Pitesti, were selected to be used as biological tools for testing the efficiency of controlled cultivation.

P. ostreatus (Jacquin ex Fries) Kummer is a mushroom species with a high potential to grow on lignocellulose wastes and form mushroom fruiting bodies during their biological cycles (Sanchez, 2010).

P. eryngii (DC.) Quél., commonly known as "king oyster mushroom" is an edible mushroom species living saprobic or weakly parasitic on roots of herbaceous plants. *P. eryngii* is able to degrade lignin selectively when growing on non-woody lignocellulosic materials being of biotechnological interest for biopulping (Carlile and Watkinson, 1996; Camarero *et al*., 1998).

Methods used in experiments

The stock cultures were kept in viable state on malt extract agar (MEA) slants at 25ºC, for 5-7 days, and then being stored at 4ºC.

In order to get the inoculum for growing the mycelia of mentioned mushroom species, the pure cultures were inoculated in 250-mL flasks containing 100 mL of malt extract broth (MEB) as liquid medium (20% malt extract, 20% peptone and 2% yeast extract as solution in pure water up to 100%) and then kept at 23º C, on rotary shaker incubator at 110 rev min^{-1} for 12 days to get the mycelia biomass in order to be used as inoculum for mushroom cultivation (Petre *et al*., 2014).

Preparation of substrates for edible mushroom cultivation

Before starting the bioprocess of mushroom cultivation, all lignocellulosic wastes, mainly composed by trunks, branches and leaves belonging to apple, plum and apricot trees were collected from the fruit tree farms. Then, all woody dried materials were chopped and split in relatively equal sized fragments of 3-5 cm.

There were set up five variants of mushroom cultivation substrates made of lignocellulosic wastes belonging to apple, plum and apricot trees, mixed with cereal grain wastes from milling industry, such as wheat bran (5% w/w) and barley bran (5% w/w).

Beside these five substrate variants, it was used the pure cellulose (Merck) as control. All the natural ingredients were added with the role of enhancing the processes of growth and development of the mycelium of fungi used, such as, in order to stimulate enzymatic activity of fungal species, as well as processes of growth and development of mycelia biomass, according to Table 1.

Table 1. The composition of substrate variants used for controlled cultivation of mushrooms

Substrate variants	The composition of substrates
S1	Apple branches 60%, apple leaves 15%, barley bran 5%, wheat bran 5%
S2	Plum branches 60%, plum leaves 15%, barley bran 5%, wheat bran 5%
S3	Apricot branches 60%, apricot leaves 15%, barley bran 5%, wheat bran 5%
S4	Apple branches 20%, plum branches 20%, apricot branches 20%, barley bran 5%, wheat bran 5%
S5	Apple leaves 25%, plum leaves 25%, apricot leaves 25%, barley bran 5%, wheat bran 5%
Control	Pure cellulose (Merck)

Main stages of edible mushroom cultivation

In the first stage, all variants of substrates for mushroom cultivation were soaked in tap water–based synthetic medium containing 5 g/L yeast extract for 20 h at room temperature. After leaching, 1 kg of the substrate was placed in polypropylene gas-permeable bags for sterilization by autoclaving at 123°C for 50 min (Saddler *et al*., 1993; Wainright, 1992; Sanchez, 2010).

After cooling at room temperature (23-25°C), in the second phase of the experiments, the bags containing lignocellulosic wastes of apple, plum and apricot trees were inoculated with 10% (wet weight) of mycelium from pure cultures of the species *P. eryngii* and *P. ostreatus* by using the hood with sterile air laminar flow for aseptic handling of biological materials during the inoculation. Immediately after inoculation of substrates with the pure mushroom cultures of mentioned species, the inoculated plastic bags (three replicates for each strain/substrate) were placed in growth chambers type Memmert IPP 110 to be kept at a constant temperature of 23°C, during the incubation period lasting between 20 and 30 days, depending on the cultivated mushroom species (Van der Twell, 1994; Stamets, 2000).

In the next stage of cultivation, all the inoculated bags were exposed during three days at 4°C to induce a cold shock necessary for the stimulation of carpophores formation.

Then, the filled bags with inoculated substrates were kept in the fruiting room at 15-18°C, having the relative humidity around 90%, the intake air volume by 3 shifts/h and under

illumination of about 1,500 luxes (Arjona *et al*., 2009; Cohen *et al*., 2002; Stamets, 2000).
However, when the first primordia appeared, the bags were removed from the blocks formed by the mycelium colonization on the whole surface and inside the substrate volume.
Thus, the carpophores of both mushroom species emerged outside the plastic bags and they were collected during three consecutive flushes (Figures 1 and 2).

Figure 1. *P. eryngii* primordia grown on substrate S1

Figure 2. Bunch of *P. ostreatus* primordia, developed on the substrate S2

During the formation of carpophores of both mushroom species, there were registered three periods of 5-7 days, corresponding to cyclic occurrence of fruit bodies related to esch mushroom species in question (Petre *et al.*, 2014).
Data regarding the total weight of *P. ostreatus* carpophores emerged in bags containing all five types of cultivation substrates were regularly recorded and are presented in Table 2.

Table 2. The carpophore yield during the cultivation of *P. ostreatus* and *P. eryngii*, on five cultivation substrates

Mushroom species	Substrate variant	Carpophore yield (g/kg substrate)				
		Flush I	Flush II	Flush III	Total yield (g)	BE (%)
P.ostreatus	S1	570	260	145	975	97.5
	S2	510	225	135	870	87.0
	S3	470	215	145	830	83.0
	S4	450	215	125	790	79.0
	S5	420	205	120	745	74.5
P.eryngii	S1	560	250	140	950	95.0
	S2	495	230	120	850	84.5
	S3	450	250	125	825	82.5
	S4	430	220	110	760	76.0
	S5	410	190	115	715	71.5

RESULTS AND DISCUSSIONS

Regarding the controlled cultivation of both mushroom species on each one of all five substrate variants, it can be noticed in Table 2 the registered results as carpophore yield (g/kg substrate), as well as the values of biological efficiency.
Biological efficiency, often referred to as BE, is simply a way to calculate the effectiveness of a mushroom strain and substrate combination when growing mushrooms. It is a measure that was originally developed by the button mushroom industry in order to grade certain strains of mushrooms.
By definition**,** 100% biological efficiency occurs when 1 kg of fresh mushrooms is harvested from 1 kg of dry substrate, over multiple flushes.
BE = (weight of harvest/weight of dry substrate) x 100%.
Biological Efficiency (BE) was estimated as being the weight ratio between the fresh carpophores and dry substrate, multiplied by 100. In this way, the species *P. eryngii* cultivated on substrate variant S1 as well as *P. ostreatus* grown on the same substrate appeared to be the most productive mushroom/substrate variant ratio, compared with the rest of cultivation variants, the best values of BE being registered between 95% and 97.5%.
Both cultivated mushroom species were more productive especially in the first flush on the substrate S1, due to their high biological efficiency. The results regarding the weight of carpophores, revealed that the highest production of carpophores was registered in the first flushes of mushroom cultivation on the

first substrate variants, which were recorded as the most significant results (Petre *et al*., 2014). At the same time, the most productive substrates were found to be S1, S2, followed, finally, by the substrate S3. During this phase, the carpophores were harvested as being the most representative specimens of *P. eryngii* grown on all five types of substrates, as shown in the Figures 3, 4, 5 while those belonging to *P. ostreatus* are presented in Figures 6, 7, 8.

Figure 3. *P. eryngii* carpophores grown on substrate S1

Figure 4. *P. eryngii* carpophores grown on substrate S2

Figure 5. *P. eryngii* carpophores grown on substrate S2 (detail)

Figure 6. *P. ostreaus* carpophores grown on substrate S1

As it is shown in the Figures 6, 7 and 8, the carpophores of *P. ostreatus* have developed unusual shapes of their fruit bodies due to the low levels of air moisture correlated with a reduced oxygen level of the atmosphere inside the growth chamber during a period of over 30 hours, as a temporary disfunction of water sprayer.

Figure 7. *P. ostreatus* carpophores developed on substrate S2

Figure 8. *P. ostreatus* carpophores grown on substrate S3

According to the main results of this research work, the procedure of recycling the fruit tree wastes by using them as growing sources for controlled cultivation of edible mushroom species *P. ostreatus* and *P. eryngii* was established (Figure 9).

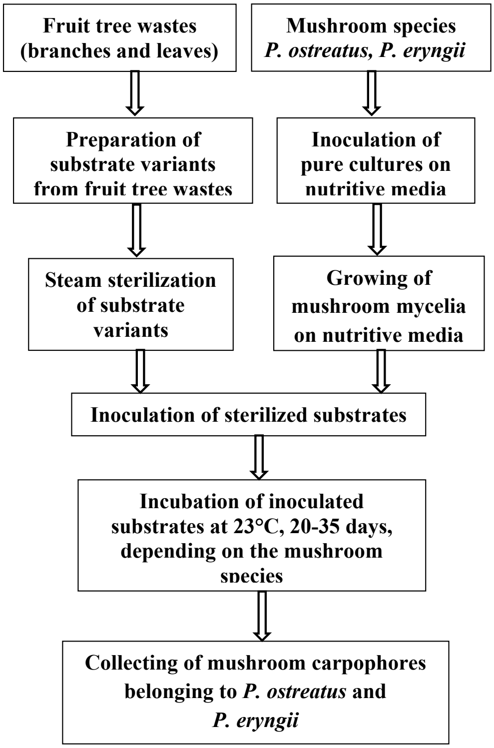

Figure 9. The scheme of efficient recycling of fruit tree wastes by controlled cultivation of edible mushroom species

CONCLUSIONS

The results regarding the weight of carpophores revealed that the highest production was registered in the first flushes of mushroom cultivation on the substrate variants.
The most significant results and most productive substrates were found to be S1, S2, followed, eventually, by the substrate S3.
Consequently, based on the carried out experiments, it was settled down the biotechnology for recycling the fruit tree wastes of apple, plum and apricot by controlled cultivation of mushroom species *P. ostreatus* and *P. eryngii*.

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INVASIVE NATIVE PLANTS IN ANTHROPOGENIC ECOSYSTEMS FROM OLTENIA, ROMANIA

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Abstract

Oltenia is located in the southwestern part of Romania, between the Danube, the Olt and the Southern Carpathians. The anthropogenic ecosystems in this part of the country are represented by those areas where human intervention is partial or total. These are confined from the plain to the Sub-Carpathian depressions. In Oltenia, there are to be found anthropogenic ecosystems comprising orchards, vineyards, protected crops (vegetables and flowers), as well as those of human settlements (rural and urban). Some spontaneous species found in these ecosystems are native plants that have shown an increased invasive potential in recent years. The analysis of data collected from the field shows an affinity of some species for certain anthropogenic ecosystems (ex. Calamagrostis epigeios (L.) Roth, Daucus carota L., Elymus repens (L.) Gould - for the orchards; Stellaria media (L.) Vill., Lamium purpureum L., Veronica hederifolia L., V. polita Fr., Senecio vernalis Waldst. et Kit. – for the viticultural ecosystems; Digitaria sanguinalis (L.) Scop., Cirsium arvense (L.) Scop., Sinapis arvensis L., Portulaca oleracea L. – for vegetable crop areas); Cardaria draba (L.) Desv., Capsella bursa pastoris (L.) Medik., Hordeum murinum L., Onopordum acanthium L. - for the human ecosystems. On the other hand, there are certain species found in almost all types of anthropogenic ecosystems: ex. Cynodon dactylon (L.) Pers., Convolvulus arvensis L.

Key words: *crops, invasive, native plants, Oltenia, Romania.*

INTRODUCTION

The plant world is the result of a long evolution, closely related to changes in the configuration of land, seas, relief, living conditions, as well as to the penetration of new species from neighboring areas, the migration or disappearance of old species and the evolution of other species (Cosmulescu, 2008).
Anthropogenic ecosystems have a specific structural and functional diversity that reflects the variety of ecological conditions, especially those related to climate and soils. The category of anthropogenic ecosystems within Oltenia includes orchards, vineyards, vegetable and flower farming areas, as well as those in the proximity of rural and urban settlements.
The anthropogenic ecosystems within Oltenia are characterized by a floristic variability during the vegetation period, which depends on latitude, altitude, exposure, microclimate conditions, soil type, water regime, temperatures, etc. If the distribution of anthropogenic ecosystems in Oltenia is regarded in the framework of the Romanian vegetation areas and vegetation levels, we can state that the former are encountered from the steppe area to the nemoral level (that of deciduous forests).
The spontaneous flora of the anthropogenic ecosystems located in this part of Romania is varied, with a high phytodiversity especially characteristic to the neglected or abandoned ecosystems.
Data on the presence of these species in different areas of Oltenia can be found in numerous floristic and vegetation studies (Buia, 1952; Păun, 1964; Costache, 2005).
Some of them are non-indigenous adventitious species (Dihoru, 2004; Costache and Răduțoiu, 2005; Răduțoiu, 2011).
The influence of climate changes during recent times has led to important modifications in the floristic composition of these ecosystems, resulting in the significant development of some native species that show invasive potential.
Invasive species are a form of “biological pollution” (McNight, 1993).
Invasive plants have drawn the attention of specialists since the 1980s, this fact being proved by the publication of several specialized papers (e.g. Drake et al., 1989; Groves & di Castri, 1991; di Castri et al., 1990; Pyšek,

1995; Pyšek et al., 2003; Carey et al., 1996; Răduțoiu & Costache, 2008; Răduțoiu & Stan, 2013).
Worldwide cultivated and spontaneous plants are affected by different biotic and abiotic constrainers, limiting their yielding capacity and adaptability in the areas affected by climate change effects (Paraschivu et al., 2020; Paraschivu et al. 2021; Velea et al., 2021; Durau et al., 2021; Paraschivu & Cotuna, 2021).
Useful information on the floristic composition of anthropogenic ecosystems can also be obtained by means of drones, which are already used in collecting data for forestry and agriculture (Călina et al., 2020).

MATERIALS AND METHODS

Numerous fieldtrips were made to different anthropogenic ecosystems in Oltenia in order to conduct this study, namely to observe the syndynamics of the spontaneous vegetation in these areas. In addition to the invasive alien species identified in the studied areas, there were observed native species that show an invasive potential in certain anthropogenic ecosystems. This has been identified especially in relation to the crops that are not properly maintained. Plant identification was performed by using the specialized literature (Ciocârlan, 2009; Sârbu et al., 2013).
The presentation of each analyzed plant comprises: the scientific name, the common name, the botanical family to which it belongs, the lifespan, the anthropogenic ecosystems where it was identified in numerous specimens, the manner of spread that favored its abundant development and possible biological control measures.

RESULTS AND DISCUSSIONS

Following the research conducted over several years within various anthropogenic ecosystems, there have been identified native plants that successfully compete with alien ones for the status of invasive species in Romania.
The degradation of certain anthropogenic ecosystems has enabled the installation and development of numerous native species that have noticeably proliferated, some becoming invasive, due to the fact that they have benefited from the reduced competition from these areas.
Significant elements in the category of native species that show invasive plant potential for different anthropogenic ecosystems in Oltenia are presented below.
Calamagrostis epigeios (L.) Roth - Wood small-reed. (Fam. Poaceae) - is a plant native to Central Europe, unpretentious to the substrate on which it grows, being found in numerous orchard ecosystems, tailings and ash dumps, neglected or abandoned orchards located in the plain region and up to the Sub-Carpathian hills. The presence of a very well-developed rhizome system and the high capacity of fruit spreading are some aspects that lead to an abundant development, which is almost exclusive on the areas where it begins to grow. It is used for grazing only in the early stages of vegetation because the mature plant has a well-developed sclerenchyma on the vegetative organs and an abundant hairiness that makes it undesirable for the herbivores.
Repeated mowing is recommended before the fruit ripening stage, in order to deplete the rhizomes in the soil.
Capsella bursa pastoris (L.) Medik. - Shepherd's purse. (Fam. Brassicaceae).
It is an annual spring or autumn species, native to Europe, frequently encountered in the rural and urban ecosystems, within orchards, but also in vegetable gardens. Due to a long anthesis that can sometimes last throughout the yearly vegetation period, to the massive seed production and to a low forage value, the plant easily spreads and even becomes monodominant on certain areas. Although it is known as a medicinal plant, in Oltenia it is regarded as a harmful weed and it is not used by the locals.
Destruction before fruit development is recommended in order to control this plant.
Cardaria draba (L.) Desv. - Whitetop. (Fam. Brassicaceae).
It is a perennial species, native to Europe, with invasive potential; in bloom, it contributes to the aesthetics of the place where it vegetates and it emits a pleasant scent. It has a high ecological plasticity, which gives it a significant resistance to environmental conditions.

The large number of specimens found along the transport network within rural and urban areas is due to the great number of seeds produced by phytoindividuals of this species and to the high propagation capacity through the shoots on the roots (Dihoru, 2004). In some anthropogenic ecosystems it is considered a harmful weed. There are many active substances that can fight this species.

Cirsium arvense (L.) Scop. - Creeping thistle. (Fam. Asteraceae).

It is a quarantine weed, native to all of Europe, Western Asia and Northern Africa and it is found in almost all anthropogenic ecosystems in Oltenia, from the plain region to the vegetable gardens located near the sheepfolds in the mountains. The massive presence of the plant on certain areas is favored by the easy spreading of its fruits and by the vegetative reproduction through the buds on the roots, the creeping thistle being among the few species that develop shoots from the roots. It is collected only during the vegetative stage by some locals, being used for pig feeding.

Repeated cutting off at ground level is recommended before flowering, in order to deplete the underground part of the plant, as well as to significantly reduce the number of seeds.

Daucus carota L. - Wild carrot. (Fam. Apiaceae).

It is an annual winter plant, native to temperate regions of Europe and Southwestern Asia and naturalized in North America and Australia. In Oltenia, it is invasive especially in the orchard ecosystems located within the Piedmont and the Sub-Carpathian hills.

The development of a large number of plants is favored by the irrational grazing conducted on these areas and by the easy fruit spreading (either through the wind or through domestic animals, to which it readily attaches). Mowing before fruit development in conjunction with rational grazing could mitigate the spread of this species.

Digitaria sanguinalis (L.) Scop. - Hairy crabgrass. (Fam. Poaceae).

It is an annual summer species, probably native to Europe (Pl@ntNet), known as the "common weed" in almost every country in the world; it is readily recognized by its inflorescence. Due to this plant's preference for areas characterized by high temperatures during the growing season, it is invasive only in the plain region of Oltenia, in vegetable crops grown on light-textured, xeric, weakly acidic soils. In the anthropogenic ecosystems of the Getic Piedmont and of the Sub-Carpathian hills, this species is present through isolated specimens.

Elymus repens (L.) Gould - Couch grass. (Fam. Poaceae).

It is a plant native to most of Europe, Asia, the Arctic biome and to the Northwestern Africa. It is frequently encountered in poorly maintained orchard and vineyard anthropogenic ecosystems, as well as on the edge of the well-kept ones and on the outskirts of rural settlements. The perennity of this species is mainly due to the very well-developed rhizome system, which is the origin of stolons. The plant eliminates any species with which it initially coexists and it forms monodominant surfaces. As it is a plant that resists rather well to water stress, some of the local people use it in animal feed during the dry periods of the year. It is sensitive to many herbicides.

Hordeum murinum L. - Wall barley. (Fam. Poaceae).

It is an annual species, common in most regions of Europe, which develops massive populations in the same typical areas as *Cardaria draba*. The wild rodents that collect the fruits for the unfavorable periods of the year and the ease with which the ripe fruits attach on animals or human clothing represent important contributing factors to the spread of this plant. Accidentally and in a small number of specimens, the plant is also encountered in orchards, vineyards or vegetable crop ecosystems.

It is used in the plain region of Oltenia as fodder for cattle during the dry periods of the year.

Lamium purpureum L. - Purple dead-nettle. (Fam. Lamiaceae). It is an annual species, native to Europe and Asia, which becomes invasive in almost all vineyard ecosystems in Oltenia and sometimes even in the vegetable gardens. In spring, before the development of the foliar apparatus of the crop plants, it gives the aspect of the grass layer within the cultivated areas. The development of a large number of plants in certain anthropogenic ecosystems in Oltenia is also favored by the long anthesis, which lasts from March to October. The only advantage that *Lamium purpureum* can bring to the coexisting crop plant consists in attracting the pollinating insects.

Onopordum acanthium L. - Scotch thistle. (Fam. Asteraceae).

It is a biennial plant, native to Europe and Western Asia, characteristic of the ecosystems located near the rural and urban settlements. It prefers nitrophilous areas, which it colonizes until full flowering, due to its robust general aspect. On the areas where it settles, the plant becomes monodominant and it eliminates almost all the species with which it coexists in the first stages of development. Its abundant hairiness, the presence of thorns on the edges of laminas and the easy spreading of the fruits, make this plant an "enemy" of the diversity of places where it grows.

It is invasive only in the plain and piedmont region of Oltenia; otherwise, the species is present only through sporadic individuals.

Portulaca oleracea L. - Common purslane. (Fam. Portulaccaceae).

It is an annual plant, with extensive distribution, which is present in almost all anthropogenic ecosystems in Oltenia, but it has the highest frequency in vegetable crop ecosystems, where it sometimes becomes invasive and develops vigorous specimens. The specialized literature includes these surfaces in the association *Portulacetum oleracei* Felföldy 1942 (Sîrbu, 2004). It prefers light-textured soils, especially the sandy ones. The massive presence in certain areas is facilitated by several factors, i.e.: the large number of seeds that a specimen can produce; the significant germination properties of the seeds of this plant, which can be preserved for a period of three - four years; the high resistance to diseases and pests due to the protective wax layer that covers the vegetative organs. In some cases, this plant is known to have antifungal properties (Banerjee & Mukherjee, 2002).

The local people avoid consuming this plant, although it is a rich source of alpha-linoleic acid, an essential omega 3 fatty acid, of vitamins C, E, proteins and negligible fats.

Senecio vernalis Waldst. et Kit. - Eastern groundsel. (Fam. Asteraceae).

It is an annual species, native to Southern and Eastern Europe, as well as to Western and Central Asia. It is able to colonize different types of anthropogenic ecosystems (Brandes, 2003; Hantsch et al., 2013; Kostov & Pacanoski, 2007; Schmiedel et al., 2014; Stevanovič et al., 2007). It is present within an area extending from the Oltenia Plain to the Sub-Carpathian hills, with a higher frequency in the plain region, where it often develops in numerous specimens, especially within the vineyard ecosystems.

During the vernal season, this plant dominates the lanes between the vine rows and it is accompanied by the few spring bulbous plants (e.g. *Ornithogalum boucheanum* (Kunth) Asch., *Gagea villosa* (M. Bieb.) Sweet, *G. lutea* (L.) Ker Gawl.), or by ephemeral vernal species (*Veronica hederifolia* L., *V. polita* Fr., *Stellaria media* (L.) Vill., etc.).

Two of the main reasons that explain the invasive character of this plant in certain vineyard ecosystems within Oltenia are represented by the easy spreading of fruits at full maturity, coupled with the presence of winds during fruit development. In the Southwestern part of Oltenia, it was also identified near the rural and urban areas, especially on surfaces with sandy or gravelly substratum. In the other anthropic ecosystems, it appears under the form of isolated individuals that add to the chromatics of the places.

Sinapis arvensis L. - Charlock mustard. (Fam. Brassicaceae).

It is an annual weed, native to Eurasia, which multiplies through the numerous seeds it produces (between 200 and 1,800 per plant). A large part of the seeds of this plant are included in the soil along with the tillage, forming a genuine "seed bank". The above-mentioned aspect, together with the high germination capacity and the plant's endurance in the competition with other species, support the idea that *Sinapis arvensis* L. will remain a major issue for the anthropogenic ecosystems in which it grows, causing significant damage. In vegetable crops consisting of plants related to it, this weed is a vector for viruses and fungi (Mulligan & Bailey, 1975). The size of the populations of this species is mainly influenced by climate, as it prefers periods with higher temperatures.

Stellaria media (L.) Vill. - Chickweed. (Fam. Caryophyllaceae).

It is an annual, ephemeral plant, native to the entire European continent. It is typical of the ecosystems located near the rural and urban settlements, but it is also encountered in other anthropogenic ecosystems within Oltenia, especially in the vineyards and vegetable crops, where it becomes exclusive. The specialized literature describes a vegetal association based on this species (*Stellarietum mediae* Prodan 1939, Hadač 1969) (Prodan, 1939). Sîrbu (2004) mentions that the phytocenoses dominated by this species do not harm the vineyards of Cotnari, Iași and Huși; nonetheless, in the anthropogenic ecosystems of Oltenia, the surfaces edified by *Stellaria media* significantly reduce the water regime in the soil, which has consequences on the vegetative and reproductive apparatus of the host plants.

The spring works conducted before fruit development can significantly reduce the areas where this plant is invasive.

Veronica hederifolia L. - Ivy-leaved speedwell. (Fam. Scrophulariaceae).

