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

SCIENTIFIC PAPERS
SERIES B. HORTICULTURE

Volume LXV, No. 1

2021
BUCHAREST

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PUBLISHERS:

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

CERES Publishing House
Address: 29 Oastei Street, District l, Bucharest, Romania
Phone: + 40 317 90 23, E-mail: edituraceres@yahoo.com, Webpage: www.editura-ceres.ro

To be cited: Scientific Papers. Series B. Horticulture, Vol. LXV, No. 1, 2021

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

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

SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLES GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANSCAPE ARHITECTURE

MISCELLANEOUS

FRUIT GROWING

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CONTENT OF MINERAL ELEMENTS IN THE LEAVES OF 'WILLAMETTE' AND 'MEEKER' RASPBERRY CULTIVARS

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Abstract

The scientific experiment was conducted during the period of 2018-2019 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 mineral elements in the leaves was found during the phenophases, such as full blossoming and fruit harvesting. The results show that 'Willamette' had the highest nitrogen content in the leaf samples in the variant of 0.50 m (3.37%) in 2019 during the phenophase of full blossoming of plants. The phosphorus amount had the highest value in the variant of 0.50 m in 'Meeker' cultivar, as it was 0.27% in the phenophase of full blossoming and 0.28% in fruit harvesting phenophase in the first experimental year. Of the studied elements, the highest content of potassium was reported during the fruit harvesting phenophase in 2018 for 'Willamette' and 'Meeker' at shorter planting distances (0.70%).

Key words: *raspberries, cultivars, agricultural techniques, mineral composition in the leaves.*

INTRODUCTION

Raspberry is a fruit species of great economic importance due to its high productivity and economic efficiency. It is widely used in the foothill and mountain regions of Bulgaria, where soil and climate conditions are largely favourable for its development. However, these areas are characterized by poorly productive, acidic and nutrient-poor soils, which requires the determination of nutrient content in both soil and leaves. Leaf diagnostics is a widely used method in fruit growing, which establishes a direct dependence between the mineral composition of the leaves and the growth and reproductive manifestations of plants. With its application it is possible to determine and control the need for different types of fertilizers. The concentration of mineral elements in leaves largely depends on growth conditions, abiotic or biotic stress, the content of mineral elements in the soil and agrotechnical methods of cultivation (Chaplin & Martin, 1980; Prive & Sullivan, 1994; Hargreaves et al., 2008; Koumanov et al., 2009; Dresler et al., 2015). The nutrient content of leaf tissues may change during the growing season (John et al., 1976). The most important mineral nutrients that stimulate yields for raspberry production are nitrogen (N) and potassium. Nitrogen determines vegetative growth, while potassium increases the cold tolerance and drought resistance of plants. The average content of both elements is usually at a similar level and can be up to eight times higher than the content of phosphorus (P) (Kowalenko 2005; Buskiene and Uselis 2008). Hart et al. (2006) reported that in raspberries, N content varies depending on the yield, growth and age of the plants, soil type, rainfall and cultivar. However, plant growth is an initial indicator of adequacy of N. Wright and Waister (1980) state tht the nutrient content of leaves decreases during the period of plant growth and during fruit formation.

According to Gorbanov (2018), the optimal values for the content of the three nutrients are as follows, for nitrogen in the range of 2.8 to 3.5%, phosphorus - 0.3-0.5% and potassium - 2.0-2.5%.

The objective of the present research is to study the content of some mineral elements in the leaves of raspberry cultivars in different agricultural techniques of plant cultivation.

MATERIALS AND METHODS

The experiment was conducted in the period 2018-2019, in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The objective of the study is the widely distributed raspberry cultivars, such as ‘Willamette’ and ‘Meeker’, which are distinguished by high fruitfullness. The area is maintained in black fallow in the intra-row spacings and with naturally grassed row spacing. Fertilization was carried out in the intra-row area to achieve optimal values of the individual nutrients for raspberry plants. They are grown at an altitude of 460 m, eastern exposure with drip irrigation applied. The variants of the experiment are the following:

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

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

In both variants the row spacing was 3.00 m.

Leaf samples were taken during the phenophases of full blossoming and fruit harvesting. The samples from the leaves were naturally dried, ground and prepared for analysis, and the following was laboratory tested:

- nitrogen content (according to the Keldal method, BDS - EN ISO 5983);
- phosphorus content - spectrophotometric (Colorimetric method of Guericke and Kurmis, AOAS, 2007);
- the content of potassium (by the method of Atomic absorption spectrophotometry, AOAS, 2007).

The methodology for studying plant resources in fruit plants was used to report the indicators (Nedev et al., 1979). Data processing was performed by the methods of two-way factor analysis of variance (Lidanski, 1988), using software product MS Excel-2010.

RESULTS AND DISCUSSIONS

During the first experimental year the nitrogen content (%) in the raspberry leaves during the period of full blossoming of the plants in the tested cultivars was in the range 0.91-1.09%. It is noteworthy that in ‘Willamette’, the element has a higher value in the second planting variant, and in the ‘Meeker’ the element had a higher value in the first planting variant (Table 1). The differences between cultivars and variants for the nitrogen content in the leaves were statistically unproven. According to Kessel (2003) and Methodology (2010), the optimal nitrogen level in raspberry leaves is 2-3.5%. Anonymous (1996) states that the critical level of nitrogen in the leaves is < 2.00%, the optimal - 2.75%, and the excess nitrogen is at > 4.0%. The present study showed that ‘Willamette’ and ‘Meeker’ contained insufficient amounts of nitrogen - 2-3 times lower than necessary.

Table 1. Mineral composition of raspberry leaves in 2018 during the period of full blossoming of plants

Cultivars/indicators	N (%)	P (%)	K (%)
‘Willamette’- 0.50 m	0.91	0.23	0.60
‘Willamette’- 0.30 m	1.09	0.21	0.60
‘Meeker’- 0.50 m	1.07	0.27	0.45
‘Meeker’- 0.30 m	0.96	0.21	0.45
x ± SE	0.034	0.012	0.037
St. Dev.	0.098	0.033	0.104
VC %	9.71	13.33	23.11
Minimum	0.91	0.21	0.45
Maximum	1.09	0.27	0.60
Level of significance among the variants (P)	n.s	n.s	n.s
Level of significance among the cultivars (P)	n.s	$p < 0.05$	$p < 0.05$

The phosphorus content (%) was higher at longer planting distances, 0.23% at Willamette (0.50 m) and 0.27% at Meeker (0.50 m), respectively. Both cultivars had the same content of the element at a shorter planting distances (0.21%). The difference between the cultivars regarding the phosphorus content in the leaves was statistically significant ($p < 0.05$), and between the agricultural techniques it was unproven. According to Kessel (2003), the optimal level of phosphorus in raspberry leaves is 0.30%. These values indicate that symptoms of insufficient phosphorus were observed in the present study.

The potassium content in leaves was 0.60% in both variants with ‘Willamette’ and 0.45% in both variants ‘Meeker’. The difference between the cultivars in terms of potassium content in the leaves was statistically significant ($p < 0.05$). Kessel claims that the optimal level of potassium in raspberry leaves is between 1-2%. According to Anonymous (1996), the optimal potassium content is 1.5%. These values indicate that symptoms of potassium deficiency have been observed.

Table 2. Mineral composition of raspberry leaves in 2018 during the period of fruit harvesting

Cultivars/indicators	N (%)	P (%)	K (%)
‘Willamette’ - 0.50 m	0.98	0.25	0.60
‘Willamette’ - 0.30 m	1.15	0.22	0.70
‘Meeker’ - 0.50 m	0.85	0.28	0.60
‘Meeker’ - 0.30 m	0.86	0.26	0.70
x ± SE	0.07	0.01	0.02
St. Dev.	0.19	0.023	0.06
VC %	17.95	9.62	9.85
Minimum	0.85	0.22	0.60
Maximum	1.15	0.28	0.70
Level of significance among the variants (P)	n.s	n.s	p < 0.05
Level of significance among cultivars (P)	p < 0.05	p < 0.05	n.s

Table 2 presents the results of the nutrients in the leaf samples of the raspberry cultivars during the fruit harvesting period. The data show that the nitrogen content was higher in the variants of ‘Willamette’ in the period of full blossoming and had the highest value (1.15%) in the second variant. A significant reduction of the element from the first variant was observed in ‘Meeker’, with a reported value of 0.85%. A lower one was also reported for the second variant of the cultivar - 0.86%. In the statistical processing of data, it became clear that in terms of nitrogen content in the leaves, the differences between the cultivars were significant ($p < 0.05$).

With regard to phosphorus, an increase in its content in the leaves was found during the fruit harvesting phenophase. Its values ranged from 0.22% for ‘Willamette’ 0.30 m to 0.28 for ‘Meeker’ from the other variant. The difference between the cultivars was statistically proven ($p < 0.05$).

During the fruit harvesting period, the potassium content in the leaves compared to the phenophase of full blossoming increased only in ‘Meeker’ and was respectively 0.60% in the first variant and 0.70% in the second variant of the cultivar. The difference between the cultivars in terms of phosphorus content in the leaves was statistically significant ($p < 0.05$).

In the second experimental year in the full blossoming phenophase, the nitrogen content in the leaf samples was reported to be 2.72% for ‘Meeker’ at a planting distance of 0.30 m to 3.37% for ‘Willamette’ 0.50 m (Table 3). In all variants of the genotypes, the element was in higher quantities compared to the previous year of the same phenophase. According to Anonymous (1996), the nitrogen content in the leaves of the studied varieties of raspberries is in optimal values. Mathematically, the differences were proved between the cultivars ($p < 0.05$).

Table 3. Mineral composition of raspberry leaves in 2019 during the period of full blossoming of plants

Cultivars/indicators	N (%)	P (%)	K (%)
‘Willamette’ - 0.50 m	3.37	0.18	0.36
‘Willamette’ - 0.30 m	2.94	0.15	0.38
‘Meeker’ - 0.50 m	2.92	0.12	0.32
‘Meeker’ - 0.30 m	2.72	0.09	0.37
x ± SE	0.16	0.02	0.01
St. Dev.	0.46	0.04	0.02
VC %	16.2	31.34	6.58
Minimum	2.72	0.09	0.32
Maximum	3.37	0.18	0.38
Level of significance among the variants (P)	n.s	n.s	n.s
Level of significance between cultivars (P)	p < 0.05	n.s	n.s

The phosphorus content was highest in 'Willamette' (0.18% and 0.15%) and significantly less in 'Meeker' (0.30 m) - 0.09%. Potassium levels were approximately the same in both genotypes and variants. The highest level was found in 'Willamette' (0.30 m) (0.38%), while the lowest content was found in 'Meeker' - (0.32%). Statistically, the differences are unproven between the variants and the cultivars.

The content of mineral elements in the leaves of raspberries during the fruit harvesting phenophase is presented in Table 4. A significant reduction of nitrogen in the variants was reported in both cultivars. Overall, the presence of the element has approximately the same values, which varied from 2.06% in 'Meeker' (0.30 m) to 2.66% in 'Willamette' (0.50 m). Mathematical differences between cultivars and variants in terms of nitrogen content are unproven.

The phosphorus content (%) in the raspberry leaves was 0.11% for 'Willamette' (0.30 m) and higher 0.16% for the other variant of the cultivar. In 'Meeker' the values are almost the same in both variants - 0.14% (0.30 m), 0.15% (0.50 m). The differences between the cultivars and the variants for the phosphorus content in the leaves are statistically unproven.

It is noteworthy that the amounts of potassium are higher at short planting distances and are respectively 0.37% for 'Willamette' and 0.36% for 'Meeker'. The lowest value of the element was found in 'Meeker' (0.50 m) - 0.32% There was no evidence regarding the potassium content in raspberry leaves during the fruit harvesting phenophase between cultivars and variants. Kessel claims that the optimal level of potassium in raspberry leaves is between 1.0-2.0%. These results show insufficient potassium levels.

Table 4. Mineral composition of raspberry leaves in 2019 during the period of fruit harvesting

Cultivars/indicators	N (%)	P (%)	K (%)
'Willamette' - 0.50 m	2.66	0.16	0.36
'Willamette' - 0.30 m	1.98	0.11	0.37
'Meeker' - 0.50 m	2.37	0.15	0.32
'Meeker' - 0.30 m	2.06	0.14	0.36
x ± SE	0.09	0.01	0.01
St. Dev.	0.27	0.03	0.02
VC %	10.92	17.42	6.99
Minimum	1.98	0.11	0.32
Maximum	2.66	0.16	0.37
Level of significance among the variants (P)	n.s	n.s	n.s
Level of significance between cultivars (P)	n.s	n.s	n.s

CONCLUSIONS

A study was made on the dynamics in the values of the nutrients, such as nitrogen, phosphorus and potassium in leaf samples of 'Willamette' and 'Meeker' raspberry cultivars.

The results of the study show that 'Willamette' and 'Meeker' contain an insufficient amount of nitrogen, which was 2-3 times lower than the allowed in the first year. In the second year, the content of the element, due to the applied fertilization, was significantly higher in the variants of the two genotypes in the two phenophases. Only 'Willamette' reached optimal values in the period of full blossoming (3.37%) in the first variant.

In both experimental years, the phosphorus was lower than the reference values for the studied crop.

The highest potassium content was reported during the fruit harvesting period in the variants with the shorter planting distance of the plants in both genotypes in the first experimental year. In general, the values of the element are low and below the allowable.

During the two-year period, a higher percentage of statistical difference between the values of the nutrients was reported between the cultivars. An exception is observed in the potassium content between the variants in the first year of the fruit harvesting phenophase.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE IMPACT OF THE CROWN MANAGEMENT SYSTEM ON THE GROWTH AND FRUCTIFICATION OF CHERRY TREE VARIETIES IN A HIGH-DENSITY CULTIVATION SYSTEM

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Abstract

This work examines the growth and fruiting of modern cherry plantations according to the vigour of the variety-rootstock association, crown shape and planting distance. The impact of the crown formation system on the growth and fruiting of 'Ferrovia', 'Kordia', 'Regina', 'Stella', 'Skeena', 'Bigarreau Burlat', 'Lapins', 'Early Star', 'Samba' and 'Black Star' cherry varieties grafted on Gisela-6 and MaxMa 14 rootstocks in various combinations and at diverse planting distances was studied. During the first eight years of fruiting, the trees of 'Skeena' and 'Ferrovia' varieties, which had an improved slender spindle shape of their crowns, yielded the highest harvests. The yield produced by the variety of trees which had been planted at the distance of 5 x 1.5 m (18942-20074 kg/ha) was the highest; the smallest harvest was produced by the varieties planted at the distance of 5 x 2.5 m. During the ninth year of vegetation, the 'Kordia' and 'Regina' varieties grafted on the MaxMa 14 rootstock produced record harvests of 19221-19314 kg/ha. On average over three years, the 'Kordia', 'Regina' and 'Skeena' varieties were more productive as compared with the 'Ferrovia' and 'Stella' varieties.

Key words: *Cerasus avium, variety, rootstocks, crown shape, planting distance*.

INTRODUCTION

Small spindle crowns planted at small distances allow for more efficient use of solar energy in the process of producing large crops of qualitative fruit, the increase in the productivity of manual pruning and fruit harvesting work, and the achievement of a high degree of mechanization of technological work. For orchards of cherry trees the crowns of which are no larger than 3 m prove to be much more productive (Robinson et al., 2013; Cimpoies, 2018).

Numerous researches on tree management systems have been carried out due to the existence of a large variety of biological material (variety-rootstock), numerous planting distances and ecological conditions (Long Lynn et al., 2014). As a result of these studies, it has been revealed that systems of high-density orchards in which the trees have narrow small-volume crowns must be promoted; if there are favourable conditions for the intensification of technological processes, they produce optimal crops from a biological and technical point of view (Gjamovski et al., 2016; Long et al., 2005; Long, 2003; Sumedrea et al., 2014).

The range of low- and average-vigour rootstocks (Gisela, Krymsk, CAB, Weiroot, P-HL and Edabriz Table) allow for the development of high density cherry tree plantations of spindle crown trees, high yield orchards and low product costs (Aglar & Yildiz, 2014; Aglar et al., 2016; Gyeviki et al., 2008; Long, 2003; Usenik et al., 2010; Vercammen, 2002).

The orchards of trees the crowns of which are of (natural) high thin spindle shape are easier to manage as compared to the orchards in which the trees have thick and bulky crowns. In this case, it is easier to do the mechanized pruning, using special platforms to assist the process, in order to reduce labour costs and to improve fruit quality (Babuc, 2012; Long, 2014; Musacchi et al., 2015).

Considering all these facts, the impact of the crown management system on the performance of 'Ferrovia', 'Kordia', 'Regina', 'Stella', 'Skeena', 'Bigarreau Burlat', 'Lapins', 'Early Star', 'Samba' and 'Black Star' cherry tree varieties, which had been grafted on the Gisela 6 and MaxMa 14 rootstocks of average vigour, in different ecological conditions and planted at different distances was studied.

Thus, the purpose of the researches was to increase the productivity of cherry tree plantations in the Republic of Moldova, by identifying highly productive crown shapes.

MATERIALS AND METHODS

The researches into the formation of highly productive shapes of cherry trees' crowns were carried out in the southern and central orchards of the Republic of Moldova. The crown of the trees was formed in the shape of a Natural Crown Ameliorated with low volume and Slender Spindle Ameliorated.

Location, planting distance, crown shape
Experiment 1. The orchard was planted by the "ProdCar" Ltd in the village of Negureni, the district of Telenesti, in the spring of 2010. The cherry trees of the 'Adriana', 'Ferrovia' and 'Skeena' varieties had been grafted on the Gisela 6 vegetative rootstock at a planting distance of 4 x 2 m.

Experiment 2. The plantation was started at the "Terra-Vitis" Ltd in the village of Burlacu, the district of Cahul, in the southern orcharding area of the Republic of Moldova in the spring of 2010. The cherry trees of the 'Bigarreau Burlat', 'Ferrovia' and 'Lapins' varieties had been grafted on the Gisela 6 vegetative rootstock at a planting distance of 5 x 1.5 m, 5 x 2 m and 5 x 2.5m.

Experiment 3. The experiment was organized at the "Vindex-Agro" Ltd in the district of Orhei. The orchard was planted in 2011. The cherry trees of the 'Ferrovia', 'Kordia' and 'Regina' varieties had been grafted on the Gisela 6 rootstock at a planting distance of 4 x 2.5 m.

Experiments 4 and 5. The researches were organized in the central orcharding area of the Republic of Moldova, at the "StarAgroGroop" Ltd in the village of Ustia, the district of Criuleni. **Experiment 4** was performed using the 'Kordia', 'Regina', 'Stella', 'Ferrovia' and 'Skeena' varieties which had been grafted on the MaxMa 14 rootstock. The trees had been planted in the autumn of 2012 at a planting distance of 5 x 3 m; the crown of the trees had a Natural Crown Ameliorated with low volume.

Experiment 5 was conducted in the autumn of 2015 using the 'Early Star', 'Samba' and 'Black Star' cherry varieties planted at a distance of 4 x 2 m which had been grafted on the Gisela 6 rootstock. The crown of the trees had a Slender Spindle Ameliorated.

Research methodology. The experiments were conducted according to the multifactorial principle using four randomized groups of 8 representative trees each (Moiseychenko et al., 1994). The interaction between the planting distance and the crown shape, the basic factors that determine the early fruiting, the harvest and the fruit quality, were studied. Morphological descriptions, biometric measurements and the statistical processing of results were made. The difference between the variants had a margin of error of 5% (Dospekhov, 1985).

Cultural management of the plantation. The agrotechnical measures in orchards were carried out in accordance with the agrotechnical guidelines in force. In the orchards in the village of Negureni in the district of Telenesti, the village of Burlacu in the district of Cahul and the village of Malaiesti in the district of Orhei, there is a weather station which determines the state of the environment and plants. In the orchards of the "ProdCar" Ltd, the "Vindex Agro" Ltd and the "StarAgroGroop" Ltd drip irrigation is utilized. To monitor soil moisture, Watermark sensors are installed at a depth of 20, 40 and 60 cm on each plot. The water is distributed through mains with drippers fixed at 40 cm from the ground in the direction of the row. In experiment 2 the soil was cultivated; in experiments 1 and 3, during the first two years after the trees had been planted, the soil was cultivated; in the following years the space between the rows was artificially grassed. At the "StarAgroGroop" Ltd the soil in the orchards was artificially grassed. The weeds on the ground between the rows (2-2.5 m wide), which grew naturally and artificially, were mowed when necessary and left as mulch. On the ground along the rows of trees herbicides were applied, or it was twice or three times weeded with a rotary tiller.

RESULTS AND DISCUSSIONS

As a consequence of the researches into the obtaining of early large and qualitative harvests, trees that have improved crowns which can be planted more densely are grown in orchards, the growth processes through the utilisation of irrigation and fertilization have been optimized, the pruning has been minimized, and branch inclination to induce early fruiting is applied (Balan, 2015; Ivanov & Balan, V., 2017; Balan et al., 2018; Ivanov, I., Balan et al., 2018).

During the growth and fruiting period, the length and width of the crowns of ‘Adriana’, ‘Ferrovia’ and ‘Skeena’ cherry tree varieties, grafted on Gisela 6 and planted at a distance of 4 x 2 m, were in full development (Table 1).

Table 1. Length and width of the cherry tree crowns according to the variety and crown shape, cm. (Gisela 6 rootstock, planting distance - 4 x 2 m, tree age - 3-5 years old, “ProdCar” Ltd)

Variety	Length of the crown (cm)			Width of the crown (cm)		
	2012	2013	2014	2012	2013	2014
Natural Crown Ameliorated with low volume						
‘Adriana’	132	168	201	110	157	225
‘Ferrovia’	150	173	215	160	173	224
‘Skeena’	120	146	195	124	146	198
Slender Spindle Ameliorated						
‘Adriana’	120	168	195	124	157	214
‘Ferrovia’	172	222	220	190	222	245
‘Skeena’	135	165	200	142	164	195
LSD 5%	-	27	32	-	52	63

Therefore, in the 3rd vegetation year, the trees of the ‘Ferrovia’ variety, the crowns of which were of improved slim spindle shape, recorded the highest value of the crown length (172 cm). In the 5th year, the cherry trees merged in a row, their crown length being of 195-220 cm. The width of their crowns also increased as they became older, namely from 110-190 cm in the 3rd year to 195-245 cm in the 5th year of vegetation. Over the years, the ‘Ferrovia’ variety had proven to be more vigorous compared to the ‘Adriana’ and ‘Skeena’ varieties, but the growth values were not distinctly significant.

Analysing the values of the gradual crown enlargement of cherry trees grafted on Gisela 6 rootstock and planted at a distance of 4x2 m, it can be stated that they reached the optimal level during the fruiting and growing period of the trees.

The ‘Adriana’, ‘Ferrovia’ and ‘Skeena’ varieties, grafted on Gisela 6, began to bear fruit in the fourth year after their planting, when a harvest of 625-1562 kg/ha was reaped (Table 2). The ‘Ferrovia’ variety proved to be more productive compared to the ‘Adriana’ and ‘Skeena’ varieties. It yielded 1125-1562 kg/ha. In the second fruiting year, the mentioned varieties yielded better harvests, namely 4250-5000 kg/ha. Once the trees got older, viz in 2016, the fruit harvest tripled, namely the ‘Adriana’ variety yielded 11875-13000 kg/ha, the ‘Ferrovia’ variety - 13250-14125 kg/ha, and the ‘Skeena’ variety - 16000 kg/ha. In the seventh year after their planting, the cherry trees yielded twice as many fruits as compared to the previous year, namely the ‘Adriana’ variety produced 21875-22500 kg/ha and the ‘Skeena’ variety - 26250-28000 kg/ha.

Table 2. The yield of cherry trees, kg/ha (Gisela 6 rootstock, planting distance – 4 x 2 m, the age of the trees - 4-12 years old, “ProdCar” Ltd)

Variety	Years								Average yields (2013-2020)
	2013	2014	2015	2016	2017	2018	2019	2020	
Natural Crown Ameliorated with low volume									
‘Adriana’	625	4375	11875	21875	10875	12958	13375	9319	10659
‘Ferrovia’	1125	4875	13250	24750	15750	15222	10791	14277	12505
‘Skeena’	625	4250	16000	26250	16875	17583	17042	16652	14409
Slender Spindle Ameliorated									
‘Adriana’	875	4500	13000	22500	10750	14820	13125	10819	11298
‘Ferrovia’	1562	5000	14125	24500	12700	15388	13541	16638	12931
‘Skeena’	375	4375	16000	28000	14000	17500	17416	18986	14581
LSD 5%	-	435.2	971.8	1315.2	1429.1	1423.6	2305.7	1314.8	-

The ‘Ferrovia’ and ‘Skeena’ varieties, grown using both tree formation systems, showed a distinctly significant crop increase as compared to the ‘Adriana’ variety. In the following year (2017) the fruit harvest decreased remarkably, namely the ‘Adriana’ variety yielded only 10750-10875 kg/ha, the ‘Ferrovia’ variety - 12700-15750 kg/ha, and the ‘Skeena’ variety - 14000-16785 kg/ha. In the following years, the fruit harvests were of 9316-18986 kg/ha; the

'Ferrovia' and 'Skeena' varieties yielded higher crops. Analysing the yielding performance during the eighth, ninth and tenth years of the tree vegetation (2017-2020), it can be mentioned that the values are mean for the plantations of cherry trees which are grafted on the rootstock of medium vigour Gisela 6, as compared to the data presented by other authors (Miter et al., 2012; Long et al., 2014).

The cherry trees which had been planted at the distance of 4 x 2.5 m and reached the age of four or five, had a crown length of 129-231 cm; when they reached the age six, their crowns occupied the reserved space in the direction of the row (249- 262 cm). We have to mention that, the distance of 2.5 m between the cherry trees in a row grafted on Gisela 6 was too great, because the trees occupied the space reserved for their crowns only in the 6th vegetation year (Table 3). In the 4th vegetation year the width of the crown was 139-190 cm; in the 6th vegetation year, it was 235-250 cm, but these values are not statistically proved. So, the width of the crown reached the optimal necessary level (250 cm) to assure a high degree of sunlight utilisation and the easy movement of tractors and other agricultural machinery.

The data in Table 4 show the impact of the crown shape on the yield of 'Ferrovia', 'Kordia' and 'Regina' varieties, which were grafted on Gisela 6 and planted at the distance of 4 x 2.5 m, during the periods of their growth, fruiting and full fruiting. The results of the research into the crown formation system are of interest both in terms of precocity and the yield in the first 7 fruiting years.