It represents an annual weed, native to Europe, tropical and temperate Asia, as well as North Africa (Pyšek et al., 2012); it is found in all anthropogenic ecosystems in Oltenia, especially in the viticultural and vegetable farming ecosystems. It prefers skeletal, light, nutrient-rich soils. Among all the annual species of this genus, this plant shows the highest resistance to control measures. In mild winters it can easily survive.

The good propagation of the plant is favored by the high viability of the seeds (about ten years), as well as by the fact that they germinate both in spring and autumn, at low temperatures. It forms genuine "seed banks" in the soils on which it grows.

Veronica polita Fr. - (Fam. Scrophulariaceae).

It is an annual species, common in ruderal and segetal areas within Oltenia. It is an Eurosiberian Southern-temperate species (https://www.brc.ac.uk/plantatlas/plant/Veronica-polita). It is found in almost all anthropogenic ecosystems in Oltenia, but it is invasive only in the vineyards and vegetable crops, mainly within open and sunny areas, where it forms colonies by reseeding. The plant also benefits from early blooming, which takes place before it develops the creeping stems, as it prefers the cold weather from the beginning of spring.

The first flowering specimens in 2022 were observed in February.

The analysis of the invasive native species from the anthropogenic ecosystems within Oltenia reveals the distinct predominance of annual plants (with a value of about 70%) (Figure 1).

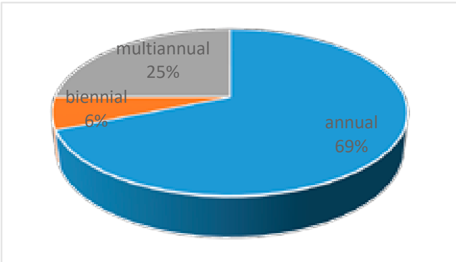

Figure 1. Lifespan analysis of invasive native flora in the anthropogenic ecosystems of Oltenia

CONCLUSIONS

The present paper analyses sixteen species of native vascular plants that are invasive in various anthropogenic ecosystems from Oltenia.

Their number is much smaller as compared to that of the alien species.

The impact exerted by the invasive native species encountered in the anthropogenic ecosystems of this Romanian territory is reflected in the economic loss (manifested either by declining productivity, or by increasing the costs necessary for their control), as well as in the ecological loss. Among these, the annual species are the most aggressive in the areas where they settle, because of the genuine "seed banks" that they form in the soil and of the high germination capacity of their seeds over a longer period of time (sometimes circa ten years - e.g. *Veronica hederifolia*).

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VEGETATION DAMAGE TO AGRICULTURAL CROPS IN OLTENIA, ROMANIA

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Abstract

The agricultural crops within Oltenia occupy significant areas, especially at the level of the Oltenia Plain and of the Piedmont Hills. The rest of the areas where agricultural crops are located account for less extensive surfaces (at the top of the Getic Piedmont and of the Sub-Carpathian hills). In recent years, much of the agricultural land in this part of the country has been cultivated with precision agriculture, but in some areas conventional agriculture is still maintained. The comparative analysis of the lands where conventional versus precision agriculture is practiced highlights the existence of a different spontaneous vegetation, which on certain surfaces is harmful to agricultural crops. If we add to these the increasing recent expansion of invasive alien species, we can say that the production of these surfaces is significantly affected. Vegetation harmful to agricultural crops in Oltenia is included in 12 associations. The phytocenoses of the association Stellarietum mediae Hadac 1969 have the largest distribution, but the phytocenoses of the association Setario-Sorghetum halepensis Ștefan et Oprea 1997 cause significant damage.

Key words*: agricultural crops, Oltenia, Romania, vegetation.*

INTRODUCTION

The crops within Oltenia are of major importance in the regional economy, as they cover representative surfaces, especially in the plain region, where favorable soils are located (e.g. chernozem). Studies on the soils on which this type of vegetation develops are present in various specialized works (e.g. Călina & Călina, 2019). At the level of the Getic Piedmont, of the hills and of the Sub-Carpathian depressions within Oltenia, the agricultural surfaces are less represented.

In recent years, precision farming has been practiced in almost all agricultural lands in this part of Romania, but there remain certain areas where conventional agriculture is still maintained. On these surfaces, there has been noticed the installation of a spontaneous vegetation that competes with crop plants for the main factors: water, air, light, heat, etc. (Chirilă et al., 2002). Sometimes, the competition is so powerful that it can lead to a decline in the agricultural production or it can even compromise it. There can be stated that in such situations we are dealing with a "harmful vegetation".

Studies on this category of vegetation can be found in several specialized works (Bujorean et al., 1956; Păun, 1966; Păun et al., 1975, 1979; Răduțoiu et al., 2009). The plant species that give the physiognomy of the phytocenoses included in the associations that the present paper analyzes have been studied by numerous researchers; they have realized floristic inventories of these plants from different areas of Oltenia (Anghel et al., 1972; Popescu, 1975; Chirilă et al., 1998; Chirilă, 2001; Chirilă et al., 2002; Ciocârlan et al., 2004; Răduțoiu, 2009), or they have conducted observations on the degree of weed development within crops (Păun & Pop, 1970; Păun & Popescu, 1983). Most of them are classified as "segetal weeds".

Both spontaneous and cultivated plants are affected by various biotic or abiotic stress factors that make them more vulnerable to present climate changes (Durău et al., 2021; Paraschivu et al., 2020, 2021; Paraschivu & Cotuna, 2021; Velea et al., 2021).

Globally, around 30,000 plant species are known to cause damage to the crops in which they grow; out of these, 2,000 bring actual economic damage - which represents about 6.6% (Chirilă et al., 2002). In Romania, of the total number of species that cause damage to agricultural crops, about 16-23% (132 weeds) induce significant damage from an economic point of view (Chirilă et al., 2002).

MATERIALS AND METHODS

The data presented in this paper are the result of an extensive field and laboratory work that took place over a period of about 15 years. The information gathered over the years has allowed us to observe the syndynamics of this type of vegetation.

The different pedo-climatic conditions within the Oltenia Plain, the Getic Piedmont and the Sub-Carpathian Depressions, enabled the installation of a various vegetation in the cultivated lands characterized by conventional agricultural practices. The cultivated areas where precision farming is practiced lack spontaneous plants or they are characterized by the presence of rare specimens, which do not affect the crop plants.

Field trips were conducted in order to identify those phytocoenosis that belong to the spontaneous vegetation within crops and cause crop damage. The field trips enabled us to seize all vegetation phases, which, in turn, allowed us to correctly identify plants, as well as to observe all vegetation stages that are necessary for a correct classification in the superior cenotaxonomic systems.

During our field trips, we followed as much as possible the stage of the vegetation, its dynamics in time and space, as well as the successional directions of phytocoenosis within some territories.

Various bibliographic sources have been used for the cenotaxonomic classification of the analyzed vegetation (Sanda et al., 2001; Sanda, 2002; Sanda et al., 2007). The description of the associations that we included in this type of harmful vegetation was conducted on the basis of the collected field data, corroborated with the existing information in the specialized literature. The following information is presented for each association: the local characteristics, the differences observed between the information identified in the field and that present in the specialized literature, as well as the crops that have been affected in the framework of each type of vegetation.

RESULTS AND DISCUSSIONS

The vegetation characterized as “harmful” for the crops within Oltenia is included in the following cenotaxonomic system:

Cl. Artemisietea vulgaris Lohmeyer, Preising. et R. Tüxen ex von Rochow 1951; Ord. Onopordetalia acanthii Br.-Bl. et R. Tüxen ex Klika et Hadač 1944; Al. Brachyaction ciliatae Pop I. et Vițalariu Gh. 1971: *Ambrosietum artemisiifoliae* Vițalariu 1973; Cl. Stellarietea mediae R. Tüxen, Lohmeyer et Preising ex von Rochow 1951, Ord. Centauretalia cyani R. Tüxen, Lohmeyer et Preising. in R. Tüxen 1950 Al. Caucalidion lappulae (R. Tüxen 1950) von Rochow 1951: *Adonido-Delphinietum consolidae* Br.-Bl. 1970, Al. Veronico-Euphorbion Sissingh ex Passarge 1964: *Lamio-Veronicetum politae* Prodan 1939, Kruseman et Vlieger 1939, *Lathyro-Avenetum fatuae* Passarge in Passarge et Jurko 1975; Ord. Chenopodietalia albi R. Tüxen (1937) 1950, Al. Panico - Setarion Sissingh in Westhoff et al. 1946: *Digitario-Setarietum pumilae* Felföldy 1942 corr. Borhidi 1996, *Echinochloo-Setarietum pumilae* Felföldy 1942 corr. Mucina in Mucina et al. 1993, *Setario-Galinsogetum parviflorae* R. Tüxen 1950 em. T. Müller et Oderdorfer in Oberdorfer 1983, *Setario-Sorghetum halepensis* Ștefan et Oprea 1997, *Stellarietum mediae* Prodan 1939, Hadač 1969; Ord. Eragrostietalia J. Tüxen ex Poli 1966, Al. Amarantho – Chenopodion albi Morariu 1943: *Digitario-Portulacetum oleracei* (Felföldy 1942) Timar et Bodrogk 1955, *Tribulo-Tragetum* Soó et Timar in Timar 1954; Cl. Artemisietea vulgaris Lohmeyer, Preising. et R. Tüxen 1950, Ord. Agropyretalia repentis Oberdorfer et al. 1967, Al. Convolvulo-Agropyrion repentis Görs 1966: *Convolvulo-Agropyretum repentis* Felföldy 1943.

Based on the data collected from the field and corroborated with information from the specialized literature, the analysis of the vegetation included in the associations presented above highlights the following aspects (for each association):

Ambrosietum artemisiifoliae Vițalariu 1973

The presence of the *Ambrosia artemisiifolia* species on the Romanian territory was first reported at the beginning of the 20th century (1908), in Orșova (after Vițalariu, 1973 in Țopa & Boșcaiu, 1965) and the high risk of spreading that this plant may present was intuited since 1971 (Vicol, 1971); two years later, the above-mentioned association was described. At the beginning, the phytocenosis of this association were known only near the railways, but now they are found in different crops within Oltenia (e.g. watermelon, Figure 1, corn, sunflower). The floristic composition of the areas dominated by *Ambrosia artemisiifolia* always includes: *Erigeron canadensis*, *Galinsoga parviflora*, *Setaria pumila*, *Convolvulus arvensis*, *Digitaria sanguinalis*, *Cirsium arvense*, *Cynodon dactylon*, *Eragrostis minor* and *Plantago scabra*. Ruderal species are found only on the edges of crops. Although in terms of floristic composition, the common ragweed-dominated phytocenosis within crops are much poorer in species than those described in ruderal sites (56 species), they still cause significant damage to crops because of the aggressiveness mainly shown by the nucleus of allogeneic species. On these surfaces, the coverage values are around 95-100%.

Figure 1. *Ambrosietum artemisiifoliae* from watermelon culture

Adonido-Delphinietum consolidae Br.-Bl. 1970

It is mainly found within cereal crops (wheat, barley, oats, and rye) (Pedrotti, 2021). Numerous annual species are present in the composition of these phytocenosis (over 75%). This vegetation is harmful within crops that grow on leached, dry chernozems and which are characterized by conventional agricultural practices. The following species register high abundance-dominance: *Consolida regalis*, *Centaurea cyanus*, *Agrostemma githago*, *Anthemis arvensis*, *Myosotis arvensis*, *Viola arvensis*, *Cirsium arvense*, *Sonchus arvensis, Ranunculus arvensis, Lithospermum arvense, Papaver rhoeas*. It also develops well on the fallow lands that have been cultivated with corn in the past. Most of the species in these phytocenosis belong to the "segetal weeds" category. The area characteristic for the association is located in the south of Olt, Dolj and Mehedinți counties.

Lamio-Veronicetum politae Prodan 1939, Kruseman et Vlieger 1939

It is a pioneer association found in poorly maintained or unmaintained vegetable gardens, fallow lands and vineyards (Sanda et al., 2001, 2002, Huțanu, 2004, Răduțoiu et al., 2009). At the national level, it is known from different regions (Mititelu, D., 1970; Mititelu L. 1974; Burduja & Diaconescu, 1976; Diaconescu, 1978; Sîrbu, 2004; Răduțoiu, 2008). During the vernal season and sometimes at the beginning of the summer, it is harmful for the crops in which it settles (Răduțoiu, 2008).

Lathyro-Avenetum fatuae Passarge in Passarge et Jurko 1975

It is frequently found within the cereal crops from certain settlements located in the western part of Dolj County (Figure 2) and Mehedinți County, where conventional agriculture is still practiced. With the change of the crop type, the physiognomy of these phytocenosis also changes, being replaced by those dominated by species of the genus Veronica (e.g. *Veronica hederifolia*, *V. persica*, *V. polita*). Along with the species characteristic of the association, there are also found: *Vicia cracca*, *Valerianella locusta*, *Lathyrus tuberosus*, *Senecio vulgaris*, *Veronica polita*, *Centaurea cyanus*, *Fallopia convolvulus*, *Polygonum aviculare*, *Myosotis arvensis*, *Anagallis arvensis*, *Veronica persica*, *Convolvulus arvensis*, *Cirsium arvense*, *Sonchus arvensis*, *Sinapis arvensis*, *Veronica arvensis*. In very rare cases, we have also encountered: *Viola arvensis*, *Vicia angustifolia*, *Galium aparine*, *Lapsana communis*).

Digitario-Setarietum pumilae Felföldy 1942 corr. Borhidi 1996

The phytocenosis of this association are harmful to the crops cultivated on permeable sandy-clayey soils within the Oltenia Plain. They are found in vineyards, especially on the lanes between the vine rows (Oprea, 1998; Sîrbu, 2004; Răduțoiu, 2009), or in other crops that are present on this type of soil within the eastern part of Romania (Ștefan et al., 1987; Mititelu & Huțanu, 1996). The physiognomy of these places is given by *Digitaria sanguinalis,* which occupies the largest area of these lands along with *Setaria pumila* and *Portulaca oleracea*. Besides these species, there are also encountered: *Sonchus arvensis*, *Amaranthus retroflexus*, *Cirsium arvense*, *Convolvulus arvensis*. In the rainy years, these phytocenosis account for a coverage of almost 100%.

Figure 2. *Lathyro-Avenetum fatuae* from Dolj county

Echinochloo-Setarietum pumilae Felföldy 1942 corr. Mucina in Mucina et al. 1993

It is a common association in almost all Romanian regions, including in Oltenia (Soó, 1947; Păun et al., 1975; Coroi, 2001), namely in gardens and crops that allow for hoe handling (especially corn) and are neglected; it causes significant damage in these ecosystems. It prefers nutrient-rich soils. A certain stratification is also noticed within the phytocenosis of this association: the first layer consists of the species *Amaranthus powellii*, *Cirsium arvense*, *Echinochloa crus-galli* and *Chenopodium album*, the second layer is represented by *Mentha arvensis*, *Digitaria sanguinalis*, *Papaver rhoeas*, *Anthemis arvensis*, *Thlaspi arvense*, *Gypsophila muralis*, while the third layer includes representatives of the species: *Plantago major*, *Trifolium repens*, *Fallopia convolvulus* and *Convolvulus arvensis*.

Setario-Galinsogetum parviflorae R. Tüxen 1950 em. T. Müller et Oderdorfer in Oberdorfer 1983

It is frequently encountered in the Oltenia Plain, in potato or watermelon crops, on dry soils with a high content of sand or gravel, where it causes considerable damage. Although the floristic composition of these phytocenosis does not include many species, the coverage is very high because of the significant abundance-dominance of the species (Figure 3): *Galinsoga parviflora*, *Oxalis fontana*, *Polygonum persicaria*, *Veronica persica*, *Chenopodium album* and *Bassia scoparia*.

Figure 3. *Setario-Galinsogetum parviflorae* from the plain of Oltenia

Setario-Sorghetum halepensis Ștefan et Oprea 1997

Although the dominant species in the phytocenosis of this association is perennial, from a cenotaxonomic viewpoint it is characteristic of the annual vegetation types (Biondi et al., 2014; Mucina et al., 2016). It is harmful to corn and vegetable crops within the Oltenia Plain and the Getic Piedmont. *Sorghum halepense* does not form compact phytocenosis in the Sub-Carpathian depression and hills within this part of the country.

It is important to monitor the vegetation edified by *Sorghum halepense*, because on certain areas located in Oltenia it negatively affects Natura 2000 habitats (Figure 4).

Figure 4. *Setario-Sorghetum halepensis* from the Jiu Corridor (ROSCI0045)

Stellarietum mediae Prodan 1939, Hadač 1969

It is an association described by Prodan from the Transylvanian gardens (Prodan, 1939). At present, it is known from almost all areas of the country (Popescu et al., 1984; Vițălariu & Horeanu, 1989; Sanda et al., 2001; Sanda, 2002; Răduțoiu, 2008), especially from ruderal places. In certain areas, it is mentioned within vineyards (Sîrbu, 2004; Răduțoiu, 2008). It is especially harmful during springtime, in the crops where conventional agriculture is practiced. It competes with crop plants for nutrients and soil water supply. Along with species of the genus Veronica (*V. polita*, *V. persica*), *Lamium amplexicaule*, *L. purpureum*, and *Capsella bursa-pastoris*, it sometimes accounts for coverage values of 100% (Figure 5).

Figure 5. *Stellarietum mediae*
from the level of the Getic Piedmont

Digitario-Portulacetum oleracei (Felföldy 1942) Timar et Bodrogk 1955
Phytocenosis belonging to this association have been identified in gardens located in the main river floodplains within the Oltenia Plain and within the Getic Piedmont - to a lesser extent, as well as in vineyards (Grigore, 1968; Spiridon, 1970; Păun et al., 1975; Burduja & Horeanu, 1976; Coste, 1998; Coroi, 2001). It grows on nutrient-rich soils with a high content of sand or gravel. It prefers well-lighted and irrigated places.
Along with the dominant species (*Portulaca oleracea*), which accounts for a coverage of over 75% (Figure 6), there are encountered: *Veronica persica*, *Polygonum aviculare*, *Hibiscus trionum*, *Sinapis arvensis*, *Setaria pumila*, *S. viridis*, *Chenopodium album*, *Anagallis foemina*, *Solanum nigrum*, *Cynodon dactylon*. The annual species predominate, while the perennials are present in small number (e.g. *Cirsium arvense*, *Convolvulus arvensis*, *Chondrilla juncea*). It causes considerable damage to crops grown in conventional agriculture.

Figure 6. *Digitario-Portulacetum oleracei*

Tribulo-Tragetum Soó et Timar in Timar 1954
The phytocenosis of this association prefer places with high temperatures and increased light conditions (Figure 7).

Figure 7. *Tribulo-Tragetum*
from the Jiu meadow

It is found in the southern part of Oltenia, in various agricultural crops grown on sandy soils (Simeanu et al., 2019), or in abandoned vineyards (Bagi, 1990). It causes crop damage especially during dry years. The floristic composition of the association highlights the presence of species resistant to water stress (*Anthemis arvensis*, *Arenaria serpyllifolia*, *Crepis tectorum*, *Cynodon dactylon*, *Digitaria sanguinalis*). *Sedum caespitosum* (Cav.) DC., a rare Mediterranean species in Oltenia, was mentioned from some phytocenosis located in the Ciupercenii Vechi area (Simion, 2017).

Convolvulo-Agropyretum repentis Felföldy 1943

This association is present in abandoned vineyards, or in those that were neglected for a long period of time. After several years, there is to be noticed an evolution towards the floristic composition of the neighboring meadows, edified by *Poa pratensis* or *Agrostis stolonifera*. Once it has been settled, it is very difficult to control this type of vegetation because of the well-developed rhizome system of the dominant species and of the root buds that are characteristic for *Convolvulus arvensis*. Along with the two species that give the name of the association (Figure 8), there are also encountered: *Cynodon dactylon*, *Cirsium arvense*, *Polygonum aviculare*, *Cardaria draba*, and *Setaria pumila*.

Figure 8. Physiognomy of phytocenosis of the *Convolvulo-Agropyretum repentis* association

CONCLUSIONS

The analyzed associations, which were included in the commonly called "harmful vegetation", are largely dominated by annual species. Some of them are present in various agricultural crops: *Digitario-Portulacetum oleracei* (Felföldy 1942) Timar et Bodrogk 1955, *Digitario-Setarietum pumilae* Felföldy 1942 corr. Borhidi 1996, *Echinochloo-Setarietum pumilae* Felföldy 1942 corr. Mucina in Mucina et al. 1993, *Lamio-Veronicetum politae* Kruseman et Vlieger 1939; other associations are mainly present in certain crops: *Ambrosietum artemisiifoliae* Vițalariu 1973 (in watermelon, corn crops); *Convolvulo-Agropyretum repentis* Felföldy 1943 (in vineyards); *Lathyro-Avenetum fatuae* Passarge 1975 (in cereal crops); *Setario-Sorghetum halepensis* Ștefan et Oprea 1997 (in maize crops); *Stellarietum mediae* Hadac 1969 (in crops that allow hoe handling and in vineyards); some associations are characteristic of a certain soil type: *Tribulo-Tragetum* Soó et Timar in Timar 1954 - on sandy soils.

This type of vegetation is an important source of "weeds" for the neighboring crops, where precision farming is practiced, as well as for other types of natural vegetation located nearby.

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PECULIARITIES OF THE LINDEN LEAF AREA IN RELATION TO THE LEAF POSITION ON THE SHOOT

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Abstract

The study evaluated the linden leaf area (LA), and found models to describe the leaf area variation in relation to the leaf position on the shoot (Lp) and shoot parameters (CSL, cumulative shoot length). Leaves were studied on annual growth shoots, from the Cenad Forest Protected Area, Timis County, Romania. Leaf area (LA) was determined by scanning (SLA) and calculation based on leaf parameters (L, w) and correction factor (Cf = 0.31, optimal value found), based on relation of type MLA = L × w × Cf. The fit level between MLA and SLA (as reference) was described by a linear equation, and confirmed by R^2 = 0.985 and RMSEP = 4.97363. The variation of SLA and CSLA (cumulative scan leaf area) in relation to the position of the leaf on the shoot (Lp) and cumulative shoots length (CSL) was evaluated by regression analysis and described by equations, 3D models and in the form of isoquants, under conditions of statistical safety (R^2 = 0.960, R^2 = 0.999, p <0.001). Some leaves have been found as deviations of SLA from the theoretical model and can be considered as foliar indicators (FI) for growth conditions during the shoot vegetation period.

Key words: *annual shoot; correction factor; foliar indicators; leaf area; linden; model.*

INTRODUCTION

The typology of shoots and leaves, respectively the development of the associated physiological processes, varies primarily in relation to the plant species (Jing et al., 2016; Gleason et al., 2018; Rawat et al., 2021).
Elements of characterization of shoots and leaves in different plant species have been studied in relation to their genetic determination (Byrne, 2006; Busch et al., 2011); with the vertical gradient of nutrients in the leaves (eg. N) and the growth of shoots (Weih & Rönnberg-Wästjung, 2007); with the functionality of arboreal plants under the influence of nutrients (Zhang et al., 2018); with complexity of shoot structure, and leaf dimension (Watanabe, 2015); with increasing shoots and biomass production (Zavistanovicz et al., 2021); with some vegetative and generative processes (Saouab & Amraoui, 2020); with the allocation of biomass and its structure in relation to light (Liu & Su, 2016); with leaf thermoregulation elements and shoot architecture (Bridge et al., 2013); with the vigor of growing shoots in relation to ecological disturbances (Florence, 1996; Au et al., 2019).
Specific aspects of shoots and leaves have been studied in species of economic interest, such as fruit trees, fruit shrubs, vines, or ornamental plants.
Thus, the articulation of shoots and leaves in fruit trees was studied based on allometric relationships and specific patterns, in relation to different elements of influence (Baïram et al., 2017; Wang et al., 2020).
In the case of vines, studies have been conducted in relation to plant nutrition (Sala & Blidariu, 2012) and in relation to the adoption of genotypes and cultivation technologies (Dobrei et al., 2009, 2016).
The development of shoots has been studied in some ornamental plants (eg. rose) in relation to specific maintenance work by cutting (Erwin et al., 1997). The influence of the architecture and status of some shoots on the photosynthetic processes and the production of flowering shoots was evaluated (Zhang et al., 2020). Shoots in relation to growth, development and stress tolerance in ornamental plants have also been studied (Toscano et al., 2020).
Leaf traits were taken into account in the comparative analysis of different species (woody, grassy) in relation to influencing

factors (phylogenetic factors, ecological factors) (Gratani, 2014; Li et al., 2015; An et al., 2021).

The leaves of plants, in different species, have been studied in relation to water, water regime and water loss in the leaves (Zhang et al., 2015; Leuschner et al., 2019), in relation to light (Atherton et al., 2017; Kaitaniemi et al., 2018), nutrient content and associated processes (Huang et al., 2015; Jokar et al., 2021), response to stress factors (Al-Namazi & Bonser, 2020; Reimer et al., 2021; Seleiman et al., 2021), and in relation to pathogen attack (Drienovsky et al., 2017).

The present study analyzed biometric elements and parameters, and physiological indices of linden shoots, *Tilia tomentosa* Moench., in order to evaluate the variation of the leaf area in relation to the position of the leaves on the shoot.

MATERIALS AND METHODS

The biological material was annual linden shoots, *Tilia tomentosa* Moench. The shoots samples were taken in July (2021), in four repetitions, from the area of the Cenad Forest Protected Area, Timis County, Romania.

The parameters studied were represented by the length of the internodes, on the basis of which the cumulative shoots length (CSL) was calculated by summing the length of the internodes, in chronological order on the shoot.

At the level of the leaves, the dimensions (length - L and width - w) were determined on the basis of which the measured leaf area (MLA) was found. To accurately measured leaf area (MLA) it was necessary to find a correction factor (Cf). To find out Cf, a working model proposed by Sala et al. (2015), which ensures obtaining Cf with high precision, was used. Measured leaf area (MLA) was determined based on the relation (1), which took into account the dimensional parameters of the leaves (L and w) and the correction factor (Cf).

$$\mathrm{MLA} = \mathrm{L} \times \mathrm{w} \times \mathrm{Cf} \qquad (1)$$

In order to verify the accuracy with which the MLA was found, the scanned leaf area (SLA), which was considered the reference leaf area, was determined in parallel for each leaf. The calculation accuracy of MLA, in relation to a set of values of the correction factor (Cf), was evaluated based on the error calculated between MLA and SLA. The optimal value for Cf was considered to be in the case of minimum error value (ME) between MLA and SLA. To confirm the Cf, the RMSEP safety parameter, equation (2) was used.

$$\mathrm{RMSEP} = \sqrt{\frac{1}{\mathrm{n}} \sum_{j=1}^{\mathrm{n}} \left(\mathrm{y}_{\mathrm{j}} - \hat{y}_{j}\right)^{2}} \qquad (2)$$

The cumulative scanned area (CSLA) was calculated by summing the leaf area of each leaf, in successive order on the shoot.

The analysis and data processing was done to evaluate the statistical safety of the data set obtained, but also to evaluate the presence of the variance in the data set (ANOVA test).

Linear regression analysis was used to assess the degree of fit between MLA and SLA, and the level of statistical safety was assessed based on R^2 and the parameter p (<95%). Multiple regression analysis was used to evaluate the variation of SLA and CSLA in relation to leaf position on shoot (Lp) and cumulative shoot length (CSL), as direct action, and as interaction.

PCA (correlation) was used to evaluate the distribution of leaf samples, L1 to L15, according to the association with the parameters considered (CSL, CSLA, SLA, MLA). Cluster analysis and SDI were used to assess the degree of similarity and association of the leaf sample (L1 to L15) in relation to the values recorded for the parameters considered. The Cophenetic coefficient was used to evaluate statistical safety.

PAST software (Hammer et al., 2001), and Wolfram Alpha (2020) software were used for analysis, data processing and generation of 3D models and in the form of isoquants, and some calculations were made in EXCEL (module of mathematical and statistical calculations).

RESULTS AND DISCUSSIONS

Based on the length dimensions of each internodes the cumulative values (CSL) were calculated on the total length of the linden

shoots. The determined values were between 6.3 cm specific for L1, and 129.5 cm, as the sum of the individual values, L1 to L15. The values for the scanned leaf area (SLA) were between 42.449 cm^2 for the L1 leaf and 5.053 cm^2 for the L15 leaf, with the maximum value recorded for the L5 leaf. The cumulative foliar surface registered increasing values from 42.449 cm^2, value afferent to the leaf L1 up to 1275.333 cm^2, with a variable rhythm of ridges, in relation to the contribution of each leaf, in their order on the shoot length (L1 to L15).
The measured leaf area obtained based on the dimensional parameters of the leaves (L and w) was calculated using the value of the correction factor (Cf), based on a relation of the type of equation (1).
The correction factor was found according to the model proposed by Sala et al. (2015). The values recorded for the determined parameters are presented in Table 1. The ANOVA single factor test, in conditions of Alpha = 0.001, confirmed the safety of the recorded data and the presence of the variance in the data set (Table 2).

Table 1. Values of the parameters determined at linden shoot

Leaf sample	CSL	SLA	CSLA	MLA
L1	6.3	42.449	42.449	40.622
L2	13.5	78.947	121.396	77.860
L3	23	97.984	219.38	93.828
L4	31.7	125.061	344.441	119.090
L5	41	133.225	477.666	129.642
L6	49.5	130.825	608.491	126.341
L7	58.7	117.910	726.401	117.050
L8	68	107.611	834.012	111.191
L9	78.35	108.251	942.263	114.278
L10	89.85	109.006	1051.269	115.472
L11	101.25	87.871	1139.14	96.565
L12	111.35	78.840	1217.98	89.241
L13	120.25	36.108	1254.088	38.363
L14	125.55	16.192	1270.28	16.508
L15	129.05	5.053	1275.333	4.957

Table 2. ANOVA test, single factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5327627	3	1775876	35.38764	5.81E-13	6.229585
Within Groups	2810276	56	50183.49			
Total	8137902	59				

Alpha=0.001

For the calculation of the measured leaf area (MLA) based on the dimensional parameters of the leaves, the optimal value for the correction factor (Cf) was initially determined. The theoretical value falls within the range (0.1), and the finding of the optimal value was made by calculating a series of CF values, determining the MLA, and finding the minimum error compared to the SLA which was considered the reference value. Thus, the value Cf = 0.31 was found, in the conditions of the working samples, and the values of the average of the calculated errors, as the difference MLA - SLA, are presented in Table 3.
As a statistical safety parameter, RMSEP, relation (2), was determined. The minimum error (ME = 1.04 cm^2) was recorded in the case of the value of Cf = 0.31, and the value RMSEP = 4.973634, confirmed the value found for the correction factor (Cf).

Table 3. Values calculated for Cf and MLA and statistical safety parameters

Cf	SLA	MLA	ME	RMSEP
0.26	85.02	72.19	-12.84	15.19424
0.27		74.96	-10.06	12.33626
0.28		77.74	-7.28	9.60363
0.29		80.51	-4.51	7.14172
0.30		83.29	-1.73	5.33915
0.31		86.07	1.04	4.97363
0.32		88.84	3.82	6.30043
0.33		91.62	6.60	8.56632
0.34		94.40	9.37	11.2159
0.35		97.17	12.15	14.03349
0.36		99.95	14.93	16.93545

Simple regression analysis was used to find the level of match between MLA, calculated on the basis of L, w and Cf, and the SLA obtained by scanning.

Equation (3) was obtained, which describes the fit between MLA and SLA in conditions of high statistical safety, $R^2 = 0.985$, $r = 0.993$, $p < 0.001$. The graphical distribution of MLA vs SLA values and regression line are shown in Figure 1.

$$MLA = 0.9834x + 2.4533 \quad (3)$$

where: x – SLA (cm^2)

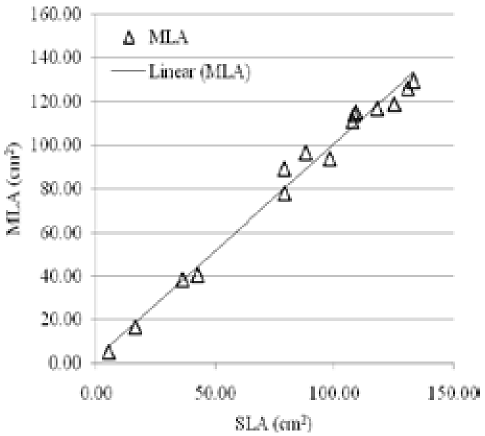

Figure 1. Graphic expression of the fit line, MLA vs SLA, linden leaves

The PCA analysis led to the diagram in Figure 2, in which the distribution of the leaf samples, L1 to L15, was obtained, depending on the association with the considered parameters (CSL, CSLA, SLA, MLA), as biplot.

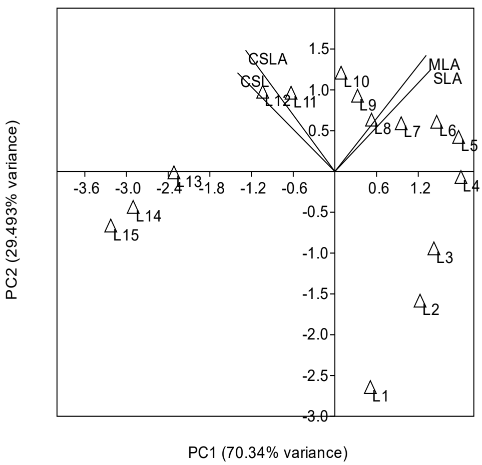

Figure 2. PCA diagram on leaf sample distribution (L1 to L15) in relation to reference parameters, as biplot

It was found the independent positioning of some leaves (positions L1 to L4, and L13 to L15) compared to the parameters taken into account. Leaves in positions L12 and L13 were associated with cumulative shoot length (CSL) and cumulative leaf area (CSLA) parameters. The leaves on positions L5 to L10 were associated with individual leaf surface parameters (SLA, MLA). PC1 explained 70.34% of variance, and PC2 explained 29.493% of variance (Figure 2).

The cluster analysis led to the dendrogram in Figure 3. It was found the formation of two distinct clusters, with several sub-clusters each, which include leaf samples (L1 to L15) depending on the degree of similarity in relation to the parameters considered, in statistical safety conditions (Coph.corr = 0.762).

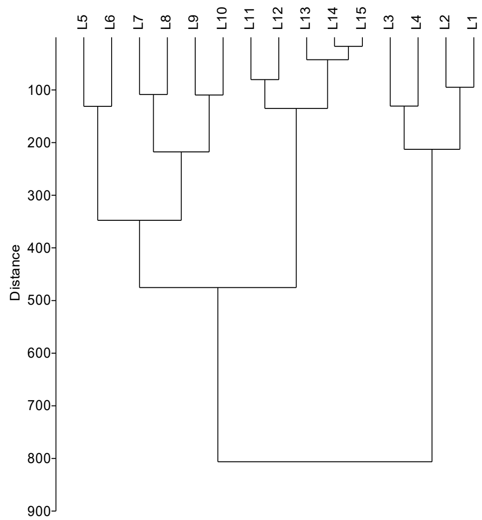

Figure 3. Leaf sample association dendrogram, by degree of similarity, based on Euclidean distances

The obtained SDI values are presented in Table 4. A high degree of similarity was recorded in leaves L14 and l15 (SDI = 17.184), followed by leaves L14 and L13 (SDI = 34.126).

These leaves are grouped in a common cluster in the dendrogram, [L13 (L14, L15)] (Figure 3), and in the PCA diagram they are in an independent position in relation to the parameters considered, as biplot. They are in the apical position on the shoot, with the lowest values for SLA.

Table 4. SDI values for linden leaf sample

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15
L1		94.885	193.65	323.77	454.73	580.89	694.31	799.75	908.08	1017.2	1103.1	1181.8	1217	1234.1	1240.1
L2	94.885		101.53	232.18	365.12	493.56	609.2	716.05	824.75	934.24	1021.7	1101	1139.2	1157.7	1164.3
L3	193.65	101.53		130.72	263.74	392.74	509.2	616.6	725.36	834.92	923.14	1002.7	1042.6	1061.9	1069
L4	323.77	232.18	130.72		134.21	264.81	382.99	491.29	599.89	709.41	798.92	878.89	921.81	942.53	950.51
L5	454.73	365.12	263.74	134.21		131.16	250.15	358.76	467.02	576.36	666.58	746.73	791.75	813.56	822.19
L6	580.89	493.56	392.74	264.81	131.16		119.33	227.97	335.99	445.28	535.72	615.94	662.2	684.8	693.94
L7	694.31	609.2	509.2	382.99	250.15	119.33		108.66	216.99	326.48	416.52	496.71	543.25	566.33	575.83
L8	799.75	716.05	616.6	491.29	358.76	227.97	108.66		108.79	218.4	307.92	388.1	435.44	459.31	469.36
L9	908.08	824.75	725.36	599.89	467.02	335.99	216.99	108.79		109.62	200.04	280.36	331.6	357.57	368.93
L10	1017.2	934.24	834.92	709.41	576.36	445.28	326.48	218.4	109.62		93.035	172.78	230.91	260.09	273.42
L11	1103.1	1021.7	923.14	798.92	666.58	535.72	416.52	307.92	200.04	93.035		80.33	140.15	171.28	185.94
L12	1181.8	1101	1002.7	878.89	746.73	615.94	496.71	388.1	280.36	172.78	80.33		76.142	110.24	127.09
L13	1217	1139.2	1042.6	921.81	791.75	662.2	543.25	435.44	331.6	230.91	140.15	76.142		34.126	51.08
L14	1234.1	1157.7	1061.9	942.53	813.56	684.8	566.33	459.31	357.57	260.09	171.28	110.24	34.126		17.184
L15	1240.1	1164.3	1069	950.51	822.19	693.94	575.83	469.36	368.93	273.42	185.94	127.09	51.08	17.184	

The variation of the SLA in relation to the position of the leaves on the shoot was described by a polynomial equation of degree 3, equation (4), in conditions of statistical safety, according to $R^2 = 0.960$, $p < 0.001$.
The variation of the cumulative scanned leaf area (CSLA) was described by a polynomial equation of degree 3, equation (5), also in statistical safety conditions, $R^2 = 0.999$, $p < 0.001$).

$$SLA = 0.099x^3 - 4.3783x^2 + 43.024x + 7.7569 \quad (4)$$

$$CSLA = -0.5787x^3 + 10.069x^2 + 66.754x - 42.546 \quad (5)$$

where: SLA – scanned leaf area;
CSLA – cumulative scanned leaf area;
x – leaf position on the shoot.

Multiple regression analysis was used to evaluate the variation of the SLA according to the position of the leaves on the shoot (Lp) and the cumulative length of the shoot (CSL). Equation (6) was obtained which described the variation of SLA as a function of Lp (x-axis) and CSL (y-axis), under conditions of $R^2 = 0.990$, $p < 0.001$, $F = 204.2354$.
The SLA variation according to Lp and CSL is presented in the form of a 3D model in Figure 4, and in the form of isoquants in Figure 5.

$$SLA = ax^2 + by^2 + cx + dy + exy + f \quad (6)$$

where: SLA – Scan leaf area; x – Lp – leaves position (no); y – CSL – Cumulative shoot length (cm); a, b, c, d, e, f – coefficients of the equation (6); a= 39.90880651; b= 0.55981845; c= 71.89687748; d= -4.58511570; e= -9.70627938; f= 0

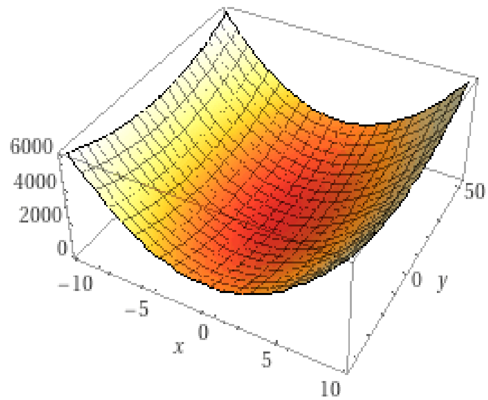

Figure 4. 3D model of SLA variation in relation to Lp (x-axis) and CSL (y-axis) in linden

The CSLA variation depending on the position of the leaves (Lp) and the cumulative length of the shoot (CSL) was described by equation (7) under conditions of $R^2 = 0.999$, $p < 0.001$, $F = 5855.883$.
A 3D graphic model was obtained (Figure 6) and in the form of isoquants (Figure 7) which represented graphically the variation of CSLA according to Lp (x-axis) and CSL (y-axis).

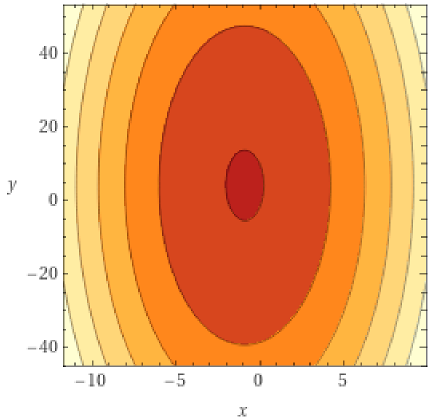

Figure 5. Model in isoquant format of SLA variation in relation to Lp (x-axis) and CSL (y-axis) in linden

$$\mathrm{CSLA} = ax^2 + by^2 + cx + dy + exy + f \qquad (7)$$

where: CSLA - Cumulative Scan Leaf Area; x – Lp – leaves position (no); y – CSL – Cumulative shoot length (cm); a, b, c, d, e, f – coefficients of the equation (1); a = -12.15325041; b= -0.39940623; c= -237.28979892; d= 42.24825265; e= 4.53261605; f= 0

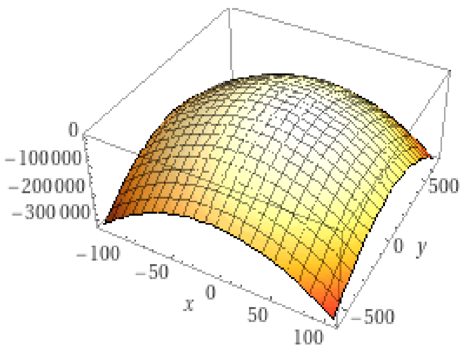

Figure 6. 3D model of CSLA variation in relation to Lp (x-axis) and CSL (y-axis) in linden

The value determined for the correction factor (Cf = 0.31) facilitated the obtaining with high precision of the measured leaf area (MLA), in relation to the SLA. Linear equation (3) described the fitting between MLA and SLA in statistical safety conditions, which confirmed the accuracy of the work. The validation of leaf area values, or of associated leaf area indices, determined by different methods, models and techniques, has been communicated in many studies, by means of specific coefficients such as R^2, RMSEP, coefficient of variation (CV), or other appropriate statistical parameters (Kumar & Sharma, 2013; De Carvalho et al., 2017; Yang et al., 2022).

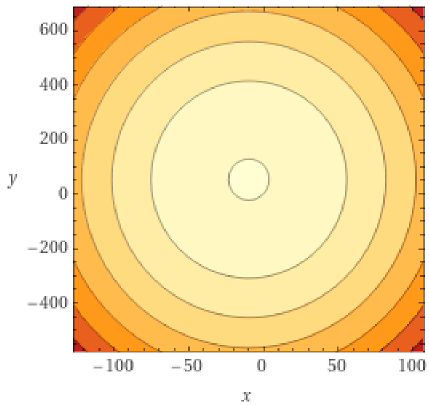

Figure 7. Model in isoquant format of variation of CSLA in relation to Lp (x-axis) and CSL (y-axis) in linden

The variation of SLA and CSLA was described in relation to the position of the leaves on the shoot (Lp) and the cumulative length of the shoot (CSL) by equations obtained on the basis of regression analysis, in conditions of statistical safety. The information obtained is useful for evaluating the LA variation in relation to the length of the shoots and the position of the leaves, and present importance through the models obtained. Based on leaf area, some studies evaluated cumulative leaf area index and leaf area index profile, in relation to biomass, biomass carbon and growth rate of some plant species (eg. pine) determined by LiDAR techniques (Beets et al., 2011). The study authors reported statistical safety values of the statistical parameters used to confirm data validation, and appreciated that LiDAR regression equations could be improved by integrating additional location and crops data.

Some studies have used models to describe the branch architecture and leaves based on allometric relationships, in order to predict the leaf area at different genotypes of fruit trees (eg. apple) in statistical safety conditions (Baïram et al., 2017). The authors reported that refining the models to increase predictive safety was made possible by focusing more on the skeletal structure of the tree crown (shoots length, spurs).

Structural equation models were used, in terms of statistical certainty ($p < 0.0001$), in studies to evaluate traits associated with "hydraulics, biomechanics and the spectrum of leaf economy" considered as factors with variable and independent contribution to growth and branch thickening (Gleason et al., 2018).

In the present study, from the detailed analysis of the graphical distribution, as well as from the obtained equations, it was found the deviation of the values of the leaves L7 and L8 in relation to the theoretical model, given by the function (4), fact that can be associated with vegetation conditions and the growth of those leaves. Such morphological elements can be considered as indicators for the expression of stress conditions during the vegetation period of the plants. The approach used in this study can be adapted to the study of species of high economic interest and can provide information for adapting cutting technologies, maintenance works in order to ensure a balance between vegetative growth / fruiting.

CONCLUSIONS

The present study facilitated the finding of the correction factor (Cf) and the determination of the leaf area by measurement of linden leaves in high statistical safety conditions, confirmed by the value of the coefficient R^2 of the fit equation between MLA and SLA, and by the RMSEP statistical parameter.

It was possible to obtain models describing the variation of the cumulative leaf area (CSLA) in relation to the position of the leaves on the shoot and cumulative shoot length (CSL).

The obtained equations and the graphical models highlighted the positioning of some leaves as a deviation from the theoretical model of variation of LA, which leads to the possibility of considering such cases as indicators of plant growth and development in relation to certain environmental factors and conditions of influence.

The approach models can be adapted to species of economic interest (fruit trees, ornamental species) in relation to maintenance works for vegetative growth, fruiting or floral elements and indicators of ornamental quality.

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MORPHO-ANATOMICAL CONSIDERATIONS ON THE SPECIES *LIMONIUM TOMENTELLUM* (BOISS.) O. KUNTZE FROM ROMANIA

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Abstract

Morphological and anatomical studies are of great importance to species of the genus Limonium, because they are essential for their correct differentiation. Limonium tomentellum (Boiss.) O. Kuntze. represents a plant of great sozological importance for Romania and it is mentioned in humid, halophilous meadows. A few specialized works include floristic and vegetation studies that mention this species, but data on the morphology and especially on the anatomy of the vegetative organs of this taxon are rare or absent.
The analysed vegetal material originated in the classic sampling location given for this species in Romania (Bratovoieşti - Dolj County). The specimens were collected in full bloom.
The roots of the specimens under study are taproots, vary in length, being comprised between 70 and 120 cm, and they have a secondary structure. At the level of the central cylinder, the stem has sclerenchyma, where the vascular bundles are located, being placed on three concentric circles. This tissue replaces the fundamental parenchyma. The leaves display an isolateral structure, their petiole having a semi-cylindrical shape, with a strongly convex lower part and a slightly concave upper part that extends on the two edges with a triangular formation with pointed end. It has six large steles towards the abaxial face and ten smaller steles towards the adaxial face, while each of the two extensions of the petiole contains two small steles that are placed one below the other; therefore, it is a polystelic petiole.

Key words: *characters, Limonium tomentellum, morpho-anatomy, Romania.*

INTRODUCTION

The first data regarding the halophilic species within Romania are published by Prodan (1922). Subsequently, with the appearance of specialized field guides (Prodan, 1939; Ciocârlan, 2000, 2009; Sârbu et al., 2013), important information related to the taxonomy, chorology, morphology, anatomy and ecology of these species also appears (Dihoru, 1990; Popescu et al., 2000; Oprea, 2005; Simeanu, 2005).
An inventory of the species characteristic for the saline environments in northern Romania is published by Țopa (1939) and a material that includes all species that vegetate on lands with different salt content within Romania is published by Grigore (2008).
Due to their adaptive heterogeneity in saline soils, halophytes are grouped into two categories, i.e. obligate and facultative halophytes (Țopa, 1954; Braun-Blanquet, 1964; Ciocârlan, 2000, 2009). Those in the category of facultative halophytes are further divided into: preferential, supportive and accidental (Popescu et al., 2000).
The plants characteristic of Romanian saline environments belong to five botanical families: Plumbaginaceae, Frankeniaceae, several genera in the Poaceae, Chenopodiaceae and Tamaricaceae families.
The Plumbaginaceae is a cosmopolitan family that has many representatives in the temperate areas of the Northern Hemisphere, preferring dry or salty surfaces (Kubitzki, 1993).
At the European level, the Plumbaginaceae family (order Plumbaginales) includes eight genera: Plumbago, Ceratostigma, Acantholimon, Armeria, Limonium, Goniolimon, Limoniastrum and Psylliostachys (Moore in Tutin et al., 1972). Only four genera (Plumbago, Armeria, Limonium and Goniolimon) with fourteen species are present in the spontaneous flora of Romania. Out of these, the *Limonium* genus has the best representation (six taxa) (Ciocârlan, 2009).