Table 3. The length and width of the cherry trees' crowns according to the variety and crown shape, cm (Gisela 6 rootstock, planting distance – 4 x 2.5 m, tree age - 4-6 years old, "Vindex-Agro" Ltd)

Variety	**Length of the crown**			**Width of the crown**		
	2014	**2015**	**2016**	**2014**	**2015**	**2016**
Natural Crown Ameliorated with low volume						
'Ferrovia'	129	192	258	175	234	241
'Kordia'	175	210	255	190	238	243
'Regina'	162	215	262	139	252	250
Slender Spindle Ameliorated						
'Ferrovia'	136	209	260	154	198	250
'Kordia'	162	231	249	148	229	220
'Regina'	170	238	260	132	241	235
LSD 5%	27	32	14	30	25	19

The trees started to bear fruit in the 4th vegetation year yielding 400-500 kg/ha. In the 2nd fruiting year, the trees yielded a harvest of 4600-5000 kg/ha, not statistically assured. Starting with the 3rd fruiting year, the 'Ferrovia' and 'Kordia' varieties yielded poorly - 12310-13290 kg/ha and 11270-12830 kg/ha respectively (statistically assured data). In 2017 and 2020, the harvest decreased significantly due to unfavourable climatic conditions during their blossom, namely it was foggy, rainy and cold.

The crops were larger than 10,000 kg/ha only in two years out of seven, namely in 2016 and 2019. During the first 7 years of tree fruiting, the 'Ferrovia' variety yielded 8193-8308 kg/ha, the 'Kordia' variety - 7650-8314 kg/ha, and the 'Regina' variety - 7208-7877 kg/ha.

The trees, the crown of which was formed after the improved thin spindle shape pattern, yielded larger crops not statistically assured.

Table 4. The yield of cherry trees, kg/ha (Gisela 6 rootstock, planting distance – 4 x 2.5 m, tree age - 4-10 years old, "Vindex-Agro" Ltd)

Variety	**Years**							**Average yields (2014-020)**
	2014	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	
Natural Crown Ameliorated with low volume								
'Ferrovia'	500	5000	12310	7900	10875	13733	7033	8193
'Kordia'	400	4600	11270	7500	7600	13811	8367	7650
'Regina'	500	4800	10380	7800	7600	12366	7010	7208
Slender Spindle Ameliorated								
'Ferrovia'	500	4900	13290	7933	11778	12938	6815	8308
'Kordia'	400	4700	12830	8876	8944	13321	9124	8314
'Regina'	400	5000	11890	7573	11289	11997	6990	7877
LSD 5%	-	845	529	824	675	315	783	-

The growth and fruiting of the 'Bigarreau Burlat', 'Ferrovia' and 'Lapins' varieties were studied in accordance with the crown management system as well as the planting distance (Tables 5 and 6). It was found that, regardless of the planting distance, during the period of tree growth, the growth of ‘Ferrovia’ trees was faster as compared to the ‘Bigarreau Burlat’ and ‘Lapins’ varieties. Thus, in the 5th vegetation year, the crowns of the trees of ‘Ferrovia’ variety reached a length of 172-264 cm. The trees that had been planted at the distance of 5 x 2.5 m reached the highest length of their crowns.

The 4 and 5 year-old trees of ‘Bigarreau Burlat’, ‘Ferrovia’ and ‘Lapins’ varieties, which had been planted at the distance of 5 x 1.5 m, occupied the entire reserved area in a row. Obviously, as the distance between the trees in a row increases from 1.5 m to 2.5 m, the time to occupy the space reserved for the tree crown also increases. The ‘Bigarreau Burlat’ and ‘Ferrovia’ varieties joined in the row in the 4th vegetation year, and the ‘Lapins’ variety - in its 5th year of vegetation.

Analysing the values of the crown length and width of the cherry trees of the ‘Bigarreau Burlat’, ‘Ferrovia’ and ‘Lapins’ varieties, it can be stated that they depend on the age of the trees, the distance between rows and between the trees in a row, and differ insignificantly from one variety to another and from one crown shape to another. Thus, for example, the crown of the ‘Bigarreau Burlat’ variety which had a naturally improved small volume shaped crown, reached a length of 125-155 cm when planted at a distance of 1.5 m in a row, and 170-258 cm when planted at a distance of 2.5 m in a row.

The same legitimacy was observed in the ‘Ferrovia’ and ‘Lapins’ varieties, i.e., regardless of the shape of the crown, as the distance between the trees in a row increased, the length of the crown increased too. The width of the crown of the ‘Ferrovia’ tree variety planted at a distance of 5 x 2.5 m was 180-182 cm in the 3rd year of vegetation, and 250-264 cm in the 5th year of vegetation. Basically, in the 5th vegetation year, the crowns of cherry trees reached the optimal length, typical for intensive orchards, in order to use the solar energy necessary to produce large yields of qualitative fruit.

Table 5. The length of the crown of cherry trees according to variety, planting distance and crown shape, cm (Gisela 6 rootstock, tree age - 3-5 years old, “Terra-Vitis” Ltd)

Planting distance, m	**‘Bigarreau Burlat’ variety**			**‘Ferrovia’ variety**			**‘Lapins’ variety**		
	2012	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
Natural Crown Ameliorated with low volume									
5 x 1.5	125	145	155	135	165	172	115	145	155
5 x 2	162	200	221	160	185	214	135	165	209
5 x 2.5	170	190	258	182	210	264	160	170	250
Slender Spindle Ameliorated									
5 x 1.5	130	162	155	127	170	170	125	170	170
5 x 2	162	190	230	180	195	217	137	162	228
5 x 2.5	191	210	265	180	190	250	152	190	261
LSD 5%	-	38	19	-	27	16	-	42	24

The varieties ‘Bigarreau Burlat’, ‘Ferrovia’ and ‘Lapins’ began to bear fruit during the 4th vegetation year. The trees which had been planted at a distance of 5 x 2.5 m yielded a crop of 320-400 kg/ha, and 666-1066 kg/ha when planted at a distance of 5 x 1.5 m. During the 2nd year of fruiting, the yield of the orchard increased directly in proportion to the density of the trees, constituting 3720-6822 kg/ha. In the 3rd fruiting year (2014), the trees planted at a distance of 5 x 1.5 m yielded the statistically confirmed largest crop (5478-6822 kg/ha), and the trees planted at a distance of 5 x 2.5 m yielded the smallest crop (3720-4200 kg/ha). During the fruit-bearing period, the yield of the trees doubled or even tripled as compared to the previous period. Thus, in the 6th vegetation year, the cherry trees grafted on Gisela 6 produced a crop of 8000-12864 kg/ha, and in the 7th year they produced 14616-20074 kg/ha of fruit.

The analysis of the yields, produced by the ‘Bigarreau Burlat’, ‘Ferrovia’ and ‘Lapins’ cherry tree varieties grafted on Gisela 6, indicates that the harvest changed depending on the age of the trees, the planting distance and the shape of their crown. Thus, during the period of full fruiting of trees, the cherry yield did not increase in proportion to the number of trees per hectare. The yield of the trees that had been planted at a shorter distance was higher and statistically confirmed. The trees the crowns of which were formed in the shape of an improved slim spindle achieved higher productivity (15632-20074 kg/ha) as compared to the trees that had a naturally improved small volume shaped crown (14616-18555 kg/ha), but it wasn't always statistically confirmed.

The 'Ferrovia' tree variety yielded the largest crop (20074 kg/ha) in the 7th year of its vegetation. It should be noted that the 'Bigarreau Burlat' and 'Lapins' varieties behaved similarly from the statistical point of view. As expected, the trees planted at a distance of 5 x 1.5 m yielded the largest crops (18942-20074 kg/ha); the smallest quantity of fruit was produced by the trees planted at a distance of 5 x 2.5 m (15632-16904 kg/tree). The cause of that phenomenon lay in the number of trees per hectare in the two planting schemes.

Table 6. The width of the cherry trees' crown according to variety, planting distance and the shape of the crown, cm (Gisela 6 rootstock, tree age - 3-5 years old, "Terra- Vitis" Ltd)

Variety	Planting distance, m	Natural Crown Ameliorated with low volume			Slender Spindle Ameliorated		
		Year 2012	Year 2013	Year 2014	Year 2012	Year 2013	Year 2014
'Bigarreau Burlat'	5 x 1.5	120	147	258	116	140	245
	5 x 2	120	159	250	125	158	262
'Ferrovia'	5 x 1.5	132	150	254	128	162	261
	5 x 2	110	140	262	140	160	250
'Lapins'	5 x 1.5	130	165	254	140	169	260
	5 x 2	120	172	262	140	180	271

As regarding the optimization of the area under trees, it can be said with certainty that the maximum width between rows is equal to the sum of the planting distance between trees per row plus two meters required for the movement of the tractors (Balan V., 2015; Babuc V., 2012). Therefore, the crown parameters of the cherry trees varieties grafted on the medium vigour Gisela 6 rootstock show that the distance of 2.5 m between trees in a row is large, and if the trees are planted at 2 m apart in the row, the distance between rows is optimal, i.e. 4 m (2 m + 2 m).

Table 7. The yield of cherry trees according to variety, planting distance and crown shape, kg/ha (Gisela rootstock 6, tree age - 4-7 years old, "Terra- Vitis" Ltd)

Variety	Planting distance, m	Natural Crown Ameliorated with low volume				Slender Spindle Ameliorated			
		Year 2013	Year 2014	Year 2015	Year 2016	Year 2013	Year 2014	Year 2015	Year 2016
'Bigarreau Burlat'	5 x 1.5	799	5962	11410	17982	711	6339	12209	18942
	5 x 2	400	4893	9150	15160	500	5120	11820	16160
'Ferrovia'	5 x 1.5	931	6397	12716	18155	1066	6822	13823	20074
	5 x 2	700	5260	12350	15920	800	5580	13130	17810
'Lapins'	5 x 1.5	666	5478	12277	18555	666	5799	11864	19382
	5 x 2	500	4580	9400	17240	500	5010	9900	18210
LSD 5%		275	647	1375	1284	275	647	1375	1284

Analysing the fruit harvest data presented in Table 7, it is evident that the grafting of cherry trees on the Gisela 6 rootstock and their planting at shorter distances, allow for the obtaining of medium-sized trees, the stimulation the early fruiting, the caring for the trees from ground level, thus reducing the pruning and harvesting costs by increasing the pruning and harvesting productivity.

In the 8th vegetation year, the 'Ferrovia', 'Kordia', 'Regina', 'Skeena' and 'Stella' cherry varieties grafted on the MaxMa 14 rootstock produced a yield ranging from 4181 kg/ha in the 'Ferrovia' variety up to 15702 kg/ha in the 'Skeena' variety (Table 8).

The 'Kordia' (10944 kg/ha), 'Skeena' (15702 kg/ha) and 'Stella' (912861 kg/ha) varieties yielded the largest crops. In the 9th year of vegetation, the 'Kordia' and 'Regina' varieties yielded record crops of 19221-19314 kg/ha; the 'Ferrovia' (4113 kg/ha) and 'Stella' (7992 kg/ha) varieties yielded the smallest crops. In the 10th year of vegetation, the 'Regina' variety produced the highest yield (12055 kg/ha); the ‘Ferrovia’ (2731 kg/ha) and ‘Kordia’ (2910 kg/ha) varieties produced the lowest yield.

Table 8. The yield of cherry trees, kg/ha (MaxMa 14 rootstock, planting distance - 5 x 3 m, the shape of a Natural Crown Ameliorated with low volume, tree age - 8-10 years old, "StarAgroGroop" Ltd)

Variety	Years			Average yields (2018-2020)
	2018	2019	2020	
‘Ferrovia’	4181	4113	2731	3675
‘Kordia’	10944	19314	2910	11056
‘Regina’	5766	19221	12055	12347
‘Skeena’	15702	9168	9058	11309
‘Stella’	12861	7992	5574	8809
LSD 5%	1238	1835	934	

During the fruiting period of the trees, the ‘Skeena’ variety produced a more constant yield compared to the ‘Ferrovia’, ‘Kordia’, ‘Regina’ and ‘Stella’ varieties. On average over the 3 years, the ‘Kordia’ (11056 kg/ha), ‘Regina’ (12347 kg/ha) and ‘Skeena’ (11309 kg/ha) varieties were more productive as compared to the ‘Ferrovia’ (3675 kg/ha) and ‘Stella’ (8809 kg/ha) varieties.
‘Early Star’ and ‘Black Star’ varieties, grafted on the Gisela 6 rootstock, began to bear fruit in the 4th vegetation year, and the ‘Samba’ variety - in the 5th year of vegetation. In 2019, the ‘Early Star’ variety yielded a crop of 7012 kg/ha, and the ‘Samba’ variety - 16820 kg/ha. The ‘Black Star’ variety produced an intermediate quantity of fruit, namely 10750 kg/ha. In 2020, the fruit harvest decreased considerably, namely the ‘Early Star’ and ‘Samba’ varieties produced only 3625-4463 kg/ha. The ‘Black Star’ variety proved to be more resistant to late spring frosts and produced a crop of 9875 kg/ha. On average over 3 years, the ‘Samba’ variety proved to be the most productive, namely 8037 kg/ha.

Table 9. The yield of cherry trees, kg/ha (Gisela 6 rootstock, planting distance - 4 x 2 m, the shape of a Slender Spindle Ameliorated, tree age - 4-6 years, "StarAgroGroop" Ltd)

Variety	Years			Average yields (2018-2020)
	2018	2019	2020	
‘Early Star’	2945	7012	4463	4807
‘Samba’	3667	16820	3625	8037
‘Black Star’	0	10750	9875	6875
LSD 5%	992	874	1013	

CONCLUSIONS

The relatively long period of time for the researches, the rootstock associations, the crown shapes and the planting distances used to study the growth and fruiting of the cherry trees, as well as the analysis of the research material allow for the following conclusions:
The crown size of the cherry trees grafted on the Gisela 6 rootstock and planted at a distance of 4 x 2 m, reached the optimal level once the trees entered the period of growth and fruiting. In the first 8 fruiting years, the ‘Skeena’ (14581 kg/ha) and ‘Ferrovia’ (12931 kg/ha) varieties, the trees of which had an improved slim spindle shaped crowns, produced the highest yield.
At the age of 6, the crown of the cherry trees planted at a distance of 4 x 2.5 m occupied the reserved space in the direction of the row (249-262 cm). During the first 7 fruiting years, the ‘Ferrovia’ variety yielded 8193-8308 kg/ha, the ‘Kordia’ variety - 7650-8314 kg/ha, and the ‘Regina’ variety - 7208-7877 kg/ha. The trees which had an improved slim spindle shaped crown produced larger quantities of fruit, but these data were not statistically confirmed.
The ‘Bigarreau Burlat’ and ‘Ferrovia’ trees varieties planted at a distance of 5 x 1.5 m, 5 x 2 m and 5 x 2.5 m, fused in the direction of the row in the 4th vegetation year, and the ‘Lapins’ variety - in the 5th year of vegetation. The trees planted at a distance of 5 x 1.5 m produced better yields (18942-20074 kg/ha), and the trees planted at a distance of 5 x 2.5 m produced the poorest yields (15632-16904 kg/ha).
In the 9th year of vegetation the ‘Ferrovia’, ‘Kordia’, ‘Regina’, ‘Skeena’ and ‘Stella’ cherry tree varieties grafted on the MaxMa 14 rootstock, and ‘Kordia’ and ‘Regina’ varieties grafted on the MaxMa 14 rootstock yielded a crop of 19221-19314 kg/ha. On average over 3 years, the ‘Kordia’ (11056 kg/ha), ‘Regina’ (12347 kg/ha) and ‘Skeena’ (11309 kg/ha) varieties were more productive as compared to the ‘Ferrovia’ (3675 kg/ha) and ‘Stella’ (8809 kg/ha) varieties.
The crown shapes tested on cherry trees did not have a significant impact on the yield formation of the studied varieties, since the naturally improved small volume crown and the improved slim spindle crown were suitable for the formation of cherry trees grafted on

medium vigour Gisela 6 and MaxMa 14 rootstocks in a high-density fruit tree cultivation system.

ACKNOWLEDGMENTS

This study was supported by the National Agency for Research and Development (NARD), project 18.817.05.29A "*The improvement of the maintenance technologies of super-intensive cherry and apple orchards; the development of fruit quality formation techniques in Europe*." Project director - Valerian BALAN, Habilitated Doctor of Agriculture, professor.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDIES ON THE INFLUENCE OF QUALITY ON BIOMETRIC AND PHYSIOLOGICAL INDICES IN APPLES (*MALUS DOMESTICA* L.)

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Abstract

The Apple is one of the basic components in modern human nutrition. They are almost the only food ready prepared in nature, which can be eaten without other additives, either fresh or processed. Apple ranks first among fruit species grown in the temperate zone. The fruits are resistant to handling and transport. In this experiment we studied the biometric and physiological indices of twogapple varieties with different origins from western Romania zone. Compared to the imposed standards on diameter, both imported and domestic varieties fall into the Extra category, except for the Golden Varciorova variety which falls into category I. In order to observe the starch/glucose content following the AI test, it was found that all the samples show indicator 9, which reveals the increased amount of glucose, so that all varieties fall into the category of overripe fruits. Compared to the standard values regarding the maturity degree for apple varieties, it is observed that all the local varieties, both Golden and Idared, Arad and Varciorova fall into the Excellent category, according to the Brix index.

Key words*: diameter; weight; firmness; glucyds; pH; dry matter.*

INTRODUCTION

The importance of the apple lies in its biological peculiarities. The existence of a large number of varieties, with staggered ripening in various seasons and the ability to keep fresh winter varieties for a long time, ensures the consumption of fresh fruit almost all year round, about 10 months out of 12 of a year (Thompson, 2008). With a high storage capacity and good handling resistance, fruits can be easily transported over long distances. Due to their technological properties, apples are a raw material with a high share in the food industry (Barreira et al., 2019). Thus, from a chemical point of view, apples contain on average: 84.5% water, 14.1% sugars, 0.2% pectic substances, 0.6% fatty substances, 90 IU vitamin A, 0.02 mg% vitamin B2, 0.1 mg% vitamin B1, 7 mg% vitamin C, 7 mg% calcium, 10 mg% potassium as well as small amounts of aluminum, manganese, sulfur, cobalt and others (Belding, 2008). Fruit quality depend on the species, variety, technological factors and climate (Vâtcă et al., 2020). In everyday life, the term "ripe" fruit refers to changes in the fruit, which makes it good to eat (Nyasordzi et al., 2013). Such changes normally include "softening" due to enzymatic breakdown of the cell wall (Puia et al. 2017), starch hydrolysis, accumulation of sugars, and the disappearance of organic acids and phenolic compounds, including tannins (Farneti et al, 2015). The main physiological change that indicates the level of maturity of the fruit is the internal production of the phyto-hormone Ethylene (C_2H_4) a naturally generated hormone, which causes a cascade of biochemical reactions (Stoian et al., 2019) involved in the ripening process (Thompson, 2008). Ethylene produced at the time of fruit ripening causes the fruit to ripen, ie it makes it softer, produces volatile compounds (aroma and flavor), converts starch into glucose, develops skin color and breaks down green chlorophyll (Pineiro and Rios, 2007). The basic physiological processes in fruits are respiration, perspiration and circulation of substances (Dobrei et al, 2006). In apples, pears, peaches and partially in other fruits, respiration decreases continuously as it ripens to a minimum that coincides with the stage of maximum cell stretching (Alexa et al. 2018). After this stage, the respiratory intensity increases to a maximum and then decreases again until cell death (FAO, 2006). During storage, the fruit is released through respiration, heat and CO_2. The decrease of the temperature and of the O_2 concentration up to 3% makes the physiological functions of the fruits to be carried out with minimum intensity (Denver and Jensen, 2014; Camen et al., 2016). By breathing and perspiration the fruits lose weight during storage (Palmer et al., 2002). Dry matter content had been shown to influence directly the post-storage atributes and represents an important assessment of apple fruits characteristics (Zhang et al., 2019). Firmness is a primary measure of apple fruit texture, the key determinant of apples quality (Saei et al., 2011). Despite the well-developed understanding of the process of firmness loss in storage, there is very limited information concerning the causes of fruit quality variation in the market-place. Nowadays few trials are made to characterize the biometric parameters of apples after ripening. This experiment sets out to examine quality indices of two apples cultivars.

MATERIALS AND METHODS

The biological material was represented by two cultivars of apples, taken from the agricultural market in the west of the country, namely 'Golden', and 'Idared' from different areas of culture (Italy, Hungary, Romania: Arad and Vârciorova). We took into study a number of 5 apples from each cultivar in 5 replicates for each area and analysis. During the experiment the following quality parameters were studied: fruit's dimensions respectively thediameter, and weight following Table 1, starch-iodine test following Figure 1, firmness, pH of vegetable juice, total sugar content, dry matter.

Table 1. The minimum diameter and weight values for apples determination

	Extra	Category I	Category II
DIAMETER			
Large fruited varieties	≥ 65 mm	≥ 60 mm	≤ 60 mm
Other varieties	60 mm	55 mm	50 mm
WEIGHT			
Large fruited varieties	110 g	≤ 90 g	≥ 90g
Other varieties	90 g	80 g	70 g

In order to determine maturity, the samples were subjected to the AI test (Chu, 2000), the interpretation of the results being based on the comparison of the results with the standard starch-iodine (AI) diagram.

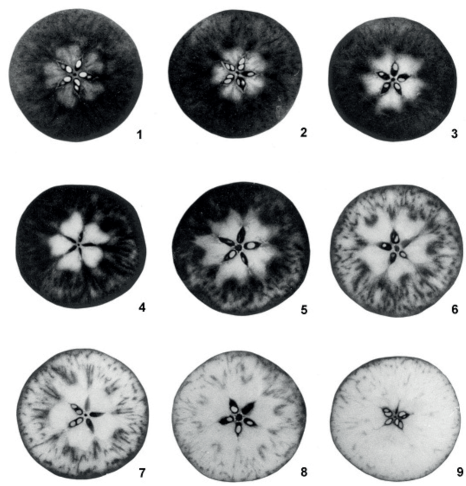

Figure 1. The degree of maturity of the apples according to the AI test (Chu, 2000); Dark areas indicate starch, open areas indicate glucose

To determine fruit firmness we used a digital penetrometer, removing the two small disks of peeled fruit in the middle of the dry flower stems and opposite sides of the apple. Total sugar content was achieved with digital refractometer, Brix degrees (Table 2) was equate to the percentage depending on the temperature determination (for each 10°C we increase the percentage of total soluble percentage with 0.5%) (Camen et al., 2011).

Table 2. Maturity varieties of apple based on Brix index (soluble glucose)

Brix guide	Low	Weak	Good	Excellent
All varieties	< 11	11	12	13
Honeycrisp	< 12	12	13	> 14

The percentage of dry fruit pulp was determined using Kern thermobalance. Prior to analysis, all the fruits were kept at 4°C to avoid their accentuated degradation until the moment of analysis. The analysis were made at room temperature. Statistical analysis was perforemed for each parameter values together, Anova and LSD with Rstudio program, agricolae package.

RESULTS AND DISCUSSIONS

For all genotypes studied, a linear relationship existed between the maximum and minimum size of fruit. The large variation in fruit size (diameter as well as weight) indicates that apples should be sorted to improve quality. This standard governs apples of the *Malus domestica*. L. varieties intended for fresh delivery. Thus, a minimum diameter and weight are required.

Compared to the imposed standards on diameter, both imported and domestic varieties fall into the Extra category, except for the 'Golden' cultivar from Vârciorova which has a diameter of 62.63 mm, thus falling into category I. Regarding the weight, both studied varietes belong to the category of large fruits with values in the range between 128.66 ('Golden' from Vârciorova), framed as a value between minimum and average weight from other studies (Blazek and Hlusickova, 2007; Musacchi and Serra, 2017) and 204.05 grams ('Idared' from Italy) (Table 3) framed as a value between average and maximum weight from the study of Blazek and Hlusickova in 2007. In terms of fruit weight, all varieties are falls into the Extra category.

Table 3. Experimental results regarding biometric indices for the studied apple genotypes (mean ± S.E.)

Genotype/Place of origin	Genotype code	Diameter (mm)	Weight (g)
'Golden'/Italy	GI	72.00 ± 2.60	193.42 ± 9.47
'Golden'/Hungary	GH	71.75 ± 1.38	198.98 ± 9.21
'Golden'/Arad	GA	68.13 ± 0.88	160.24 ± 5.41
'Golden'/Vârciorova	GV	62.63 ± 1.50	128.66 ± 7.98
'Idared'/Italy	II	76.25 ± 1.44	204.05 ± 8.26
'Idared'/Hungary	IH	75.25 ± 3.89	193.80 ± 13.82
'Idared'/Arad	IA	70.25 ± 2.69	179.63 ± 26.26
'Idared'/Vârciorova	IV	65.75 ± 2.46	132.28 ± 14.93

The fruits 'Idared' from Italy and Hungary have ideal size for serving as desert apples as a study conducted in Canadian territories reported the range between 7.4 and 7.6 cm as being ideal (Hampson et al., 2002). Usually the lines of sorting machines determine these size classes and usually smaller apples size usually give a lower price of the fruit.

As a rule, fruits with an AI indicator of 3-4, compared to the AI diagram, are suitable for long-term storage in rooms with a controlled atmosphere, apples with an AI indicator of 4-6 are best suited for long-term storage short in rooms with a controlled atmosphere, and fruits with an indicator of 6, 7, 8, 9, should be placed in regular cold stores or sold immediately. In order to observe the starch/glucose content after performing the AI test, it was found that all samples show indicator 9, which reveals the increased amount of glucose, so that all varieties fall into the category of overripe fruits (Figure 2).

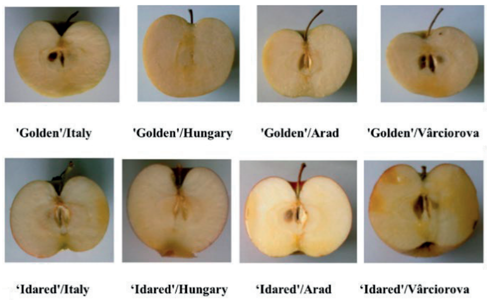

Figure 2. The results of the AI (starch-iodine) test in the studied apples

By ripening the fruit, the pulp is softer, and the best indicator is firmness. The cell walls of the apple pulp are bound to each other by pectin, which is activated by calcium, which forms a very important component of the cell walls of the fruit pulp (Asgharzade et al. 2012). Calcium levels in fruits contribute to both the integrity and durability of the fruit tissue, including its firmness (Saei et al., 2011). In addition to the variety/genetic characteristics, the level of calcium in the fruit is also one of the most important factors that determine the firmness of the fruit.

The determinations regarding the firmness of the fruits highlighted the variety with the lowest value, namely 'Golden' from Arad, with 4.24 lbr, as well as the variety with the highest value of 'Idared' from Italia firmness, with 8.09 lbr (Figure 3). In the study of Blazek and

Hlusickova from 2007, both 'Golden' and 'Idared' cultivars complied their firmness values respectively in average of 8.1 and 8.2 kg/cm^3.