Studies on Limonium species are present in several specialized papers (Fraine, 1916; Arisz et al., 1955; Pignatti, 1971, 1972; Erben, 1993; Czerepanov, 1995; Dolores et al., 2005).
Based on a vegetal material collected from the Poltava region (Ukraine), Orlova and Kotelevsky (2016) conducted certain studies concerning the intensity of physiological processes that take place in the vegetative organs of the *Limonium tomentellum* species (intensity of respiration, perspiration and photosynthesis). Following these analyses, it was found that the intensity of the above-mentioned processes varies depending on temperature, humidity and light.
Limonium tomentellum (Boiss.) O. Kuntze (syn. *L. alutaceum* (Stev.) O. Kuntze, *L. czurjukiense* (Klok.) Lavr. ex Klok., *L. tschurjukiense* (Klok.) Lavr. ex Klok., *Statice tschurjukiensis* Klok., (Czerepanov, 1995), *Statice tomentella* Boiss.) is a very rare taxon in the spontaneous flora of Romania and it is known from several places in Oltenia (Dihoru et Dihoru, 1994; Dihoru et Negrean, 2009; Păun et al., 1970; Răduțoiu 2013, 2014; Răduțoiu et al., 2016, 2018). It belongs to the halophyte plant category.
The specialized literature (Dihoru et Negrean, 2009) supplies a morphological description of this taxon, but it is incomplete. Data on the anatomy of the vegetative organs of this plant are absent at the national level. Few papers in the field literature deal with the anatomy of the vegetative organs of Plumbaginagee species (Fraine, 1916; Rao & Das, 1968, 1981).
The aim of this research is to examine the anatomical features of the root, flowering stem and leaves of this species, in order to identify new characters that would contribute to a better differentiation from other species and to explain its adaptation to the substrate on which it grows.

MATERIALS AND METHODS

The analysed plant material originated in the classic sampling location given for this species in Romania (Bratovoieşti - Dolj County). The specimens were collected in full bloom.
The observations were conducted in a differentiated manner, i.e. the root system was analysed on a number of 20 plants, while the other elements were analysed on 100 specimens; both fresh and preserved material was used for these observations. Comparable segments were cut from the roots, stems and leaves of different specimens.
The root system was analysed through the total exhumation method. The length and the width of the vegetative organs were measured with a ruler. The anatomy of these organs was studied in cross sections, under a microscope. The epidermis was studied in tangential sections.
The microscopic measurements were performed with the ocular micrometer and a Nikon microscope was used for photography.
The following terms were used in the text: V_M = the maximum individual value, V_m = the minimum individual value, X = the arithmetic mean of the individual values.

RESULTS AND DISCUSSIONS

The analysis of the morphological and anatomical characters in the specimens collected from this species revealed the elements presented below.
The root is a taproot; first order lateral roots have an irregular appearance, grow parallel to the soil surface and display branches (Figure 1). The length of the root is characterised by the following values: V_M = 120 cm; V_m = 70 cm; X = 91.8 cm.

Figure 1. Shows the aerial parts of plant

In cross section, the root has a secondary structure (Figure 2). The phellem is multilayered, it is 90 μm thick and the outer layers are dead and partially exfoliated. The phellogen is single-layered, consists of slightly elongated cells in a tangential direction and it has an average thickness of 4.5 μm. The phelloderm is multilayered, its cells are regularly arranged in radial rows, just like those of the phellem, and the tangential walls of the first four - five layers of cells are collenchimatized. No strict delimitation can be made between the outer layers of the phelloderm and the outer part of the primary cortex, just as no delimitation can be made between the inner primary cortex and the phloem.

The primary cortex is made up of ovoid and spheroid shaped cells, with spaces of variable dimensions in between them, which make up the root aerenchyma. In the median area of the primary cortex there are four nodules made up of sclereids (or mineral salt deposits).

The primary phloem is not perceived as being distinct from the secondary one; together they are 78.75 μm thick. The size of the phloem vessels is characterised by: V_M= 13.5 μm; V_m = 6.75 μm; X = 10.57 μm. The cambium consists of one - two layers of cells that are elongated in a tangential direction. The well-developed secondary wood consists of xylem vessels, xylem parenchyma and xylem fibres, grouped in bundles of varied dimensions, generally located on the outer part of the secondary wood. The xylem vessels are not regularly arranged and have a diameter of: V_M = 38.25 μm; V_m = 15.75 μm; X = 27 μm. The medullary parenchyma is missing and the medullary area of the root is occupied by small xylem vessels that belong to the primary wood (Figure 2).

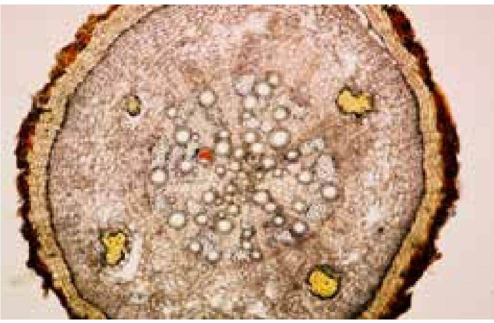

Figure 2. Root structure

The above-ground **stem** develops from a short rhizome, it is strongly branched starting with the lower third part and it has a length of: V_M = 73 cm; V_m = 34 cm; X = 56.1 cm.

On the cross section, the stem displays a primary structure (Figure 3) and has an average thickness of 1485 μm. The outward part of the stem displays a single-layered epidermis, which is 22.5 μm thick and has a 2.25 μm thick cuticle on its exterior side. The epidermal cells are tangentially elongated and they have cutinized walls. Many epidermal cells are transformed into elongated unicellular hairs.

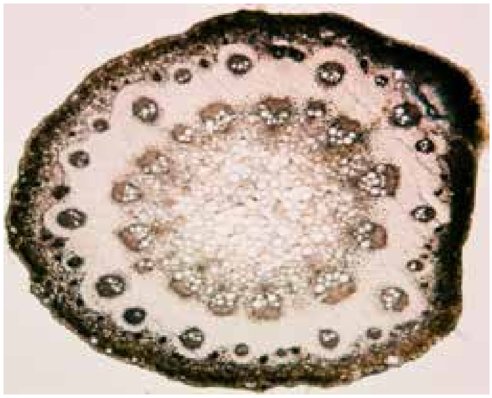

Figure 3. Stem structure

The cortex is 101.25 μm thick. The first three-four layers of cells located under the epidermis are radially elongated, arranged in palisades, with no spaces between them, with numerous chloroplasts inside. As we approach the central cylinder, the cortex cells become ovoid and then spheroidal in shape, containing few chloroplasts and small spaces between them. The starch sheath is not obvious.

The central cylinder is well developed and it does not display an obvious pericycle. The vascular bundles are arranged on three concentric circles, in the sclerenchyma, which occupies the place of the fundamental parenchyma; the circle located in the outer area of the central cylinder is made up of small bundles, the middle circle consists of larger bundles and the inner circle consists of much larger beams of the open collateral type.

The diameter of the xylem vessels in the outer circle is: V_M = 6.75 μm; V_m = 2.25 μm; X = 4.05 μm; that of those in the middle circle is: V_M = 18 μm; V_m = 6.75 μm; X = 11.7 μm, while that of the vessels in the inner circle is: V_M = 27 μm; V_m = 9 μm; X = 20.25 μm.

The diameter of the phloem vessels of the bundles located in the outer circle is: V_M= 4.5 μm; V_m = 2.25 μm; X = 3.15 μm; that of those located in the middle circle is: V_M= 4.5 μm; V_m = 2.25 μm; X = 3.6 μm, while the diameter of those in the inner circle is: V_M = 6.75 μm; V_m = 2.25 μm; X = 5.4 μm.

The outer circle displays vascular bundles that are completely surrounded by sclerenchyma, while others are only partially surrounded, the outer side remaining at the border with the parenchymal part that connects with the cortex. The middle circle is completely placed in the sclerenchyma and the bundles in the inner circle are incompletely surrounded by the sclerenchyma. The sclerenchyma cells located under the protoxylem have slightly thickened walls.

The central area of the stem is occupied by the medullary parenchyma, which consists of spheroidal cells with spaces of different sizes between them.

The leaves are arranged in a rosette at the upper part of the short rhizome and they are oblong-lanceolate, with the lamina gradually narrowing towards the petiole.

The basal ones are characterised by length values of: V_M = 32.1 cm; V_m = 11 cm; X = 17.13 cm and the width values of: V_M = 5.3 cm; V_m = 1.7 cm; X = 3.54 cm.

The adaxial epidermis has cells with straight and thick lateral walls, while the cuticle has numerous streaks on the outer walls, which represent its wrinkles. The values characteristic for the length of the epidermal cells are: V_M = 47.25 µm; V_m = 33.75 µm; X = 37.35 µm and those characteristic for the width are V_M = 27 µm; V_m = 13.5 µm; X = 22.05 µm.

The stomatal apparatus is of diacytic type, the stomata having length values of: V_M = 31.5 µm; V_m = 27 µm; X = 29.7 µm and density values of: V_M= 191.1 stomata/mm^2; V_m = 127.4 stomata/mm^2; X = 161.35 stomata/mm^2. The trichomes are unicellular, elongated and they start from a common cell, as a group of 4-6 elements arranged in a rosette shape (Figure 4). The values corresponding to the length of the trichomes are V_M = 292.5 µm; V_m = 117 µm; X = 184.5 µm.

The abaxial epidermis has epidermal cells with straight and thick lateral walls, and the cuticular streaks on the outer walls are less obvious than in the case of the adaxial epidermis. They are not regularly arranged and have length values of: V_M = 60.75 µm; V_m = 45 µm; X = 54 µm, while the width values are of: V_M = 36 µm; V_m = 22.5 µm; X = 28.57 µm. The stomata are of diacytic type, having length values of: V_M = 31.5 µm; V_m = 27 µm; X = 29.25 µm and density values of: V_M = 127.4 stomata/mm^2; V_m = 84.92 stomata/mm^2; X = 112.52 stomata/mm^2. Trichomes are unicellular, elongated and they start from the same cell in groups of 4-7, rarely fewer; they are arranged in a rosette and have length values of: V_M = 234 µm; V_m = 108 µm; X = 170.55 µm.

Figure 4. Trichomes unicellular

The leaf lamina is of isolateral structure type (Figure 5) and it is 301.5 µm thick. The single-layered adaxial epidermis consists of tangentially elongated cells, it is 22.5 µm thick and has a 2.7 µm thick cuticle.

Figure 5. Leaf structure

The adaxial palisade parenchyma consists of three layers of cells that are radially elongated and do not display spaces between them; the substomatal chamber is located under the stomata. The lacunar parenchyma consists of cells that are slightly elongated in tangential direction and have small spaces between them.

The abaxial palisade parenchyma consists of two, rarely three layers of radially elongated cells. The abaxial epidermis is single-layered, it

is 20.25 μm thick and has a 2.25 μm thick cuticle on the outside. Beneath the stomata there is the substomatal chamber.
At the level of the midrib there are eight vascular bundles of open collateral type, of which four large ones are placed towards the abaxial face of the leaf and four small ones towards the adaxial face. All vascular bundles are surrounded by a sclerenchyma ring, which is much more developed around the large vascular bundles. The diameter of the phloem vessels within the four large vascular bundles has the following values: V_M = 6.75 μm; V_m = 2.25 μm; X = 3.83 μm, while that of the xylem vessels from the same vascular bundles is characterised by: V_M = 24.75 μm; V_m = 9 μm; X = 16.42 μm.
On the cross section, the **petiole** has a semi-cylindrical shape (Figure 6), with a strongly convex lower part and a slightly concave upper part that extends on the two edges with a triangular formation with pointed end.

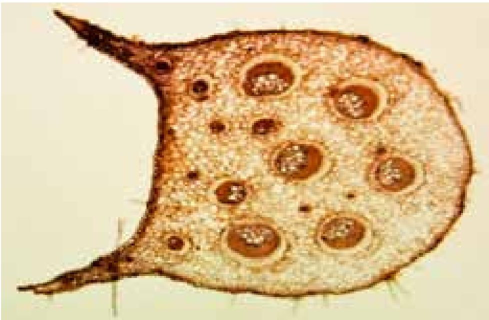

Figure 6. Petiole structure

On the outside, the petiole has a single-layered epidermis that consists of cells with cutinized walls; it has an average thickness of 18 μm, the cuticle being 0.9 μm thick. Among the cells of the epidermis there are elongated unicellular hairs that become sharp at the tip.
The cortex is multi-layered and consists of spheroidal and ovoid cells with large spaces between them. The petiole is polystelic, having six large steles towards the abaxial face and ten smaller steles towards the adaxial face, while the two extensions of the petiole contain two small steles that are placed one below the other. The large steles and partially the small steles are surrounded by a multilayered sclerenchyma ring. On the outside of the phloem and xylem there is located the single-layered endoderm, which consists of cells with thickened walls, combined with cells with thin walls. The cambium is to be noticed between wood and phloem. Each large stele is characterised by the presence of numerous xylem vessels that are not orderly arranged (Figure 7).

Figure 7. The structure of the conducting beam

The diameter of the xylem vessels has the following values: V_M = 18 μm; V_m = 6.75 μm; X = 13.05 μm. The xylem vessels are accompanied by xylem parenchyma.
The phloem is made up of vessels with a diameter characterised by the following values: V_M = 6.75 μm; V_m = 2.25 μm; X = 4.5 μm and of phloem parenchyma.

CONCLUSIONS

The branching of the root near the soil surface is due to the high humidity of the areas where this plant grows. The secondary structure of the root derives from the activity of the phellogen and of the one - two layers of tangentially elongated cells that belong to the cambium.
The flowering stem is strongly branched starting with the lower third part. It has a primary structure and numerous elongated unicellular hairs on the outside.
The leaves have an elliptical elongated shape and they are arranged in a rosette, at the basal part of the flowering stem. Their structure is isolateral and they have numerous elongated unicellular hairs that start from the same cell in groups of four to seven, rarely fewer.
The petiole has a semi-cylindrical shape, with a strongly convex lower part and a slightly concave upper part, which extends on the two edges with a triangular formation that sharpens towards the tip. The petiole is polystelic; it displays six large steles towards the abaxial face and ten smaller steles towards the adaxial face, while the two

extensions of the petiole contain two small steles that are placed one below the other.

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PRELIMINARY SURVEY FOR MAPPING THE DISTRIBUTION OF SPONTANEOUS GOJI BERRY SHRUBS IN ROMANIA

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Abstract

So far in Romania, based on literature, online resources, and personal reports, the solanaceous shrubs of Lycium barbarum L. (syn. L. halimifolium Mill.) were recorded in Bucharest and 37 counties, from a total of 41. During the field trips between July 2021 and February 2022, made by the authors and researchers in the frame of the ProtectGoji citizen science-based project, new data about the locations where the wild goji berry bushes are encountered were gathered and some of these records have already been introduced and validated in the iNaturalist database.
The present study records of spontaneous goji berry plants included Bucharest and 18 counties, as follows: Arad, Brăila, Brașov, Buzău, Caraș-Severin, Călărași, Cluj, Constanța, Galați, Giurgiu, Ialomița, Iași, Ilfov, Prahova, Olt, Teleorman, Tulcea and Vrancea. Of these, Bucharest and 14 counties (Arad, Brașov, Buzău, Caraș-Severin, Călărași, Cluj, Constanța, Galați, Giurgiu, Iași, Ilfov, Olt, Tulcea and Vrancea) were previously mentioned in the dedicated literature or online databases, whilst Ialomița, Brăila, Prahova and Teleorman represent new occurrences for wild goji distribution. To the best of author's knowledge, there are still no records or published data concerning distribution of wild Lycium shrubs in Covasna, Dâmbovița, Hunedoara and Sălaj, although some goji plantations have been reported in some of these counties.
The aim of this survey was to design a preliminary map of distribution for wild Lycium spp. in Romania, considering the increasing importance in terms of ornamental, nutritional and therapeutic value, but also for plant protection reasons, to prevent the spread of new alien species in the commercial fields via the reservoirs of wild goji berry plants.

***Key words**: Lycium sp., citizen science, distribution map, spontaneous, Romania.*

INTRODUCTION

Lycium barbarum L., syn. *L. halimifolium* Miller and *L. vulgare* Dunal, is a solanaceous plant of Asian origin, mainly China, cultivated as an ornamental and medicinal plant, that was introduced in most of the Euromediterranean area, Central and Western Asia, throughout Canada and USA, and some parts of South America and Australia (EPPO, 2022). It was deliberately introduced in Romania, where is characterized as an invasive, locally abundant (Anastasiu & Negrean, 2009) and distributed in all nine historic regions of the country: Maramureș, Transilvania, Crișana, Banat, Oltenia, Muntenia, Moldova, Bucovina, and Dobrogea (Oprea, 2005).

According to Sîrbu & Oprea (2011), the fences sea buckthorn ("cătina de garduri" in Romanian) is known in Romania since the 19th century, when it was mentioned in Moldova (cultivated next to fences and subspontaneous), Banat (hedges) and Transylvania.

Spontaneous goji berry has received different Romanian common names, depending on the geographical region, e.g. in Transylvania it was called "licină" or "liţian", in Bucovina "rachipară", "răchișoară" or "zăhărică", being described as a thorny shrub, poisonous, with long, thin and flexible branches, with purple flowers and elongated red berries and fruits (Coteanu et al., 2010).

The tradition of using goji berry for nutritional purposes and as medicine is due to the fruit's nutraceutical qualities, that were well documented in the past (Asănică et al., 2016; Clapa et al., 2021). The increasing demand for goji berry has become undeniable, as evidenced by numerous *Lycium* plantations in Europe, including in Romania (Mencinicopschi et al., 2012; Ciceoi et al., 2021). Some papers recently reported data about new varieties and locations with cultivated *Lycium* plants, however little is known about wild goji berry distribution in Romania.

Today, goji berry is the name used mainly to the fruits of two goji species, *Lycium barbarum* and *L. chinense*, grown in central and western China and Tibet. The wild goji grows also in different parts of Europe and Western Asia, including Romania. The retailers may distribute both products under the generic name of "goji", either involuntarily or out of a desire to mislead consumers and the lack of knowledge in differentiate the fruit origin might have a direct impact on consumers. The safety of *Lycium* fruits was considered uncertain after the detection of three tropane alkaloids, such as atropine and hyoscyamine in fruits of *L. barbarum* (India) and scopolamine in *L. halimifolium* at concentrations higher than the toxic dose (Barceloux, 2008; Qian et al., 2017). According to Befu (2015), the Romanian wild goji berry has smaller fruits and become bitter, which is not the case for the cultivated varieties. As goji is increasingly successful on the Romanian market, the differences between the cultivated goji and the wild goji must be clearly defined and made available for the consumers.

Recently, wild goji berry drew attention to the plant protectionists, since these shrubs are hosting an invasive alien mite, *Aceria kuko* that might affect also other economic solanaceous species, as the sweet pepper (Agrointel, 2017; Ciceoi et al., 2021).

Although not exhaustive, the aim of this work was to investigate the distribution of wild goji berry bushes in Romania, which could be of great interest for conservation biologists and potentially interested people in consuming fruits for health benefits.

MATERIALS AND METHODS

To build up the distribution map of spontaneous goji plants in Romania, online databases, as iNaturalist (inaturalist.org), Global Biodiversity Information Facility (gbif.org), Plants Database (garden.org), etc., citations databases and offline resources as Oprea (2005), Anastasiu & Negrean (2009), Sîrbu & Oprea (2011) were consulted. In addition, in the frame of ProtectGoji citizen science-based project (Ciceoi et al., 2021), some locations with wild *Lycium* bushes were collected from field trips during July 2021 to February 2022 period, while other observations and photos were provided by iNaturalist database and Facebook groups, such as "Plants from Romania" (*Plante din Romania*). Where possible, GPS coordinates (DMS) for each location were given.

Overlapping the literature review with personal study records, a map was compiled.

RESULTS AND DISCUSSIONS

Out of a total of 41 counties of Romania, the presence of *Lycium* sp. has been mentioned so far in 37 counties and in Bucharest (Figure 1). The new data allowed to note that wild goji shrubs have not been recorded in Covasna, Dâmbovița, Hunedoara and Sălaj counties. However, there are documented several *Lycium* plantations in Dâmbovița, Hunedoara (Ciceoi et al., 2021) and Sălaj (Agerpres, 2014) counties.

The citizen scientist's records of wild goji berry shrubs during the study period included Bucharest and 18 counties (Arad, Brăila, Brașov, Buzău, Caraș-Severin, Călărași, Cluj, Constanța, Galați, Giurgiu, Ialomița, Iași, Ilfov, Olt, Prahova, Teleorman, Tulcea and Vrancea) (Table 1) (Figures 2-4).

On the one hand, Bucharest and 14 counties (Arad, Brașov, Buzău, Caraș-Severin, Călărași, Cluj, Constanța, Galați, Giurgiu, Iași, Ilfov, Olt, and Tulcea and Vrancea) were previously documented in the above-mentioned resources as locations for wild *Lycium* sp. shrubs.

According to iNaturalist database, *Lycium barbarum* was introduced in Brașov and Cluj counties via anthropogenic means.

On the other hand, Ialomița, Brăila, Prahova and Teleorman counties represent new distribution records for the growth of spontaneous *Lycium* genus in Romania, compared to already published data.

More research is needed in order to obtain a detailed map of the wild goji berry distribution on the Romanian territory.

Figure 1. Wild goji berry distribution in Romania: literature review and present study records

Figure 2. *Lycium* sp. from Eforie Sud, Constanța county, August 2021 (photo by Mala Stavrescu-Bedivan)

Table 1. Citizen scientist's occurrence of wild *Lycium* sp. in Romania

County	Location and/or GPS coordinates
Arad	Near the railway embankment, 46°10'46.4"N 21°20'26.5"E
Brăila	Baldovinești; Bărăganu; Cazasu; Câineni-Băi; Constantinești; Corbeni; Grădiștea; Lacu Sărat; Mărul Roșu-Lacu Rezii; Muchea; Oprișenești; Plășoiu; Plopu; Racovița; Spiru Haret; Surdila Găiseanca; Șuțești; Tudor Vladimirescu; Tufești; Ulmu (cemetery); Vișani; Viziru; Vultureni; at the exit of Însurăței towards Slobozia (44°54'19.8"N 27°35' 22.3"E); Brăila city (Catholic Cemetery, Sf. Maria Cemetery, Sf. Constantin Cemetery, Sf. Mina Cemetery, Danube cliff, Great Garden Park)
Brașov	Turcheș, Săcele (iNaturalist)
Buzău	Râmnicu-Sărat (45°24'29.2"N 27°02'56.8"E)
Caraș-Severin	Măcești; Șușca (44°46'59.0" N 21°32' 32.1"E)
Cluj	Gruia; Turda (iNaturalist)
Constanța	Eforie Sud, viran area (44°02'02.9"N 28°39'08.3"E) (iNaturalist)
Galați	Between villages Vasile Alecsandri and Braniștea
Giurgiu	Comana Natural Park: – Crânguri (44°12'24.4"N 25°58'46.8"E, 44°12'30.5"N 25°58'48.5"E), – Singureni (44°13'19.9"N 25°57'30.1"E), – Călugăreni (44°11'09.5"N 26°00'28.8"E), – Vlad Țepeș (44°07'46.0"N 26°08'12.0"E; 44°07'40.0"N 26°08'16.0"E), – Pietrele (44°03'38.0"N 26°06'55.0"E); Iepurești (44°15'55.2348"N 25°51'59.2776"E); Goleasca (44°21'24.8"N 25°35'39.1"E); Mihăilești (44°19'36.1"N 25°54'16.4"E); Greaca (44°06'38.0"N 26°19'27.0"E, 44°06'22.0"N 26°18'39.0"E, 44°06'36.0"N 26°18'59.0"E, 44°06'35.0"N 26°19'14.0"E)
Călărași	Călărași (Facebook)
Ialomița	At the exit of Slobozia towards Drajna Nouă, 44°32'55.2"N 27°23'11.5"E
Iași	Oprișeni (47°07'51.3"N 27°49'04.8"E) (iNaturalist); near Ion Corvin commune (44°05'01.4"N 27°48'02.2"E)
Ilfov	Near Dinamic Parc, Lacul Morii Str.
Olt	Ibănești; Lunca; 44°38'06.7"N 24°22'36.4"E
Prahova	At the entrance of Blejoi commune, 44°59'15.0"N 26°00'53.3"E; Fântânele (45°00'35.1"N 26°22'30.8"E)
Teleorman	Turnu Măgurele (43°45'37.3"N 24°51'46.0"E; 43°45'35.7"N 24°51'47.4"E; 43°45'52.9"N 24°52'42.4"E; 43°45'58.7"N 24°52'44.0"E).
Tulcea	At the exit of Cataloi, towards Brăila (45°07'51.3"N 28°45'21.9"E); near the railway, between Cataloi and Tulcea (45°07'50.7"N 28°45'21.6"E); irrigation canal, before entering Tulcea city (45°07'51.3"N 28°45'21.9"E); Babadag Str. (45°09'41.5"N 28°47' 13.0"E); Intrarea Marmurei Str. (iNaturalist); Slava Rusa (Facebook)
Vrancea	Dumbrăveni (45°32'17.2"N 27°06'23.1"E); Obrejița (45°30'18.5"N 27°04'41.1"E)
București	On the fences of a childcare nursery, Militari district, sector 6 (44°26' 20.148"N 26°1'14.0916"E); Tineretului Park, sector 4 (44°24'18.7"N 26°06'31.6"E); Văcărești Natural Park (44°24'11.5194"N 26°8'2.8896"E)

Figure 3. *Lycium* sp. from Singureni, Giurgiu county, September 2021 (photo by Cristina Pelcaru)

Figure 4. Wild *Lycium* sp. bushes (arrows) in Teleorman county, February 2022 (photo by Marian Lincă)

CONCLUSIONS

The present work draws attention to the importance of mapping the distribution of spontaneous goji plants in Romania, a country where there has been a growing interest for setting up new *Lycium* plantations. This study revealed that spontaneous goji berry shrubs have been reported so far in several online and offline resources, but the available data are still scarce. The number of reports for Romania is increasing on open access platforms, as iNaturalist, following the activity inside the ProtectGoji citizen science-based project.