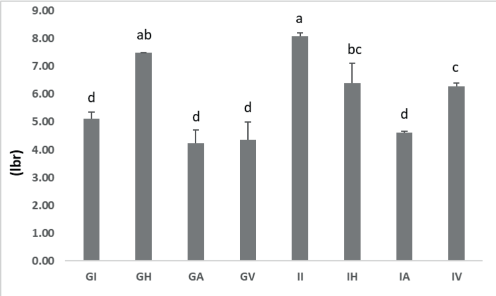

Figure 3. Experimental results on fruit firmness in studied apples; (LSD test, $p < 0.05$); GI–'Golden'/Italia; GH–'Golden'/Hungary; GA–'Golden'/Arad; GV–'Golden'/Vârciorova; II–'Idared'/Italy; IH–'Idared'/Hungary; IA–'Idared'/Arad; IV–'Idared'/Vârciorova

Foreign varieties, with a greater firmness, can be kept for a longer period of time, compared to domestic varieties. The LSD test indicate a superior significance of 'Idared' Italy variety firmness compared to all other varietes except 'Golden' Hungary variety. 'Idared' Hungary has good firmness with significant values compared to GI, GA, GV and IA. For 'Golden' variety, the highest firmness is achieved in the plants orginated from Hungary, with signifficant differences to the other 3 ones. 'Idared' originated from Italy have a signifficant higher firmness than all the other 3 varities, but there is no signifficant difference between the cultivars from Hungary and Vârciorova. Compared to the standard values regarding the degree of maturity for apple varieties, it is observed that all local varieties, both 'Golden' and 'Idared', Arad and Vârciorova fall into the Excellent category, according to the Brix index. Almost all varieties from Italy and Hungary fell into the Good category, with a percentage of over 12% (Table 3) except 'Golden' weak variety from Italy with 11.63%.

Regarding the content of soluble carbohydrates, the 'Golden' Vârciorova variety had the best percentage of glucose for about 14.20%, compared to 'Golden' Hungary with 12.35% (Figure 4). Another comprehensive study found low soluble solids to 'Idared' 11.9% BRIX (Blazek and Hlusickova, 2007) and also aur resuls are according to remarcable contents of solible solids found to 'Golden' cultivar respectively 13.8 % BRIX indicating a high fruit quality (Blazek and Hlusickova, 2007).

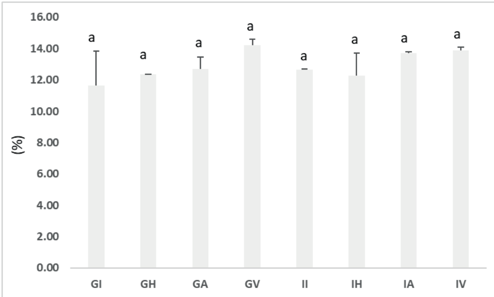

Figure 4. Experimental results on the content of soluble glucose in the studied apple varieties (% BRIX); (LSD test, $p < 0.05$); GI-'Golden'/Italia; GH-'Golden'/Hungary; GA-'Golden'/Arad; GV-'Golden'/Vârciorova; II-'Idared'/Italy; IH-'Idared'/Hungary; IA-'Idared'/Arad; IV-'Idared'/Vârciorova

These parameters were tasted with ANOVA to explore their significance level (Table 4).

Table 4. Parameters significance level analized with ANOVA

Parameter	Variety	*p* value
Weight	6.22	$p < 0.001$
Diameter	3.67	$p < 0.001$
Soluble glucose	0.84	ns.
Acidity	12.47	$p < 0.001$
Firmness	14.26	$p < 0.001$
Dry matter	3.04	$p < 0.05$
Residuals	NA	NA

Soluble glucose parameter did not differ significantly between varieties; a low level of significance was observed to dry matter parameter.

From the point of view of acidity, the 'Golden' Arad and 'Golden' Vârciorova varieties have the highest acidity with a value of 4.33 and 4.37, respectively, while at the opposite pole belongs the 'Idared' Italia variety, with a pH of 3.68 (Figure 5).

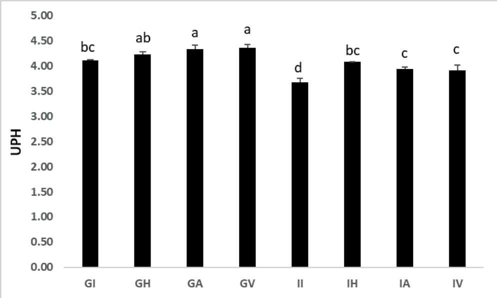

Figure 5. Experimental results on juice acidity (pH) in the studied apple varieties); (LSD test, $p < 0.05$); GI-'Golden'/Italia; GH-'Golden'/Hungary; GA-'Golden'/Arad; GV-'Golden'/Vârciorova; II-'Idared'/Italy; IH-'Idared'/Hungary; IA-'Idared'/Arad; IV-'Idared'/Vârciorova

The lowest value regarding the amount of dry matter was registered for the 'Golden' Italy variety with 13.69%, and the highest value, 'Idared' Vârciorova with 18.63% (Figure 6).

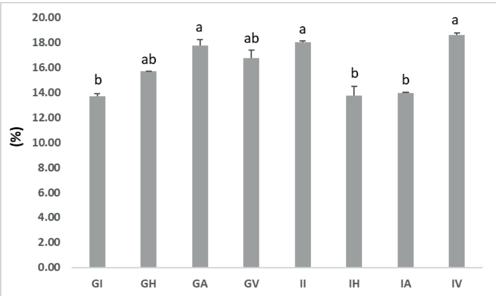

Figure 6. Experimental results on the percentage of dry matter in the studied apple varieties (%); (LSD test, $p < 0.05$); GI-'Golden'/Italia; GH-'Golden'/Hungary; GA-'Golden'/Arad; GV-'Golden'/Vârciorova; II-'Idared'/Italy; IH-'Idared'/Hungary; IA-'Idared'/Arad; IV-'Idared'/Vârciorova

Significant dry matter values were registered for GA, II and IV in comparison with GI, IH and IA. The cultivar 'Golden' usually suffer mutation and generally it has a decreased dry matter content, less acidity, also produces less russet fruits (Musacchi and Serra, 2017). We observed some contradictory results and the only explanation could be that in Arad, 'Golden' cultivar is best fitted to the environmental conditions and accumulates a significantly high dry matter. Our studies are also in the compliance with other results that present several improvements of the standard cultivars as 'Golden' and 'Idared' are (Blazek and Hlusickova, 2007; Faby, 1987; Stehr, 1996; Meyer, 2001)

CONCLUSIONS

Both cultivars fall into the Extra category, except for 'Golden' from Vârciorova which falls into category I in terms of diameter.

In terms of fruit weight, all varieties fall into the Extra category.

All varieties fall into the category of overripe fruits following the AI test.

According to the Brix index, both cultivars 'Golden' and 'Idared' degree of maturity from Arad and Vârciorova fall into the Excellent category. Cultivars from Italy and Hungary fell into the Good category, with over 12% BRIX.

Regarding the biometric and physiological indices, from the two cultivars, it can be seen that 'Idared' from Italia, 'Idared' from Hungary and 'Golden' from Hungary had high values in diameter, weight and firmness, while 'Golden' from Arad and 'Golden' and 'Idared' from Vârciorova had the highest content of soluble carbohydrates, pH and dry matter.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

NUTRIENTS AND CONTAMINANTS CONTENT IN THREE ORGANIC EDIBLE ROSES

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Abstract

This study aims to present the influence of the organic technology applied to three edible English roses on nutrients and contaminants content. Fresh petals from Falstaff (F), Brother Cadfael (B) and Crown Princess Margareta (C) cultivars and three variants of jam using different ingredients were analysed and determined the content of minerals and contaminants. The results showed significant differences between experimental variants regarding nutrients content. Cu was found at low levels, with a maximum of 2.307 mg kg^{-1}, important result for the organic protection scheme. The highest values were of K found in all variants, in the fresh petals 2143.40-2625.89 mg kg^{-1} (C), 2378.77-3103.65 mg kg^{-1} (F) and 2176.13-2903.97 mg kg^{-1} (B) and in the jam variants 538.77-616.045 mg kg^{-1} (C), 544.14-685.22 mg kg^{-1} (F) and 486.02-729.38 mg kg^{-1} (B). Important quantities of Ca and Mg were found in fresh petal variants. All the analysed samples of fresh rose petals and rose petal jam had contaminants traces bellow the standard maximum limits. Important quantities of minerals were found, expressing the positive influence of the applied organic technology.

Key words: *edible rose, minerals and contaminants content, rose petal jam.*

INTRODUCTION

Roses are one of the most present ornamental plants in human life. Together with beauty, elegance, fragrance, they have a specific role in medicine, food and cosmetics industry, requiring special standards.

An organic technology for climbing edible roses was established between the 2015-2017 period and registered at the State Office for Inventions and Trademarks (Butcaru et al., 2018a). Organic technology' influence on plant development and production (Butcaru et al., 2020), on fresh rose petal and rose petal jam biochemical compounds (Butcaru et al., 2019; Butcaru et al., 2018b; Butcaru et al., 2017) were analysed.

Monitoring and analysing the specific amount of contaminants and minerals in the final products, respectively in rose petals were important aspects of organic technology for edible roses.

Potassium, in the plant, stimulates the production of quality flowers and increases resistance to diseases, pests, drought and frost; regulates the water regime in the cell; has a role in the transport and storage of organic reserve substances (Hessayon, 2005; Madjar & Davidescu, 2009). For human health, potassium is needed for the maintenance of total body fluid volume, acid and electrolyte balance, and normal cell function. Reduced potassium consumption has been correlated with hypertension and cardiovascular diseases and appropriate consumption levels could be protective against these conditions (WHO, 2012).

Calcium, in the plant, gives tissues resistance, is involved in fruit ripening, root development, detoxification of the plant from ions and radicals that occur in metabolic processes (Hessayon, 2005; Madjar & Davidescu, 2009). For humans, calcium is an essential nutrient that plays a vital role in neuromuscular function, many enzyme-mediated processes and blood clotting, as well as providing rigidity to the skeleton (WHO & FAO, 2004).

Magnesium is involved in the process of photosynthesis, promotes the absorption and translocation of phosphorus in energy transfer

(Hessayon, 2005; Madjar & Davidescu, 2009). In the human body, 30-40% of the total level of magnesium is found in muscles and soft tissues, 1% is found in extracellular fluid, and the rest in the skeleton (WHO, 2012).
Iron enhances the green color of the leaves; contributes to the plant health. It has a role in the synthesis of auxins, implicitly in the development of roots (Hessayon, 2005; Madjar & Davidescu, 2009). It is important for electron transport in some enzymes and it is associated with enzymes in chlorophyll formation (Lohry, 2007). Regarding human health, iron serves as an oxygen carrier to the tissues, as a transport medium for electrons within cells and as an integrated part of important enzyme systems in various tissues (WHO & FAO, 2004).
Zinc has a protective role for auxins; it is involved in seed formation (Hessayon, 2005; Madjar & Davidescu, 2009). As a plant nutrient, Zn is involved in numerous plant metabolic and physiological processes. Zn can negatively affect plant metabolic reactions both at low (Lohry, 2007) as well as at higher levels (Shadid, 2017). Zinc toxicity can induce a deficiency of other minerals like Mg, Fe or Mn (Tripathi et al., 2015). Zn is an important mineral for human health, one-third population of the world suffering from Zn deficiency (Shadid, 2017) related to poor immune system, poor physical and mental growth (Sharma et al., 2013).
Copper has a specific role in plant metabolism (Hessayon, 2005; Madjar & Davidescu, 2009). In excess is bound to phytochelatins. A deficiency interferes with protein synthesis (Lohry, 2007). In the human body, copper has a role in the immune defense mechanism (WHO, 2012).
In-plant metabolism, most elements interact with each other, for example (Zn-Fe) the metabolic functioning of Fe in plants is connected in a way with the supply of Zn; (Fe-Mn) are positively correlated in their metabolic functions, (Fe-Mo) at a marginally adequate level, Mo enhanced Fe uptake, and at higher levels depressed Fe uptake; (Cu-Mo) - antagonism; (Cu-Zn) - zinc induce a copper deficiency in several crops (Ronen, 2007); higher levels of Ca limit Fe uptake.
For some of the contaminants analysed (Be, V, Co, Ni, As, Sr, Mo, Ag, Cd, Sb, Ba, Ti, Pb, Th, U, Al) the international regulation established the maximum limit (Regulation 1881/2006).
Arsenic has been ranked number one pollutant from the top twenty toxic substances. In the plant, interferes with numerous plant metabolic processes, leading to decreased biomass, affects growth and chlorophyll synthesis, DNA breakage, membrane leakage, enzyme inactivation, affect on RUBISCO. Human exposure to high levels of As via contaminated foods is linked to respiratory diseases, diabetes, reproductive disorders, liver and cardiovascular problems, gastrointestinal, high blood pressure and cancer of skin, liver, bladder, kidney and lungs (Shadid, 2017).
Lead at higher levels can induce several toxic effects in plants. Decreased seed germination, inhibition of chlorophyll synthesis and plant biomass, affect the physiological parameters are some of the effects. For human health, lead (the second toxic substance) can lead to mental impairment in children under 15, affect bones, heart, kidneys, intestine, nervous and reproductive systems (Shadid, 2017).
Cobalt, at low levels, promotes plant growth and possibly can, indirectly, bring biotic stress resistance. Higher levels are toxic, damaging various physiological and biochemical activities. The excess of cobalt induces Fe deficiency. For humans, exposure to cobalt can lead to lung disease, being considered carcinogenic (Shadid, 2017).
In plants, nickel is part of numerous enzymes including ureases, and low levels induce positive effects. But, high levels of nickel lead to toxic effects. It is also recognized as a carcinogen for humans (Shadid, 2017).
This study aims to present the influence of the organic technology applied to three edible climbing English roses cultivated in a specially designed orchard on nutrients and contaminants content.

MATERIALS AND METHODS

The research has been carried out in the Experimental Field of Faculty of Horticulture and in the Research Centre for Studies of Food and Agricultural Products Quality within USAMV Bucharest.
In 2015, an organic rose culture of a total area of 1,350 m^2 with three climbing edible

cultivars: 'Falstaff' (F), 'Brother Cadfael' (B) and 'Crown Princess Margareta' (C) was established and an organic technology was applied. On the row, the soil was mulched with wool and wood chips and for irrigation, a drip system was used. The inter-row was kept grassy through repeated mowing. Different bio-stimulators, fertilizers and plant protection specific strategies were used (Butcaru et al., 2018a).

Three variants of jam were made from each cultivar, using different ingredients like lemon, sea buckthorn and ginger and analyzed. To determine the influence of the applied organic technology on the content of minerals and contaminants in fresh rose petals and petal rose jam (Table 1), specific analyses were made.

The micro and macro elements content from the rose petals and rose petal jam has been determined according to AOAC Official Method 2015.01. The results were reported in mg/100 g and mg kg^{-1} according to European legislation - Regulation (EU) No. 1169/2011.

Sample preparation for ICP-MS analysis 0.250g ± 0.0001 g of samples were weighted with an analytical balance in Teflon recipients, then 8 ml concentrated ultrapure HNO_3 (65%) and 2 ml H_2O_2 30% were added and were subjected to mineralization by microwave digestion (30 min.) with ETHOS UP microwave digestion system, then the clear solutions were transferred quantitatively into the volumetric flasks (50 ml) and made up with Milli-Q ultrapure water (2 repetitions) (Milestone ETHOS UP Digestion Apps, Zovinka & Stock, 2010)

The Agilent Series ICP-MS spectrometer with quadrupole analyzer 7700x and MassHunter Workstation software (Agilent Technologies) was used in the analysis.

The calibration curve was performed with ICP-MS multi-element calibration standard (1000 mg/L of Fe, K, Ca, Na, Mg; 100 mg/L of Sr; 10 mg/L of Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U in 5% HNO_3) (Sannac et al., 2017; Pacquette et al., 2015; Liba et al., 2014).

Table 1. Sample codification

Code	Explanation	Code	Explanation	Code	Explanation
15 C	Crown Princess Margareta, control	13F	Falstaff, control	14B	Brother Cadfael, control
1C	Crown Princess Margareta, wood chips mulch	2F	Falstaff, wood chips mulch	3B	Brother Cadfael, wood chips mulch
26 C	Crown Princess Margareta, wool mulch	27F	Falstaff, wool mulch	25B	Brother Cadfael, wool mulch
D1-C-l	Crown Princess Margareta rose petal jam with lemon	D2-F-l	Falstaff rose petal jam with lemon	D3-B-l	Brother Cadfael rose petal jam with lemon
D4-C-g	Crown Princess Margareta rose petal jam with ginger	D5-F-g	Falstaff rose petal jam with ginger	D6-B-g	Brother Cadfael rose petal jam with ginger
D7-C-s	Crown Princess Margareta rose petal jam with sea-buckthorn	D8-F-s	Falstaff rose petal jam with sea-buckthorn	D9-B-s	Brother Cadfael rose petal jam with sea-buckthorn

RESULTS AND DISCUSSIONS

The obtained results reflected the influence of the organic technology applied, especially of the fertilization and protection scheme (Singh et al., 2017). The contaminants were generally below the legal limits (Table 2).

Vanadium ranged between 0.004-0.039 mg kg^{-1} (C), 0.015-0.026 mg kg^{-1} (F), 0.003-0.011 mg kg^{-1} (B) in the fresh petals. The processed ones were below 0.005 mg kg^{-1} except for Falstaff with higher values.

Cobalt ranged between 0.002 - 0.018 mg kg^{-1} (C), 0.004-0.021 mg kg^{-1} (F) and 0.00-0.018 mg kg^{-1} (B) in fresh petals with traces in the processed ones. The wool mulched variants presented zero or low values. Dos Santos et al. (2017) obtained values between 0.001-0.01 mg kg^{-1} for the same species.

At Nickel, all the rose petal jams had 0 values. For the fresh petals, it ranges between 0.420-0.687 mg kg^{-1} (C), 0.149-0.491 mg kg^{-1} (F) and 0.021-0.809 mg kg^{-1} (B). The control has higher values at C and B. Dos Santos et al. (2017) obtained values between 0.04-0.2 mg kg^{-1}.

Arsenic was generally at 0 or very low values (bellow 0.05). For the C were registered 0.026 mg kg^{-1} on the control row and 0.010 mg kg^{-1} on the wool mulched row. Dos Santos et al. (2017) obtained also bellow 0.02 mg kg^{-1} values.

Strontium ranged between 0.28-0.81 mg kg^{-1} (C), 0.22-0.58 mg kg^{-1} (F) and 0.12-0.58 mg kg^{-1} (B) in fresh petals with lower values in the processed ones.

Cadmium in fresh petals was between 0.001-0.004 mg kg^{-1} (C), 0.002-0.006 mg kg^{-1} (F) and 0.001-0.002 mg kg^{-1} (B), with maximum 0.001 mg kg^{-1} in jam, all below 0.2. Dos Santos et al. (2017) registered also values bellow 0.001 mg kg^{-1}.

Barium was between 0.146-0.747 mg kg^{-1} (C), 0.393-0.941 mg kg^{-1} (F) and 0.128-0.499 mg kg^{-1} (B) in fresh petals. Some of the processed variants had higher values than in the fresh petals.

Lead traces were quantified between 0.0-0.404 mg kg^{-1} (C), 0.006-0.079 mg kg^{-1} (F) and 0.0-0.070 mg kg^{-1} (B). Some of the jam variants had higher values (lemon and ginger at F and B). Generally, all of them were below 0.30 mg kg^{-1}.

Aluminium was found only in the control and wood dust fresh petal samples, 5.05-15.55 mg kg^{-1} (C), 14.85-24.28 mg kg^{-1} (F), 0.44-13.17 mg kg^{-1} (B) significant higher on the mulched row. Rose petal jam with sea-buckthorn at Falstaff had a higher value.

Interesting results were also found in mineral content (Table 3).

Chromium was significant higher in the fresh petals, with lower content on the wool mulched variants, 0.038-0.103 mg kg^{-1} (C), 0.0-0.145 mg kg^{-1} (F) and 0.018-0.250 mg kg^{-1} (B). Dos Santos et al. (2017) registered in the rose petal analyzed below 0.02 mg kg^{-1} quantities.

The higher content of iron was found in fresh petals on the control row (at Falstaff similar to wood chips mulch), varying between 9.968-53.846 mg kg^{-1} (C), 10.8-25.003 mg kg^{-1} (F) and 7.147-47.979 mg kg^{-1} (B). The jam variants had lower values. Rop et al. (2012) obtained in fresh petals of *Rosa odorata* 3.55 mg kg^{-1}. Dos Santos et al. (2017) presented values between 5.0-7.24 mg kg^{-1}. In a rose jam, Albuquerque et al. (2013) obtained values bellow 0.02 mg kg^{-1}.

Copper had similar values in the three rose varieties with a maximum of 2.307 mg kg^{-1} at Falstaff (control). The jam variants had significantly lower values. Rap et al. (2012) obtained 2.28 mg kg^{-1} and Dos Santos et al. (2017), 1.08-1.67 mg kg^{-1}. In a rose jam, values bellow 0.06 were obtained by Albuquerque et al. (2013).

Table 2. Influence of the applied organic technology on the contaminants content (mg/kg) in fresh petals and rose petal jam

Sample	V	Co	Ni	As	Sr	Cd	Ba	Pb	Al
15C	0.023 ±0.002	0.017 ±0.000	0.687 ±0.006	0.026 ±0.003	0.807 ±0.012	0.004 ±0.002	0.747 ±0.002	0.404 ±0.009	5.046 ±0.229
1C	0.004 ±0.001	0.018 ±0.000	0.492 ±0.015	0.000 ±0.000	0.761 ±0.000	0.001 ±0.002	0.146 ±0.003	0.000 ±0.000	15.551 ±0.093
26C	0.039 ±0.004	0.002 ±0.001	0.420 ±0.024	0.010 ±0.005	0.279 ±0.003	0.001 ±0.002	0.283 ±0.000	0.036 ±0.008	0.000 ±0.000
D1-C-l	0.001 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.109 ±0.005	0.001 ±0.000	0.326 ±0.013	0.008 ±0.002	0.000 ±0.000
D4-C-g	0.003 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.081 ±0.002	0.000 ±0.000	0.122 ±0.005	0.000 ±0.000	0.000 ±0.000
D7-C-s	0.005 ±0.001	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.084 ±0.000	0.001 ±0.000	0.063 ±0.005	0.000 ±0.000	0.464 ±0.656
13F	0.019 ±0.002	0.004 ±0.002	0.367 ±0.024	0.002 ±0.003	0.218 ±0.008	0.002 ±0.000	0.605 ±0.014	0.079 ±0.003	14.850 ±0.669
2F	0.015 ±0.001	0.021 ±0.000	0.491 ±0.022	0.000 ±0.000	0.577 ±0.002	0.006 ±0.002	0.393 ±0.008	0.007 ±0.003	24.284 ±0.337
27F	0.026 ±0.001	0.005 ±0.001	0.149 ±0.001	0.006 ±0.005	0.525 ±0.005	0.004 ±0.001	0.941 ±0.030	0.006 ±0.001	0.000 ±0.000
D2-F-l	0.005 ±0.001	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.206 ±0.000	0.000 ±0.000	0.396 ±0.010	0.212 ±0.002	0.000 ±0.000
D5-F-g	0.011 ±0.001	0.002 ±0.000	0.000 ±0.000	0.004 ±0.000	0.317 ±0.003	0.001 ±0.001	0.525 ±0.016	0.068 ±0.001	0.000 ±0.000
D8-F-s	0.009 ±0.000	0.004 ±0.000	0.000 ±0.000	0.001 ±0.001	0.114 ±0.002	0.000 ±0.000	0.051 ±0.002	0.000 ±0.000	34.530 ±0.889
14B	0.009 ±0.001	0.007 ±0.001	0.809 ±0.035	0.003 ±0.004	0.581 ±0.009	0.001 ±0.001	0.499 ±0.001	0.070 ±0.002	0.441 ±0.624
3B	0.011 ±0.001	0.018 ±0.000	0.021 ±0.011	0.006 ±0.004	0.252 ±0.009	0.001 ±0.000	0.128 ±0.010	0.022 ±0.005	13.167 ±2.022
25B	0.003 ±0.001	0.000 ±0.000	0.278 ±0.028	0.000 ±0.000	0.117 ±0.000	0.002 ±0.002	0.184 ±0.002	0.000 ±0.000	0.000 ±0.000
D3-B-l	0.002 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.025 ±0.000	0.001 ±0.000	0.226 ±0.011	0.091 ±0.001	0.000 ±0.000
D6-B-g	0.003 ±0.000	0.006 ±0.001	0.000 ±0.000	0.000 ±0.000	0.134 ±0.003	0.001 ±0.001	0.782 ±0.077	0.084 ±0.002	0.000 ±0.000
D9-B-s	0.004 ±0.001	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.001 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000

Table 3. Influence of the applied organic technology on the minerals content (mg/kg) in fresh petals and rose petal jam

Sample	Cr	Fe	Cu	Zn	Se	Na	Mg	K	Ca
15C	0.08 ±0.01	53.85 ±2.57	1.91 ±0.00	9.19 ±0.04	0.04 ±0.05	45.62 ±1.12	294.00 ±0.09	2143.40 ±1.08	430.21 ±4.13
1C	0.10 ±0.01	16.97 ±3.47	1.38 ±0.00	39.52 ±0.10	0.16 ±0.23	12.56 ±0.11	330.66 ±0.55	2369.02 ±9.56	1252.31 ±4.34
26C	0.04 ±0.01	9.97 ±1.25	1.50 ±0.02	2.50 ±0.03	0.00 ±0.00	0.00 ±0.00	324.27 ±1.94	2625.89 ±12.26	119.04 ±3.30
D1-C-l	0.09 ±0.01	8.23 ±2.08	0.16 ±0.00	0.59 ±0.06	0.00 ±0.00	13.68 ±3.81	51.49 ±1.23	538.77 ±12.96	226.50 ±8.44
D4-C-g	0.00 ±0.00	2.54 ±0.46	0.14 ±0.01	0.00 ±0.00	0.00 ±0.00	24.62 ±1.27	63.43 ±0.69	616.05 ±5.61	0.00 ±0.00
D7-C-s	0.01 ±0.01	0.90 ±1.21	0.16 ±0.00	0.27 ±0.07	0.00 ±0.00	0.32 ±0.45	49.72 ±0.74	560.25 ±3.24	68.56 ±3.03
13F	0.14 ±0.01	24.71 ±1.06	1.44 ±0.00	17.26 ±0.18	0.04 ±0.03	21.89 ±1.48	288.49 ±2.51	2378.77 ±23.36	326.22 ±7.14
2F	0.12 ±0.00	25.00 ±0.72	2.31 ±0.01	26.73 ±0.08	0.27 ±0.18	23.14 ±0.72	382.03 ±1.81	3103.65 ±17.96	676.88 ±3.30
27F	0.00 ±0.00	10.80 ±1.15	1.24 ±0.01	16.00 ±0.12	0.00 ±0.00	6.20 ±2.63	312.45 ±5.17	2608.78 ±35.45	316.60 ±8.68
D2-F-l	0.00 ±0.00	5.01 ±1.44	0.27 ±0.01	1.22 ±0.05	0.00 ±0.00	7.03 ±1.29	56.65 ±2.38	544.14 ±5.25	62.75 ±6.38
D5-F-g	0.00 ±0.00	9.12 ±1.11	0.23 ±0.00	0.00 ±0.00	0.00 ±0.00	45.64 ±2.44	75.38 ±0.71	685.22 ±3.41	148.38 ±2.97
D8-F-s	0.11 ±0.00	4.73 ±1.03	0.18 ±0.01	0.08 ±0.00	0.00 ±0.00	0.00 ±0.00	57.87 ±1.13	599.98 ±5.86	43.82 ±3.61
14B	0.25 ±0.01	47.98 ±1.83	1.81 ±0.01	12.00 ±0.12	0.03 ±0.04	79.48 ±2.30	247.94 ±2.77	2176.13 ±18.48	299.92 ±7.06
3B	0.10 ±0.02	17.95 ±2.53	1.62 ±0.04	16.14 ±0.31	0.28 ±0.37	15.76 ±4.16	305.01 ±7.35	2863.82 ±58.92	370.88 ±10.46
25B	0.02 ±0.00	7.15 ±3.27	1.33 ±0.00	5.25 ±0.00	0.00 ±0.00	0.55 ±0.53	300.35 ±0.44	2903.97 ±7.68	132.72 ±0.91
D3-B-l	0.05 ±0.01	4.31 ±0.11	0.13 ±0.00	0.00 ±0.00	0.00 ±0.00	110.03 ±0.20	41.97 ±0.06	486.02 ±0.79	0.00 ±0.00
D6-B-g	0.00 ±0.00	4.89 ±0.76	0.22 ±0.00	0.44 ±0.03	0.00 ±0.00	16.67 ±1.50	67.78 ±0.77	729.38 ±8.75	42.77 ±1.76
D9-B-s	0.00 ±0.00	0.00 ±0.00	0.12 ±0.00	0.05 ±0.03	0.00 ±0.00	0.00 ±0.00	45.70 ±0.38	581.53 ±5.05	0.00 ±0.00

Regarding the Zinc content, the highest values were registered in the control fresh petals and the lowest on the wool mulched variants, varying between 2.499-39.525 mg kg^{-1} (C), 16.004-26.725 mg kg^{-1} (F) and 5.249-16.141 mg kg^{-1} (B). The jam variants had much lower values. Rap et al. (2012) obtained 4.55 mg kg^{-1} and Dos Santos et al. (2017), 2.34-3.03 mg kg^{-1}. In a rose jam, values bellow 0.2 mg were mentioned by Albuquerque et al. (2013).

Selenium was found only in fresh petal, in the control (higher) and the wood chips mulched variants, 0.038-0.164 mg kg^{-1} (C), 0.036-0.266 mg kg^{-1} (F) and 0.027-0.275 mg kg^{-1} (B). In a rose jam, values between 5.0- 16 μg were reported by Albuquerque et al. (2013).

Significant quantities of Na were found in the rose petal jams, especially in D5-F-g and D3-B-l. In the fresh petals, all the wool mulched variants had significantly lower values. Rap et al. (2012) obtained 76.61 mg kg^{-1}. In a rose jam, values bellow 7.0 mg were obtained by Albuquerque et al. (2013).

Significant quantities of Magnesium were found in the fresh petals, similar for control and wool mulched rows, 294.0-330.656 mg kg^{-1} (C), 288.493-382.029 mg kg^{-1} (F) and 247.94-305.013 mg kg^{-1} (B). The jam variants had lower values, between 41.968-75.375 mg kg^{-1}. Rap et al. (2012) obtained 141.83 mg kg^{-1} and Dos Santos et al. (2017), 277-326 mg kg^{-1}. In a rose jam, values between 3.0-10 mg were obtained by Albuquerque et al. (2013).

Significant quantities of potassium were found in all variants, in the fresh petals 2143.40-2625.89 mg kg^{-1} (C), 2378.77-3103.65 mg kg^{-1} (F) and 2176.13-2903.97 mg kg^{-1} (B) and in the jam variants 538.77-616.045 mg kg^{-1} (C), 544.14-685.22 mg kg^{-1} (F) and 486.02-729.38 mg kg^{-1} (B). The wood chips variants, for the fresh petals and the lemon variants, for rose petal jam variants had lower values. Rap et al. (2012) obtained 1969.11 mg kg^{-1}. In a rose jam, values between 8.0-25 mg were obtained by Albuquerque et al. (2013).

Important quantities of calcium were found in fresh petal variants, 119.04-1252.31 mg kg^{-1} (C), 316.60-676.88 mg kg^{-1} (F) and 132.72-370.88 mg kg^{-1} (B). The highest values were in the control followed by wood chips variants. Rap et al. (2012) obtained 275.15 mg kg^{-1} and Dos Santos et al. (2017), 132- 301 mg kg^{-1}. Some of the jam variants presented 0 values. Albuquerque et al. (2013) reported values bellow 7.0 mg in rose petal jam.

	Co	Ni	As	Sr	Cd	Ba	Pb	Al	Cr	Fe	Cu	Zn	Se	Na	Mg	K	Ca
V	0.22	0.45	0.62	0.43	0.47	0.43	0.22	0.07	0.10	0.37	0.57	0.19	0.06	(-0.14)	0.61	0.56	0.12
Co		0.54	0.37	0.75	0.59	0.16	0.20	0.54	0.45	0.60	0.73	0.79	0.86	0.04	0.67	0.62	0.79
Ni			0.49	0.79	0.55	0.31	0.34	0.14	0.68	0.87	0.84	0.57	0.27	0.26	0.74	0.68	0.57
As				0.56	0.41	0.42	0.75	(-0.07)	0.10	0.66	0.47	0.04	0.02	0.13	0.39	0.33	0.13
Sr					0.65	0.47	0.40	0.22	0.45	0.78	0.75	0.73	0.43	0.16	0.70	0.60	0.79
Cd						0.52	0.26	0.28	0.18	0.53	0.71	0.50	0.41	0.02	0.66	0.62	0.43
Ba							0.48	(-0.22)	0.04	0.45	0.30	0.13	(-0.14)	0.19	0.28	0.24	0.09
Pb								(-0.16)	0.05	0.58	0.19	(-0.10)	(-0.13)	0.33	0.04	(-0.03)	(-0.01)
Al									0.47	0.15	0.29	0.46	0.54	(-0.19)	0.28	0.25	0.41
Cr										0.72	0.57	0.47	0.38	0.37	0.42	0.40	0.43
Fe											0.76	0.44	0.30	0.41	0.58	0.53	0.45
Cu												0.71	0.61	0.06	0.95	0.94	0.62
Zn													0.73	(-0.04)	0.77	0.71	0.95
Se														(-0.04)	0.59	0.59	0.67
Na															(-0.09)	(-0.11)	(-0.03)
Mg																0.99	0.67
K																	0.58

Figure 1. Pearson Correlation matrix

Strong correlations were observed between elements Co-Se, Ni-Fe, Ni-Cu, Cu-Mg, Cu-K, Zn-Ca, Mg-K. Some of the correlations are similar to De Saedeleer et al. (2010) and Dobrin et al. (2018).

CONCLUSIONS

All the analyzed samples of fresh rose petals and rose petal jam had contaminants traces bellow the standard maximum limits. Important quantities of minerals were found, expressing the positive influence of the applied organic technology. Very strong correlations observed between minerals should be confirmed through the following researches.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDIES REGARDING THE STORAGE CAPACITY AND THE DYNAMICS OF THE MAIN PHYSICAL-CHEMICAL CHARACTERISTICS OF SOME CHERRY FRUITS VARIETIES, UNDER DIFFERENT STORAGE PARAMETERS

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Abstract

The studies carried out and shown in the present paper were performed for four cherry varieties: 'Hedelfinger', 'Stella', 'Boambe de Cotnari' and 'Durone di Vignola'. The storage capacity, the weight losses and the quality depreciations were measured as well. The fruits were stored in the following environment condition variants: V1 = modified atmosphere, at 2-3°C and 85-90% RH; V2 = modified atmosphere at 24 - 25°C and 80-85% RH; V3= cold storage, at 2-3°C and 75-80% RH; V4 = room temperature conditions at 24 – 25°C and 70-75% RH. The results obtained have proven the advantage of storing fruits in modified atmosphere, the storage durations ranging from 12 days for the 'Boambe de Cotnari' variety to 18 days for the 'Durone di Vignola' variety. The 'Durone di Vignola' variety has proven to have been the best preserved at the end of the storage period, the total losses recorded being of 2.3% in room temperature conditions after 8 days and only 9.2% after 18 days of storage in modified atmosphere.

Key words: *cold storage, modified atmosphere, storage capacity, total losses.*

INTRODUCTION

Cherries are much appreciated fruits, firstly for their earliness (some varieties - in the May month) when most other fresh fruits are not available and also for their nutritional and technological value given by the many possibilities of processing (Chira et al., 2002).
During trading and distribution up to the moment the produce arrives at the final consumer, the quality may change quite rapidly, depending on the circumstances during storage and transport, thereby affecting both the possibilities of selling the produce and the price that can be obtained (Asănică et al., 2003; Chira et al., 2008).
Cherry fruits belong to the category of the perishable horticultural products and have a reduced storage capacity of only a couple of days. For this reason, there are studies carried out through which the extension of the storage period is attempted and at the same time the decrease of the quantitative and qualitative losses this type of fruit undergoes (Chira et al., 2014). This is the area of interest of the research carried out and put forward in the present paper.
A deep concern is represented by the storage of the cherry fruits in modified atmosphere conditions, which is obtained with low costs and can be kept constant throughout the entire storage period (Aglar et al., 2017, Çelikel F.G et al., 2003, Singh Sandhya, 2010). The research results carried out worldwide have given proof the superiority of this storage method in comparison with keeping fruits in normal cold storage conditions (Goliáš et al., 2007, Mangaraj et al., 2009, Sypula et al., 2018).
The research conducted within the Faculty of Horticulture in Bucharest has aimed to highlight the main aspects (the development of the main phisical-chemical characteristics of fruits during storage, weight losses, quality losses, etc.) regarding the response behaviour of certain cherry varieties in modified atmosphere conditions in comparison to storage in cold or ambiental conditions.

MATERIALS AND METHODS

The research conducted for the present paper has taken under study four cherry varieties: Hedelfinger, Stella, Boambe de Cotnari and

Durone di Vignola, grown in 10-year-old orchard situated in the Bucharest area.

The cherry fruit varieties' characteristics are as follows:

1. The 'Hedelfinger' variety of German origin is very productive, displays winter-hardiness, is vigorous and has a long lifespan. It blossoms late and has a good production even in unfavourable years with late frosts. The fruits are very big, weighing between 8.5 and 9.5 grams, have a ruby-red hue and turn dark red when overripe. The pulp is firm (stony), succulent, sweet, with medium acidity. The fruits do not crack, not even in very rainy years.
2. The 'Stella' variety of Canadian origin is very productive and bears fruit early. The fruits are medium-large, weighing between 7 to 8 grams, with a pink-reddish hue. The pulp is firm (stony), of a red colour, with a pleasant bitter- sweet taste.
3. The 'Boambe de Cotnari' variety of Romanian origin is very resistant to frost and to the main diseases. It is productive and bears fruit annually. The fruits are medium-large, weighing between 7 to 8 grams, they are bicolor (yellow and red) and resistant to transportation. The pulp is yellowish-white, firm (stony) sweet and with low acidity.
4. The 'Durone di Vignola' variety of Italian origin has large to very large fruits, weighing around 9.5-10.5 grams. Their colour is dark red. The pulp is firm (stony), with a specific and very pleasant taste and is also dark red.

It is a self-pollinated variety so it requires the presence of other varieties in the vicinity for pollination. It has low resistance to the attack of the *Monilinia laxa.*

The optimal time of fruit harvesting was determined by phenological and physic-chemical criteria.

The fruits were harvested in 2020, at the full ripeness stage, making sure that cherry fruits would not continue the ripening process after harvesting.

Both after harvesting and at the end storage period, fruits were physical-chemical analysed, taking in consideration weight losses and depreciation.

The fruits were stored in the following conditions:

V1 = modified atmosphere, at 2-3^{0}C and 85-90% RH;

V2 = modified atmosphere, at 24-25^{0}C and 80-85% RH;

V3= cold storage conditions, at 2-3^{0}C and 75-80% RH;

V4 = ambient conditions, at 24-25^{0}C and 70-75% RH.

The cherry fruits were stored in specific conditions immediately after harvest and cooling down, but only the first and extra quality fruits, in accordance with Regulation (EC) 543/2011.

The experimental variants for each variety consisted of 3 repetitions. Each repetitions consisted of 2 kg of packaged fruits.

Based on the results of worldwide research and due to the limited practical possibilities of obtaining modified atmosphere different from the ambient one, the research was carried out using a gaseous composition of 3% O_2 and 5% CO_2.

This type of modified atmosphere was achieved by covering the wrappers containing fruit with a low-density semipermeable polyethylene film (LDPE), with a thickness of 15µ (microns), figures 1, 2, 3, and 4. The modified atmosphere was obtained after seven days, by the natural fruits respiration.

During the respiration process the O2 content decrease and the CO2 content increase and the constant level of the gas concentration is assured by using the low-density semipermeable polyethylene film.

The measurements and analysis made after harvesting and at the end of storage period tracked the following:

- the evolution of the main physical-chemical characteristics of the fruits (total soluble solids, total titratable acidity and the content of ascorbic acid). The content of the total soluble solids was measured using the Atago electric refractometer. The total titratable acidity was measured by titration with a NaOH 0,1N solution. The results, expressed in percentage of malic acid was calculated with the specific formula for the Total titratable acidity. The content or ascorbic acid was measured using the iodometric method.
- establishing the weight losses and quality depreciation, by quantity and percentage;
- determining the temperature and relative air humidity of the cherries storage environment, using the Hanhart thermo hygrometer;

- determining the concentration of the main component gases (O_2 and CO_2) in the wrappers with modified atmosphere, using the Oxybaby gas analyser.

Figure 1. ‘Hedelfinger’ variety, in cold storage under modified atmosphere (own source)

Figure 2. ‘Stella’ variety, in ambient storage conditions (own source)

Figure 3. ‘Boambe de Cotnari’ variety, in cold storage conditions (own source)

Figure 4. ‘Durone di Vignola’ variety, in cold storage under modified atmosphere (own source)

RESULTS AND DISCUSSIONS

Physical-chemical results measured immediately after harvesting (Table 1) have highlighted differences between varieties for all analysed characteristics.

Table1. The main physical-chemical characteristics of the cherry fruits at harvesting

Variety	Weight average -g-	Total Soluble Solid -%-	Total titratable acidity -%-	Ascorbic acid mg/100g
Hedelfinger	9.5	13.8	0.87	8.5
Stella	8.5	14.2	0.82	9.2
Boambe de Cotnari	8.5	13.0	0.85	8.7
Durone di Vignola	10.5	14.0	0.72	9.0

Source: own determination

Thus, the total soluble solids shown values between 13% for 'Boambe de Cotnari' variety and 14.2% for 'Stella' variety. The total titratable acidity values oscillated between 0.72% for the 'Durone di Vignola' variety and 0.87% for 'Hedelfinger', while the ascorbic acid content oscillated between 8.5 mg/100 g for 'Hedelfinger' and 9.2 mg/100g for 'Stella'.

After determining the main physical-chemical characteristics of the cherry fruits, these were stored in different conditions, as shown in the material and method section. Fruits were stored for many days, just before they lose their commercial value suitable for market sold they were again analysed.

Regarding the duration of the storage (Table 2) it can be seen that it was longer for cold storage conditions under modified atmosphere (V1). The storage period was developed between 12 days for the 'Boambe de Cotnari' variety and 18 days for 'Durone di Vognola'.

Cherries kept in ambient conditions (V4), were stored for the shortest period, ranging from 5 days for the 'Boambe de Cotnari' variety and 8 days for 'Durone di Vignola'.

For the fruits stored in modified atmosphere (V2) the storage period ranged from 6 days for the 'Boambe de Cotnari' variety to 9 days for 'Durone di Vignola'. Storage in cold storage V3) increased the commercial period of valorisation by one day, compared withV2, therefore it ranged from 7 days for the 'Boambe de Cotnari' variety to 10 days for 'Durone di Vignola'.

The weight losses determined at the end of storage period varied widely depending on the variety, and particularly on the storage conditions. The main reduced losses were recorded for V2 variant, with values ranging between 4.2% for 'Durone di Vignola' and 5.5% for 'Boambe de Cotnari', after 9 and 6 storage days, respectively. The most important weight losses were recorded at the end of storage period in ambient conditions (V4), the values oscillating between 9.8% for 'Durone di Vignola' and 11.4% for 'Boambe de Cotnari'.

Regarding the weight losses, the significant data is the daily average losses weight. From this perspective it can be seen that for all the varieties the lowest values were recorded for V1, as for example 0.38% for 'Durone di Vignola' and 0.72% for 'Boambe de Cotnari', while for V4 the highest values were recorded, as for example 1.22% for 'Durone di Vignola' and 2.28% for 'Boambe de Cotnari', respectively.

For the V2 and V3 storage conditions, intermediary values were recorded for all of the analysed varieties, after 7 days of storage.

During storage some of the fruits rotted mainly as a consequence of the *Monilinia laxa* attack (Figure 5).

Figure 5. 'Hedelfinger' variety at the end of storage period in ambient conditions with symptom of *Monilinia laxa* - brown mould (own source)

The obtained results have proven once again the advantage of storage in cold conditions and in modified atmosphere (V1). For this storage variant, the daily average ranged between 0.13% for 'Durone di Vignola' and 0.4% for 'Boambe de Cotnari'. The highest number of rotten fruit was recorded in the case of ambient room storage conditions (V4), the daily average ranging between 1.68% for 'Durone di Vignola' and 3.8% for 'Boambe de Cotnari'.

A relatively good behaviour from fruits rotting point could be observed at V3, were daily average looks like: 0.44% for 'Durone di Vignola', 0.58% for 'Stella', 0.72% for 'Hedelfinger' and 0.91% for 'Boambe de Cotnari'.

The total losses recorded were calculated from the sum of weight losses and quality depreciations due to rot. Thus, the lowest values were recorded for the V1, as for example 0.51% for 'Durone di Vignola' and 1.12% for 'Boambe de Cotnari', while the highest values could be seen for V4, as for example 2.9% for 'Durone di Vignola' and 6.8% for 'Boambe de Cotnari'.

At the end of storage period the main physical-chemical characteristics of the cherry fruits were determined.

From the results obtained (Table 3) is can be seen that the total soluble solids values were higher compared to those obtained immediately after harvest for all varieties, irrespective of the storage conditions, due to the water losses transpiration rate.

Table 2. The cherry fruits storage capacity, under different storage conditions (mean ± SD; n = 3)

VARIETY	Storage conditions (variant)	Storage duration -days-	Weight losses - % -		Rotted fruits - % -		Total losses - % -	
			Total	Daily average	Total	Daily average	Total	Daily average
HEDELFINGER	V1	14	8.10 ± 0.03	0.58	3.70 ± 0.49	0.26	11.80 ± 0.51	0.84
	V2	7	5.20 ± 0.16	0.74	13.20 ± 0.47	1.88	18.40 ± 0.47	2.62
	V3	8	6.70 ± 0.19	0.84	5.80 ± 0.60	0.72	12.50 ± 0.49	1.56
	V4	6	10.90 ± 0.34	1.82	18.40 ± 1.44	3.06	29.30 ± 1.59	4.88
STELLA	V1	16	7.80 ± 0.21	0.48	3.20 ± 0.48	0.20	11.00 ± 0.68	0.68
	V2	8	5.00 ± 0.60	0.62	12.30 ± 0.74	1.54	17.30 ± 1.07	2.16
	V3	9	6.20 ± 0.40	0.69	5.20 ± 0.50	0.58	11.40 ± 0.90	1.27
	V4	7	10.50 ± 0.69	1.50	17.20 ± 0.70	2.46	27.70 ± 0.49	3.96
BOAMBE DE COTNARI	V1	12	8.60 ± 0.56	0.72	4.80 ± 0.38	0.40	13.40 ± 0.94	1.12
	V2	6	5.50 ± 0.56	0.92	13.80 ± 0.74	2.30	19.30 ± 1.29	3.22
	V3	7	7.20 ± 0.34	1.03	6.40 ± 0.66	0.91	13.60 ± 0.85	1.94
	V4	5	11.40 ± 0.56	2.28	19.00 ± 0.56	3.80	30.40 ± 1.12	6.08
DURONE DI VIGNOLA	V1	18	6.80 ± 0.56	0.38	2.40 ± 0.32	0.13	9.20 ± 1.20	0.51
	V2	9	4.20 ± 0.41	0.47	10.80 ± 0.53	1.20	15.20 ± 0.35	1.67
	V3	10	4.60 ± 0.56	0.46	4.40 ± 0.68	0.44	9.00 ± 1.23	0.90
	V4	8	9.80 ± 0.60	1.22	13.50 ± 0.67	1.68	23.30 ± 1.21	2.90

V1 = modified atmosphere, at 2-3^{0}C and 85-90% RH
V2 = modified atmosphere, at 24-25^{0}C and 80-85% RH
V3 = cold storage, at 2-3^{0}C and 75-80% RH

Table 3. The main physical - chemical characteristics of the cherry fruits at the end of storage period

Variety	Variant	Storage Duration -days -	Total soluble solid - % -	Titratable acidity -% malic acid-	Ascorbic acid - mg/100 g fresh product -
HEDELFINGER	V1	14	14.2	0.80	7.9
	V2	7	14.5	0.73	8.3
	V3	8	14.3	0.70	8.1
	V4	6	14.7	0.68	7.8
STELLA	V1	16	14.7	0.70	8.7
	V2	8	14.8	0.67	8.9
	V3	9	14.9	0.65	8.9
	V4	7	15.1	0.64	8.6
BOAMBE DE COTNARI	V1	12	13.4	0.82	8.1
	V2	6	13.7	0.75	8.5
	V3	7	13.5	0.72	8.3
	V4	5	13.9	0.70	8.0
DURONE DI VIGNOLA	V1	18	14.4	0.67	8.3
	V2	9	14.8	0.62	8.7
	V3	10	14.6	0.60	8.5
	V4	8	14.9	0.60	8.2

The higher values of total soluble solid were recorded for V4, as for example 13.9% for 'Boambe de Cotnari' and 15.1% for 'Stella'. The values of the total titratable acidity decreased during storage for all the varieties and in all storage conditions. The decrease was more accentuated in the V4 conditions than in V1, due to the intense metabolism of cherries stored in room temperature conditions in comparison to those stored in modified atmosphere.

The same tendency could be observed in the case of ascorbic acid content.

The values of ascorbic acid content have decreased in comparison to the harvesting date, but the oscillations were not as significant for each variety and storage variant.

CONCLUSIONS

As a consequence of keeping cherry fruits in cold storage and modified atmosphere, achieved with the aid of the low-density semipermeable polyethylene film (LDPE), superior results are obtained compared to storage in normal refrigerator conditions or at room temperature.

The storage period of cherry fruits in cold storage and in controlled atmosphere varied between 12 days for 'Boambe de Cotnari' and 18 days for 'Durone di Vignola', while at room temperature fruits could only be stored for 5 days for 'Boambe de Cotnari' and 8 days for 'Durone di Vignola'.

The weight losses recorded at the end of storage were by 24.6% ('Boambe de Cotnari' variety) to 30.6% ('Durone di Vignola' variety) lower in modified atmosphere under cold storage in comparison to storage at room temperature.

The quality depreciations due to the fruits rotting at the end of storage period were by 25.2% ('Boambe de Cotnari' variety) to 17.8% lower in modified atmosphere under refrigeration in comparison to room temperature storage.

The total losses determined at the end of storage period were by 44% ('Boambe de Cotnari') to 39.5% ('Durone di Vignola') lower in modified atmosphere under cold storage than in room temperature storage.

As a consequence of the metabolic activity during the cherries storage the main physical-chemical characteristics have not recorded a downward trend, with the exception of the total soluble solids, which increased due to concentration as a result of the water loss through transpiration rate. The decreases were more significant for the fruits stored in room temperature conditions.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE EFFECT OF FOLIAR APPLICATION WITH ORGANIC AND INORGANIC PRODUCTS ON THE BIOCHEMICAL QUALITY INDICATORS OF HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.)

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Abstract

Foliar fertilization is applied quite frequently in combination with soil fertilization. The objective of this study was to evaluate the influence of two foliar organic fertilizers and one chemical on the biochemical quality of fruit of highbush blueberry (Vaccinium corymbosum L.). The experiment was performed on a farm in the meadow Argeş, in 2020 on a three-year plantation and was presented in a randomized block design, with three repetitions and four fertilization treatments:control (untreated), Poly-Feed 19–19–19 + ME (10 kg/ha), Algacifo 3000 (2 L/ha) and ERT 23 Plus (1 L/ha), repeated four times at every 10-14 days, from the formation of the bud to the beginning of fruit ripening. The blueberry varieties studied were: 'Blueray', 'Duke', 'Elliott' and 'Hannah's Choice'. Data were recorded on the following biochemical quality indicators: total dry matter content, soluble dry matter, total titratable acidity, sugar content, vitamin C, total anthocyanin content and polyphenols. The study showed that organic fertilizers had a superior effect on the biochemical quality of the fruit than the chemical one.

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

INTRODUCTION

In recent years, there has been a rapid increase in the sale of organic food (Ochmian & Kozos, 2014), with consumers willing to pay a higher price for organic products due to the fact that they are healthier and more nutritious (Saba & Messina, 2003). Although organic farming is traditionally practiced in Europe, the modern ecological movement began around the 1920s, being highly valued in the 1970s due to greater knowledge of the adverse effects of fertilizers and pesticides used in conventional practices (Hurtado-Barroso et al., 2019).In addition to the production of healthy food, organic farming also contributes to the protection of the natural environment (Milivojevic et al., 2012).

Excessive use of chemicals has had the effect of destroying the physiochemical properties of the soil, reducing friendly predators and increasing residual hazards to both human health and the environment. The use of beneficial microbial inoculants together with organic manure is considered an alternative requirement for crops. Technological approaches to the use of organic fertilizers and biofertilizers in agriculture have proven to be effective means of increasing crop yields (Thakur, 2017).

Blueberry (*Vaccinium corymbosum* L.) is a popular commercial crop in Europe (Kader et al., 1996), with an annual production of 136,495 tons in 2019. In Romania, blueberry (*Vaccinium corymbosum*) was brought in 1968 by Stefan Nicolae (Botez et al., 1984). Interest in these fruits has increased in recent years, so that in 2019, in our country,a blueberry production of 610 tons was recorded (FAO, 2021). Foliar fertilization is applied quite frequently in combination with soil fertilization.