Currently, several online platforms make available for conservation biologists information regarding the geographical distribution of *Lycium* plants. This may contribute to a better understanding of the potential for future use of goji plants but also to the need for protection against anthropogenic impact.

The Distribution Map of *Aceria kuko* in Romania, an open sources map for accurate data on the national distribution of goji berry gall mite, will include the dataset on spontaneous goji berry shrubs in Romania.

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NUTRITIONAL CHARACTERIZATION OF ORGANIC SEABUCKTHORN POMACE

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Abstract

Seabuckthorn is recognized worldwide as a valuable berry with real benefits in human health. It is being analysed in detail for its introduction as a functional ingredient in various sectors of the food industry. The most popular industrial product of sea buckthorn fruit is sea buckthorn juice, but significant amount of pomace results after fruit processing as waste. The purpose of this paper is to characterize fresh, dried, and ground sea buckthorn pomace (as powder) that could be further used as functional ingredients. In terms of nutritional characterization, antioxidant activity, total phenolic content and ascorbic acid content were determined. The results show that sea buckthorn pomace presents important nutritional value and can be further processed and used as a functional ingredient in various industrial applications.

Key words: *sea buckthorn, functional ingredient, pomace, sea buckthorn powder.*

INTRODUCTION

Seabuckthorn (*Hippuphae rhamnoides* L.) is a world-renowned plant for both its high content of compounds (over 100 types), and for its ease of adaptation to climate and soil types (Gâtlan & Gutt, 2021), belonging to *Eleagnaceae* family (Mihova & Ivanova, 2020). Seabuckthorn is a thorny deciduous plant widely grown in Eurasian countries (Sharma & Kalkal, 2018), which is important from both economic and ecological point of view, being a low-demanding, wind-pollinated, and winter hardy tree (Kumar et al., 2021a). Sea buckthorn is a multipurpose plant with many uses starting from the control of soil erosion to use as horse fodder, a food component, and also in tea, drugs and cosmetics. Different parts of this plant are considered a good source of bioactive substances (Kant et al., 2012) and used in traditional medicine for a long time (Sne et al., 2013; Zhao et al., 2017; Wei et al., 2019; Luntraru et al., 2022). Seabuckthorn presents long lanceolate leaves, having characteristic silver hairs on the underside. It blooms between late April until early May, producing a great number of small green and brown flowers that grow together, forming clusters. In time, round yellow / orange berries are formed. The ripening season is in the fall, in September. Each fruit contains a small, elongated, grooved stone covering an oily seed (Fu et al., 2014; Bartish et al., 2002; Korekar et al., 2013). Approximately 150 species, subspecies and varieties of seabuckthorn have been identified; they differ in shrub habitat, appearance of berries and their value in use (Ciesarova et al., 2020). The development of research and interest in this plant led to the taking of seabuckthorn from the spontaneous flora and introduction into crops. With easy adaptability and good profitability, the industrialization of fruit was of great interest. Seabuckthorn berries are attractive due to their nutritional properties, being scientifically recognized for their content in vitamin C, carotenoids, flavonoids, sterols and tocopherols (Maheshwari et al., 2011; Olaru & Popa, 2019; Ciesarova et al., 2020). They are known in traditional medicine for anti-inflammatory properties, antitoxic effect, positive effect on hair and skin regeneration and condition (Tkacz et al., 2019). Different parts of the plant vary in composition of antioxidants, which has shown a positive biological, physiological, and medicinal effect of seabuckthorn. The medicinal properties of seabuckthorn were reported in various research studies, properties such as antimicrobial, antioxidative, hepatoprotective, anti-carcinogenic, antiulcerogenic, antihypertensive, radioprotective, anti-inflammatory and

immunomodulatory, which can be attributed to the content of various bioactive compounds which cand be found in different parts of the plant, like leaves, berries and seeds (Sharma & Kalkal, 2018).
Generally, the scientific interest concentrates on the ascorbic acid content of berries, juice, and leaves (Chandra et al., 2018); phytosterols, such as cycloartenol, campesterol (Zheng et al., 2017); citrostadienol, sitosterol (Hao et al., 2019); carotenoids, among which lycopene, lutein, zeaxanthin, α-carotene, β-carotene, γ-carotene can be found (Tudor et al., 2020; Pop et al., 2015); tocopherols (Bittová et al., 2014); flavonoids, isorhamnetin, and quercetin (Guo et al., 2017), or polyphenolic compounds, such as gallic acid in leaves and berries, and lower amounts of caffeic acid, p-coumaric acid, and ferulic acid (Bittová et al., 2014). Also, seabuckthorn pomace valorization is of great interest nowadays, due to the presence of different valuable compounds with heath promoting properties (Sharma et al., 2022; Hussain et al., 2021). Studying the nutritional aspects of sea buckthorn by-products could lead to new opportunities for obtaining nutraceuticals and natural functional food at low prices. Effective valorisation of agri-food industrial wastes/by-products targets to contribute to an enhanced economy and also to minimize the negative impact on the environment, with positive effect on ensuring food security (Bhat, 2021; Kumar et al., 2021b). Therefore, the aim of this study was to characterize the waste from seabuckthorn juice processing taking into study three organic seabuckthorn varieties.

MATERIALS AND METHODS

Experimental design

Three varieties of organic seabuckthorn were analysed in this study, namely Mara, Clara and Sorana. The seabuchthorn pomace was analysed from a nutritional point of view in different stages of processing: fresh (Clara_Fresh, Mara_Fresh, Sorana_Fresh), dried (Clara_Dried, Mara_Dried, Sorana_Dried) and in the form of powder (Clara_Powder, Mara_Powder, Sorana_Powder). Organic seabuckthorn fruits were processed into juice and the remaining pomace was further used in this research. The drying process was conducted at 50°C for 12h resulting dried pomace, and the powder was obtained by dried pomace grinding (Figure 1). All analysis were performed within Food Biotechnology Laboratory of the Faculty of Biotechnologies, USAMV Bucharest.

Figure 1. Aspect of the three varieties of organic seabuckthorn in different stages of processing

Antioxidant activity
The effect of antioxidant activity on 1,1-dipheny l-2- picrylhydrazyl (DPPH) was estimated according to the procedure described by Villaño et al. (2007), with some modification presented further. Briefly, 10 g of sample was macerated in 50 ml ethanol (75%) for 48 h in the dark, at room temperature. For each measurement, 0.05 ml filtered extract solution was added to 1.95 ml DPPH ethanolic solution and thoroughly homogenized, and incubated in dark at room temperature for 30 min. Sample absorbance was measured at 515. Results were expressed as quercetin equivalents (QE) per 100g D.W.

Ascorbic acid content
The content of ascorbic acid was determined by extracting 10 g of sample in 100 ml of 2% oxalic acid. The extract was filtered and 2 ml from the extract solution, 1 ml oxalic acid 2%, 5 ml buffer solution, 2 ml indophenol (2, 6-Dichlorophenol Indophenol) and 20 ml xylene, were placed in a centrifuge tube and centrifuged for 20 min at 5°C and 9000 rpm. The absorbance of the samples was measured 500 nm and the results were expressed as mg ascorbic acid / 100g D.W.

Total polyphenolic content
Total content of polyphenols (TP) was determined using the Folin-Ciocalteu method. Briefly, for each measurement, 1.58 ml distilled water, 20 µl filtered extract solution (10 g of fruit macerated in 50 ml ethanol (75%) for 48 h in the dark at room temperature), and 100 µl Folin-Ciocalteu reagent were mixed and then 300 µl Na_2CO_3 (20%) was added. The solutions were mixed and stored in the dark at room temperature for 2 hours. Sample absorbance was measured at 765 nm. Total polyphenol concentration was expressed as mg/L Gallic acid equivalents (GAE) per 100g D.W.

Statistical analysis
All determination was performed in duplicate. The obtained data was statistically analysed using Microsoft Excel 2017. In all tests, it was considered the significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Antioxidant activity
Figure 2 presents the results obtained for the antioxidant activity of the tested samples. Fresh seabuckthorn samples presented the highest antioxidant activity (1436.33-2567.55 QE/ 100 g D.W.), while for the dried pomace and seabuckthorn powder lower values were obtained, most probably due to the heating during drying process.

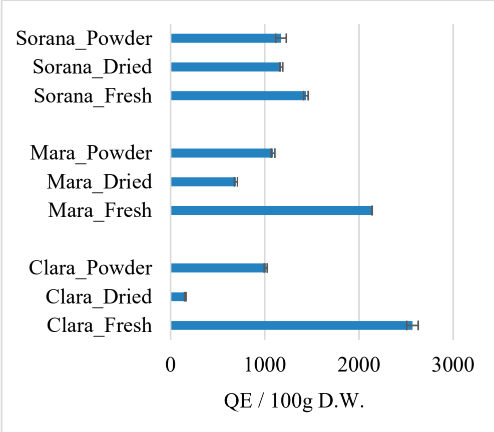

Figure 2. Antioxidant activity of tested samples

Therefore, for Sorana variety, the values obtained for antioxidant activity were similar for both dried pomace (1175.75 QE/100 g D.W.) and for the powder sample (1171.83 QE/100 g D.W.). Mara variety sample presented an antioxidant activity of 693.39 QE/100 g D.W. for dried pomace and 1087.55 QE/100 g D.W. for the powder sample. Significant difference between the values for this parameter was observed in case of Clara variety, where for the dried pomace a value of 155.18 QE / 100g D.W. was obtained, while for the powder sample the value was of 1009.95 QE/100 g D.W. The results obtained regarding the antioxidant activity of the tested samples showed similar values between varieties, especially in the case of powder samples (1009.95-1171.83 QE/100 g D.W.).

Ascorbic acid content
As shown in Figure 3, the highest amount of ascorbic acid was determined in fresh seabuckthorn pomace samples (28.43-48.34 mg /100 g D.W.), the values decreasing after drying processing for Mara and Sorana varieties, but being close for both dried pomace and seabuckthorn powder.
Clara variety maintained similar values for all tested variants, namely 25.49 mg/100 g D.W. for the dried pomace, 27.49 mg/100 g D.W. for the

powder sample and 28.43 mg/100 g D.W. for the fresh pomace sample. Regarding this parameter, the values obtained for dried pomace and powder samples were similar for all three studied organic seabuckthorn varieties.

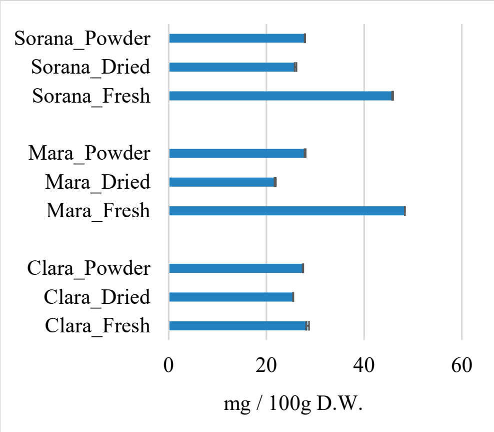

Figure 3. Ascorbic acid content of tested samples

Total polyphenolic content

Regarding the total polyphenolic content of three varieties (Figure 4), the highest values were obtained for the pomace samples processed into powder (2418.18-3019.80 mg GAE/100 g D.W.), most probably due to the high content of polyphenols present in seabuckthorn seeds.

For the fresh pomace, similar results were obtained, the polyphenolic content values being of 432.98 mg GAE/100 g D.W. for Mara variety, 508.80 mg GAE/100 g D.W. for Clara variety and 613.41 mg GAE/ 100 g D.W. for Sorana variety.

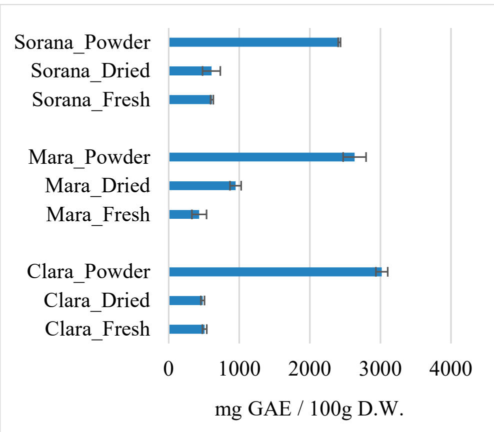

Figure 4. Total polyphenolic content of tested samples

Same trend was observed in case of dried pomace, for which values of 481.56 mg GAE/100 g D.W. (Clara variety), 605.84 mg GAE/100g D.W. (Sorana variety) and 947.89 mg GAE/100 g D.W. (Mara variety) were obtained.

CONCLUSIONS

The aim of this study was to characterize organic seabuckthorn pomace from a nutritional point of view, before and after processing.

The results showed all samples presented antioxidant activity both in both fresh form and after drying (dried pomace and powder), their values being slightly influenced by the drying process, but not significant.

Ascorbic acid was also determined in the studied seabuckthorn pomace.

Polyphenolic content was higher in powder samples, most probably due to the presence of grinded seeds.

The results of this study shows that seabuckthorn waste represents valuable by-products that can be further processed and used in various industrial application, such as food industry, phytochemicals extraction, pharmaceuticals and cosmetics.

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RESEARCH ON THE BIOLOGY OF *LOBESIA BOTRANA* (DENIS AND SCHIFF.) IN THE CONTEXT OF THE NEW CLIMATIC CONDITIONS IN MOLDOVA, ROMANIA

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Abstract

This paper reports the results obtained from the analysis of capture data using the TERASEYA FLY device for Lobesia botrana males (Denis and Schiffermüller). The research was carried out in the region of Moldova, within two plantations belonging to two representative wine basins in the country, being the Odobesti Vineyard and the Husi Vineyard. In the study areas, the vineyards (of Vitis vinifera L.) are cultivated mostly with quality wine varieties, predominantly in the Odobesti vineyard we meet the Muscat Ottonel variety, and in the Husi vineyard we meet the Fetească regala variety, which have also been the subject of research our.The main aim of the study was to investigate the dynamics and biology of L. botrana species inside vineyards and to assess the effect of new climate change on pest distribution. Pest prediction and warning models have been developed and tested to monitor the life cycle of L. botrana, integrating both biological and climatic information. The timing of the first appearance of adults and the hatching of the first eggs can be predicted by predictive models based on the temperature requirements of the stages.Unfortunately, the models of prediction based on the calculation of the sum of daily degrees of temperature are empirical and their robustness strongly depends on the environment in which they were validated. The alternative forecasting techniques are currently being developed, such as pest assessment in the study area using these modern digital farming techniques.

Key words: *vine moth, climatic conditions, pheromone atraBot.*

INTRODUCTION

So much has been written and talked about in the vineyard, from Hippocrates and Columela, from Pliny and Horatius to the present day, specialists and writers, doctors and hygienists, scientists and thinkers of all times, revealing the properties of grapes and they brought the wine a well-deserved praise. Poets, writers, painters and sculptors praised grapes and wine. Thus, over the centuries, vines and wine have made their direct contribution to the development of civilization and have contributed directly or indirectly to the progress of mankind, obviously bearing the imprint of the stages of development of human society. The viticulture was one of the main occupations of the Geto-Dacians, most of them alive, occupying the hilly slopes of Moldova, Transylvania and Oltenia, with natural conditions suitable for obtaining quality wines.

Grapes are of great importance from a nutritional, therapeutic, economic and social point of view. Grape production is affected by a number of pathogens and pests, which in the absence of means of control cause significant production losses and quality depreciation. *Phylloxera* sp., first reported in France (1863), destroyed almost all of France's viticulture between 1863 and 1900. The restoration of the new plantations, by using grafted cattle, opened a new stage of modern viticulture. (Tomoioaga et al., 2006)

In order to obtain high yields, both quantitative and qualitative, an important role is played by plant protection, to signal in time the main pathogens and pests and to establish the most effective measures to prevent and control diseases and pests of vineyards.

In order to ensure a good phytosanitary condition of the vineyards, it is necessary to apply integrated control measures by harmoniously combining agrotechnical, physical, mechanical, chemical and biological methods (use of entomophagus, use of biological products, creation of new resistant varieties, etc.). (Tălmaciu et al., 1994)

MATERIALS AND METHODS

Density of moth populations was estimated by visual inspections during vegetation on pheromone traps and laboratory analyzes of collected samples. Thus, the complex of vine moths that characterize the viticultural ecosystem in 2021 was established and inventoried, in the Huși and Odobesti viticultural centers, at the Muscat Ottonel and Fereasca Regala varieties.

During the research, the surveillance of the evolution of the moth generations was made on the basis of the catches registered on the pheromone traps of the atraBOT (Filip, 2003) type through the Teraseya Fly surveillance system (Figure 1), for the capture of the males of the *Lobesia botrana* species. Pheromone traps were produced and procured from the Romanian Institute for Chemistry Raluca-Ripan Cluj-Napoca. The Teraseya Fly capture system together with the pheromone traps were installed at the end of April and the beginning of May, the norm being 2 traps/ha, this Teraseya Fly system is an innovative and bold product designed for monitoring harmful insects in an agricultural crop.

Figure1. Capture system Teraseya Fly

The device is capable of attracting insects, capturing and storing them alive, photographing and recognizing them using advanced image processing software algorithms, and transmitting information to a data server.

Being bold in what it aims at, however, the product must be robust to withstand all conditions in the field, be self-sufficient in energy and provide information in a way that is easy for the user to understand.

The data are recorded and centralized in real time, and the replacement of the capsules impregnated with synthetic pheromone was done at an interval of 4-5 weeks.

The observations regarding the different parameters of the biological cycle of the grape moth were made in natural conditions, the climatic data being recorded in real time by the same Teraseya Fly device.

The placement of the combat experiments in the field was done according to the norms of experimental technique.

The establishment of the treatment warning terms was made on the basis of biological, ecological (Figure 2) and phenological criteria. For this purpose, the following were taken into account: the lower threshold for the development of the grape moth (*Lobesia botrana*) t_0 = 12°C, the flight curve of the adults, the phenology of the host plant, as well as the warning graph prepared on the database ecological and biological.(Mitrea et al., 2000)

Figure 2. Weather station

For each generation of the pest, only one treatment was applied 1-3 days after the registration of the maximum flight curve established with the help of pheromone traps of the type: 'atraBot' produced by the Romanian Institute for Chemistry "Raluca-Ripan" Cluj-Napoca.

The issue of the toxicity of second-generation insecticides to warm-blooded animals had to be reconsidered and therefore measures were taken to ban, or severely limit, as appropriate, the use of certain types of second-generation insecticides.

The research revealed a third generation of insecticides, designed to eliminate the major deficiencies of those of the previous generation, including endohormones and exohormones of insects.

Exohormones, also known as pheromones, play an important role in the life of insects, causing certain behaviors in finding food, of the opposite sex, as well as in cases of danger. The beginning of pheromone chemistry took place in 1959 and spread very quickly in England, the USA, Italy and Germany, etc. (Ciochia, 1986; Ciochia et al., 1993)

In our country, the study of pheromones began in 1973 at the Cluj-Napoca Institute of Chemistry, in recent years there has been great progress in the application of pheromones in the rationalization of chemical treatments in large production units in several counties. Even the method of direct combat marked a promising beginning in our country (Ghizdavu et al., 1983; Ghizdavu & Bunescu, 1991).

RESULTS AND DISCUSSIONS

Following the analysis of the obtained data, we noticed that the moth, in the two wine centers, has 3 generations annually. The first generation develops in May-June, the second July-August and the third August-May. It winters in the form of a chrysalis (stern) in a cocoon of white silky threads in the bark of the stems or under the leaves. Butterflies appear in the first or second decade of May. The flight is twilight. After 10-11 days of feeding, copulation and spawning take place. Eggs are laid alone or in small groups on flower buds, shoots, leaves. It is noteworthy that the larvae cause great damage to various varieties of vines. (Boguleanu, et al. 1980)

Among the sensitive varieties are: Coarna neagra, Coarna albă, Afuz-Ali, Muscat Ottonel. among the least attacked are: Perla de Csaba, Riesling. The larvae of the first generation bear fruit buds, wrapping the attacked parts in silk threads, in the form of nests. Sometimes they bear fruit inside the shoots. A larva can destroy 60-80 buds. The second and third generation larvae attack the grapes. The grains, attacked during the growing season, turn brown, wrinkle and fall off, and those affected in the ripening phase can be attacked by phytopathogens.

Observations on the number of males captured were performed once a week, and the data were entered tabularly and graphically, in order to compose the flight curve. These highlighted the beginning of the flight, the rescheduling of the third generation with the maximum flight and the cessation of the flight.

In order to have a dynamic record of the adult numerical density, after each weekly centralization, the captured males were removed from the trap device, so that the results could be interpreted both by the cumulative values for the whole period and by the partial values of each observation. The replacement of the capsule with pheromones was done monthly.

*DYNAMICS OF THE FLIGHT OF VINE MOLY IN THE VINEYARD PLANTATION FROM THE **HUȘI** WINE CENTER*

From the analysis of the data presented in Table 1, which represents the evolution of the vine moth in 2021, in the vineyard in Huși, is found as the species *Lobesia botrana* was present in the specific climatic conditions of this year.

Throughout the vegetation period, with the location of the TERASEYA FLY system, daily catch records were made, but the centralization of the data was done every 7 days in order to carry out the treatment warning scheme for each generation.

Following the 28 recordings, three peaks of the flight curve were recorded (Figure 3), data on which the treatment warnings were issued for each generation of the moth.

Table 1. The evolution of the capture of the *Lobesia botrana* Den et Schif moth., in the vine plantation from Huși, the year 2021

Date of observation	Number of males captured	Date of observation	Number of males captured
13.04	**1**	20.07	**76**
20.04	**5**	27.07	**41**
27.04	**11**	3.08	**68**
4.05	**32**	10.08	**89**
11.05	**71**	17.08	**141***
18.05	**125***	24.08	**89**
25.05	**92**	31.08	**42**
1.06	**56**	7.09	**37**
8.06	**14**	14.09	**25**
15.06	**18**	21.09	**13**
22.06	**52**	28.09	**3**
29.06	**195***	5.10	**1**
6.07	**111**	12.10	**0**
13.07	**81**	19.10	**0**

* treatment warning

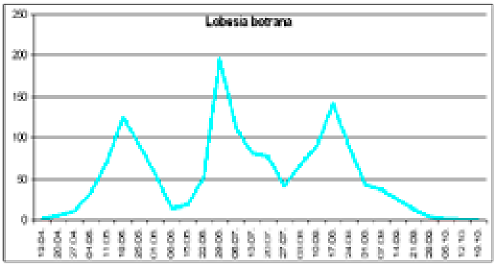

Figure 3. Appearance graph and flight curve of *Lobesia botrana* species in 2021 in Huși wine center

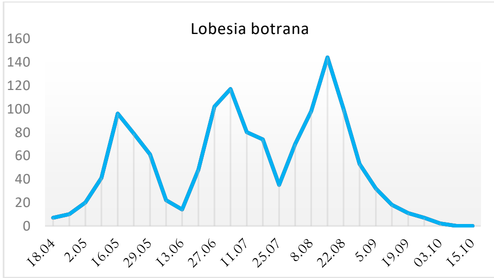

Figure 4. Appearance graph and flight curve of *Lobesia botrana* species in 2021 in Odobesti wine center

*DYNAMICS OF THE FLIGHT OF VINE MOTH IN THE VINEYARD PLANTATION FROM THE **ODOBEȘTI** WINE CENTER*

Following the data recorded and processed in Table 2, where the evolution of the vine moth in 2021 is represented, in the vineyard from Odobești, it is observed that the species *Lobesia botrana* Den et Schiff. was present in the specific climatic conditions of this year.

Following the location of the experience, respectively the installation of the TERASEYA FLY device, and with its help, adult capture data were recorded daily, and the data was centralized at 7 days in order to carry out the treatment warning scheme for each generation.

Following the 28 recordings, three peaks of the flight curve were recorded (Figure 4), dates on which the treatment warnings were issued for each generation of the moth.

Table 2. Evolution of the capture of the *Lobesia botrana* Den et Schif moth., in the vineyard from the Odobesti wine center, year 2021

Date of observation	Number of males captured	Date of observation	Number of males captured
11.04	**1**	18.07	**74**
18.04	**7**	25.07	**35**
25.04	**10**	1.08	**70**
2.05	**20**	08.08	**98**
9.05	**41**	15.08	**144***
16.05	**96***	22.08	**100**
23.05	**79**	29.08	**53**
29.05	**61**	5.09	**32**
6.06	**22**	12.09	**18**
13.06	**14**	19.09	**11**
20.06	**48**	26.09	**7**
27.06	**102**	3.10	**2**
4.07	**117***	10.10	**0**
11.07	**80**	12.10	**0**

* treatment warning

AREA OF BIOLOGICAL ACTIVITY OF THE SPECIES LOBESIA BOTRANA DEN ET SCHIF

The general researches of ecology consisted in following the influence of the climatic factors on the development stages of the grape moth (*Lobesia botrana* Den et Schif.), Its development constants were determined in the climatic conditions of the area.These refer to:

- lower biological threshold (t_0) = 12°C
- the threshold of prolificacy (O) = 16.2°C
- lower thermal optimum (O_1) = 26.7°C
- optimal thermal optimum (O_2) = 31.1°C
- upper biological threshold (T) = 35.6°C
- thermal constant of the species (k) = 384°C

Thermal constant (k) and the lower biological threshold (t_0) was established by his method Blunk (1914, 1923) using the relationship:

$$\mathbf{k = X_n (t_n - t_0)} \text{ where:}$$

k = thermal constant;
X_n = duration of development (in days);
t_n = development temperature;
t_0 = lower biological threshold;
$t_n - t_0$ = effective temperature.

Following the results obtained regarding the biology of grape moths, it results that the area of biological activity (ZAB), of the *Lobesia botrana* species was differentiated as follows in the two research centers:

From Table 3 it can be observed that in 2021 in the stationary Huși the area of biological activity was between April 13 - September 20, totaling 160 days and a sum of the effective temperature of 1354.4°C

Table 3. Calculation of the area of biological activity (ZAB) of the grape moth (*Lobesia botrana*) in the Huși wine center in 2021

Month	Xn (days)	Precipitations (mm)	Temperat $t_n(^0C)$	Efective temperat. t_n-$t_o(^0C)$	S t_n-t_o	ZAB
January	31	2,3	0,3	-	-	DHP 01.I-12.IV
February	28	1,9	4,8	-	-	
March d1	10	2,8	11,2	-	-	
March d2	10	1,6	9,9	-	-	
March d3	10	3,8	8,1	-	-	
March	31	8,2	9,7	-	-	
April d1	10	10,1	6,7	-	-	
April d2	10	23,8	11,7	-	-	
April d3	10	11,7	12,3	0,3	3	ZAB 13.IV-20.IX
April	30	45,6	10,2	-	**3**	
May d1	10	0	17,9	5,2	52	
May d2	10	4,5	24,1	12,1	121	
May d3	11	7,7	19,3	7,3	80,3	
May	31	12,2	20,4	-	**253,3**	
June d1	10	28,5	25,5	13,5	135	
June d2	10	1,6	25,3	13,3	133	
June d3	10	19,8	21,6	9,6	96	
June	30	49,9	24,1	-	**364**	
July d1	10	11,3	19,8	7,8	78	
July d2	10	8,5	23,2	11,2	112	
July d3	11	67,2	25,1	13,1	144,1	
July	31	87	22,7	-	**334,1**	
August d1	10	67,3	22,6	10,6	106	
August d2	10	7	19,9	7,9	79	
August d3	11	0,1	20	8	88	
August	31	74,3	20,8	-	**273**	
September d1	10	18,5	19,9	7,9	79	
September d2	10	15	14,9	2,9	29	
September d3	10	14	13,9	1,9	19	DHE 21.IX-30.IX
September	30	47,5	16,2	-	**127**	
Octomber d1	10	3	11,3	-	-	DHT 01.X-31.XII
Octomber d2	10	10,4	11,1	-	-	
Octomber d3	11	15	10,3	-	-	
Octomber	31	28,4	10,9	-	-	
November	30	30,2	6,8	-	-	
December	31	11,2	2,9	-	-	
S t_n-t_o in 2021 = 1354,4 ºC						
DT: 01.01.-12.04 + 21.09-30.09 + 01.10.31.12.= 172 days						
ZAB: 13.04 – 20.09 = 160 days						
t0= 12°C						
DHP: 01.01 – 12.04 =102 days						
DHT: 01.10-31.12 = 92 days						

The general ecology research consisted in following the influence of climatic factors on the development stages of the grape moth (*Lobesia botrana* Den et Schif.) its development constants were determined in the climatic conditions of the Odobești wine center area.

Following the results obtained regarding the biology of the grape moth, it results that the area of biological activity (ZAB), of the *Lobesia botrana* species was thus differentiated in the two research centers.

From Table 4 it can be observed that in 2021 the area of biological activity was between April 13 and September 20, totaling 160 days and a sum of the effective temperature of 1383.7°C.

Table 4. Calculation of the area of biological activity (ZAB) of the grape moth (*Lobesia botrana*) in the Odobești wine center in 2021

Month	Xn (days)	Precipitations (mm)	Temperatt$_n(^0C)$	Efective temperat. t_n-$t_o(^0C)$	S t_n-t_o	ZAB
January	31	47	0,6	-	-	DHP 01.I-11.IV
February	28	4,4	1,6	-	-	
March d1	10	3,6	9,2	-	-	
March d2	10	41,8	6,6	-	-	
March d3	10	1,4	12,2	-	-	
March	31	46.8	9,3	-	-	
April d1	10	4,8	7,4	-	-	
April d2	10	28	9,0	-	-	
April d3	10	8,2	10,2	-	-	ZAB 12.IV-03.X
April	30	41	9,9	-	-	
May d1	10	0,8	15,7	3,7	37	
May d2	10	6,8	16,9	4,9	49	
May d3	11	15,2	19,8	7,8	85,8	
May	31	22,8	17,5	-	**171,8**	
June d1	10	20,6	18,1	6,1	61	
June d2	10	98,4	18,5	6,5	65	
June d3	10	15,6	24,3	12,3	123	
June	30	134,6	20,3	-	**249**	
July d1	10	19,6	23,4	11,4	114	
July d2	10	15,0	25,3	13,3	133	
July d3	11	5,4	26,7	14,7	161,7	
July	31	40,0	24,3	-	**408,7**	
August d1	10	4,4	24,9	12,9	129	
August d2	10	10,8	22,9	10,9	109	
August d3	11	29,8	23,2	11,2	123,2	
August	31	45	22,9	10,9	361,2	
September d1	10	0	17,1	5,1	51	
September d2	10	5,6	19,2	7,2	72	
September d3	10	0,6	13,6	1,6	16	
September	30	6,2	16,7	4,7	**139**	
Octomber d1	10	0,6	17,4	5,4	54	DHT 04.X-31.XII
Octomber d2	10	8	15,4	-	-	
Octomber d3	11	0,6	17,5	-	**54**	
Octomber	31	9,2	16,8	-	-	
November	30	12	12,9	-	-	
December	31	81,6	5,1	-	-	
S t_n-t_o in 2021 = 1383,7 ºC						
DT: 01.01.- 10.04 + 04.10-31.12= 189 days						
ZAB: 11.04 – 3.10 = 156 days						
t_0= 12°C						
DHP: 01.01 – 10.04 =100 days						
DHT: 04.10-31.12= 89 days						

On October 10, the average air temperature dropped further, which led to the continuation of the rest of the crisalids and their entry into the autumn-winter diapause. This break lasted 100 days from October 10 to December 31. The spring- winter diapause covered the period from January 1 to April 10, totaling 100 days.

The climatic conditions in 2021 were favorable in terms of the biology of the *Lobesia botrana* species, a situation in which the pest recorded 3 generations / year.

For a long time, pest control has been done by mechanical and chemical means. The long and intensive use of insecticides has revealed a number of negative aspects. In addition to ecosystem pollution, a major drawback is the lack of selectivity of chemicals, which leads to biocenotic imbalances, in some cases dramatic.

CONCLUSIONS

Among the vine pests, grape moth remains the main pest in Moldovan vineyards.

The grape moths, but especially *Lobesia botrana* is the species that has shown its presence and has been considered a key pest, which is why it has been monitored using AtraBot pheromone traps.

Between April and September, the population of *Lobesia botrana* exceeded the PED of 100 catches / trap / week for all 3 generations in the two stations, Huși and Odobești.

Currently, the evolution of the pest in the two stationary was marked by three peaks of its evolution, taking into account the evolution of climatic conditions recorded in the Huși and Odobesti stationary.

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GREYISH OAK (*QUERCUS PEDUNCULIFLORA* K. KOCH) SMART FORESTS FROM DOBROGEA'S PLATEAU

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Abstract

Dobrogea Plateau is situated in southeast Romania, being bordered by Danube's Meadow and Delta at West and North. The climate is temperate-continental, with reduced precipitations (around 400 mm/year) and a silvosteppe and steppe vegetation. Climate-Smart Forestry (CSC) is a branch of the forest's smart management that focuses on the response of forests towards climatic changes. In the present article, this is adapted to Greyish oak. Greyish oak smart forests are characterised by advanced ages (61-70 years) and even-aged stand structures. These forests are situated at altitudes between 51-100 m and 250-300 m, and on west and east expositions. The purpose study showed that Greyish oak can be included in the category of smart forests, by taking into account 13 elements (flora, soil and forest type) and stand conditions (pruning, vitality, diameter and average height, functional group, litter). As Greyish oak resists well to drought and grows relatively well, the species is important for improving the climate and conserving forests from the silvosteppe.

Key words: *age, altitude, greyish oak, smart forests, structure.*

INTRODUCTION

Dobrogea Plateau is situated in southeast Romania, being bordered by the Danube's Meadow and Delta.

It is a hill region where fragmentation has sometimes led to the development of pinnacles, large valleys and basins. The region amounts to approximately 10 400 km^2, meaning 4.3% of Romania's territory.

The relief is mainly plain (over 50%) or slightly reclined. Exceptions are represented by cliffs marked by a surface slope of up to 90%, petrographic or structural steeps, susceptible to active modelling. The exposition of slopes with an important role in activating phenomena and processes is more diverse in the north area of Dobrogea.

The climate is temperate-continental, with reduced precipitations (around 400 mm/year). All these data suggest that Dobrogea has a semiarid temperate climate, situated at the limit of the transition temperate climate (both as geographic location, precipitation and other meteorologic elements) (Ielenicz M., 2003).

Drought is the most extended and pregnant meteorological phenomena (both in time and in space) in Dobrogea's climate. This is the main argument for its semi aridity and also the most visible component of the image inhabitants form about this area. Even though Dobrogea is the warmest territory from Romania, this does not exclude frost phenomena.

In Dobrogea, silvosteppe is situated as a layer over the zonal steppe. Two other levels are developed over the silvosteppe: sub Mediterranean soft oak forests (present in Dobrogea and in south Banat); nemoral forest level with a holm and mixed holm inferior sublevel that is strongly influenced by the sub mediterranean one. Two areas distinguish themselves in the steppe area: graminee steppe (very narrow) and graminee and dicotyledonous steppe. Two subareas can also be found: north silvosteppe with mesophile oaks (*Quercus robur*); and south silvosteppe (sub mediterranean) with xerophile oaks (*Quercus pubescens*, *Q. pedunculiflora*) (Doniță et al., 2005). Eutric cambisol, luvisol and preluvisol are the specific soils from this area (Spârchez et al., 2017; Oneț et al., 2019; Crișan et Dinca., 2020).

Quercus Genus (oaks) is one of the most important trees from an economic and ecologic perspective. Widespread in the north hemisphere, it includes approximately 350-500 species. In Romania, it is represented by 7 species, occupying approximately 16% of the country's forest surface in the year 2012 (Beldeanu, 2004; Apostol et al., 2017; Dincă L., Breabăn I.G. 2020).

The first mention from Romania regarding the presence of Greyish oak was made by the renowned botanist Al. Borza, in 1936. The species appears in the silvosteppe from Oltenia, Muntenia, Dobrogea and South Moldavia, in both fields and low hills of 50-300 m (Curtu et al., 2009; Doniță et al., 2004).

Pedunculate oak is considered to be the most important species from Romania's forest steppe (Enescu, 1993).

Greyish oak (*Quercus pedunculiflora*) has more accentuated xerophyte characteristics than pedunculate oak and Hungarian oak, allowing it to vegetate better on dry steppe soils (Stănescu et al., 1997).

Greyish oak (*Quercus peduculiflora* K. Koch), together with other oak species such as Pubescent oak (*Q. pubescens* Willd.), Pedunculate oak (*Q. robur* L.) and Sessile oak (*Q. petraea* (Mattuschka) Liebl.) are of interest for the afforestation of degraded fields (Enescu, 2015; Constandache et al., 2016; Dincă et al., 2018; Silvestru-Grigore et al., 2018; Vlad et al., 2019).

Studies realized in Romania have shown that oak has declined in the extra-carpathian Romanian area from 1900 up to present days. This also includes Greyish oak (Nechita, 2019; Clinovschi, F. 2004).

The Climate-Smart Forestry Concept (CSC) appears as an important step in promoting the objectives of long lasting forests, as well as a response towards climatic changes (Bowditch et al., 2020). A smart silviculture is needed from a climatic perspective in order to increase the total surface of forests and to avoid land clearings.

Forests ensure numerous ecosystem services that are essential for both environment and climate. In addition, they conserve and protect biodiversity, protect against climatic changes, produce wood and non-wood products, offer water and recreational functions (Nabuurs et al. 2015; Tognetti, 2017; Vasile et al., 2018; Nabuurs et al. 2018; Dincă et al., 2018; Cântar et al., 2019; Pleșca et al., 2019; Tiwary et al., 2020; Verkerka et al., 2020; Leskinen et al., 2020; Tudor et al., 2020; Toressan et al., 2021).

Furthermore, Greyish oak acorn is considered as one of the most important forest products, followed by linden and locust (Cioacă et Enescu, 2018; Dincă L. and Dincă M., 2020).

Greyish oak is a slivosteppe species that prefers a warm climate and has a slow growth especially during its youth as well as a reduced productivity.

The species has a very high ecoprotective value for conserving forests from the silvosteppe. Numerous old oak forests exist in Dobrogea's Plateau (Vechiu et al., 2021). However, they can be affected by intensive grazing (Hinkov et al., 2019).

The purpose of this article is to identify and describe Greyish smart oak forests from Dobrogea's Plateau in order to protect them.

MATERIALS AND METHODS

The material used in this paper is represented by Greyish oak elements extracted from forest management plans realized during 1993-2007 for 10 forest districts (Amenajamente silvice). The extremely large number of values (2751 stand elements) ensures a good statistical representation.

In total, 13 parameters specific to the stands or site were taken into consideration (Table 1). Each analysed parameter has obtained a grade from 1 to 5, namely: 1 = very low; 2 = low; 3 = medium; 4 = high; 5 = very high. The degrees took into account the Greyish oak's ecological requests. A hierarchy of Greyish oak stands was obtained by adding all these values. The largest values were framed in the smart forest category.

Table 1. Grade obtained based on the site and stand characteristic

Nr crt	Characteristic	Grade				
		1	2	3	4	5
1	Soil type	1215; 1703; 9102; 9604	1210; 2205	1204; 1701; 2401	1301; 1302; 2201; 3101	1201; 1401
2	Station type	6112; 9110	6151; 7120	9210; 9220	7430; 9530	9320
3	Litter	1	2	3	4	5
4	SUP	Q	V	A	K	E, M
5	Functional group + Functional category	1,3G ; 2,1C	1,3A; 2,1B	1,2B	1,2A; 1,2E; 1,5L	1,5C; 1,5D; 1,5H
6	Average diameter (cm)*	2-8	10-14	16-18	20-22	24-70
7	Average H (m)*	1-6	7-9	10-11	12-13	14-23
8	Production class	5	4	3	2	1
9	Pruning	0.2-0.3	0.4	0.5	0.7	0.6
10	Vitality	5	4	3	2	1
11	Structure	1	2	3	4	
12	Crown density	0.2-0.3	0.4-0.5	0.6; 0.9	0.8	0.7
13	Distance to road	1-3	4-6	7-11	12-22	23-90

*For these characteristics, the entire value range was divided in 5 categories, 1 = the smallest 5 = the highest. The category division was realized so that the analyzed biometric characteristics are respected. In addition, a balanced division was intended as number of values for each category.

The meaning of some terms used in Table 1 is rendered below:

Soil type: 1201 = chernozem; 1204 = rendzic chernozem; 1210 = vertic-stagnic chernozem; 1215 = lithyc-rendzic chernozem; 1301 = cambic chernozem; 1302 = cambic-vermic chernozem; 1401 = phaeozem; 1701 = rendzina; 1703 = lithyc rendzina; 2201 = preluvisol; 2205 = stagnic preluvisol; 2401 = luvisol; 3101 = eutric cambisol; 9102 = calcic lithosol; 9604 = lithyc erodisol.

Station type (TS): 6112 = Hill oak, rockland and excessive erosion; 6151 = Hill Quercus (cer, gârniță) small edaphic Bi eutricambosol; 7120 = Hill Quercus on strongly eroded slope in B non-chalk sedimentary small edaphic eutricambosol; 7430 = Hill Quercus with oak, high edaphic Bm-s eutricambosol; 9110 = strongly eroded silvosteppe in limestone sedimentary; 9210 = External silvosteppe with soft oak, Bm chernozem on loess; 9220 = External and extrazonal silvosteppe in xerophyte oak steppe, Bm-i, weak chernozem on loess; 9320 = Average silvosteppe of xerophyte oak stands, Bs, clay chernozem on loess; 9530 = Internal silvosteppe of mezo xerophyte-xerophyte Quercus, Bs, degraded chernozem on fine clay.

Litter: 1 = missing litter; 2 = thin interrupted litter; 3 = thin continuous litter; 4 = normal continuous litter; 5 = thick continuous litter.

Production/protection subunits (SUP): A = Regular forest, normal assortments; K = Seed reservations; E = Reservations for integrally protecting nature; J = quasi-selection system forest; M= Forests under the extreme conservation regime; Q = simple locust thicket; V = Forests with recreation functions through hunting.

Functional group (GF) and functional category (FCT): 1,2A = Forests located on cliffs, debris, on fields with depth erosion, on fields with a slope higher than 35 degrees and on flysch, sand or gravel with a slope higher than 30 degrees; 1,2B = Forests composed of entire parcels, bordering public roads of high interest or normal railroads from areas with rugged relief (fields with slopes higher than 25 degrees and in danger of landslides); 1,2E = Forest plantations realized on degraded fields; 1,3A = Steppe forests, from the limit between steppe and silvosteppe; 1,3G = Bodies of dispersed forests, with surfaces under 100 ha, situated in plain areas; 1,5C = Natural reservetions; 1,5D = Scientific reservations; 1,5H = Forests established as reservations for the production of forest seeds and the conservation of the forest genofond; 1,5L = Forests located in reservation protection areas (buffer areas); 2,1B = Forests destined to produce thick trees with superior timber quality; 2,1C = Forests destined to produce mainly average and slim trees for cellulose, rural constructions and other usages.

Vitality: 1 = very vigorous; 2 = vigorous; 3 = normal; 4 = weak; 5 = very weak.
Structure: 1 = even aged stand; 2 = relatively even aged stand; 3 = relatively uneven aged stand; 4 = uneven aged stand.

RESULTS AND DISCUSSIONS

The final grades for the analysed stands vary from 22 to 54. Smart forests are the ones that have grades higher than 50, namely 29 stands (6% of all stands).
The percentage is similar with the one obtained by other species situated in the smart forest category (Dincă et al., 2019; Dincă & Dincă, 2019; Blaga et al., 2019; Dincă & Breabăn, 2019).
The main characteristics of Greyish oak smart forests from Dobrogea's Plateau are rendered in Table 2.

Table 2. The characteristics of the smart greyish oak forests from Dobrogea's plateau

Nr crt	OS	Age (years)	Participation percentage (%)	Current growth (m^3/year/ha)	Relief	Configuration	Exposition	Field slope (g)	Altitude (m)
1	Casimcea	70	7	0.9	31	P		10	265
2	Casimcea	110	2	0.1	31	P		10	265
3	Casimcea	85	3	0.2	31	O	NE	36	260
4	Baneasa	120	6	0.3	33	P	NE	10	155
5	Constanta	70	3	0.4	12	P	V	6	60
6	Babadag	80	3	0.2	32	O	NE	6	160
7	Ciucurova	70	1	0.1	30	P	E	15	212
8	Constanta	90	1	0.1	12	P		0	70
9	Constanta	80	1	0.1	12	P	SV	6	70
10	Constanta	65	1	0.1	53	P		0	35
11	Babadag	105	4	0.1	32	P	E	8	155
12	Babadag	100	2	0.1	52	P	E	13	230
13	Babadag	70	5	0.3	43	P		0	145
14	Casimcea	70	5	0.6	31	O	V	15	275
15	Casimcea	160	2	0.1	31	O	V	15	275
16	Casimcea	85	8	0.5	30	O	S	15	270
17	Casimcea	120	2	0.1	30	O	S	15	270
18	Ciucurova	80	3	0.2	32	O	V	20	210
19	Baneasa	55	4	0.7	31	P	N	20	65
20	Baneasa	60	2	0.2	30	F	N	30	55
21	Baneasa	50	7	1.1	33	O	V	15	75
22	Baneasa	55	5	0.8	31	P	V	10	25
23	Constanta	60	2	0.3	31	P	E	15	80
24	Constanta	75	9	0.5	43	P		0	150
25	Babadag	60	1	0.1	32	O	V	16	170
26	Babadag	70	4	0.2	32	P	E	8	155
27	Casimcea	100	1	0.1	11	P		0	235
28	Ciucurova	50	3	0.8	32	P	V	12	190
29	Ciucurova	80	4	0.3	30	P	S	15	240

Location
From a geographic perspective, Greyish oak smart forests are present in the following forest districts: Casimcea, Băneasa, Babadag, Ciucurova, Constanța, Cerna, Măcin, Niculițel, Cernavodă (Figure 1).
The age of these smart forests is diverse, varying between 50 and 100 years. The majority of stands are situated in the 50-60 year category and between 61-70 years (Figure 2).
The altitude where Greyish oak appears in Dobrogea's Plateau ranges between 38 m in Constanța and 275 m in Casimcea. Our analysis shows that 24% of the surface occupied by these stands is situated at altitudes between 51-100 m and 250-300 m (24%) Figure 3).
Participation percentage in the stand's composition. It has been observed that this species is included in the stand's composition in different percentages. The composition is mixed (intimate + groups = 42%) and in groups (14%) (Figure 4).
The structure of Greyish oak stands is especially even-aged (52%), relatively even-aged (34%) and relatively uneved-aged (14 %) (Figure 5).

Figure 1. Distribution of smart Greyish oak stands from Dobrogea's Plateau

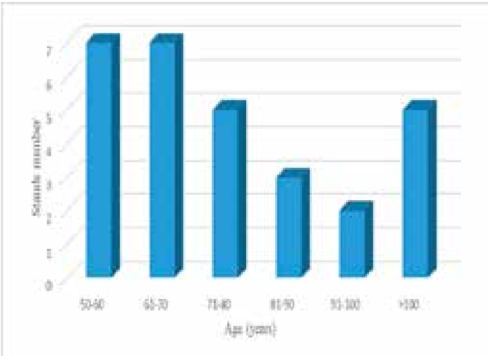

Figure 2. Distribution of smart Greyish oak forests in Dobroudja Plateau on ages

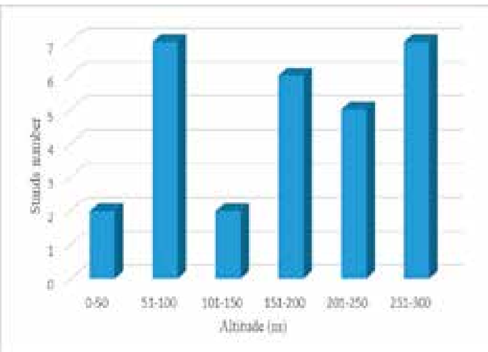

Figure 3. Distribution of smart *Quercus pedunculiflora* in Dobrogea Plateau on altitudes

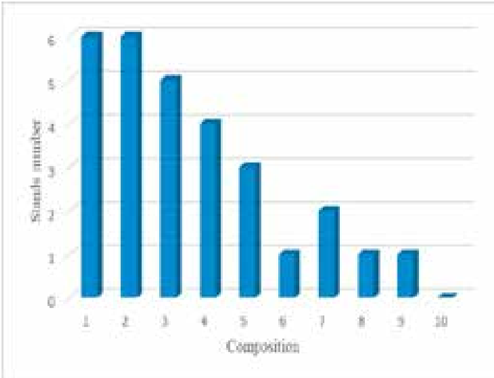

Figure 4. Participation percentage in stands compositions of smart *Quercus pedunculiflora* from Dobrogea Plateau

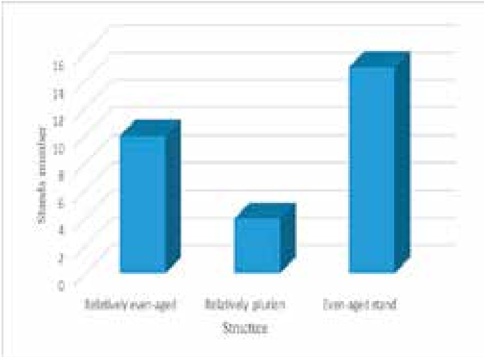

Figure 5. Distribution of smart *Quercus pedunculiflora* in Dobrogea Plateau on structure categories

The inferior slope is **the relief** form characteristic for these stands, occupying only 28% of the total surface of studied stands (Figure 6).