Organic and inorganic fertilizers have a significant beneficial effect on global food production and are an indispensable component of many agricultural systems (Hernandez et al., 2014).

Fruit quality is influenced by a number of genetic factors (species, variety), environmental factors (climatic conditions: latitude, light exposure, soil conditions, production period) and agronomic factors (cropping system, organic or conventional fertilization, stress and the period of fruit growth and ripening) (Di Vittori et al., 2018).

Blueberries are low in calories. According to Mladin (1992), 100 g of fresh fruit contain between 10.4-15% dry matter, 6.07-10.53% total sugars, 0.49-1.16% organic acids, 14.08-48.5 mg/100 g vitamin C, 0.236-0.481% tannoid substances, 0.343-0.643% pectic substances and 0.30-0.62% protein.

Blueberries have also been reported to be rich in anthocyanins, flavonoids with a high antioxidant capacity (Kalt et al., 2020; Okan et al., 2018), anti-inflammatory, antimicrobial, renoprotective, ophthalmic-protective, hepato-protective, gastro-protective, anti-osteoporotic and anti-aging role (Patel, 2014).

Due to the rich content of flavonoids, phenolic acids (Kalt et al., 2020; Reque et al., 2014), consumption of blueberry products has benefits in preventing the development of obesity, chronic inflammation, type 2 diabetes (Shi et al., 2017), cardiovascular disorders, neurodegenerative diseases and cancer (Routray et al., 2011).

It has been observed that blueberries produced from organic crops contain significantly higher amounts of phytonutrients than those produced from conventional crops (Wang et al., 2008). Rembiałkowska et al. (2003) reported a higher content of micro- and macronutrients, beneficial bioactive compounds (flavonoids, anthocyanins and vitamin C) in fruits obtained from organic crops. Asami et al. (2003) also showed higher levels of total phenols found consistently in organically grown crops compared to those produced by conventional practices. Hallmann and Rembiałkowska (2007) found that blueberries in organic crops are characterized by high levels of organic acids and sugars. However, Häkkinen and Törrönen (2000) reported similar flavonol and phenolic acid contents to some varieties grown conventionally or by organic techniques.

An analysis of the content of secondary metabolites in organic products stated that, in terms of nutritional composition, it is not yet possible to conclude that an organic production system is better than a conventional system (Barański et al., 2017).

The aim of this study was to evaluate the influence of foliar organic fertilizers and inorganic fertilizers on the biochemical quality of blueberry fruits with tall bush (*Vaccinium corymbosum* L.).

MATERIALS AND METHODS

The experience took place on a farm in the Argeș meadow (44° 54′N, 24° 52′E), Romania, on a three-year-old blueberry crop. The experimental field was located, on flat ground, brown-clay soil with a loam-clay texture in the first 60-70 cm, and in depth the texture becomes sandy. Along the rows of plants, the soil was improved by adding acid peat, 30 t/ha. The planting was done on billets covered with black polyethylene. The plants were irrigated using two lines of polyethylene drip tubes located along the row near the base of the plants and covered with polyethylene. Irrigation was applied from mid-May to late September. Groundwater was about 1.5 m.

When harvesting the fruit, the soil showed the following properties, at a depth of 0-20 cm: pH (1: 2.5 H_2O) = 5.67, total nitrogen (N) = 0.11%, P-P_2O_5 = 80.00 ppm, K-K_2O = 80.59 ppm, C = 2.67%, H = 4.60%. At a depth of 20-40 cm, these parameters had the following values: pH = 5.97, C = 0.77%, H = 1.33%, (total N) = 0.09%, P-P_2O_5 = 52.14 ppm, K-K_2O = 52.35 ppm. Soil samples were collected from the row of plants with an agrochemical direct push soil sampler. Sulfur was used to lower the pH of the soil.

The experimental project was bifactorial. Factor A, the blueberry with high bush (*Vaccinium corymbosum* L.) had 4 levels: four varieties of blueberry frequently cultivated in Romania ('Blueray', 'Duke', 'Elliott' and 'Hannah's Choice') planted 3 m away between rows and 0.8 m distance per row (resulting in 4385 plants ha^{-1} density). The varieties were chosen according to their popularity and ripening season. The experiment was organized in randomized blocks. Factor B, the fertilizer used with four fertilization treatments: control (untreated), Algacifo 3000 - extracts of brown seaweed *Macrocystis integrifolia* with betaines of vegetal origin (2% organic nitrogen, 10% organic carbon, 50% organic substance) (2 L/ha), ERT 23 Plusseaweed extracts (*Macrocystis integrifolia*), folic acid, glycine betaine (1.5% organic nitrogen, 11% organic carbon, 6.1% K_2O, 10% betaines) (1 L/ha) and inorganic product Poly-Feed 19-19-19 + ME (10 kg/ha), repeated four times every 10-14 days, from bud formation to early fruit ripening and 3 replicates for each treatment. There was a three-story space between rehearsals. The fruits were harvested manually at the optimal stage of maturity.

Biochemical analyzes and laboratory determinations consisted of the determination of the total dry matter content, the soluble dry matter content, the titratable acidity, vitamin C, total sugar, total polyphenols and anthocyanin pigments. All biochemical determinations were performed in three repetitions for each variant of fertilization and repetition of each variety.

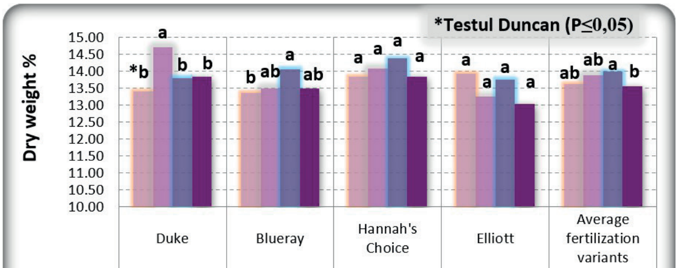
The total dry weight content was determined by gravimetric method (drying 10 g of fruit tissue at 105°C up to constant weight) according to Gergen (2004). Vitamin C content was estimated by the iodometric method and expressed in mg/100 g FW Gergen (2004). The soluble dry matter content was measured with a Kern digital refractometer and expressed in units of Brix degrees. The total sugar content was estimated by the Fehling-Soxhlet method, 1968 (JAOAC, 1968). Titratable acidity was determined by volumetric method using NaOH 0.1N (Gergen, 2004).

Determination of total polyphenols was performed spectrophotocolorimetrically, with Folin-Ciocâlteu reagent (Singleton et al., 1999) using gallic acid as standard and were expressed as mg GAE/100 g FW. Methanol (70%) was used as the solvent for the extraction of polyphenols. The level of total anthocyanin pigments in fruits was performed by the Fuleki method (1968). This method consists in extracting anthocyanins with suitable extractive solutions and measuring the absorbance of the extract, spectrophotocolorimetric at the wavelength λ = 535 nm. The determined total anthocyanins were expressed as cyanidin-3-glucoside mg/100 g fresh fruit (FW). Absorbances were measured with a PG instruments T70 spectrophotometer.

Statistical analysis was performed with an IBM SPSS 20 program. ANOVA and Duncan Multiple Range tests were used. The values were considered statistically significant at $p \leq 0.05$. Three independent samples were performed for each determination and the resulting data were used to obtain mean values and standard deviations for all tests.

RESULTS AND DISCUSSIONS

Total dry weight content (DW). In the experiment, the total dry weight content had an average value of 13.77% (Table 1). The highest value of the total DW content was recorded in 'Duke', V2 (14.71%) and 'Hannah's Choice', V3 (14.39%) and the lowest value in the 'Elliott' variety, V4 (13.04%), and 'Duke' variety, V1 (13.4%) (Figure 1). Organic and conventional foliar fertilization did not significantly influence the total dry weight content of the fruit of the 'Elliott' and 'Hannah's Choice' varieties in terms of the Duncan test, the series of values being homogeneous. The total dry weight content of the fruit was significantly influenced by the cultivar ($F(3.13) = 3.73$; $p = 0.013$) with an influence of only 8%. The variety and the fertilization variant have accumulated the effect ($F(9.13) = 2.16$; $p = 0.029$) with a statistically assured variation of only 13.2%.



	Duke	Blueray	Hannah's Choice	Elliott	Average fertilization variants
V1	13.402	13.370	13.839	13.938	13.64
V2	14.714	13.501	14.091	13.249	13.89
V3	13.798	14.054	14.392	13.760	14.00
V4	13.848	13.500	13.841	13.038	13.56

Figure 1. Influence of the fertilization variant on the total dry matter content of the fruits, depending on the variety

The average values of the fertilization variants show statistically assured differences between the organic variant V3 (14.0%) and conventional variant V4 (13.56%).

The results obtained are consistent with the data in the literature. Ostrowska and Ściążko (1996) reported a total dry matter content in blueberry fruit between 12.8-15.09%.

Radunzet et al. (2017) noted that DW is influenced by variety, fruit size, cultivation technologies and climatic conditions

Total soluble solids (TSS) content. The average value of 12.35ºBrix (Table 1) of the TSS content reached a maximum of 15.01ºBrix for the 'Blueray' variety, the organic leaf variant V3 and a minimum of 9.87ºBrix for the 'Elliott', control variant V1 (Figure 2). Prior et al. (1998) found a much wider range of soluble solids content in blueberry varieties (*Vaccinium corymbosum*) 10.0-19.0%. The cultivar significantly influenced the TSS content of blueberry fruits ($F(3.13) = 19.46$; $p = 0.000$ indicating that 31.3% of the variation in TSS content could be explained by the cultivar effect). The 'Blueray' cultivar had the highest TSS content in fruit, compared to the other cultivations studied as shown by Skupień, (2006). The fertilization variant had a statistically assured influence on the TSS content of blueberry fruits ($F(3.13) = 13.94$; $p = 0.000$, with an influence of 24.6% of the value of the TSS content of fruits).

The fertilization variant follows approximately the same trends as in the case of the average effect (Figure 2).

Also, a significant influence of the variety-applied foliar fertilizer interaction with the cultivar was observed ($F(9.13) = 2.82$; $p = 0.005$, with a variation of 16.5% on the TSS content). According to Strik et al. (2017) the TSS content of blueberries is affected by climatic conditions, fertilizers and varieties. The average values of the TSS fruit quality indicator show statistically significant differences in terms of a significant increase, from the point of view of the Duncan test, in the variants with organic foliar fertilizers V2 (12.98ºBrix) and V3 (13.15ºBrix) compared to the conventional variant V4 (12.12ºBrix). The control variant showed significantly lower TSS content values compared to the three foliar fertilization variants. In the 'Duke' variety, only the organic variant V2 showed significant increases in the TSS content of the fruit.

In the 'Hannah's Choice' variety, foliar fertilization did not significantly influence the TSS content of the fruit, observing the presence of a single homogeneous series of (Figure 2). Skupień (2006) showed similar results, explaining that this indicator is more influenced by environmental conditions.

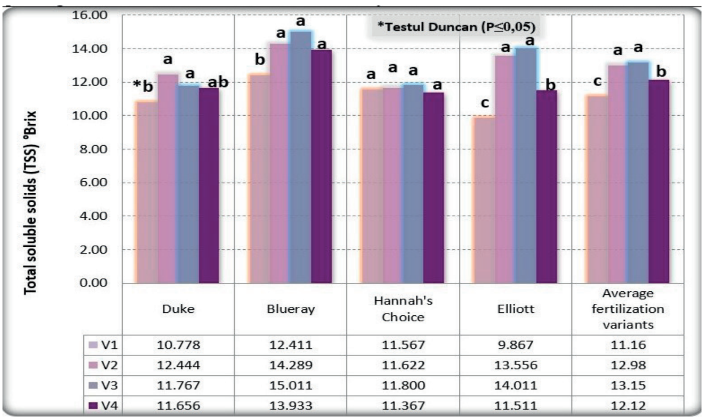


Figure 2. Influence of the fertilization variant on the content of total soluble substance of the fruits, depending on the variety

Titratable acidity content expressed as malic acid. The average malic acid content of blueberry fruits 0.92% (Table 1) ranged from 0.70% ('Elliott', V4) to 1.41% ('Blueray', V3) (Figure 3). Statistically assured differences were observed between varieties ($F(3.13) = 498.39$; $p = 0.000$, where 92.1% of the variation of the malic acid content could be explained by the cultivar effect). In the present study, on average, the 'Bluecrop' variety had the highest content of malic acids (1.36%) compared to other varieties. Similar results were reported by Skupień, (2006).

In two of the varieties ('Elliott' and 'Hannah's Choice') there are statistically assured differences between the variants with organic foliar fertilizers and the conventional one. Figure 3 shows a homogeneous series in the case of the average effect of foliar fertilization variants and 'Duke' and 'Blueray' varieties. Foliar fertilization did not significantly influence the evolution of the total acidity of the fruits.

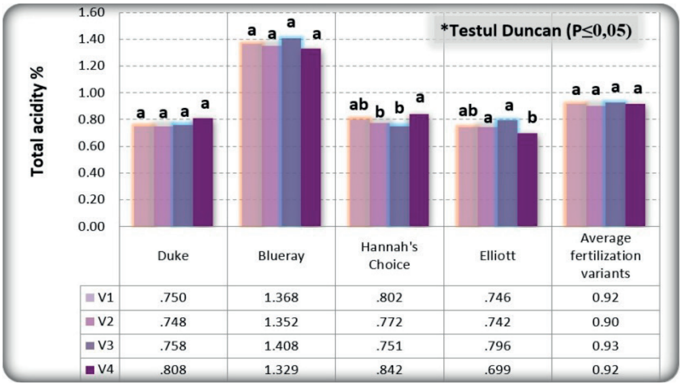


Figure 3. The influence of the fertilization variant on the content of total organic acids of the fruits, depending on the variety

The sugar content is an important biochemical indicator used to determine the quality of sweet fruits (Okan et al., 2018). The sugar content of the fruits (Figure 4) varied between the minimum values for the 'Blueray' (9.59%) and 'Duke' varieties (9.60%) and the maximum values for the 'Elliot' variety (11.84%) with an average of 10.38% (Table 1). The sugar total content was significantly influenced by the cultivar (F(3.13) = 10.42; p = 0.000, with a 19.6% influence on the sugar total content). The fertilization variant significantly influenced the sugar content of blueberry fruits by 18.3%. The cumulative effect of the two factors was statistically ensured with an influence of only 14%. The average values of the fertilization variants show a significant increase of the total sugar content of the blueberry fruits. The highest values of sugar content were obtained in the case of the version with chemical fertilizers V4 and in the version with organic fertilizers V3.

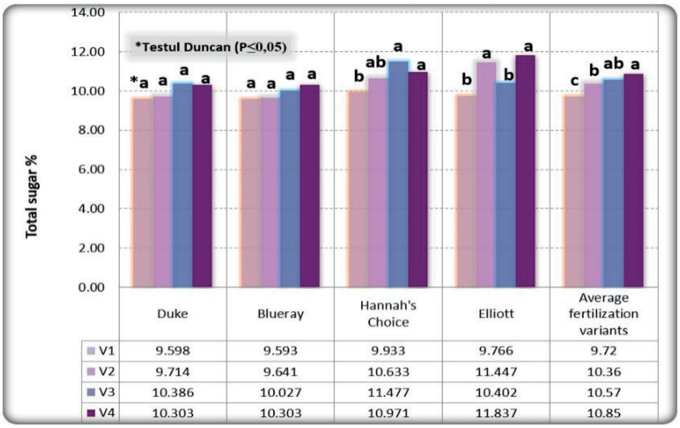


Figure 4. The influence of the fertilization variant on total sugar content of the fruits, depending on the variety

Vitamin C content. Vitamin C content ranged from 18.40 mg/100 g ('Blueray', V1) to 13.79 mg/100 g ('Duke', V2) (Figure 5), with an average of 16.28 mg/100 g (Table 1). The vitamin C content was significantly influenced by the cultivar ($F(3.13) = 86.50$, $p = 0.000$, with a 67% influence on the vitamin C content). 'Blueray' and 'Elliott' cultivars had significantly higher vitamin C values than 'Hannah's Choice'. Fertilization variants did not show a statistically assured variation in vitamin C content. Figure 5 shows the content of vitamin C in fruits in the control variant V1 and in the version with inorganic foliar fertilizer V4 is higher than in the variants with organic fertilizers V2 and V3. The 'Elliott' and 'Hannah's Choice' varieties show a homogeneous series between fertilization variants. Foliar fertilization did not show a statistically significant variation in these two varieties.

	Duke	Blueray	Hannah's Choice	Elliott	Average fertilization variants
V1	14.082	18.396	16.220	17.796	16.62
V2	13.788	17.070	15.840	17.462	16.04
V3	14.356	17.671	15.676	16.649	16.09
V4	14.561	17.306	16.106	17.522	16.37

Figure 5. The influence of the fertilization variant on vitamin C content of the fruits, depending on the variety

Total anthocyanins (TA) content. The content of total anthocyanins expressed as cyanidin-3-glucoside in the studied blueberry varieties recorded an average value of 228.28 mg/100 g FW (Table 1). The highest value was recorded by the 'Elliott' variety, V3 (377.78 mg/100 g FW) and the lowest by the 'Blueray' variety V1, (124.81 mg/100 g FW) (Figure 6). The total anthocyanin content varied significantly between varieties ($F(3.13) = 799.44$, $p = 0.000$, 94.9% of the variation in anthocyanin content could be explained by the cultivar effect), the fertilization variant ($F(3.13) = 24.8$, $p = 0.000$ had an influence of 36.8%). The combined effect of the two factors significantly influenced the total anthocyanin content ($F(9.13) = 9.19$, $p = 0.000$ by 39.2%). In the case of the fertilizer control variant, approximately the same trends are maintained as in the case of the average effect.

A significant increase compared to the conventional fertilization variant of the total anthocyanin content is observed for the 'Elliott' and 'Hannah′s Choice' varieties for organic fertilizer V3 and for the 'Duke' variety for organic fertilizer V2. At the 'Blueray' cultivar there are no statistically assured differences between the fertilization variants, from the point of view of the Duncan test, the series of values being homogeneous.

The application of organic and conventional foliar fertilizers significantly influenced the total anthocyanin content of fruits to the studied varieties.Blueberries produced from organic culture contained significantly higher amounts of anthocyanins than those produced from conventional culture.

Similar results of the total anthocyanin content were obtained by Okan et al. (2018) which reported a variation from 43.03-295.06 mg/ 100 g FW. For highbush blueberries, Mazza and Miniati (1993) have reported a range of 25 to 495 mg/100 g anthocyanins. Wang et al. (2008) found significantly higher average anthocyanin values on organic farms in comparison with conventional farms.

Total phenolics (TP) content. The TP content recorded an average value of 344.01 mg GAE/100 g FW (Table 1).

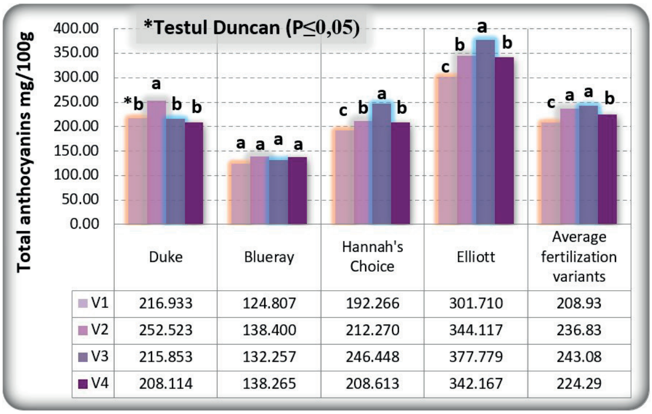
Analyzing the average effect of foliar fertilizers applied on the content of total polyphenols in fruits, there is a significant increase in foliar application of organic fertilizers (variants V2 and V3) (494.89 mg GAE/100 g FW and 499.13 mg GAE/100 g FW) compared of untreated control V1 and conventional fertilizer V4 (Figure 7).

And in the 'Elliott' variety the content of total polyphenols increased significantly in the organic variant V3 compared to the other fertilization variants. The effect is not maintained in all varieties.

The figure 7 shows the presence of a single homogeneous series (a) in the 'Duke' variety.

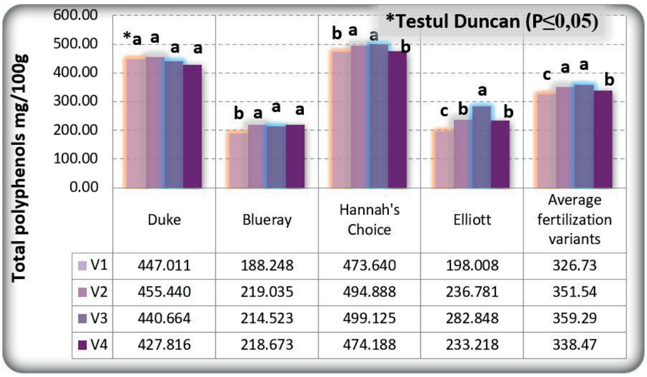
On average, it is observed that the effect of the fertilization variant materialized in a minimum value at control V1 (326.73 mg GAE/100 g FW), the highest effect being observed at variant V3 (359.29 mg GAE/100 g FW).

In the 'Blueray' variety, there is an interaction of the two experimental factors (variety and fertilization variant) in the sense of increasing the total polyphenol content of all fertilization variants compared to martor. The variety had a significant influence of TP (F(3.13) = 1891.35, p = 0.000 with 97.8%). Also, the fertilization variant significantly influenced the TP content (F(3.13) = 19.87, p = 0.000 with 31.8%).



	Duke	Blueray	Hannah's Choice	Elliott	Average fertilization variants
V1	216.933	124.807	192.266	301.710	208.93
V2	252.523	138.400	212.270	344.117	236.83
V3	215.853	132.257	246.448	377.779	243.08
V4	208.114	138.265	208.613	342.167	224.29

Figure 6. Influence of the fertilization variant on the total anthocyanin content of the fruits, depending on the variety



	Duke	Blueray	Hannah's Choice	Elliott	Average fertilization variants
V1	447.011	188.248	473.640	198.008	326.73
V2	455.440	219.035	494.888	236.781	351.54
V3	440.664	214.523	499.125	282.848	359.29
V4	427.816	218.673	474.188	233.218	338.47

Figure 7. Influence of the fertilization variant on the total polyphenol content of the fruits, depending on the variety

According to Kim et al (2013), total phenolic content ranged from 170.9 to 523.8 mg GAE/100 g FW in the high bush blueberry fruits. Okan et al. (2018) reported a total blueberry polyphenol content of 215.12 mg GAE/100 g FW. Lopez et al. (2016) reported high values for total polyphenols (450 mg GAE/100 g) for the 'Duke' variety fruits.

Table 1. Statistical descriptors for DW (%), TSS content (°Brix), Total acidity (%), Total sugar (%) Vitamin C (mg/100g FW), TP (mg/100g FW) and TA (mg/100g FW), on *Vaccinium corymbosum* L., 'Duke', 'Blueray' 'Hannah's Choice' and 'Elliott' cultivars, Arges County (2020)

	Dry weight (%)	**TSS (°Brix)**	**Titratable acidity (%)**	**Total sugar (%)**	**Vitamin C (mg/100 g FW)**	**TA (mg/100 g FW)**	**TP (mg/100 g FW)**
Mean	13.77	12.35	0.92	10.38	16.28	228.28	344.01
Std. Deviation	0.80	1.08	0.12	0.97	1.11	29.85	40.61

Collectively, the data presented by Wang et al. (2008) suggest that different cropping systems can significantly affect the quality of blueberry fruits. Significant differences were evident between the two cultivation practices (organic and conventional). Blueberries produced from organic culture contained significantly higher amounts of phytonutrients than those produced from conventional culture.

From Table 2 it can be observed that in our experiment the total dry matter content present distinct significant positive correlation with total polyphenols content (r = 0.268**) and present significant negative correlation with vitamin C (r = -0.188*). Total soluble solids present distinct significant positive correlation with titratable acidity (r = 0.655**) and respectively with vitamin C content (r = 0.246**). It showed a distinct significant negative correlation with total polyphenols content (r = -0.351**) and with total anthocyanins content (r = -0.449**).

Alsoit can be observed that the titratable acidity present distinct significant negative correlation with total sugar content (r = -0.260**), with total polyphenols content (r = -0.555**) and with total anthocyanins content (r = -0.693**) respectively. Similarly, Senica, et al. (2018) reported that organic acids were negatively correlated with polyphenols, which means that acids decrease when polyphenols rise.Itshowed a distinct significant positive correlation with vitamin C content (r = 0.394**). Total sugar content present distinct significant positive correlation with total anthocyanins content (r = 0.324**).

According toGralec et al. (2019) results, we obtained a nonsignificant correlation between phenolics and anthocyanins (r = -0.018).

Table 2. Pearson correlations coefficients for the biochemical quality indicatorsfor the studied blueberry varieties

	Total dry matter	*Total soluble solid*	*Titratable acidity*	*Total sugar*	*Ascorbic acid*	*Total Polyphenols*	*Total Anthocyanins*
Total dry matter	x	0,082	-0.099	-0.087	-0.188*	0.268**	-0.042
Total soluble solid		x	0.655**	-0.199*	0.246**	-0.351**	-0.449**
Titratable acidity			x	-0.260**	0,394**	-0.555**	-0.693**
Total sugar content				x	0,060	0.065	0.324**
Ascorbic acid					x	-0.671**	-0.020
Total Polyphenols							-0.018

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

The application of organic foliar fertilizers had a positive effect in the sense of accumulation of bioactive compounds compared to conventional fertilizers. There was a significant increase in the content of total dry substance, the total polyphenols, anthocyanins and total soluble substances in fruits following the applications of organic foliar fertilizers.

The accumulation of bioactive species is influenced by the cultivar and type of fertilization.

The application of foliar fertilizers is beneficial in terms of increasing the biochemical qualities of blueberries.

An additional study is needed to determine which of the used foliar fertilizers has a superior effect on the accumulation of bioactive compounds in fruit.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTS OF PEG 6000 STRESS ON STRAWBERRY (*FRAGARIA* × *ANANASSA* DUCH.) *IN VITRO* PROPAGATION

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Abstract

The aim of the study was to assess the drought tolerance of two varieties of strawberry, Tecla and Hecker, according to their reaction by morphometric characteristics to the presence of PEG 6000 in different concentrations in culture media (0 g/L - control, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L). The results show that cv. Hecker exhibited the lowest value of dry weight (66.08 ± 12.17) at 50 g/L PEG concentration. The highest concentrations of PEG 6000 (40 g/L and 50 g/L) considerably decreased the DW in the plantlets in both Tecla and Hecker cvs. High concentrations of PEG 6000 decreased significantly the plantlets' length and proliferation rate in both cultivars. A dramatic decrease (71.77%) in average height was recorded in cv. Hecker at the highest concentration of PEG 6000 (50 g/L) when compared to the control, from 8.68 ± 0.13 cm to 2.45 ± 0.19 cm (PEG 6000 50 g/L). These results suggest that PEG 6000 disrupted the water metabolism in both strawberry cultivars.