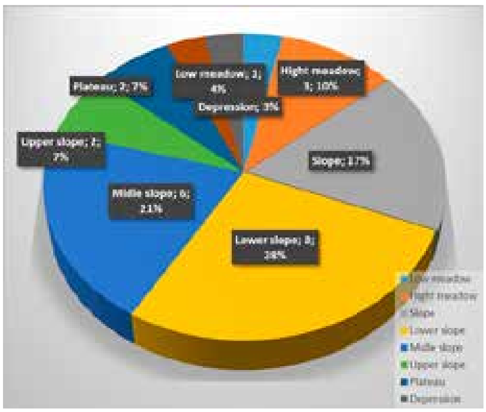

Figure 6. Relief forms characteristic for smart *Quercus pedunculiflora* stands from Dobrogea's Plateau

East and west expositions are characteristics for smart Greyish oak forests from Dobrogea's Plateau (Figure.7)

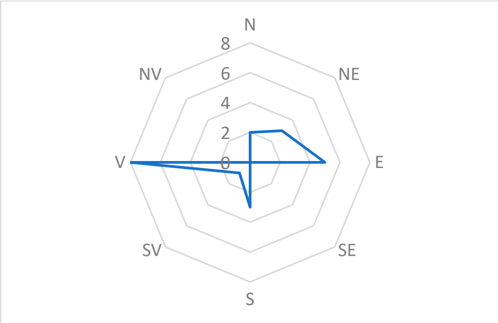

Figure 7. Distribution of smart *Quercus pedunculiflora* forests in Dobrogea Plateau on expositions

The field slope ranges between 6g and 36g, with most fields characterised by small slopes of 6g-15g. The slope acts in a close connection with exposition and altitude, influencing environment conditions.

CONCLUSIONS

The age of smart Greyish oak stands ranges between 50 and 100 years, being situated at altitudes between 51-100m and 250-300m, on West and South expositions. The stands' structure is especially even-aged, being located at relatively small distances from forest roads (between 1-10 km). In Romania, Greyish oak occupies small surfaces from the forest fund, being however widespread in the South silvosteppe. Identifying these Greyish oak stands is extremely important both for their protection, as well as for applying proper silvicultural measures.

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COMPARATIVE STUDY OF TEXTURAL PROPERTIES OF ORIGINAL BREADS CONTAINING VEGETABLES POWDERS AND ROMANIAN COMMERCIALLY AVAILABLE RYE BREAD

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Abstract

Functional and personalized foods have generated a growing interest for the general public and nutritionists. Bread is one of the most popular food being a versatile option for developing functional and personalized assortments. Vegetables powders of spinach, grape seeds, tomato seeds, carrot, tomato fruits, beetroot or broccoli have been added to bread dough to improve its phenolic content, textural properties, storage and shelf life. The present research is comparing the textural properties of three assortments of commercially available Romanian sliced rye bread (FT, SV, CR), which is a healthier alternative to wheat bread, with three assortments of manufactured white bread containing lyophilized powder of hawthorn (P2) and rosehips berries (P3) and grapeseed powder (P4). Cohesiveness was significantly different between the original assortments and the commercial ones however the cohesiveness of the P2, P3 and P4 bread assortments were not significantly different. No significant difference was also observed for the cohesiveness of the FT, SV and CR rye breads. The springiness index was not significantly different between all tested breads (P = 0.198), however the chewiness of P4 was significantly different compared to the commercial assortments but not significantly different compared to P2 and P3. The lack of difference between the springiness index of the two groups of beads indicates that the proposed original bead assortments can be a healthier alternative to rye breads, thanks to their exceptional phenolic content and mineral concentrations.

Key words*: bread, lyophilized hawthorn and rosehips berries, grapeseed powder, textural properties, phenols, minerals.*

INTRODUCTION

Functional and personalized foods have generated a growing interest for the general public but also for the medical world. Bread is the most consumed food but also one of the most versatile foods that can be personalized and fortified, responding to the needs of a wide range of people from those with gluten intolerance to those with cardiovascular disease or diabetes. The incorporation of different vegetable powders to bread improved nutritional values such as fiber content, polyphenol content, antioxidant levels, and at the same time can improve its rheological properties.

Similary, Junejo et al. (2021) reported that adding spinach powder to bread has dramatically improved the textural (hardness and chewiness) and antioxidant (free radical diphenylpicrylhydrazyl and fluorescence recovery after photobleaching) properties of bread.

These results indicated that incorporation of spinach powder enriched the functional and nutritional properties of bread that may help to design healthier food products. The addition of lupine and flaxseed to the wheat bread improved its nutritional profile (Wandersleben et al., 2018).

Tomato seeds flour is an important source of proteins and has been reported to contain high levels of minerals by Sogi et al. (2002) therefore its addition to bread or bakery products will improve the nutritional value of these products. The texture profile analysis performed on bread enriched with tomato seeds flour revealed that tomato seeds flour addition has significant effects on the texture parameters of bread samples; the hardness, cohesiveness, chewiness and elasticity of the bread increases (Mironeasa et al., 2016). Similarly, the incorporation of pineapple core fiber (5, 10, and 15%, w/w flour basis) increased the hardness and gumminess of Chinese steam bread, while decreased the cohesiveness, specific volume and elasticity (Shiau, Wu, & Liu, 2015).

The texture analysis showed that the addition of chia seeds in bread caused a decrease in the hardness of the crumb and increased the nutritional value of the bread. Liu el al. (2018) suggested that the incorporation of oat bran into wheat flour decreases specific volume and softness of Chinese steamed bread and decreased the glycemic response.

Although the rye breads are known to have several health benefits and have similar healthiness perceptions among age groups, their consumption is limited (Sandvik et al, 2017) The present paper presents the textural properties of three original assortments of bread containing powders of lyophilized hawthorn and rosehips berries and grapeseed powder compared to commercially available rye bread in the attempt to identify healthier bread assortment with a larger acceptability among consumers.

MATERIALS AND METHODS

Preparation of original bread assortments

Three original varieties of breads were prepared by addition of lyophilized hawthorn fruit powder, lyophilized rosehip fruit powder and grape seed powder. The powders selected for bread fortification have functional properties such as maintaining blood pressure at normal values, increase resistance to mental exertion, improve peripheral and main blood circulation, prevent cardiovascular accidents, prevent hyperlipidemia, and may act as diuretic, hypotensive, astringent and vasodilator. At the same time, they have hepatoprotective, anti-inflammatory, regenerating, healing effect, prevent avitaminosis, stimulate immunity through the antiviral potency of interferon, prevent atherosclerosis and are indicated in the treatment of gout and rheumatism. These powders contain major bioactive substances such as tannins, bioflavonoids, vitamins B1, B2, B3, C, A, P, PP, anthocyanins, flavonoids, and tartaric, citric, malic, caffeic, ursolic, oxalic, nicotinic, chlorogenic acids as well as sorbitol, choline, glucose, fructose, pectin, volatile oils, triterpenes, mineral salts, and carotenoids.

The original bread recipe consists of the following basic ingredients: white wheat flour 000, water, salt, raw sugar, baking yeast, cold pressed sunflower oil. To the basic ingredients the 3 types of functional powders were added (2.5%), namely grape seed powder (P4), lyophilized hawthorn fruit powder (P2) and lyophilized rosehip fruit powder (P3)

Bead preparation: after mixing the ingredients, the dough was kneaded for 30 minutes, then the samples were left to rise at 25°C for 30 minutes. After fermentation, the samples were placed in the oven at 180°C for 60 minutes. After baking, the samples were left to cool and then packed in plastic bags to avoid dehydration.

Figure 1. Bread with addition of lyophilized hawthorn fruit powder (P2)

Figure 2. Bread with addition of lyophilized rosehip fruit powder (P3)

Figure 3. Bread with addition of grape seeds powder (P4)

Bread samples preparation for rheological analysis

All assortments of original bread were kept in the refrigerator for 12 hours before analysis. The bread was kept at room temperature for one hour before analysis. Measurements were made 4 days after bread baking

The commercial rye bread was bought from local supermarkets. Three companies producing rye bread were chosen, and their products were purchased and then coded as SV, CR, FT.
All bread assortments, including the comercial ones, were cut into cylinders with a diameter of 13 mm and height between 17 mm and 25 mm. Each sample height was measured before analyzing each bread sample. For each bread assortment were analyzed 15 samples.

Textural analysis
Textural analysis was performed using the TPA (Texture Profile Analysis) test (double compression test). The texture profile analysis was performed using a Texture Analyzer TA Plus (Llyod Instruments) within the Interdisciplinary Laboratory for the Study and Modeling of the Accumulation of Heavy Metals in the Food Chain of University of Agronomic Sciences and Veterinary Medicine.
The analyzed textural parameters were: hardness at first compression, cohesiveness, chewiness, and springiness index for all bread assortment. Double compression tests were performed at speeds of 5 mm / s to mimic the masticatory process.

Data statistical analysis
Statistical data analysis was performed using MedCalc software to compare the original and commercial bread assortments. ANOVA analysis was used to identify the significant differences between the rheological parameters measured for the selected bread assortments. The post-hoc test used is the Tukey test. When ANOVA generated p> 0.05, the Tukey test was no longer required because there were no significant differences between the samples analyzed. When ANOVA residuals were not normally distributed, ANOVA was replaced by the nonparametric tests Kruskal-Wallis.

RESULTS AND DISCUSSIONS

The textural parameters used to compare the rheological properties between the original assortments and the commercial ones are hardness (after the first compression) cohesiveness, chewiness and springiness index (Table 1).

Table 1. Hardness, cohesiveness, chewiness and springiness index for original bread assortments and commercially available rye bread

	HARDNESS FOR THE FIRST COMPRESSION (N)		COHESIVENESS		CHEWINESS (J)		SPRINGINESS INDEX	
BREAD TYPE	MEAN	SD*	MEAN	SD	MEAN	SD	MEAN	SD
P2	21.1300	8.6331	0.2827	0.0342	0.0479	0.0227	0.7563	0.1315
P3	9.0111	3.2711	0.3107	0.0492	0.0201	0.0104	0.6891	0.2274
P4	13.6417	4.5189	0.3101	0.0278	0.0346	0.0095	0.7737	0.1637
CR	30.6960	23.8040	0.2083	0.0489	0.1001	0.0675	0.7521	0.1185
SV	46.3120	25.4120	0.2101	0.0384	0.1515	0.0588	0.7885	0.0860
FT	31.5960	11.3150	0.1821	0.0279	0.0772	0.0263	0.7244	0.1371

*SD = Standard Deviation

These four parameters were choose due to the information they can provide on the internal structure of the new bread assortments as well as of the commercially available rye bread: the hardness during first compression is the maximum force required to obtain the maximum deformation established for the first compression which can be considered a measure of the response of the bread during mastication compression between molars; cohesiveness represents the intensity of the internal bonds that form the structure of the sample offering information on the internal structural modifications due to fruits and seed powder addition to the bread recipe; chewiness represents the energy needed for the complete destruction of a solid sample giving useful information on the consumers perception during mastication; the springiness index is the ability of the product to return to its original shape after the first compression and it is a good indicator of the structure response when bread is tested for freshness by hand compression.

Table 2. Testing residuals normality for ANOVA for the hardness data

Hardness - Shapiro-Wilk test for Normal distribution **(Residuals)**	W=0.7854 reject Normality (P<0.0001)

In order to accept the results of the ANOVA analysis for hardness the residuals normality (Table 2) was tested. The Shapiro-Wilk test for residuals of cohesiveness lead to rejection of Normality, therefor the nonparametric test Kruskal-Wallis was used (Table 3).

Table 3. Kruskal-Wallis test for Hardness during first compression (N)

Data	Hardness1 (N)
Factor codes	Type_of_bread
Sample size	154
Corrected for ties Ht	83.0790
Degrees of Freedom (DF)	5
Significance level	P < 0.000001

The Kruskal-Wallis test showed that hardness during compression is significantly different for the bread assortments (P < 0.000001; Table 3). To distinguish between hardness bread assortments the post-hoc test Conover was used (Table 4)

Table 4. Post-hoc analysis (Conover) for hardness during first compression

Factor	n	Average Rank	Different (P<0.05) from factor nr
CF	37	80.24	FT, P2, P3, P4, SV
FT	36	96.22	CF, P2, P3, P4, SV
P2	16	59.19	CF, FT, P3, P4, SV
P3	15	13.33	CF, FT, P2, SV
P4	16	28.56	CF, FT, P2, SV
SV	34	114.65	CF, FT, P2, P3, P4

The Conover post-hoc test for hardness during compression (Table 4) shows that all commercially available assortments are significantly different from the original breads (P2, P3, P4). Wirkijowska et al, (2020) reported that were Flaxseed flower and marc added to bread (10% and 15%) significantly increased bread hardness, however the original bread assortments presented in this study however showed a significant decrease of hardness compared to commercially available assortments of rye bread.

Table 5. Testing residuals normality for ANOVA for Cohesiveness data

Cohesiveness - Shapiro-Wilk test for Normal distribution (residuals)	W=0.9677 reject Normality (P=0.0011)

In order to accept the results of the ANOVA analysis for cohesiveness we have tested the residuals normality (Table 5).
The Shapiro-Wilk test for residuals of cohesiveness showed that Normality is rejected, therefore the nonparametric test Kruskal-Wallis is used (Table 6).

Table 6. Kruskal-Wallis test for Cohesiveness

Data	Cohesiveness
Factor codes	Type_of_bread
Sample size	154
Test statistic	88.6170
Corrected for ties Ht	88.6170
Degrees of Freedom (DF)	5
Significance level	P < 0.000001

The Kruskal-Wallis test showed that cohesiveness is significantly different for the bread assortments (P < 0.000001; table 6).
The Conover posthoc test for chewiness (Table 7) shows that CF assortment is not significantly different from SV assortment, however it is significantly different of the original breads (P2, P3, P4) and FT.
The original bread assortments cohesiveness is significantly different from the commercial assortment's cohesiveness (Table 7).
The ANOVA for chewiness showed that the normality of the residuals is rejected (Table 8).
The Nonparametric Kruskal-Wallis test is used to differentiate between bread assortments (Table 9).
The Kruskal-Wallis test showed that chewiness is significantly different for the bread assortments (P < 0.000001; Table 9).

Table 7. Post-hoc analysis (Conover) for cohesiveness

Factor	n	Average Rank	Different (P<0.05) from factor nr
CF	37	61.68	FT, P2, P3, P4
FT	36	41.47	CF, P2, P3, P4, SV
P2	16	116.75	CF, FT, SV
P3	15	129.40	CF, FT, SV
P4	16	133.44	CF, FT, SV
SV	34	65.18	FT, P2, P3, P4

Table 8. Testing residuals normality for ANOVA of chewiness data

Chewiness- Shapiro-Wilk test for Normal distribution (Residuals)	W=0.8686 reject Normality (P<0.0001)

Table 9. The Kruskal-Wallis test for chewiness (J)

Data	Chewiness
Factor codes	Type_of_bread
Sample size	154
Test statistic	100.3872
Corrected for ties Ht	100.3872
Degrees of Freedom (DF)	5
Significance level	P < 0.000001

The Conover posthoc test for chewiness (Table 10) shows that CF assortment is not significantly different from FT assortment, however it is significantly different of the original breads (P2, P3, P4) and SV.
The original bread assortments chewiness is significantly different from the commercially available bread assortment's chewiness (Table 10).
The addition of rosehip and hawthorn fruits lyophilized powders and grape seeds powder significantly decreased the bread chewiness which is a encouraging result compared with the results of Wirkijowska et al. (2020) who reported that the adition of flaxseed flour significantly increased the chewiness of bread.
ANOVA (table 12) was used to analyse the springiness index data because the Shapiro-Wilk test for Normal distribution of residuals showed that normality can be accepted (Table 11). No significant differences were observed between all bread assortments tested (P = 0.198; Table 12); therefore, the perception of freshness by hand compression may be considered similar among all the bread assortments tested.

Table 10. Post-hoc analysis (Conover) for Chewiness

Factor	n	Average Rank	Different (P<0.05) from factor:
CF	37	89.08	P2; P3; P4; SV
FT	36	82.67	P2; P3; P4; SV
P2	16	46.56	CF; FT; P3; SV
P3	15	12.47	CF, FT, P2, SV
P4	16	29.31	CF, FT, SV
SV	34	125.35	CF, FT, P2, P3, P4

Table 11. Testing data normality and equality of variances for springiness index

Data	Springiness_Index
Factor codes	Type_of_bread
Sample size	154
Levene's test for equality of error variances Levene statistic	6.490
DF 1	5
DF 2	148
Significance level	P < 0.001
Residuals Shapiro-Wilk test for Normal distribution	W=0.9877 accept Normality (P=0.1953)

Table 12. ANOVA for springiness index considering as factor the bread assortments.
Data are normally distributed and the variances are not equal. ANOVA is corrected for unequal variances

Data		Springiness_Index	
Factor codes		Type_of_bread	
Sample size		154	
ANOVA Source of variation	Sum of Squares	DF	Mean Square
Between groups (influence factor)	0.1422	5	0.02844
Within groups (other fluctuations)	2.8351	148	0.01916
Total	2.9773	153	
-ratio			1.485
Significance level			P = 0.198

CONCLUSIONS

The hardness, chewiness and cohesiveness of original assortments of bread containing lyophilized hawthorn fruit powder (P2), lyophilized rosehip fruit powder (P3) and grape seeds powder (P4) are significantly different of the same parameters measured for the commercially available rye bread assortments.
The hardness of all original assortments is significantly lower than the hardness of commercially available rye bread assortments.
The cohesiveness of original assortments of bread is significantly higher than the

cohesiveness of commercially available rye bread assortments, meaning that these original assortments of bread will crumble less. The chewiness of original assortments of bread is significantly lower than the chewiness of the commercially available rye bread assortments, for this reason the original assortments of breads will be perceived by consumers to be more tender, soft or fluffy compared to the assortments of rye bread. The original bread assortments chewiness is significantly different from the commercially available rye bread assortment's chewiness, suggesting that during mastication the original assortments of bread will be perceived as being more soft.

The springiness index for the original assortments of bread is not significantly different from the commercially assortments of rye bread, showing that the consumers will not see any difference when testing their freshness by hand compression. Therefore it can be considered that the consumer's expectation (Sajdakowska et al., 2021) will be satisfied from the point of view of the way in which they perceive the freshness of the original proposed bread assortments.

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ESTABLISHMENT OF NATIONAL INFORMATION SYSTEM OF PLANT GENETIC RESOURCES IN BULGARIA

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Abstract

Preservation and use of plant diversity is one of the global priorities for the development of sustainable agriculture in climate change conditions. During the period 1982-2021 in the National Genebank in Sadovo 53,545 accessions of cultural and wild plant species were stored. The enrichment of plant genetic resources collections in recent years has been focused on local traditional varieties, mainly by vegetable crops. The paper presents the status of conserved plant gene pool in Bulgaria in connection with optimization of the managing process of germplasm storage, study, reproduction, free exchange and use via establishment of an information system with specialized software. The created architecture follows the international standard of FAO/Bioversity. Passport data includes taxonomic description, biological status and origin of the accessions. The information system uses ontologies for facilitated free access and security of records through innovative blockchain technologies. As a result, the functionality of the documentation process as well as access to the plant gene pool to all stakeholders, in accordance with International Treaty on Plant Genetic Resources for Food and Agriculture and the Nagoya Protocol, is guaranteed.

Key words: *descriptor, documentation, EURISCO catalogue, ex situ collections, local plant genetic resources.*

INTRODUCTION

Plant genetic resources are the basis of the genetic improvement of cultivated plants, essential for food security and development of innovative bio economy (ECPGR, 2021). Agro biodiversity supports sustainable productivity of farmers under the current climate changes (Priyanka et al., 2021; Ron & Rodino, 2022). Seedbanks are collections of genetic resources which can be seen as selection of old plant materials at risk of being lost that are maintained for future use as inputs into the research processes for development of new varieties. In addition, these old crop varieties may have value in and of themselves apart from their use in the breeding process. Genebanks thus serve as both providers of valuable traits to breeding programs as well as repositories of diverse crop genetic material representing society's agricultural heritage (Rocchi et al., 2016; Weise, 2021).

The main goal of all EU programs regarding plant diversity is to improve the coordination of conservation activities in Europe and to facilitate the access to plant genetic resources and the information about them. Enrichment of genetic resources means collected, systematized and documented components of biodiversity of actual or potential value, stored in controlled conditions outside their natural habitats (*ex situ*) in order to maintain them alive, further study and rational use. The database of collections includes descriptive information about each accession. As the societies and economies become more and more driven by data exchange, the governance of information is increasingly important. In parallel, as biodiversity continues to decline the status quo is not acceptable and new solutions need to be found. Possible changes for proper genebanks quality management, guaranteeing the long-term conservation of, and immediate access to the plant genetic resources conserved in it, are recommended by Hintum et al. (2021). Bulgaria is an area particularly suitable for the cultivation of different crops. It is one of the richest in plant diversity countries in Europe.

Despite being small in size (110,910 km^2), the territory of the country includes various relief and geology, the specific microclimatic conditions and the millennium human activity determine the rich plant diversity (CBD, 2014). Over the last decades the area of Balkan Peninsula experienced genetic erosion, resulting in the loss of many traditional plant genetic resources (Knüpffer, 2016). Local varieties (landraces, old cultivars and neglected crops) have unique climatic and ecological tolerances. Therefore, the main priority for conservation at the national level is focused on the species and variety diversity in home gardens (Knüpffer, 2002; Kehlenbeck et al., 2007). The largest part of the home garden area in Bulgaria is occupied by vegetable crops of *Solanaceae* and *Cucurbitaceae* (Ivanova et al., 2021). The breeders and farmers need access to a wide diversity of genetic resources, predominantly farmers' varieties, landraces, and crop wild relatives (Ebert, 2020). Documentation units have been established in almost all genebanks worldwide. Without well-structured documentation it is not possible to make the information a useful resource. Information about the composition of collections allows statements about which species and/or regions of origin are under-represented and have to be explored. Comprehensive information management is a prerequisite for the further development of genebank collections (Weise et al., 2020).

The access to the information about the Bulgarian accessions is possible only through the international databases and that is a limiting factor for many Bulgarian stakeholders. Thus, it does not meet the requirements the collections to be visible and accessible to scientific, public, ecological and other organizations (Doychev et al., 2020).

The study aims to present the status of conserved *ex situ* plant gene pool in Bulgaria in connection with optimization of the managing process of germplasm storage and use via establishment of an information system with specialized software.

MATERIALS AND METHODS

The Institute of Plant Genetic Resources, Bulgaria is the National Coordinator in the European Program for Plant Genetic Resources (ECPGR). The National Genebank was established in 1984 and the research activities for conservation of plant germplasm are carried out according to the standards of FAO (2014).

The Information Centre for documentation of seed accessions has been established in 1982. It works according to the international standards for documentation in line to the activities for free access and guarantees the international cooperation (FAO/Bioversity, 2017).

The electronic register contains catalogue and European number of accessions, passport characteristics as country of origin, donor, ecology-geographical characterization of the collection site, biological status, type of storage: base collection (long-term), exchange collection (medium-term), work collection (short-term), *in vitro* and/or field collection, botanical garden, etc. The taxonomic description of the crops is under the nomenclature of the USDA (GRIN, 2015).

The National Genebank is nominated as a focal point in the European Search Catalogue for Plant Genetic Resources - EURISCO (http://eurisco.ecpgr.org) for Bulgaria (Weise et al., 2017).

From 2019 an integral national network for plant genetic resources with specialized software it starts to be established. Starting with the use of field books, a gradual development of electronic data base as National register, nowadays the intelligent data management system aims to improve the availability of conserved seed accessions in genebank to users (Doukovska, 2021).

RESULTS AND DISCUSSIONS

Status of ex situ germplasm collections

During the period 1954-2021 the fund of National Genebank is enriched with 53,541 accessions (Table 1).

Table 1. Status of *ex situ* collection of plant genetic resources, conserved in Bulgarian genebank

Collecting period	Acquisition method	Origin	Number of accessions
1954-2021	Introduction	Foreign	36,716
1957-2021	Expedition	Local	10,777
1978-2021	Crop breeding	Bulgarian	6,048
Total number of accessions			53,541

Currently, accessions with local and Bulgarian origin are 31% of the collection and their sustainable preservation is a main priority in connection with the climate changes.