Key words*: drought stress, polyethylene glycol, proliferation rate, strawberry.*

INTRODUCTION

Due to the climatic changes, plants are exposed to various abiotic stresses: water deficit, excess water, low temperatures, high temperatures, high light intensity, salinity and heavy metals (Sah et al., 2020; Albergaria et al., 2020; Zhang et al., 2020, Hassan et al., 2020; Arteaga et al., 2020). As a consequence of global warming, some plant species face new environmental conditions for which they are not adapted (Vuksanović et al., 2019). Drought is one of the main factors of abiotic stress that limits plant growth and productivity (Nadeem et al., 2019).
Drought stress imposes changes in important plant growth and development processes, including germination, plant size, dry matter production and distribution, flower and fruit development and plant maturity (Anjum et al., 2017).
In vitro cultures have been used to assess drought tolerance for different species: olive (*Olea europaea*) (Baccari et al., 2016), strawberry (*Fragaria ananasa*) (Salma et al., 2016), guava (*Psidium guajava* L.) (Youssef et al., 2016), chickpeas (*Cicer arietinum* L.) (Hussein et al., 2017) quinoa (*Chenopodium Quinoa* Willd.) (Telahigue and Toumi, 2017).
Plant response to hydric stress is a complex phenomenon and *in vitro* culture could be used to study the numerous physiological and biochemical changes in all plant organs, as a consequence of the reduced availability of water (Gupta et al., 2016; Peiro et al., 2020).
In vitro simulation of drought stress using chemical reagents, such as polyethylene glycols (PEG), constitutes a convenient way to assess, in controlled conditions, the effects of drought on plant growth and development (Khayatnezhad et al., 2010).
Polyethylene glycol PEG 6000 is a polyether compound derived from petroleum that is usually used to simulate drought stress in *in vitro* culture conditions (Vuksanović, 2019). The PEG 6000 is a non-penetrating and nontoxic osmotic substance, and the addition of this selective agent to the *in vitro* culture media determines variations of the water potential of plants (Sen et al., 2013; Gupta et al., 2016; Beyaz, 2019).
Genotypic differences in drought tolerance have been evaluated for various crop species (Bota et al., 2001; Herralde et al., 2001; Zhong et al., 2018) including strawberry (Hussein et al., 2017). However, there is still a lack of

information about morpho-physiological changes in different *in vitro* cultured strawberry cultivars under limited water availability (Adak and Kaynak, 2020).
Therefore, the main objective of this study was to evaluate the drought tolerance of two *in vitro* cultured varieties of *Fragaria* × *ananassa* Duch. The addition of PEG in different concentrations into the culture media determined plant morphological changes which have been quantified by morphometric analysis.

MATERIALS AND METHODS

Two varieties of strawberry were tested: Tecla and Hecker. Five shoot tips were cultured per 720 ml glass jar with 100 ml of Murashige & Skoog (1962) (MS) medium supplemented with 0.2 mg/L N6-benzyladenine (BA), 4 g/L Plant Agar and 30 g/L sugar.
The experimental design included six treatments/concentrations of PEG 6000: 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L and control with three repetitions/jar per treatment and cultivar. The PEG 6000 was added to the culture media before adjusting the pH and before autoclavation. The pH of the media was adjusted to 5.8. The media were autoclaved at 120°C for 20 minutes. After inoculation, the culture vessels were incubated in the growth room with a controlled environment (23 ± 3°C, 27-32.4 mmol·m^{-2}·s^{-1}, 16 h photoperiod). After 10 weeks of *in vitro* culture, fresh weight, dry weight of plantlets and plantlet heights were measured and the plantlets resulted by axillary shoot proliferation at the end of the culture cycle were counted and proliferation rates were calculated.
To determine the dry wheight (DW) of plantlets of each experimental group and the control plantlets, first the fresh weights (FW) of samples were measured. Next, samples were dried at 45°C for 48 h and their DW were determined.
Data Analysis
To analyse the data, ANOVA analysis was performed first to check the differences among the means. When the null hypothesis was rejected, Tukey's HSD test (P≤0.05) was performed to determine the means that were significantly different from each other. Values shown are means ± SE.

RESULTS

In this study, the *in vitro* studies for drought tolerance assessment was achieved using two strawberry cultivars and five concentrations (i.e. 10, 20, 30, 40, and 50 g/L) of polyethylene glycol (PEG).
Results showed that a high PEG concentration in the culture media had a negative effect on the growth and development of the plantlets during the *in vitro* multiplication phase on the MS medium supplemented with 0.2 mg/L BA.
The results of Tukey's HSD test (P≤0.05) show that there was a statistically significant difference in the dry weight of plantlets grown on culture media supplemented with different concentrations of PEG versus control (without PEG) in both strawberry cultivars and the DW was PEG 6000 concentration dependent.
Our results show that cv. Hecker exhibited the lowest value of dry weight (66.08 ± 12.17) at 50 g/L PEG concentration. Furthermore, the highest concentrations of PEG 6000 (40 g/L and 50 g/L) considerably decreased the DW in both cv. Tecla and cv. Hecker plantlets (Figure 1).

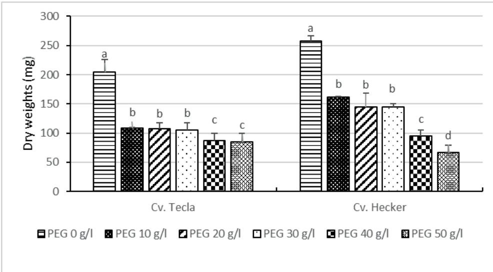

Figure 1. Effect of PEG 6000 on *in vitro* cultured strawberry cv Tecla and cv Hecker dry weights (PEG 0 g/L: MS+0.2 mg/L BA without PEG 6000 - control; PEG 10 g/L: MS+0.2 mg/L BA+10 g/L PEG 6000; PEG 20 g/L: MS+0.2 mg/L BA+20 g/L PEG 6000; PEG 30 g/L: MS+0.2 mg/L BA+30 g/L PEG 6000; PEG 40 g/L: MS+0.2 mg/L BA+40 g/L PEG 6000; PEG 50 g/L: MS+0.2 mg/L BA+50 g/L PEG 6000). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test (p≤ 0.05)

Regarding the number of plantlets it was observed that the addition of PEG 6000 into the culture media variants used for the *in vitro* plant multiplication phase led to a decrease in the number of plantlets/culture jar.

The average number of plantlets /culture jar decreased from 52.6 ± 0.29 (without PEG) to 38 ± 0.51 (50 g/L PEG) at cv. Tecla and from 38 ± 0.25 (without PEG) to 19.66 ± 0.29 (50 g/L PEG) in case of cv. Hecker. The differences between the control variant (0 g/L PEG) and the other treatments with different PEG concentrations (10-30 g/L) were statistically significant in both Tecla and Hecker cultivars (Figure 2). In the case of both cultivars, there was no statistically significant difference regarding the average number of plantlets /culture jar recorded in the variants supplemented with 40 g/L PEG and 50 g/L PEG, respectively.

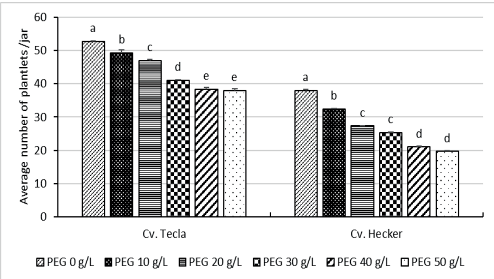

Figure 2. Effect of PEG 6000 on *in vitro* cultured strawberry cv Tecla and cv Hecker average number of plantlets/culture jar (PEG 0 g/L: MS+0.2 mg/L BA without PEG 6000 - control; PEG 10 g/L: MS+0.2 mg/L BA+10 g/L PEG 6000; PEG 20 g/L: MS+0.2 mg/L BA+20 g/L PEG 6000; PEG 30 g/L: MS+0.2 mg/L BA+30 g/L PEG 6000; PEG 40 g/L: MS+0.2 mg/L BA+40 g/L PEG 6000; PEG 50 g/L: MS+0.2 mg/L BA+50 g/L PEG 6000). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

The same decreasing trends of the values were registered at the proliferation rate/ inoculum, compared to the increase of the PEG concentrations. The proliferation rate decreased from 10.53 ± 0.99 (without PEG) to 7.60 ± 0.64 (PEG 50 g/L) in case of cv. Tecla. Hecker's results show values from 7.6 ± 0.51 (control variant) to 3.9 ± 0.26 (PEG 50 g/L) (Figure 3).

The increase in PEG concentration in the culture media was accompanied by a general decrease in the plantlets' height in both Tecla and Hecker plantlets (Figure 4). It is worth mentioning that on MS supplemented with 0.2 mg/L BA Tecla regenerants recorded higher values of proliferation rate than Hecker, but the average height of the plantlets was smaller, with an average of 5.48 ± 0.15 cm compared to 8.68 ± 0.13 cm on Hecker cultivar (Figure 5).

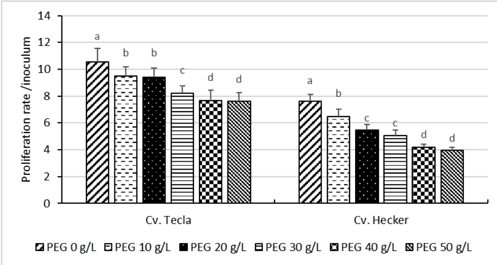

Figure 3. Effect of PEG 6000 on *in vitro* cultured strawberry cv Tecla and cv Hecker proliferation rate/ inoculum (PEG 0 g/L: MS+0.2 mg/L BA without PEG 6000 - control; PEG 10 g/L: MS+0.2 mg/L BA+10 g/L PEG 6000; PEG 20 g/L: MS+0.2 mg/L BA+20 g/L PEG 6000; PEG 30 g/L: MS+0.2 mg/L BA+30 g/L PEG 6000; PEG 40 g/L: MS+0.2 mg/L BA+40 g/L PEG 6000; PEG 50 g/L: MS+0.2 mg/L BA+50 g/L PEG 6000). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

Figure 4. *In vitro* culture of strawberry with different PEG 6000 concentrations: A - cv. Tecla and B - cv. Hecker

There were differences between the analyzed strawberry genotypes referring to their reaction to the used PEG concentrations. In both cases, there was an inverse correlation between the PEG concentration and the height of the plantlets. Our results show that high concentrations of PEG (30, 40, and 50 g/L) strongly inhibited the growth of Hecker plantlets but there was no statistically significant difference between the length of plantlets from Hecker and also Tecla cultivars (Figure 5).

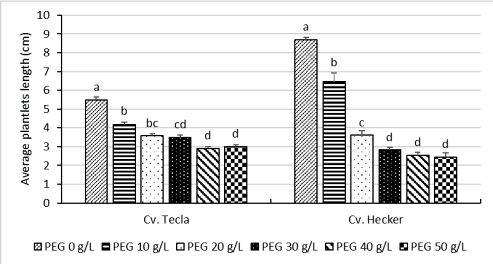

Figure. 5. Effect of PEG 6000 on *in vitro* cultured strawberry cv Tecla and cv Hecker: average plantlet height (PEG 0 g/L: MS+0.2 mg/L BA without PEG 6000 - control; PEG 10 g/L: MS+0.2 mg/L BA+ 10 g/L PEG 6000; PEG 20 g/L: MS+0.2 mg/L BA+ 20 g/L PEG 6000; PEG 30 g/L: MS+0.2 mg/L BA+ 30 g/L PEG 6000; PEG 40 g/L: MS+0.2 mg/L BA+ 40 g/L PEG 6000; PEG 50 g/L: MS+0.2 mg/L BA+ 50 g/L PEG 6000). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

In this study, the average length of Tecla plantlets grown on culture media supplemented with 50 g/L was reduced to 42.76% compared to the control variant (without PEG), while the Hecker plantlets recorded only 28.26% of the plantlets length of control variant, as can be observed in Figure 5.

DISCUSSIONS

Strawberry (*Fragaria×ananassa* Duch) is a small fruit crop of prime importance all over the world (Thokchom et al., 2019). However, like other horticultural crops, the performance of strawberry is negatively affected by environmental stress, especially water stress (Mozafari et al., 2019; Yosefi et al., 2020). The results obtained in this study were in agreement with those reported by Erdogan et al. (2016) who reported that diminishing water supply caused a gradual decrease in strawberry plant growth under water deficit stress.

Our results show that water stress, induced by the incorporation of PEG 6000 in culture media, can adversely affect the development of *in vitro* strawberry plantlets, results confirmed in previous studies performed on other plant species (Kacem et al., 2017; Islam et al., 2019). *In vitro* screening techniques are highly recommended in many studies for minimizing the impact of the changing external experimental conditions (Piwowarczyk et al., 2014). The simulation of drought conditions is relatively easy since non-penetrating and inert polyethylene glycol (PEG) induces water stress in plants (Rai et al., 2011). Until now, *in vitro* studies on the influence of water stress on the regeneration ability of cultivars from the genus *Fragaria* have received less attention (Hussein et al., 2016; Thokchom et al., 2019).

In general, the findings that PEG reduced the multiplication rate and plantlet vigour of strawberry cultivars are similar to those reported for *in vitro* drought screening of other plant species (Rai et al., 2011; Piwowarczyk et al., 2014; Ahmad et al., 2020). Our results suggest that the interaction between PEG and varieties leads to decreased plantlets length in Tecla and Hecker cultivars (Figure 5) and more profound decrease was observed in cv Hecker as compared to cv Tecla.

It is interesting to note that the intensity of multiplication rate and plantlets vigour reduction under certain levels of PEG treatment (40 and 50 g/L) was not genotype dependent, and this is in contrast with the results reported by Thokchom et al. (2019). With increasing concentration of polyethylene glycol, the plantlets' vigour declined. According to Jaleel et al. (2009) this may be a consequence of greatly suppressed cell elongation as a result of the low turgor pressure due to the low hydraulic conductivity in plant cells.

CONCLUSIONS

The results of the present study showed that PEG-drought stress as well as difference in varietal traits leads to alter different morpho-physiological characteristics of strawberry plantlets.

Different levels of PEG treatments and varieties cause certain changes in the growth attributes as well as in the DW of strawberry plantlets.

The treatments with concentrations of 20 and 30 g/L PEG 6000 had lower effects than 40 and

50 g/L PEG in all the analysed morphological parameters. Also, our study showed that Tecla showed better performance in most of the morphophysiological parameters as compared to Hecker variety.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI - UEFISCDI, project number PN-III-P2-2.1-PTE-2019-0670, within PNCDI III and 946/SMIS 14064: Institute of Advanced Horticulture Research of Transylvania.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PHENOLOGICAL TRAITS OF GOJI BERRY (*LYCIUM BARBARUM* L.), GROWTH AND REPRODUCTIVE CHARACTERISTICS OF SOME VARITIES CONDUCTED AS A TREE

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Abstract

The study was conducted in the experimental base of the Department of Fruit Growing at the Agricultural University - Plovdiv in the period 2018-2020. In vitro propagated plants of four varieties: goji berry (BioTree) of the species (Lycium barbarum L.) - JB 1, JB 2, JB 4 and JB 10 were planted at distances of 3 m x 2 m in June 2014. They were fortmed as trees with a stem height of 70-90 cm. The results of the study showed that starting of vegetation for variety JB 2 started about a week earlier than in other varieties. Mass flowering in the varieties occurs in May and damages from late frosts in goji berry are not possible. It takes 30 to 40 days from flowering to fruit ripening. At the end of the seventh vegetation, the growth of the trees has not yet weakened. With the 3 m / 2m planting scheme, the plants have not fully occupied the available space and closer planting distances are possible. From the goji berry varieties studied, the most productive was the JB 1 variety, followed by the JB 2 variety. The aim of the study was to examine the phenological, growth, reproductive and other features associated with the cultivation of goji berry, conducted as a tree under the open field conditions. The data from the study would be useful, when selecting the appropriate variety and plant densities for cultivation as well for using various agro-technological practices such as fertilization, harvesting and storage of fruits.

***Key words**: Goji berry, growth, Lycium barbarum L., phenology, yield.*

INTRODUCTION

One of the main factors determining the great diversity of the assortment of the fruit crops is the climate. The goji berry species originates from Asia and is naturally adaptable to the conditions of this continent. With the establishment of the beneficial properties of the goji berry plant (fruits and vegetative parts), studies of the suitability for growing the plant in many places around the world began. Growth and productivity of goji berries are related to climatic conditions.

The species has been identified as adaptive to the climatic conditions of Mongolia (Liu, 1999), Romania (Mencinicopshi et al., 2012). In Georgia, it is recommended for cultivation in the Shida Kartli region and in other parts of the country with similar soil and climatic conditions (Bobokashvili et al., 2017). Dzhugalov et al. (2015) studied the growth and reproductive manifestations of two *in vitro* propagated goji berry varieties (JB 1 and JB 2) under the conditions of Plovdiv region-Bulgaria and identified them as suitable for cultivation. They registered better productivity of variety JB 1 - 0.56 kg/tree with theoretical yield - 93.52 kg/dka, as in the other variety, the yields were, respectively 0.31 kg/tree and 51.77 kg/dka, respectively. The authors also found differences in terms of variety growth. Differences in growth and productivity have also been identified by (Lichev et al., 2020). Very often genetic characteristics influence different growth and reproductive characteristics. They must be taken into account when creating plantations.

The fruits appears on the current growth during the vegetation for a long period of time. The shoots are long. Due to the weeping habit of the plant when grown as a shrub, most of the fruits are contaminated and damaged, and their collection is quite time consuming. An opportunity to avoid the disadvantages associated with the cultivation of goji berry is the training of the plant as a tree. For this purpose because the plant does not form a thick stem and is quite unstable, a supporting structure is needed. There is little information related to pruning practices and planting distances (Zhang Haizhou, 2001; Chen Fang and Liu Fue, 2008). In China it has been established a standard for growing goji berries (Lee Yoon et al.). Several crowns have been described in the cultivation of the plant as a tree:

- Naturally semicircular with 5 to 8 main branches: 3 to 5 on the first level and 2 to 3 branches on the second level. The crown after formation is 1.7 m high and 1.8-2 m in diameter.
- Semicircular with 5 to 8 main branches, which are evenly distributed on the central leader branch. The tree is about 1.7 m high and 2 m in diameter.
- Conical: The tree has 16 to 20 branches with a nude central leader and a single level. After formation, the diameter of the crown is about one meter and the height about 1.7 m.

Umbrella-shaped (with one or more levels of branches, with 4 to 5 branches per level). After 4 to 5 years, the tree is expected to be about 1.6 m high, with crown diameter 1.0 m and thickness of the stem 5-6 cm.

In Canada (Penn State College), offer the formation of a conical crown. A well-shaped tree with a conical crown should have 16 to 20 semi-skeletal branches and be about 1.80 m high and 1.0-1.2 m in diameter. The other offered crown for goji berry is with a few levels of branches. Crown (with three levels), after the formation should have from 10 to 15 main branches, height from about to 2.2 m and crown width 1.5-1.8 m.

There is no information available about the phenological development of goji berry in Bulgaria. Also for the growth, reproductive and other features associated with the cultivation of goji berry, formed as a tree under open field conditions. The permanent maintenance of the average daily air temperature above 5°C in spring is considered to be the beginning of the active vegetation in fruit plants (Vandova et al., 1984). For the region of Plovdiv in assessing the agro-climatic conditions for growing some fruit crops in Bulgaria it was found that the permanent increase in air temperature above 5°C is in early March, the difference between the earliest and latest date is 42 days (Vandova et al., 1984).

The information from the study would be usefull, when selecting the appropriate variety and plant densities for cultivation as well for using various agro-technological practices such as fertilization, harvesting and storage of fruits.

MATERIALS AND METHODS

The study was performed with 4 *in vitro* propagated plants (BioTree) in the period 2018-2020. The experimental plants were planted in June 2014. They are conducted as trees with a stem height of 70-90 cm and are on a suppor-ting trellis and are cultivated with drip irrigation. Additional water with 40 l/tree per week was assured during the months of July and August.

With the winter pruning (before the beginning of the vegetation), the branches growing inwards and downwards are removed. During the vegetation the current vegetative growth is shortened when reaching a length of 30 cm. Each tree is fertilized with 300 g NPK (14:10:12), first after the winter pruning and then in May before flowering. The climate in Plovdiv is typical for the temperate climate zone with 3900° active temperature sum and with precipitat-ion in the amount of about 515 mm.

The information for the dates of occurrence of the main phenological phases (beginning of vegetation, bud burst, mass flowering and fruit ripening) is given. The indexes for the high of the trees, diameter of the stem (mm), diameter and volume of the tree crowns and the yield characteristics (kg/tree) are taken into account.

Ripe fruits are those, which are shiny and rich in color. Usually they are easily separated from the stalk. The data are processed statisticaly.

RESULTS AND DISCUSSIONS

The information about the main phenophases is presented in (Table 1). The bud burst in the studied period 2018-2020 was observed in middle of March, beginning of April. The vegetation in JB 2 variety started every year earlier than in the other cultivars. The first flowers was seen in middle of April and the mass flowering occur in June.

The first red fruits were observed in June and next month is a good time for the first picking.

Flowering and fruiting, takes place over a long period of time. Harvesting once a month usually is enough. It took about 30 minutes to harvest a tree full of fruit. The last harvest in this study has been done in early November. Even during this time, flowers still were observed on the plants.

Table 1. Main phenophases in goji berry varieties in the period 2018-2020

Variant	Vegetation			Flowering			Maturity		
Сорт	2018	2019	2020	2018	2019	2020	2018	2019	2020
JB 1	14.03	25.03	14.03	09.06	05.06	29.05	13.07	18.07	29.07
JB 2	09.03	19.03	09.03	02.06	05.06	29.05	03.07	18.07	29.07
JB 4	20.03	22.03	14.03	12.06	14.06	24.05	25.07	22.07	16.07
JB 10	20.03	22.03	14.03	12.06	14.06	20.05	25.07	22.07	16.07

The results regarding the growing characteristics are presented in Table 2. The trees of the varieties JB 4 and JB 10 are higher than those of JB 1 and JB 2. The harvest of the JB 1 and JB 2 still can be done without additional equipment, while for JB 4 and JB 10 such in future probably will be needed. The results for the diameter of the stem show that after seventh vegetation (2020) the diameter of the stem in studied varieties ranged from 15.65 mm to 31.17 mm. The thickest stem form JB 2 variety, followed by JB 1. The most unstable in terms of stem thickness are the other two varieties - JB 4 and JB 10. The supporting trellis for the studied varieties is still useful and in the future removal of the supporting structure can be done first in JB 2 than in JB 1 variety.

The diameter of the crowns in the studied varieties after seventh vegetation (2020) year, is in range 81.75-136.25 cm. Variety JB 2 formed the wider crown and the narrowest have JB 4. At the end of the seventh vegetation, at the selected planting distances of 3 m/2 m, the trees have not yet completely occupied their defined area. The winter and summer pruning is helping to maintain the width of the crowns in the desired size. The trees are shown in Figures 1-4.

Table 2. Growth characteristics of goji berry plants after 7-th vegetation (2020) year

Variety	High of the trees, cm	Diametar of the stemm, mm	Diametar of the crown, cm	Volume of the crown, m^3
JB 1	196.67	21.89	120.00	0.37
JB 2	197.50	31.17	136.25	0.58
JB 4	247.50	15.65	81.75	0.26
JB 10	250.00	19.45	116.25	0.59

Significant differences by $p < 0.05\%$

Figure 1. Tree of JB 1

Figure 2. Tree of JB 2

Figure 3. Tree of JB 4

Figure 4. Tree of JB 10

The results related to the yield characteristics are presented in Table 3. The data indicate variety JB 1 as the most productive with total yield of 4.02 kg and on average 1.34 kg per tree for the studied period 2018-2020. The variety JB 2 follows the most productive variety JB 1, in terms of average yield of 0.73 kg/tree as well with total yield of 2.20 kg. Less number of fruits in total for the period 2018-2020 and average were obtained by the varieties JB 4 and JB 10, respectively 0.49 kg and 0.16 kg. Closer planting distances could compensate the low yield to some extent in those two varieties, as well as finding proper pruning methods for them.

Table 3. Yield of fresh fruit kg/tree

Variety	2018	2019	2020	average	total
JB 1	0.66	1.71	1.65	1.34	4.02
JB 2	0.62	0.71	0.87	0.73	2.20
JB 4	0.15	0.09	0.25	0.16	0.49
JB 10	0.18	0.12	0.19	0.16	0.49

Significant differences by $p < 0.05\%$

Goji berry varieties visibly differ in shape, size (Figure 5). The largest fruit had been noticed at the JB 4 variety followed by JB 10. These two varieties are sweet when are fully ripen and can be eated immediately after picking, while JB 1 and JB 2 varieties need to be dried and then consumed because of their bitter taste.

The sweetness of the JB 4 and JB 10 varieties attracts ants and insects, so it is imperative that the fruits of these two varieties to be harvested, when they acquire the characteristic ripening color. Characteristic for the ripe fruits is the easy separation of the stalk.

The skin of the JB 2 fruit, which is the juiciest, is thickness and juice is released when the fruit is detached from the stalk. In case of poor ventilation during drying, the fruits of this variety stick together and are easy damaged when separated.

Figure 5. The mature fruit of goji berry

CONCLUSIONS

The trees of JB 4 and JB 10 varieties are higher in comparison with JB 1 and JB 2.

The vegetation starts in the middle of March, first the JB 2 variety. The flowering occurs at the end of May and beginning of June and the fruits are mature in July.

The supporting trellis for the studied varieties is still useful and the future removal of the supporting structure can be done, first in JB 2 and then in JB 1 variety.

At the end of the seventh vegetation, at the selected planting distances of 3 m/2 m, for the trees have not yet completely occupied their dedicated area.

The most productive variety is JB 1 followed by JB 2 one.

ACKNOWLEDGEMENTS

This work is supported by the Center for Research, Technology Transfer and Intellectual Property Protection at the Agricultural University - Plovdiv, under project 11-19.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GROWTH AND FRUIT BEARING OF PRIMOCANE RASPBERRY CULTIVAR 'LYULIN' IN THE TROYAN REGION

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Abstract

The scientific experiment was conducted in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The objective of the study was the primocane raspberry cultivar 'Lyulin'. The vegetative indicators, such as average number of shoots (1m^2), average height (cm) and average thickness (mm) of shoots and also some reproductive indicators, such as average fruit weight (g), and average yield per one linear meter of intra-row area were analyzed. Fruit bearing was reported from the spring-summer harvest, intermediate (formed by additional fruit twigs between two fruit bearing) and summer-autumn harvest. The largest average number of shoots were reported in the first experimental year - 26.17 items. The average shoot height reached 118 cm in the first two years (2018 and 2019). The largest average shoot thickness (8.23 mm) was measured in 2019. The highest yield in the spring-summer harvest was gathered in 2020 (1.31 kg/1 m^2), from the intermediate harvest in 2019 (0.96 kg/1m^2) and from the summer-autumn harvest of the same year (2.74 kg/1 m^2). The highest average fruit weight (3.25 g) was registered in the intermediate harvest (in 2019).