The accessions from collecting missions are 10,777 - local varieties and populations from home gardens and crop wild relatives from their natural habitats. With higher percentage from the local accessions are vegetable crops and grain legumes, followed by cereals. Emerging from unconscious selection within a population and well adapted to environmental factors, the local plant genetic resources are of great importance for transfer of valuable economic traits as tolerance to abiotic and biotic stress in creation of new varieties.

The described geographical characteristics of the collected accessions make it possible for the traditional varieties to restore in the regions of origin through the seed resources, stored in the ex situ collections in the genebank.

Traditional vegetables have considerable commercial value and high market potential to contribute the household income. In home gardens were found local varieties of tomato, pepper, cucumber, pumpkin, melon, watermelon, onion, leafy vegetables and potatoes, perfectly adapted to specific environmental conditions, with valuable qualities as early maturity and high biological content (Krasteva et al., 2013).

In the database 6,048 breeding materials are registered – lines and improved new varieties with Bulgarian origin. They are conserved in long-term conditions and the access is regulated in accordance with the principles for the protection of breeders' intellectual property rights.

There are 36,716 genotypes, introduced from abroad by international free germplasm exchange. The National Genebank conducts professional contacts with about 197 genebanks worldwide. The main partners in the exchange are established research centers such as GRIN (USDA), ICARDA (Syria), VIR (Russia), NordGen (Sweden), IPK (Germany), INRA (France), John Innes Center (UK), Suceava Genebank (Romania), etc.

The requested foreign germplasm is investigated in the country environmental conditions and used as a donor of valuable traits in breeding programs. *Ex situ* collections from cereals, grain legumes, technical, fodder, vegetable, medical and aromatic crops are maintained (Figure 1).

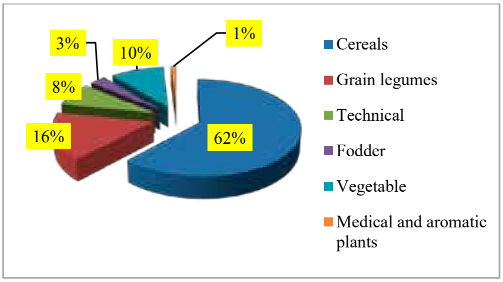

Figure 1. Distribution of plant gene pool in genebank by crop groups

Preservation of plant genetic resources in Botanical garden

A total of 450 plant species from 60 families are maintained in the Botanical Garden of the Institute of Plant Genetic Resources. Of these, eight species are Balkan endemics: *Achillea clypeolata*, *Allisoides bulgarica*, *Knautia macedonica*, *Chamaecitisus janke*, *Iris reichenbachii*, *Iris suaveolens*, *Aegilops cylindrica*, *Haberlea rhodopensis,* which also has the status of a rare species; five species of Bulgarian endemics: *Allium rhodopaeum*, *Sedum album*, *Vicia incisa, Aegilops neglecta*, *Soldanella rhodopaea;* four species of endangered plants: *Leucoyum aestivum*, *Artemisia pedemontana*, *Anemone sylvestris*, *Pyracantha coccinea*; and 11 rare species: *Meum athamanticum*, *Artemisia lerchiana*, *Artemisia pontica*, *Leontopodium alpinum*, *Leucanthemum vilgare*, *Andrachne telephioides*, *Aegilops triuncialis*, *Koeleria brevis*, *Secale cereale var. perene*, *Clematis alpina*, *Paeonia tenuifolia*. Thematic distribution of species is: essential oily, cereal grasses, fodder, decorative, protected and rare species, wild crop relatives.

International networks and databases for plant genetic resources

Bulgaria maintains the richest plant genetic resources collection in Southeast European region. According to EURISCO (data check February, 2022) the Bulgarian National Inventory comprises 69,767 accessions. The collection consists of genotypes from crop research institutes in the country and it is characterised with diverse geographical origin. The Bulgarian collection is the 7th biggest in Europe and has a share of 3,4%, after Great Britain, Russia, Germany, Ukraine, Poland and

Spain. In connection to its taxonomic composition, the preserved accessions belong to 532 genera and 1,927 plant species. The highest number of accessions is from the genera *Triticum, Hordeum, Zea, Phaseolus, Avena, Capsicum, Pisum, Arachis*. The crops with the highest number of accessions are presented in the Table 2.

Table 2. Crops with the highest number of accessions in EURISCO database (February, 2022)

Taxonomy	Number of accessions	With BGR origin
Triticum aestivum	13,175	2,909
Hordeum vulgare	6,365	303
Zea mays	4,827	1,939
Phaseolus vulgaris	3,488	1,698
Avena sativa	2,476	149
Triticum durum	2,370	1,193
Capsicum annuum	1,885	1,408
Pisum sativum	1,744	241
Triticosecale	1,461	532
Linum usitatissimum	1,461	77
Arachis hypogaea	1,373	444
Lycopersicon esculentum	1,371	534
Secale cereale	1,300	827
Cucumis sativus	1,031	95

Bulgarian National Inventory is a part from a "virtual" European Genebank Integrated System - AEGIS (Hintum, 2021). The status of the Bulgarian collection in the AEGIS database amounts to 391 accessions and it is presented by crops in the Table 3.

Table 3. Bulgarian accessions in AEGIS database (February, 2022)

Crop	Origin	Status of accession	Number of accessions
Triticum aestivum	BGR	Local	135
Triticum dicoccon	BGR	Local	26
Triticum durum	BGR	Local	126
Triticum monococcum	BGR	Local	32
Triticum spelta	BGR	Local	7
Secale cereale	BGR	Local	15
Lathyrus sativus	BGR	Local	9
Medicinal and aromatic plants	BGR	Local	41
Total number of accessions			391

Bulgarian Genebank Information System

The new intelligent system ensures the full public access to the information about the fund of Bulgarian genebank for all stakeholders and it is expected to increase the sustainable use of germplasm in breeding programs and agricultural production. Based on the analysis of the existing National register and EURISCO standard for documentation, a concept and analytical model of ontology for plant genetic resources has been developed (Stoyanova-Doycheva et al., 2020). The first version of the ontology, called GenBankOntology, of the plant genetic resources in the National genebank was developed. The ontology has been tested for consistency and integrity and the results show that it can be implemented in storage of plant genetic resources and management information system (GenBankSystem). The information system includes a database model, a system interface model and an implementation model. The database of the application has been implemented and the large part of the data of the genebank has been migrated. For this purpose, scripts were created for data migration.

The first version of GenBankSystem has been developed and it includes the implementation of the Central Register module, which is the core of the system. The system provides the access of the various users to the system and realization of the main functionalities for storage and management of plant genetic resources. The model has been created for the implementation of blockchain technology, which will be used in the functionality for security of records and germplasm exchange between different national and international organizations (Krasteva et al., 2020).

A server infrastructure on which the ontology and the relational database of the information system of the national genebank are deployed has been implemented.

The implementation of the system for storage and management of plant germplasm in the genebank and the integration of the ontology for plant genetic resources are applicable both in the institute for servicing the work in the genebank of different users and for external stakeholders from the gene pool in the ex situ collection, which is required of International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, 2009) and the Nagoya Protocol (CBD, 2011).

By reviewing and highlighting the National state of conserved plant germplasm, we attempt to unlock food resources from some neglected and underutilized species, which along with the wealth of traditional knowledge about their uses and practices, could help support sustainable agriculture while ensuring better protection of the environment and the continued delivery of its ecosystem services.

The research is implemented on three priorities. In the first stage, attention is focused on the conservation of forgotten regional and ancient cultivars of plant species, which form in the country - part of natural resources and cultural heritage (vegetables, legumes, cereals and other species). This valuable collection of genotypes presents irreplaceable genetic resources for plant breeding for their high tolerance to biotic and abiotic environmental factors.
In the second priority, attention is paid to underutilized plant species, which are widespread throughout the country and regions, but the population used them only by harvesting a part of the plants for practical use in nutrition, health and quality of life (spices, medicinal plants). The people harvest significant parts of wild populations for sale in local markets. These plant species are vital to sustainable agriculture.
The third group is focused on less-known species. It is a group that has not been cultivated in the country so far, but current climate change allows their cultivation and spread. These lesser-known species can be practically used mainly by family and young farmers for their socio-economic development. Therefore, there is a special interest in their expansion and using.

CONCLUSIONS

Genebanks are the most important sources of potentially useful genetic diversity for improving quantity and quality of agricultural yields and for adapting crops to climate changes, meeting the environmental stresses.
Genebanks are centres of information on genetic resources and have to be able to provide relevant information to every potential user and the general public.
The Bulgarian National Genebank maintains one of the largest *ex situ* collections in Europe and the richest conserved plant diversity in Southeast European region.
Documentation system, according to the international standards of FAO/Bioversity, optimizes the management of plant genetic resources in relation to their sustainable conservation and target use.
The National Information System for plant genetic resources will gradually build a "knowledge bank" and also will be a great platform for scientists looking to enhance biodiversity as a source of agricultural development.
The results obtained within the framework of the project BGPlantNet "Establishment of National Information Network GenBank – Plant genetic resources" will be used in the National Research Program "Smart Crop Production".

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REVIEW: ACTUAL APPROACHES FOR THE CRAFT BEER FERMENTATIONS

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Abstract

The present paper describes some studies regarding the potential of some conventional and non-conventional brewing and wine yeasts for their application in the craft beer process. The artisanal or the craft brewing industry has grown in the last decade. The representative microorganisms for the production of craft beers are Saccharomyces yeasts. Nevertheless, the non-Saccharomyces yeasts became important for the particular aromatic profile of craft beer, through the increased customer's experience and also for the potential health benefits such as the probiotic effect. The objectives of this paper were to screen the reported data regarding the general behavior of the microorganisms involved in craft beer production, such as the fermentative capacity to produced alcohol, flavors and the probiotic effects, including the general analytic assays. Starting from this research, studies will be opened for innovative pathways of using yeast strains isolated from vineyard and winery environment in artisanal beer production.

Key words: *Craft beer, yeast, Saccharomyces and non-Saccharomyces, brewing.*

INTRODUCTION

Beer is an ancient beverage which is the most consumed alcoholic drink worldwide according to the World Health Organization (WHO) (Neffe-Skocińska et al., 2022). The history of beer is far back in times; Babylonians and Egyptians created a simple drink made from cereals let to germinate and after a spontaneously fermentation beer was brewed (Habschied et al., 2020).
Beer is a nutritious and refreshing carbonated drink produced from four types of ingredients according to the Purity Law adopted in Bavaria in 1516: water, barley, hops, and yeast. (Protz,R, 2004). Nowadays, a new trend is visible within the global beer market and apart from so-called industrial brewing industry, a new sector has been increased during recent years, that has been named the *craft beer* industry. For the forecast period (2022-2017) the compound annual growth rate (CAGR) for this business is estimated at 14.1% (Craft Beer Market, 2022)
In order to reach the higher expectations of customer in terms of taste, aroma, flavors, alcoholic, non-alcoholic, low alcoholic, low carbohydrates, gluten free, the craft sector grew progressively. This because a small brewery is more flexible and has the possibility to create innovative beers using different techniques, raw materials, and additional ingredients.

Current trends on obtaining craft beer

Yeast has an important role in creating the distinctive aroma and flavor components which lead to an impressive variety of beer styles. Without the metabolism of yeast cells, the traditional production of alcoholic beverages would not be possible.
Beer production is divided in different styles, lager and ale being the most predominant: fermentation at the bottom on the fermenter (6-15ºC), performed by *Saccharomyces pastorianus* in lager beer production and top-fermented beer (temperatures between 16-24ºC) by the most studied yeast, *Saccharomyces cerevisiae*, producing ales. Ale beer is known for its fruity aromas, while lager beers are more neutral (Moura-Nunes et al., 2016).

Fermentation can be performed with *Saccharomyces* and *non-Saccharomyces* yeasts. Yeasts are largely responsible for the complexity and sensorial quality of fermented beverages. Choosing a pure yeast culture or a mix of strains is an important decision for achieving a final product which will have distinct characteristics appreciated by the consumers. The yeast is responsible to metabolize the sugar (maltose) in the wort into CO_2 and ethanol, but also is responsible for production through fermentation of by-products such as higher alcohols and esters, which have an important contribution to beer flavor.

It is known that *Saccharomyces* yeasts play an essential role in industrial brewing fermentation processes (Lengeler et al., 2020). Multiple studies have shown the major potential of *Saccharomyces* wild yeasts isolated from different foodstuffs to produce beer with new aromatic profile.

Postigo et al. (2021) conducted an evaluation of 141 *Saccharomyces* yeasts strains isolated from grapes and grape must. The main characteristics such as the fermentation behaviour, the production of volatile compounds, the melatonin production, and the antioxidant capacity were studied against the commercial strain *Saccharomyces cerevisiae*. The studies were carried from lab scale to industrial scale. In the end, *Saccharomyces*-G520 was indicated as a new potential yeast for the production of functional beer with healthier benefits, as this yeast can produce higher amount of melatonin and shows higher antioxidant capacity (Postigo et al., 2021).

The probiotic *Saccharomyces cerevisiae* var. *boulardii* (Scb) is a commercial strain of *Saccharomyces cerevisiae*, recently introduced in brewing industry. Mulero-Cerezo et al. (2019) used the probiotic *Saccharomyces cerevisiae* var. *boulardii* as a single yeast starter to produce craft beer with higher antioxidant activity and low alcohol content (Mulero-Cerezo et al., 2019). *Saccharomyces boulardii* is the most common and widely studied species with significant probiotic properties (Souza et al., 2022; Lazo-Velez et al., 2018). The use of *S. boulardii* in co-fermentation with *S. cerevisiae* to produce craft beers has been studied by Capece in 2018, who demonstrated that the probiotic yeast has the capacity to survive during the brewing process. The experiments performed by Capece refer to the integration of *S. cerevisiae* var. *boulardii* (S.b) in mixed cultures with *S. cerevisiae* strains for the production of beers which offer an increased content of polyphenols and higher antioxidant activity (Capace et al., 2018).

According to Datta et al. (2017), *S. boulardii* growth showed no significant difference in relation to the growth pattern presented by *S. cerevisiae* strains, although the probiotic strain showed higher tolerance to fermentative stress. *S. boulardii* had an antioxidant potential of 6 to 10 times higher than *S. cerevisiae*, with a total phenolic content 70 times higher and the total flavonoid content 20 times higher in the extracellular fraction. In addition, better results were obtained when *S. boulardii* and *Lactobacillus delbrueckii* acted together.

There is a continuous interest among research groups to achieve innovative fermentation characteristics from pure yeast cultures, that's why interspecific yeast hybrids are currently on focus for reinventing the lager yeast *Saccharomyces pastorianus* by crossing *Saccharomyces cerevisiae* with *Saccharomyces eubayanus* (Winans, 2022). Other authors, like Giannakou et al. (2021), created a novel non-GMO hybrid between *S. jurei* and *S. cerevisiae* ale yeasts to develop new starter strains with interesting flavours for the craft brewing.

Bruner et al. (2021) studied five distinct species of *Saccharomyces* from UC Davis selected from the Phaff Yeast Culture Collection, as well as an interspecies hybrid from the Fermentis company to produce beer on a pilot scale of 40 L. *S. kudriavzevii, S. mikitae, S. paradoxus, S. bayanus* and *S. uvarum* were inoculated in duplicate, with one fermenter in each pair receiving 10 g/L of dried hops during fermentation. The beers were analyzed for aroma, taste and mouthfeel, with the aim to assess the specific aroma of each yeast strain in terms of its brewing potential. All beers were spicy, probably due to the presence of phenols; the dry-hopping increased fruit notes at the same time perceived an increased bitterness and astringency.

Assessment of the potential to use mixed yeast starter cultures in brewing

The craft beer industry is interested in using new ingredients in order to develop new beer styles. According to this, the brewers' attention is focused to other, non-traditional method. Therefore, the *non-Saccharomyces* yeasts have been tested regarding their potential for aroma enhancement at the same time maintaining the specific *terroir* (Gamero et al., 2020).

Non-Saccharomyces yeasts represent a group of microorganisms with genetic diversity, specific metabolic characteristics and high potential for use in different fermentation processes.. Either *non-Saccharomyces* or *Saccharomyces* yeasts share common pathways for the central metabolism of carbon; both groups metabolize glucose through glycolysis (Figure1, Steward, G., 2016).

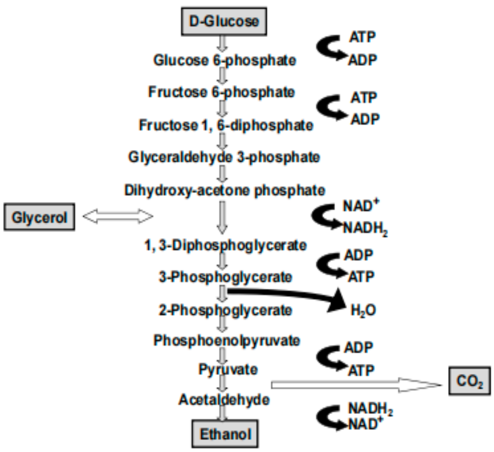

Figure 1. Obtaining of ethanol from glucose by the Embden-Meyerhof-Parnas (EMP) Pathway

Ethanol is the most important fermentation by-product, and from the technological point of view, the ethanol production capacity of yeasts is an important parameter that determines their use in fermentative processes (Steward, G., 2016).

The fermentative behavior of some non-Saccharomyces yeasts has been studied to find the most appropriate and suitable conditions for the strains to be used in the production of fermented beverages (Escalante, 2018). For a successful fermentation, a mixed culture with Saccharomyces spp. is required. It is necessary to search for a correct pitching ratio by testing different culture combinations at microscale, but also to scale-up the process to study the brewing process in real environment (Figure 2, Steward, G., 2016).

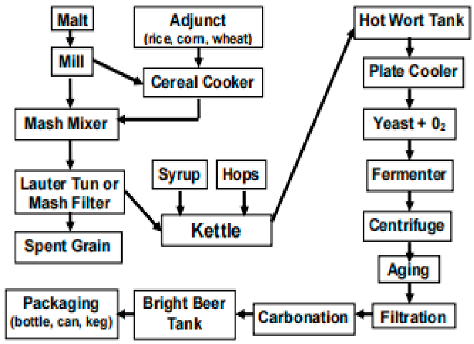

Figure 2. Beer brewing process

Non-*Saccharomyces* yeasts, such as *Dekkera animals, Naumovozyma dairenensis* and *Debaryomyces spp, Hanseniaspora, Torulaspora*, *Wickerhamomyces* have been reported with a high potential for use in beer fermentation because these yeasts can offer a diversified enzymatic apparatus and bioconversion abilities to reduce the energy value and lower the alcohol content in beer (Escalante, 2018).

Cyberlindnera saturnus TUM, a strain isolated from soil underneath an ash tree in Bavaria, was subject of a detailed studies made by Methner et al. (2022) about the influence of varying fermentation process to achieve different concentrations of flavors components in non-alcoholic beer. The trails were carried out using the surface methodology to optimize the beer with maximum content of fruitiness and minimal off-flavors and ethanol. The conclusion was that a low pitching rate combined with moderate fermentation temperature gave the optimal beer characteristics.

Torulaspora delbrueckii was explored in single strain fermentations for enhancing the aroma profile and producing a low alcohol beer. Some strains fermented all sugar, others did not succeed in this process. Important to mention is that all of them displayed an improved flavor profile characterised by "fruit/citric" notes and "full-bodies" attribute, as well and enhanced clarity and persistent foam (Gamero et al., 2020).

Fermentation of barley-sorghum wort with *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Metschnikowia pulcherrima* yeast strains were studied (Einfalt, 2021). Diferences were noticed in terms of fermentation time and ability to ferment maltose. *S. cerevisiae* showed a high fermentative power, *T. delbrueckii* and *M. pulcherrima* have been found to have low maltose fermentation abilities and to offer significantly different sensory attributes to barley-sorghum beers.

Kveik yeast was subject of a recent study realized by Kawa-Rygielska et al. (2022). Kveik yeast is an unconventional Norwegian yeast used to make bread and produce New England India Pale Beer (NEIPA). The antioxidative activity of the final product described as the total phenolic compounds of malt, hop and melanoidins were studied. These compounds influence the key quality attributes of beer: clarity, color, taste, and storage stability. The antioxidative activity of worth and beers were analyzed and the higher antioxidative potential was reached by the strain Linda Kveik, an indicator which leads to interesting sensory features to be used in the growth of the segment of unconventional beer sector.

Other current approaches in craft brewing

Beer is a worldwide beverage, and the addition of different herbs is a well know procedure used especially by microbrewers. *Artemsia vulgaris, Juniperus communis, Melissa officinalis, Brasica nigra, Coraindrum sativum* are examples of plants and plant extracts used in brewing. Lemon juice, raspberry syrup and grapes are very popular through the women population who dislike the bitterness of the beer (Habschied et al., 2020).

Ganoderma lucidum, a medical mushroom has been studied by Leskosek-Cukalovic et al. (2010), in combination with beer by adding alcoholic extract. The final product gains in natural antioxidants and can have potential medical significance.

The continuous search for new functional beer brought to attention new raw materials such as buckwheat and amaranth for creating gluten free-beer (Dabija et al., 2022). The research performed to date shows the possibility of using of these pseudo cereals in different combinations to obtain new beer varieties.

Another trend is to use mixed microbial cultures such as yeast and lactic acid bacteria (LAB) to create sour beers. The last ones were considered contaminants in beer fermentation, nevertheless are also appreciated in production of sour beer and different wild beer styles, e.g. Lambics and American Coolship Ales (Piraine et al., 2021).

Craft beer is becoming more and more popular in Romania as new microbreweries, brewpubs and craft beer shops are opening (Figure 3, www.malt.ro).

Figure 3. Craft beer styles diversity

Innovation is mandatory in today's food industry and brewing industry is also in line with this trend. Customers are more interested in choosing themselves the food and drink products, and also to consider their nutritional and beneficial effects on health. Yeast starter culture is an important decision for craft brewing industry in designing functional beers with attractive sensorial characteristics.

CONCLUSIONS

The "craft beer phenomenon" is one of the most fast growing trend in the beverages market worldwide, mainly because of the changes in consumer preferences and the interest for food and drinks with strong personality. One of the main characteristics of the sector that could be further explored is represented by the potential link with the local production of raw materials such as grains and hops, including the exploitation of the local microflora that could be used either as pure culture or in mixed cultures with commercial yeasts for craft beer fermentation. For example, the use of different yeast strains *Saccharomyces* and non-*Sacharomyces* can contribute to obtain craft beers with diverse and attractive sensory characteristics, making possible the exploitation of the *terroir* concept in the brewing industry as well.

Starting from this research, studies will be opened for innovative pathways to use yeast strains isolated from vineyard and winery environment from the Pietroasa Viticulture and Winemaking Research and Development Station in the production of craft beers.

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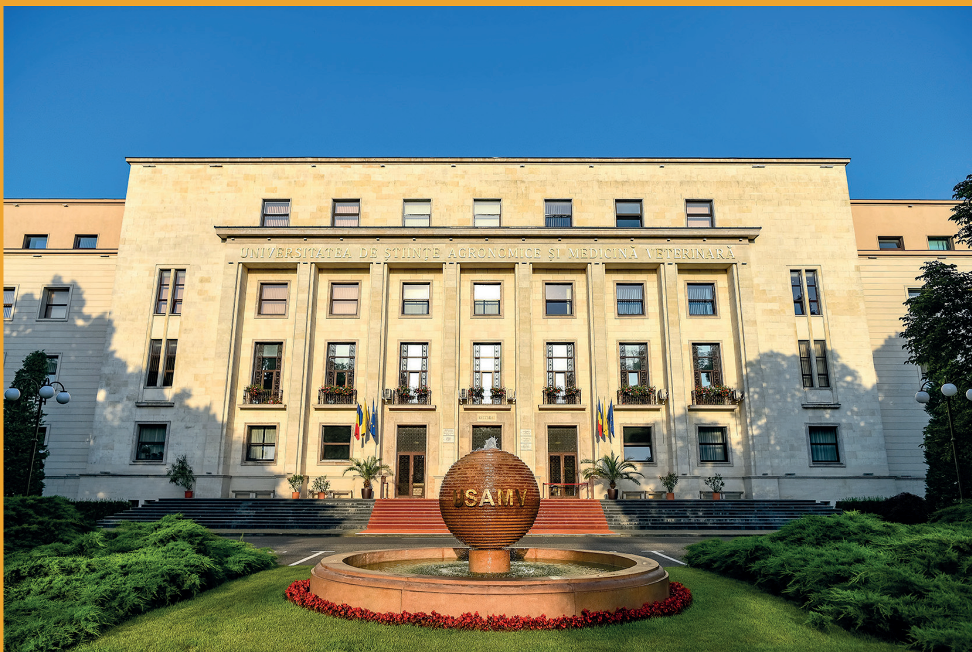

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