***Key words**: fruit weight, cultivars, raspberries, vegetative indicators, yield.*

INTRODUCTION

Raspberry is a traditional fruit crop in the mountain and semi-mountain regions of Bulgaria, where there are suitable soil and climatic conditions for its growth. A great part of these regions are characterized by predominant clay soils, with an impermeable B horizon, with a tendency to overwetting (Hristov, 1983; Dinkova et al., 2000). Finding and applying appropriate elements of cultivation technologies are extremely important for the harvesting of plantations. Another important feature is the use of cultivars suitable for specific soil and climate conditions. Through selection, cultivars with different vegetative and reproductive potential have been created, which is manifested under conditions that correspond to the maximum degree of the genotype. The selection activity created cultivars with certain specifics in their growth and fruit bearing of raspberry plants. One of the most significant advantages of primocane cultivars is the provision of fresh fruit for a longer period of time (Boycheva, 1996; 1999; Catling and Small, 2001; Boycheva and Lazarov, 2004; Knight, 2004).

The interest in the raspberry crop is determined by the attractive fruits and the quick return on investment, through early maturity and early fruit bearing. These qualities of the crop predetermine the significant share it occupies in agricultural holdings (Stavroulakis and Gerasopoulos, 1998; Kondanova et al., 2005; Zorenc et al., 2017).

The objective of the present study was to observe the correlation dependences among some vegetative and reproductive characteristics of the primocane raspberry cultivar 'Lyulin'.

MATERIALS AND METHODS

The scientific experiment was conducted during the period of 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The objective of the study was the primocane raspberry cultivar 'Lyulin'. The following indicators were observed:

- vegetative: average number of shoots per linear meter of intra-row area, average height (cm) of shoots per 1 m^2, average

thickness of shoots (mm), measured at 10 cm height from the soil surface;
- reproductive: average fruit weight (g), average yield (kg) per 1 m^2.

The experiment was set in six replications per one linear meter each.
The methodology for studying plant resources in fruit plants was used to report the indicators (Nedev et al., 1979). Data were processed according to the methodology of Lidanski (1988) using the software product MS Excel - 2010.

RESULTS AND DISCUSSIONS

In the first experimental year the average number of shoots was the highest, compared to the next two years and was 26.17 items (Table 1). The average shoot height reached 118 cm and the average thickness was 7.38 mm. Regarding the reproductive indicators, the average fruit weight in the spring-summer harvest was 2.72 g, and from the summer-autumn harvest it had a lower value - 2.17 g. A significant difference was observed in terms of the obtained average yield. The spring-summer harvest was 0.43 kg and was higher than the summer-autumn harvest - 0.71 kg.

Table 1. Vegetative and reproductive indicators in 'Lyulin' cultivar for the period of 2018-2020

Indicators Harvests	Average number of shoots per 1/m^2	Average height of shoots (cm)	Average shoots thickness (mm)	Average fruit weight (g)	Average yield (kg) per 1/m^2
2018					
Spring-summer harvest	26.17	118	7.38	2.71	0.43
Summer-autumn harvest				2.17	0.71
2019					
Spring-summer harvest	19.5	118	8.23	2.21	0.93
Intermediate harvest				3.25	0.96
Summer-autumn harvest				2.43	2.74
2020					
Spring-summer harvest	23	91	6.84	2.64	1.31
Intermediate harvest				2.87	0.82
Summer-autumn harvest				1.93	1.44

The reported correlation dependences between the vegetative and reproductive indicators for the spring-summer harvest reported a significant correlation only for the indicators average thickness with the average height of the shoots (r = 0.65) (Table 2). While in the summer-autumn harvest, the results show a high correlation between the average yield with the average height of the shoots (r = 0.73), as well as a significant correlation in the indicators of average yield with the average plant thickness (r = 0.67) (Table 3). That is, in the primocane cultivar harvest, the average yield was directly related to the average height and average thickness of the shoots.

Table 2. Correlation dependences of vegetative and reproductive indicators from the spring-summer harvest in 2018 for 'Lyulin' cultivar

	Number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Number of shoots	1			
Average height (cm)	-0.1133	1		
Average thickness (mm)	-0.10356	0.653751	1	
Avarage yield (kg)	-0.10935	0.279603	-0.25247	1

Table 3. Correlation dependences of vegetative and reproductive indicators from the summer-autumn harvest in 2018 for 'Lyulin' cultivar

	Number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Number of shoots	1			
Average height (cm)	-0.1133	1		
Average thickness (mm)	-0.10356	0.653751	1	
Avarage yield (kg)	0.357537	0.72689	0.666838	1

In the second experimental year, a significantly lower average number of shoots was reported - 19.5 (Table 1). The average height of the plants is in the same values as from the previous year - 118 cm, but a higher average thickness was registered, which was also the highest value of the entire study period - 8.23 mm. The least average fruit weight was registered in the spring-summer fruit bearing - 2.21 g. It was the highest in the intermediate harvest - 3.25 g, as in the summer-autumn the fruit bearing was 2.43 g. A significant increase in the average yield was found. From the spring-summer harvest it was 0.930 kg, from the intermediate harvest it was 0.960 kg and from the summer-autumn harvest it was 2.74 kg.
Regarding the interrelations of vegetative and reproductive indicators, we report a high negative dependence between the average yield

and the average thickness of the shoots from the spring-summer harvest (r = -0.91) (Table 4) In the intermediate harvest, the results show a significant correlation for the same indicators, but in a positive value (r = 0.57) (Table 5). While in the summer-autumn harvest a correlation was found in the average yield with the average height of the shoots (r = 0.68) (Table 6). In this fruit bearing, as from the spring-summer harvest, a negative but moderate correlation was also reported between the average yield and the average thickness of the shoots (r = - 0.44).

Table 4. Correlation dependences of vegetative and reproductive indicators from the spring-summer harvest in 2019 for 'Lyulin' cultivar

	Number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Number of shoots	1			
Average height (cm)	-0.45201	1		
Average thickness (mm)	-0.06873	0.306181	1	
Average yield (kg)	-0.07428	-0.47308	-0.91129	1

Table 5. Correlation dependences of vegetative and reproductive indicators from the intermediate harvest in 2019 for 'Lyulin' cultivar

	Number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Number of shoots	1			
Average height (cm)	-0.45201	1		
Average thickness (mm)	-0.06873	0.306181	1	
Avarage yield (kg)	0.111939	-0.42862	0.568981	1

Table 6. Correlation dependences of vegetative and reproductive indicators from the summer-autumn harvest in 2019 for 'Lyulin' cultivar

	Number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Number of shoots	1			
Average height (cm)	-0.45201	1		
Average thickness (mm)	-0.06873	0.306181	1	
Avarage yield (kg)	-0.14723	0.683449	-0.43619	1

In 2020, an increase in the average number of shoots was reported compared to the previous year and their number was 23 (Table 1). The lowest values were found for the average height of the shoots - 91 cm and their average thickness - 6.84 mm for the entire study period. The average fruit weight in the spring-summer harvest was 2.64 g, the highest was again in the intermediate harvest 2.87 g and the lowest value was in the summer-autumn harvest - 1.93 g.

In the third year, an increase in the average yield from the spring-summer harvest was reported again and reached a value of 1.31 kg. The decrease was insignificant compared to the previous year for the intermediate harvest - 0.82 kg. The average yield in summer-autumn fruit bearing was 1.44 kg, which was approximately twice lower than the second experimental year.

For spring-summer fruit bearing, a moderate correlation was reported between the average height of the shoots with their average number (r = 0.42) and significant for the average thickness of the plants with their average height (r = 0.64) (Table 7). The relationship between the average yield and the average thickness of the shoots was moderate (r = 0.31).

Table 7. Correlation dependences of vegetative and reproductive indicators from the spring-summer harvest in 2020 for 'Lyulin' cultivar

	Number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Number of shoots	1			
Average height (cm)	0.421426	1		
Average thickness (mm)	0.0187	0.636976	1	
Avarage yield (kg)	0.135442	-0.17462	0.312694	1

Table 8. Correlation dependences of vegetative and reproductive indicators from the intermediate harvest in 2020 for 'Lyulin' cultivar

	Number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Number of shoots	1			
Average height (cm)	0.421426	1		
Average thickness (mm)	0.0187	0.636976	1	
Avarage yield (kg)	0.23147	-0.23145	-0.35626	1

In the intermediate harvest, a moderate but negative correlation was registered in the indicators of the average yield with the average thickness of the shoots (r = - 0.36).
While in the summer-autumn fruit bearing a high negative correlation was reported between the average yield with the average thickness of the plants (r = - 0.73).

Table 9. Correlation dependences of vegetative and reproductive indicators from the summer-autumn harvest in 2020 for 'Lyulin' cultivar

	Number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Number of shoots	1			
Average height (cm)	0.421426	1		
Average thickness (mm)	0.0187	0.636976	1	
Avarage yield (kg)	0.209606	-0.01885	-0.73345	1

CONCLUSIONS

The average number of shoots had the highest value in the first experimental year. The best results in terms of average plant height were reported in the first two years, and their average thickness - in the second year.
The highest average fruit weight was registered in the intermediate harvest in the second year (3.25 g). An upward increase in the average yield from the spring-summer harvest was reported during each experimental year. The highest average yield was obtained from the summer-autumn harvest in the second year - 2.74 kg.
Correlation dependences show a relationship between average thickness and average shoot height in the first and third years. A high negative dependence was reported in the spring-summer harvest between the average yield with the average thickness of the shoots (r = -0.91) in the second year.
In the summer-autumn fruit bearing in the first year there was a high correlation between the average yield with the average height of the shoots (r = 0.73) and significant in the average yield with the average thickness of the plants (r = 0.67). In the second year, at the same fruit bearing, a significant correlation was reported between the average yield with the average shoot height (r = 0.68) and high, but negative in the average yield with the average plant thickness (r = -0.73).

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VEGETATIVE AND REPRODUCTIVE CHARACTERISTICS OF RASPBERRY CANDIDATE CULTIVAR 'TROYANSKI BISER'

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Abstract

The aim was to observe some characteristics dependences between vegetative and reproductive characteristics of a candidate raspberry cultivar 'Troyanski biser' at the foothill environment with over wetting soils.
The scientific experiment was conducted during the period of 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The objective of study was a candidate cultivar 'Troyanski biser' propagated through in vitro technology. The vegetative and reproductive indicators were analyzed, such as average number of shoots (1 m^2), average height (cm) and average thickness (mm) of shoots and average fruit weight (g), and average yield (kg/1 m^2). The largest average number of shoots were formed in 2018 (17.33 total number) during the experimental period. The highest average height (146 cm) was registered during the same year. The highest values for the average thickness were registered in 2019 (9.14 mm). The average yield was comparatively stable for the whole period, as it was the highest for the second experimental year (1.33 kg).

Key words*: cultivars, fruit weight, raspberries, vegetative indicators, yield.*

INTRODUCTION

Raspberry is a half shrub fruit species that belongs to the *Rosaceae* family, of genus *Rubus*. It includes 12 subgenera, the most economically important being the subgenus *Ideobatus* and especially the European red raspberry, North American red raspberry and black raspberry (Ourecky, 1975; Ellis et al., 1991).
Currently, interest in raspberries is due to a number of advantages over some of the other fruit crops. It is a cosmopolitan fruit species, widespread in the world, characterized by early maturity, early fruiting bearing, regular and high yields and a quick return on capital investment (Hristov et al., 1988; 1991; Misić and Nikolić, 2003).
The productivity of raspberries is directly dependent on the soil and climate conditions, the applied agricultural cultivation techniques and the biological qualities of the crop.
Many researchers focus their work on the study of vegetative and reproductive manifestations. In this direction, new cultivars of raspberries are created with specific qualities and requirements, consistent with the specific growing conditions to the indicators of vegetative growth, yield, fruit characteristics and other qualities of the crop.
The selection activity is diverse, expressed in the improvement of each indicator of the manifestations of plants (Stanchev et al., 1991; Stanisavljevic et al. 2002; Leposavic et al, 2015).
The objective of the present study is to observe some relationships between vegetative and reproductive indicators of the candidate cultivar 'Troyanski biser'.

MATERIALS AND METHODS

The scientific experiment was conducted during the period of 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan.
The object of the study is the candidate cultivar 'Troyanski biser', which was selected in RIMSA.
The following vegetative indicators were observed: average number of shoots per linear meter of intra-row area, average height (cm) of shoots (1 m^2), average thickness of shoots

(mm), measured at 10 cm height from the soil surface;
Reproductive indicators: average fruit weight (g), average yield (kg) per 1 m^2.
The experiment was set in six replications per one linear meter each.
The indicators are reported according to the methodology of plant resources (Nedev et al., 1979). The software product MS Excel - 2010 was used for data processing.

RESULTS AND DISCUSSIONS

In the first experimental year, the average number of shoots was the highest - 17.33 (Table 1). The average height of the shoots reached 146 cm and the average thickness was 7.45 mm. The average fruit weight was 3.16 g, the highest value of the three-year period. The average yield was 1.24 kg per linear meter.

Table 1. Vegetative and reproductive indicators in candidate cultivar 'Troyanski biser' for the period of 2018-2020

Average number of shoots per 1 m^2	Average height of shoots (cm)	Average shoots thickness (mm)	Average fruit weight (g)	Average yield (kg) per 1 m^2
2018				
17.33	146	7.45	3.16	1.24
2019				
14.33	130	9.14	2.21	1.33
2020				
16.83	86	6.97	2.85	1.29
Average				
16.16	120.67	7.85	2.74	1.29
ẋ ±SE				
0.93	17.94	0.66	0.28	0.03
St Dev				
1.61	31.07	1.14	0.48	0.05
VC %				
9.96	25.75	14.52	17.52	3.88

The results related to correlation treatments show that a moderate correlation is observed between the average height with the average number of shoots (0.36), as well as between the average yield with the average height (0.38) and with the average thickness (0.36) of the plants (Table 2). A high but negative correlation was reported at average thickness with the average shoot number (-0.77).
In the second experimental year, the average number of shoots was the lowest - 14.33. The average height was lower, compared to the first year - 130 cm, and the average thickness was the largest of the three-year period - 9.14 mm. The average fruit weight had the lowest values, compared to the whole period - 2.21 g, but the average yield was the highest - 1.33 kg.

Table 2. Correlation dependences between vegetative and reproductive characteristics of candidate cultivar 'Troyanski biser' in 2018

	Average number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Average number of shoots	1			
Average height (cm)	0.362313	1		
Average thickness (mm)	-0.76713	-0.21095	1	
Avarage yield (kg)	-0.02305	0.379517	0.363796	1

Table 3. Correlation dependences between vegetative and reproductive characteristics of candidate cultivar 'Troyanski biser' in 2019

	Average number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Average number of shoots	1			
Average height (cm)	-0.72281	1		
Average thickness (mm)	-0.02099	0.17354	1	
Avarage yield (kg)	0.218289	0.124495	-0.49716	1

Regarding the interdependence between the indicators, there is a strong negative correlation between the average height and the average number of shoots (0.72) and a significant also negative correlation between the indicators average yield and average thickness of the shoots (-0.50) (Table 3).
In the third year, the average number of shoots was high again - 16.83. The shortest average height for the reported period was 86 cm. The same downward trend was observed in the indicator of average plant thickness. - 6.97 mm. The average fruit weight occupied an intermediate value of three years - 2.85 g.
The reported yield was 1.29 kg, which shows that the values of the indicator are relatively constant for the period.

In the third experimental year, more correlations were observed between the indicators (Table 4). A moderate correlation was observed between the average height of the shoots and their average number (0.36) and between the average yield with the average thickness of the shoots (0.49). Significant correlation dependence was observed at the average thickness with the average number of shoots (0.62), as well as between the average yield with the average height of the plants (0.64 cm). A high correlation was observed between the average thickness and the average shoot height (0.78 cm).

Table 4. Correlation dependences between vegetative and reproductive characteristics of candidate cultivar 'Troyanski biser' in 2020

	Average number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Average number of shoots	1			
Average height (cm)	0.357614	1		
Average thickness (mm)	0.620549	0.78046	1	
Avarage yield (kg)	0.261007	0.639876	0.487822	1

The average for the period, the average number of shoots per linear meter was 16.16, their average height was 120.67 cm and the average thickness - 7.85 mm (Table 1).

In terms of reproductive performance, the average fruit weight was 2.74 g and the average yield was 1.29 kg. The coefficient of variation according to the indicators is as follows, for an average number of shoots it was low - 9.96%, high for the average height - 25.75% and medium for their average thickness - 14.52%.
In the reproductive indicators, it was average for the average fruit weight - 17.52% and very low in the average yield - 3.88%.
Regarding the correlation dependences, we observe a significant correlation between the average thickness of the shoots with their average number (0.62) and the average yield with the average height (0.64 cm) (Table 5). It was high between the indicators average thickness with the average height of the plants (0.78 cm).

Table 5. Correlation dependences between vegetative and reproductive characteristics of candidate cultivar 'Troyanski biser', avarage for the period of 2018-2020

	Average number of shoots	Average height (cm)	Average thickness (mm)	Avarage yield (kg)
Average number of shoots	1			
Average height (cm)	-0.05639	1		
Average thickness (mm)	-0.22423	0.408213	1	
Avarage yield (kg)	0.18156	0.664728	-0.2446	1

CONCLUSIONS

The highest number of shoots, average height and average fruit weight were reported in the first experimental year, average thickness and average yield in the second year.
High negative correlations were reported between the average shoot thickness with the average number of shoots (-0.77) in the first experimental year and between the average height with the average number of shoots (-0.72) in the second year.
In the third year, a positive correlation was found in the average thickness of the plants with their average height (0.78).
On average for the period a significant correlation was registered between the average yield and the average height of the shoots (0.66).

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON LIMITING THE MANIFESTATION OF FRUIT STORAGE DISEASES THROUGH THE USE OF TREATMENTS WITH BIOLOGICAL PRODUCTS

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Abstract

In the context of organic farming, the control of plant diseases is done by applying certain methods that, depending on the time and method of application, can be preventive and curative. Deposits are usually additional sources of infestation with diseases that begin in the orchard. This paper proposes laboratory cultures of pathogens such as Venturia inaequalis, Monilinia fructigena, Gloeosporium fructigenum, Botrytis cinerea, Penicilium expansum and Pezicula alba, in an attempt to limit the evolution of the diseases produced until the total cessation of evolution. After cultivation on MS culture medium enriched with vitamins and sucrose in a sterile manner, the pathogenic material is allowed to develop. After 6 days, treatments were made with colloidal silver and with a biopesticide solution based on essential oils made according to our own recipe. In both cases, the area of infection was considerably reduced, the best results being obtained, however, after the treatment with the LIVAL 1 Biopesticide.

Key words*: organic farming, deposit diseases, biopesticide.*

INTRODUCTION

In the chemical composition of some plants from spontaneous or cultivated flora there are some biologically active substances with antimicrobial action (Ardelean, 2000; Docea, 2008). They act either directly on pathogens, inhibiting their development (fungistatic) or killing them (fungicide), or directly on the attacked plant, which stimulates them, morphologically or functionally, an adequate system of defence (strengthening its immune system), for example, soy lecithin prevents the attack of cucumber powder by spraying the leaves of the plants weekly with a solution in a concentration of 0.15% (Ion et al., 2013).

Some studies have also shown that some species of *Streptomyces* sp. are characterized by the ability to produce substances with antibiotic action (streptomycin) and control many pathogenic fungi of the root system (*Pyrenochaeta lycopersici*, *Fusarium oxysporum, Pythium debaryanum*, *Verticillium dahliae*, *Phytophthora capsici*, etc.).

These methods are particularly important in plant protection because they capitalize on the natural (genetic) properties of plants, have no impact on the environment and are relatively inexpensive. Although the name is common to all categories of pathogens, genetic methods are very different, consistent with the characteristics of each category and species of pathogens and cultivated plants (Beers et al., 1993). Plant improvement results in new varieties with superior qualities, including increased resistance to pathogens (Ion, 2007). Resistance is not only mechanical, but also chemical, under the action of a pathogen giving rise to various substances with a defensive role. These mechanisms are genetically determined and can be manipulated by various specific techniques (Hatman, 1989; Bruma, 2004).

Chemical control, which can be carried out using:

- sodium silicate, which is used to control the species *Botrytis, Sclerotinia, Pythium*;
- compost extract, which can be used to control powdery mildew;
- potassium permanganate, which is used only for fruit trees and vines to control powdery mildew;
- products based on copper, in the form of copper hydroxide, copper oxychloride, copper (tribasic) sulphate, copper oxide, copper octanoate, which are used, for example, to control downy mildew in grape

vines or scab to pomaceous before flowering. The total dose of copper must not exceed 6 kg/ha/year;
- sulfur-based products, which are used to control powdery mildew on vines and fruit trees;
- calcium hydroxide, which is used only in fruit trees, including nurseries, to control branch ulceration (Ion, 2017).

Various products have been used in the past: Trichodermin with spores of the fungus *Trichoderma lignorum*, to control the fungi *Fusarium* sp., *Rhizoctonia solani*, *Pythium debaryanum*; Sandstone-extract from the capitulas of the plant *Helichrysum arenarium*, with stimulating effect for culture plants and pathogen against fungi *Corynebacterium michiganense* and *C. insidiosum* (Hatman, 1989; Gareth, 2005; Amzar, 2008). Phytobacteromycin extracted from *Actinomyces lavandulge*, with complex effect on bean diseases; Unanin obtained from St. John's wort, very effective against mosaic, stolbur and brown spot of tomatoes (Munteanu, 2008; Stoleru, 2008). *Clonostachys* is a fungus that acts in the soil as a saprophyte, and is characterized by a specific spiral growth. Due to the production of toxic metabolites, this fungus has a good antagonistic activity against several pathogens in the soil. It can also invade and destroy resistant forms of pathogens, such as sclerotia (Stoleru, 2008; Hatman, 1989).

Potassium permanganate. Action: disinfectant, inhibits the growth of fungi and bacteria. Use: it is used in a concentration of 0.01-0.03% (1-3 g per 10 l of water) for the treatment of seedling roots (Bălan, 2003; Munteanu, 2000).

According to Calin (1989), Duza (2008) and Toncea (2002), over 1450 species of plants with insecticidal effect grow worldwide, of which only about 50 are useful. As far as our country is concerned, too few of the 200 species credited with this action have been or are actually used for this purpose and still less studied from this point of view (Davidescu, 1994).

One way to control plant diseases is to induce and increase their own protection mechanisms (phytoalexins and elicitors), which would avoid the use of toxic components for plants (Pop, 1975).

One of the objectives of the research of new compounds with phytosanitary uses is to look for new structures from natural plant sources. Many researchers, especially from countries with increased biodiversity, have contributed to the detection of new compounds derived from medicinal plants for phytosanitary uses (Rosca et al., 2008).

MATERIALS AND METHODS

The biological material used refers to 5 pathogens that can cause storage diseases, thus causing major damage to many fruits during their storage period.

Figure 1 and Figure 2. Samples of pathogenic material taken for the experiment (*Venturia inaequalis* and *Monilinia fructigena*)

The experiment was performed in the virology laboratory of the HORTINVEST Research Center. Samples of pathogenic material were taken from pathogenic microorganisms, namely:
- *Venturia (Endosigme) inaequalis*, which produces brown or rotting spots and is manifested on the fruit by more or less circular spots, from a few millimeters to a centimeter in diameter, gray, covered by a velvety layer consisting of conidiophores and conidia mushrooms.
- Brown rot or *Moniliosis* comes in three forms: brown rot, black rot, and heart rot.
- Bitter rot, caused by the fungus *Gloeosporium fructigenum*, which infects fruits in full maturity. In the deposit, it is manifested by the appearance of large, brown spots, developed on any part of the fruit, around the lentils, but especially in the peduncular area.
- *Botrytis cinerea* manifests itself on the fruit during the growing season and continues to grow during the storage period

- *Pezicula alba* with the conidial shape *Gloeosporium album* is very common in some apple stores. The infection occurs in the orchard but continues during storage, the fungus penetrating the fruit, especially through the lentils, but also through unhealed wounds.


Figure 3 and Figure 4. Samples of pathogenic material taken for the experiment (*Gloeosporium fructigenum* and *Botrytis cinerea*)

- The blue rot, produced by the fungus *Penicilium expansum* is manifested in conditions of high humidity and temperature and lack of aeration by the appearance on the surface of the fruit of circular, brown spots, which spread rapidly and on which develop piles of conidiophores and conidia, arranged irregular, at first white, then bluish green. The pulp of infected apples and pears rots.


Figure 5 and Figure 6. Samples of pathogenic material taken for the experiment (*Penicilium expansum* and *Pezicula alba*)

The culture medium represents the physical and chemical support necessary for the growth and development of any living organism, including pathogenic ones.
The culture medium must respond through its composition, nutritional and hormonal requirements of the body.
Following our own research, the composition of the culture medium that ensures an optimal germination of pathogenic spores is presented in Table 1.

Table 1. Establishing the recipe for the optimal culture medium for the growth of pathogenic microorganisms

No.	Substance	Concentration	Quantity
1	Macro MS	10 x	100 mL
2	Micro MS	1000 x	1 mL
3	Iron chelate	200 x	5 mL
4	Vitamins MS	100 x	10 mL
5	Inositol	100 x	10 mL
6	BAP	-	2 mg/L
7	GA	-	1 mg/L
8	IBA	-	0.1 mg/L
9	Sucrose	-	30 g/L
10	Agar	-	6 g/L
Culture medium pH = 5.8 (corrected with 1 N NaOH)			

Inoculation was performed under aseptic conditions, with sterile instruments under a laminar flow hood. In each petri dish with culture medium, an essay with pathogenic biological material from the 6 pathogenic species was inoculated on the same day.


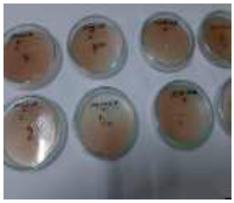

Figure 7 and Figure 8. Inoculation of the pathogenic material on culture medium

RESULTS AND DISCUSSIONS

On March 3, 2020, the inoculations were made with the culture medium established in its own recipe that supports the growth and development of any living organism. For 5 days the samples were left to incubate, then the infected areas were measured at intervals of 4 days for each pathogen, resulting in a percentage of the area of infection that was reduced after treatment. Table 2 shows the results regarding the limitation of the infection surface following the colloidal silver treatment. Thus, following the incubation of pathogens on March 5, 2020, the initial area of infection was measured. For *Pezicula alba*, with the conidial form *Gloeosporium album*, the initial infection surface was 12 mm^2, which after the treatments with colloidal silver was reduced to 4 mm^2, respectively 2 mm^2, with a reduction of the infection surface by 66.67% and 83.33%, at intervals of 4 days. For *Monilinia fructigena*, the initial infection area was 6 mm^2 which after the colloidal silver treatments was reduced to 4 mm^2 and 2 mm^2, respectively, with a reduction of the infection area by 33.33% and 66.67%, respectively, at intervals of 4 days. For *Venturia inaequalis*, the initial area of infection was 4 mm^2 which after the treatments with the initial colloidal silver was not reduced remaining 4 mm^2, then was reduced to 2 mm^2, with a reduction of the infection area by 0.0% respectively 50.00%, at intervals of 4 days. For *Penicilium expansum*, the initial infection area was 12 mm^2 which after the colloidal silver treatments was reduced to 2 mm^2 and 1 mm^2, respectively, with a reduction of the infection area by 83.33% and 91.67%, respectively, at intervals of 4 days.

Table 2. Results regarding the limitation of the infection surface following the treatment with colloidal silver

Infection area (mm^2) before and after treatment with colloidal silver					
Storage disease	Initial	After treatment with colloidal silver mm2)	After treatment with colloidal silver mm2)	After treatment with colloidal silver (%)	After treatment with colloidal silver (%)
	March 5	March 9	March 13	March 9	March 13
Pezicula 1	12	4	2	66.67	83.33
Monilinia 1	6	4	2	33.33	66.67
Venturia 1	4	4	2	0	50
Penicilium 1	12	2	1	83.33	91.67
Gloeosporium fructigenum	4	1	0.25	75	93.75
Botrytis 1	9	1	0.25	88.89	97.22
Average	47	16	7.5	57.87	80.44

For *Gloeosporium fructigenum*, the initial area of infection was 4 mm^2 which after treatments with the initial colloidal silver was reduced to 1 mm^2, then was reduced to 0.25 mm^2, with a reduction in the area of infection by 75.0%, respectively 93.75%, at intervals of 4 days.

For *Botrytis cinerea*, the initial area of infection was 9 mm^2 which after treatments with the initial colloidal silver was reduced to 1 mm^2, then was reduced to 0.25 mm^2, with a reduction in the area of infection by 88.89%, respectively 97.22%, at an interval of 4 days. At an average infection area of 47 mm^2, in the end this area was reduced by 80.44% in two applications every 4 days. The results are also observed spatially in Figure 9.

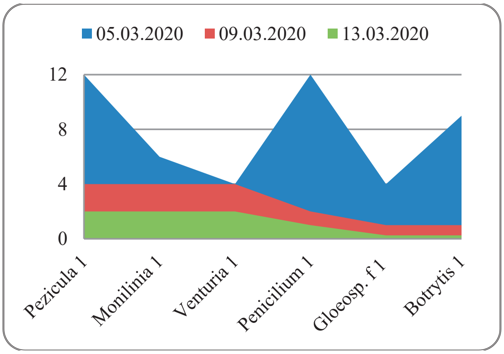

Figure 9. Results regarding the evolution of the infection surface after the colloidal silver treatment

Regarding the results obtained from the treatments with essential oils, the determinations were made after the incubation period on March 5, 2020 and are presented spatially in Figure 10.

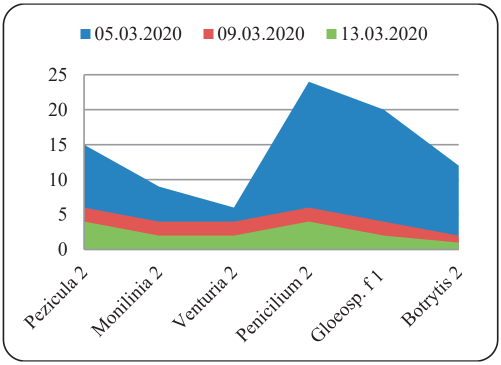

Figure 10. Graph regarding the evolution of the infection surface following the treatments with essential oils

Table 3 presents results regarding the evolution of the infection surface following the treatments with essential oils, measured at a distance of 4 days. For *Pezicula alba*, with the conidial form *Gloeosporium album*, the initial infection area was 15 mm^2, which after the treatments with essential oils was reduced to 6 mm^2 and 4 mm^2, respectively, with a reduction of the infection area by 60.00% and 73.33%, at intervals of 4 days. For *Monilinia fructigena*, the initial area of infection was 9 mm^2 which after the treatments with essential oils was reduced to 4 mm^2 and 2 mm^2, respectively, with a reduction of the infection area by 55.56% and 77.78%, respectively, at intervals of 4 days. For *Venturia inaequalis*, the initial area of infection was 6 mm^2 which, after treatment with essential oils, was reduced to 4 mm^2, then reduced to 2 mm^2, with a reduction in the area of infection by 98.0% and 66. 67%, respectively, at intervals of 4 days.

Table 3. Results regarding the evolution of the infection surface following the treatments with essential oils

Infection area (mm^2) before and after treatment with essential oils					
Storage disease	Initial	After treatment with essential oils (mm^2)	After treatment with essential oils (mm^2)	After treatment with essential oils (%)	After treatment with essential oils (%)
	March 5	March 9	March 13	March 9	March 13
Pezicula 2	15	6	4	60	73.33
Monilinia 2	9	4	2	55.56	77.78
Venturia 2	6	4	2	98	66.67
Penicilium 2	24	6	4	75	83.33
Gloeosporium fructigenum	20	4	2	80	90
Botrytis 2	12	2	1	83.33	91.67
Average	86	26	15	75.31	80.44

For *Penicilium expansum*, the initial area of infection was 24 mm^2 which after the treatments with essential oils was reduced to 6 mm^2 and 4 mm^2, respectively, with a reduction of the infection area by 75.00% and 83.33%, respectively, at intervals of 4 days.
For *Gloeosporium fructigenum*, the initial area of infection was 20 mm^2 which after the treatments with essential oils was reduced remaining 4 mm^2, then was reduced to 2 mm^2, with a reduction of the infection area by 80.0%, respectively 90.00%, at intervals of 4 days.
For *Botrytis cinerea*, the initial area of infection was 12 mm^2 which after the treatments with essential oils was initially reduced remaining 2 mm^2, then was reduced to 1 mm^2, with a reduction of the infection area by 83.33% respectively 91.67%, at intervals of 4 days.
Regarding the treatments with essential oils at an average infection area of 86 mm^2, there is a reduction of the infected area in proportion of 80.46% applied in 2 treatments at intervals of 4 days, which demonstrates a good effectiveness.

CONCLUSIONS

Medicinal plants and essential oils represent the oldest category of therapeutic remedies that has accompanied mankind throughout its historical evolution, now used in organic farming. More recently, colloidal silver is also used in integrated control and organic farming. It is already known worldwide that silver nanoparticles (AgNPs) are the most widely used and widespread nanotechnological material. Colloidal silver solutions can contain several types of material, as far as silver is concerned, because the notion of "colloidal" only defines the type of solution (a colloid) and not its content. In the case of colloidal silver treatments, at an average infection area of 47 mm^2, in the end this area was reduced by 80.44% in two applications every 4 days. Regarding the treatments with essential oils at an average infection area of 86 mm^2, there is a reduction of the infected area in proportion of 80.46% applied in 2 treatments at intervals of 4 days, which demonstrates a good effectiveness. The differences between the 2 products is not very large which concludes that both forms are equally effective.

ACKNOWLEDGEMENTS

This study was produced from the PhD thesis.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

BLUEBERRY(*VACCINIUM CORYMBOSUM*) BREEDING PROGRAMME IN THE MAIN CULTIVATING COUNTRIES

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Abstract

Cutivated blueberies (Vaccinium corymbosum) are known as some of the most important berries for health support, being highly valued for fresh consumption, having sizes two to four times larger than those of wild blueberries (Vaccinium myrtillus), and the nutrient content exceeds those of black blueberry or wild blueberry in the spontaneous flora. This species prefers temperate climate and precipitation more than 700 mm annually. Over the last one hundred years, the geographical area cultivated with the blueberry is widely expanded. European countries have gradually become interested about North American blueberry. The highbush breeding programme began in 1908. The main objectives of the breeding programme are: increased adaptability on low acid soils (pH to 6), extending the harvest season, improving the quality parameters of the fruits (weight, firmness, dry pedicelar scar, high sugar content, thick pruin layer). As a research methodology, it is used the selection of germplasm genitors, the directed hybridization, the production of biological material for nurseries, the evaluation of hybrids and of new selections. As a results of blueberry breeding programme, in the last decade, in the world over 342 blueberry cultivars were registered.

Key words: *breeding programme, highbush, blueberry.*

THE IMPORTANCE OF BLUEBERRY CROP

Blueberry, *Vaccinium* sp., is native to North America, especially Canada, the Eastern and Southern United States, and is represented by about 150-450 species in 30 subgenera (Luby et al., 1991), spread between the 45 ° and 71 ° parallels in North America, Europe and Asia, where it grows in mountainous areas and on acid soils (Bîstrova A. et al., 1968).

The Genus *Vaccinium* L. is a common and widespread dwarf shurb of the *Ericales* order, family *Ericaceae*, Subfamily *Vaccinioideae.* Blueberries are ranked under the family *Ericaceae*, subfamily *Vacciniaceae*, genus *Vaccinium*, and subgenus *Cyanococcus* which are most commonly growing in acid and infertile soil conditions (Ștefănescu et al., 2019). Commercially important species are found in the subgenera C*yancoccus*, *Vitis-Idaea* and *Myrtillus*. The highbush blueberry is derived from two main species, *Vaccinium australe* and *Vaccinium corymbosum* (Vander K., 1988). The breederes made numerous crosses within the subgenus *Cyancoccus* including the tetraploid *V. corymbosum* x the tetraploid *V. angustifolium* (Luby et al., 1991), most of the cultivated *Vaccinium* species belong to the section *Cyanococcus* subgenus (Table 1).

According to Romanian Pomology, vol. VII, published in 1968, the most important blueberry species are the following: *Vaccinium corymbosum* L., synonym *Vaccinium australe* Small. Highbush blueberry is widespread along the Atlantic coast from Florida to Maine, West and South of Michigan. It is a 1-4 m tall plant, which grows in the form of a bush with large fruits (up to 2 cm in diameter). *Vaccinium myrtillus* L. - black blueberry - is widespread in Europe and North Asia.

In Romania it is spread from the region of high hills to the alpine area, occupying large areas in spruce forests, brown soils, podzolic or primary podzols and beech forests on primary podzols, preferring acid soils. *Vaccinium uleginosum* L. (Bog bilberry, northern bilberry, western blueberry) - the wild bilberry - is spread to Europe, Asia and America. In our country it can be found in the Alpine and subalpine region, through forests, bushes, meadows, on poor soils. *Vaccinium lamarckii* Camp., synonyms *V. angustifolium* Ait., *V. angustifolioum* var. *laevifolium*, *V. pennysilvanicum* Lam. var *angustifolium* (lowbush blueberry) - the American dwarf blueberry - is widespread in North America in a large area from northeast Maine to West Virginia, south Wisconsin and Minnesota west. The selections from *V. lamarckii* Camp. are used in genetic improvement of highbush blueberry.

Table 1. Taxonomy and spread of blueberry species

Genus	Species	Ploidy	Spreading area
Cyanococcus	*V. angustifolium* Ait. (Lowbush blueberry)	4x	N-E North America
	V. ashei Reade. (Rabbiteye blueberry)	6x	S-E North America
	V. boreale Hall & Aald (Northern blueberry)	2x	N-E North America
	V. constablaei Gray (Hillside blueberry)	6x	S-E North America
	V. corymbosum L. (Highbush blueberry)	2x	S-E North America
	V. corymbosum L. (Highbush blueberry)	4x	E North America
	V. darrowii Camp (Darrow's blueberry)	2x	S-E North America
	V. fuscatum Ait. (Black highbush)	2x	Florida
	V. myrtilloides Michx. (Velvetleaf huckleberry)	2x	Central North America
	V. pallidum Ait. (Blue ridge blueberry)	2x, 4x	Mid- Atlantic North America
	V. tenellum Ait. (Small black blueberry)	2x	S-E North America
	V. elliottii Chapm. (Elliott's blueberry)	2x	S-E North America
	*V. hirsutum (*Hairy blueberry)	4x	S-E North America
	V. myrsinites L. (Shiny blueberry)	4x	S-E North America
	V. simulatum Small (Upland highbush blueberry)	4x	S-E North America
Oxycoccus	*V. macrocarpon* Ait. (Large cranberry)	2x	North America
	V. oxycoccos L. (Cranberry, Mossberry)	2x, 4x, 5x, 6x	Circumboreal
	V. erythrocarpum	2x	S-E North America and E. Asia
	V. microcarpum (Small Cranberry)	2x	Circumboreal
Vitis-Idaea	*V. vitis-idaea* L.	2x	Circumboreal
Myrtillus	*V. myrtillus* L.	2x	Circumboreal
	V. deliciosum (Cascade bilberry)	4x	N.W. North America
	V. cespitosum	2x	North America
	V. membranaceum (Mountain bilberry)	4x	W. North America
	V. ovalifolium (Oval-leaved bilberry)	2x	Circumboreal
	V. parvifolium (Red bilberry)	2x	N.W. North America
	V. scoparium (Grouseberry)	2x	N.W. North America
Pyxothamnus	*V. consanguinem (Costa Rican blueberry)*	2x	S. Mexico and Central America
	V. ovatum (California Huckelberry)	2x	N.W. North America
Bracteata	*V. bracteatum (Sea bilberry)*	2x	E. Asia, China and Japan
Vaccinium	*V. uligiosum* L. (Bog bilberry)	2x, 4x, 6x	Circumboreal

Source: from Ballington (1990), Luby et al. (1999), Suda (2003), Hancock et al. (2008) cited by Guo-Qing Song and James F. Hancock (2011)

The Order *Ericales,* Subfamily *Vaccinoidae* includes many species of berries whose fruits have benefits for health. Highbush blueberry (*Vaccinium corymbosum*) is known as one of the most important berries for fresh consumption, being two to four times larger than wild blueberry (*Vaccinium myrtillus*) and its nutrient content exceeds that of black blueberry or wild blueberry from spontaneous flora (Botez, 1984). Anthocyanins found in the blue epidermis fruits with strong antioxidant, anti-inflammatory, antibacterial effect, blueberries with a high antioxidant capacity. Nutritional value of fruits indicates that blueberries are a high source of minerals (Ca, K, Na, Mg, Zn, Fe), vitamins (A, C, B1, B2), but also protein (Table 2).

Table 2. Nutritional values per 100 g of fruit *Vaccinum myrtyllus*

Principles	Nutritional values	% RDA
Energy	57 Kcal	3%
Carbohydrates	14.49 g	11%
Protein	0.74 g	1%
Total fat	0.33 g	1%
Cholesterol	0 mg	0%
Fiber	2.4 g	6%
Vitamins		
Folați	6 μg	1.5%
Niacină	0.418 mg	2.5%
Pantothenic acid	0.124 mg	2.5%
Pyridoxine	0.052 mg	4%
Riboflavin	0.041 mg	3%
Vitamin A	54 IU	2%
Vitamin C	9.7 mg	1.5%
Vitamin E	0.57 mg	4%
Vitamin K	19.3 μg	13%
Electrolites		
Sodium	1 mg	0%
Potassium	77 mg	3%
Minerals		
Calcium	6 mg	0.5%
Iron	0.28 mg	3.5%
Magnesium	6 mg	1.5%
Manganese	0.336 mg	14%
Zinc	0.16 mg	1.5%
Micro-nutrients		
α-carotene	0 μg	-
ß-carotene	32 μg	-
Lutein-zeaxanthin	80 μg	-

Asănică A., 2015

Consumption of blueberry fruits keeps the brain healthy, because the vitamins and minerals found in the composition of these fruits nourish nerve cells, thus prolonging life. Blueberries are also called "vegetal insulin" because they can be consumed by diabetics. Many factors, including genetics, growing conditions, fruit ripening and other variables affect the nutritional levels of the fruit (Frith 1993; Gary 2004; Kuepper 2004; Hedrick 1925). The blueberry fruit content is about 83% water, 0.7% protein, 0.5% fat, 0.5% ash and 15.3% carbohydrates (Hancock et al., 2003). In processed form, blueberries are consumed as jam, marmalade, syrup, liqueur, juice, compote, frozen fruit (Ghena, 2003). The beneficial properties of blueberry were discovered already by the North American Indians, who consumed blueberry tea for body weight control. Thus, shoots and leaves of blueberry have a high content of substances, such as: thiamine, arbutin, hyminic acid, palmitic acid and can be used for medicinal purposes, to treat diseases of the stomach, small intestine, but also in the treatment of diabetes (Hedrick, 1925; Frith, 1993; Gary, 2004; Kuepper, 2004). The leaves can be used to prepare tinctures and teas and help improve visual acuity, preventing the evolution of ophthalmic diseases (Botez, 1984). First breeders performed numerous crosses within the subgenus *Cyancoccus* including the tetraploid *V. corymbosum* x the tetraploid *V. angustifolium* (Luby et al., 1991). The genetic gain plays an essential role in increasing blueberry production (Gallardo et al., 2018).

Highbush blueberry (*Vaccinium corymbosum*) together with the other commercial berry species are classified as the second most economically important berry after strawberry in the U.S., the U.S. is the world biggest blueberry producer.

Over time, it was difficult to estimate worldwide area harvested, production or consumption of *Vaccinium corymbosum*, but according to data reported in 2018 by FAOSTAT, currently the world area harvested with blueberry is 109,270 ha. Of the continents, the largest blueberry growing are America (86,718 hectares) and Europe (20,718 hectares). Asia and Africa have registered only 96 and 18 ha. In 2018, the area harvested by major blueberry growing countries is as follows: Canada (40,998 ha), USA (36,098 ha), Poland (8,089 ha), Germany (3,040 ha), France (2,393 ha), Netherlands (934 ha), Russian Federation (634 ha), Romania (183 ha, Table 3). Worldwide production of blueberry increased in recent years. In the world the largest producer of blueberries (in thousand tons) is the United States - 255,050 in 2018 followed by Canada - 164,205, Spain - 43,516 (FAOSTAT, 2018, Table 4). In Europe, important productions are obtained also by: Poland - 25,301, Germany - 12,764, Netherlands - 10,257, France - 9,127, Russian Federation - 3,300, Italy - 1,675, Romania - 684 and Switzerland - 353.

Table 3. Area harvested of blueberry in the world and in major growing countries

World/ country	**Area harvested/year (ha)**										
	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
World	74.912	72.711	76.455	82.346	83.593	87.567	94.520	104.754	103.832	103.239	109.270
America	58.651	61.048	62.945	69.069	69.133	72.305	78.483	90.857	86.271	82.990	86.718
Europe	14.933	10.352	12.124	11.872	12.795	13.456	14.339	12.128	14.995	18.224	20.407
Asia	100	100	100	110	114	90	89	105	103	97	96
Africa	11	12	13	15	15	15	16	17	18	18	18
Canada	34.109	34.848	34.277	38.493	36.929	37.569	40.443	49.977	42.570	41.569	40.998
USA	24.480	26.010	28.530	29.660	30.919	32.796	34.297	36.349	37.555	33.953	36.098
Spain	477	499	522	547	572	975	1.760	1.803	2.260	3.260	3.722
Poland	2.256	2.366	2.167	2.404	3.126	3.223	3.470	3.230	5.039	7.070	8.089
Germany	1.406	1.426	1.429	1.434	1.835	2.031	2.083	2.479	2.714	2.844	3.040
France	2.800	2.800	2.640	2.455	2.458	2.421	2.489	2.484	2.440	2.440	2.393
Netherlands	419	526	535	584	586	574	639	737	777	832	934
Russian Federation	500	500	500	500	500	500	500	500	607	529	634
Romania	808	741	735	636	544	480	546	150	128	132	183
Italy	173	171	172	172	172	173	172	173	172	172	171
Switzerland	39	42	46	55	66	73	73	76	83	93	85

FAOSTAT, 2008-2018

Table 4. Production of blueberry in the world and in major growing countries

World/ country	**Production/year (thousand tons)**										
	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
World	314.895	339.517	327.866	363.776	403.624	434.520	546.384	555.721	641.518	606.206	682.790
America	253.926	273.398	273.565	312.348	345.931	367.937	467.178	473.270	551.122	497.848	554.311
Europe	55.087	60.163	47.970	45.232	50.647	58.876	71.923	74.877	79.840	99.872	119.570
Asia	600	700	800	600	700	600	600	700	693	663	662
Africa	50	54	59	65	68	68	70	75	78	81	78
Canada	95.516	102.753	83.550	104.294	126.012	109.550	183.649	193.469	232.798	173.641	164.205
USA	158.260	169.050	188.926	201.030	212.168	246.559	262.498	254.012	269.257	235.206	255.050
Spain	6.374	6.535	6.696	6.858	7.019	11.912	21.060	21.060	24.945	35.355	45.516

FAOSTAT, 2008-2018

OBJECTIVES OF PERSPECTIVE FOR BLUBERYY BREEDING

The breeding of new cultivars will continue to play a major role in the growth of the blueberry market worldwide. In the last years, breeding programmes have obtained new cultivars that have supported the spread of blueberry into new growing areas, such as low-chill regions and the southern hemisphere (Gallardo et al., 2018). Other desirable objectives for blueberry breeding include tolerance to the high soil pH (with pH up to 6), chilling requirement, cold hardiness, early bloom, ripening interval, frost tolerance for expand the harvest season to cover regions where the production is low (Hancock et al., 2008), for obtaining mechanized harvestable plants to reduce one of the technological branch costs, fruit quality parameters improvement (high weight, crisp texture, good flavour, firm texture, dry stem scar, thick pruin layer, an optimal sugar-to acid ratio, light blue color) and the disease resistance.

RESULTS AND DISCUSSIONS

The first *Vaccinium corymbosum* plantations were established in New Jersey (1910), and soon expanded (Hancock, 2006a) to North Carolina (1920) and Michigan (1930). In the early 1900s, Dr. Frederick V. Coville showed interest in blueberry breeding, obtaining the first hybrid in 1908. His first cultivar was named 'Brook' in New Hampshire (USA). He studied the species' requirements for soil pH, the need for cold, developed some cutting methods and improving it. In his research, he collaborated with Elizabeth White, breeding numerous hybrid combinations, which resulted in over 30 selections, which later became varieties, many of them still cultivated today: 'Bluecrop', 'Weymouth', 'Blueray', 'Rubel', 'Berkeley','Coville', 'Jersey' cv.

European breeding of *Vaccinium corymbosum* began after 1920 for the first time in Netherlands. In the next years, other countries (Poland, Germany etc.) became interested by that new species. In Germany, in 1929 Dr. Hermann of the Landsberg Plant Breeding Institute (now Gorzów Wlkp.) has made great progress in blueberry breeding and cultivation. Through his research activity, as well as collaboration with other researchers, from the breeding of numerous hybrids resulted the next cultivars: 'Blauweiss - Goldtraube', 'Blauweiss - Zukertraube', 'Heerma', 'Rekord', 'Ama' and 'Gretha'. After 1980, areas harvested with highbush blueberry have increased, mainly concentrating in Germany, Poland, Spain, Romania, Italy (Strik and Yarborough, 2005).

The most important globally representative units for breeding activity are Florida Foundation Seed Producers, Mountain Blue Orchards, Fall Creek Farm and Nursery, Costa Exchange Limited, University of Georgia Research Foundation, The New Zealand Institute for Plant and Food Research, Driscoll Strawberry Associates, Prunus Persica, Berry Blue, Next Progeny, Planasa - Inovation in Plant Varieties, Research Institute for Fruit Growing, Pitești-Mărăcineni etc. (Table 5).

Part of work of some the main centers of breeding is as follows:

Fall Creek Farm and Nursery, activity began in 1978, gradually developing, with the help of a large team, dedicated to innovation in production and customer service. The breeding process carried out here was complemented by the evaluation and selection of genotypes in different areas around the world. The main objectives of the breeding programme are those related to fruit quality (size, attractiveness, shelf life) and adaptability to different areas of cultivation (high and low altitudes, climate with low and high temperatures). Over the time, the breeding process increasead resulting many varieties (Table 6).

Table 5. The main Breeding Centers

No.	Breeding Center	No. of cultivars
1	Florida Foundation Seed Producers. Inc.	49
2	Mountain Blue Orchards Pty. Ltd.	27
3	Fall Creek Farm and Nursery. Inc.	50
4	Costa Exchange Limited	23
5	University of Georgia Research Foundation. Inc.	22
6	The New Zealand Institute for Plant and Food Research Ltd.	21
7	Driscoll Strawberry Associates. Inc.	20
8	Prunus Persica Pty Ltd.	18
9	Berry Blue. LLC	15
10	Next Progeny Pty. Ltd	14
11	Research Institute for Fruit Growing. Pitești-Mărăcineni	9

Brevis (2019)

Table 6. Blueberry cultivars registered in the last decade (2009-2019)

No.	Continent/Country	No. of cultivars
1	Europe	154
2	USA	131
3	Australia	97
4	Chile	87
5	New Zealand	80
6	Mexico	77
7	South Africa	75
8	Peru	62
9	Canada	40
10	Japan	37
11	Turkey	29
12	Ukraine	21
13	Argentina	20
14	Colombia	19
15	Brazil	18
16	Morocco	18
17	Serbia	18
18	Georgia	15
19	Ecuador	10
20	Israel	9
21	Russia	7
22	Uruguay	7
23	Switzerland	6
24	Kenya	5
25	China	4
26	Estonia	4
27	Sweden	3
28	Latvia	2
29	Costa Rica	1
30	Finland	1
31	Poland	1

Brevis (2019)

In late 2019, this company launched The Fall Creek Collection, its new blueberry genetics assuring blueberry varieties for all chill levels.

Mountain Blue Orchards Pty Ltd is a centre in Australia dedicated to blueberry culture with 26 years of experience. It produces 250,000 plants annually of warm climate southern highbush and rabbiteye blueberry varieties for Australia and various export destinations. The latest varieties from Florida, Georgia and Mississippi are cultivated.

Planasa - Innovation in Plant Varieties, is another main centre with uses major part of its resources to develop new cultivars using selection breeding.

The centre began its activity in France, in 1887. Planasa has four major centres all around the world, and also smaller ones in different climatic areas to test new cultivars. It has more than 1,500 hectares in Spain, Poland, Morocco, California in the United States, Mexico and Peru (https://planasa.com/en/nursery/.

The New Zealand Institute for Plant and Food Research Ltd. - In New Zealand, the blueberry was introduced in the 1950s from North America. The blueberry breeding centres are located in North and South Island, New Zealand and a smaller programme at Dierking's Nursery in Germany. The breeding objectives of Plant & Food Research Ltd. are improving the quality parameters of the fruits (fruit size, firmness, taste, color), ripening season and disease resistance.

The Institute of Pomology and Floriculture (now the Institute of Horticulture) in Skierniewice, Poland, began the breeding programme of *Vaccinium corymbosum* L. in 2008 in the Plant Breeding Department using the first crossings of parental forms.

A few years later the first seedlings were planted in the selection plot in the Experimental Orchard in Dąbrowice. The targets of the blueberry breeding are high productivity of the shrubs, good quality of the berries, both internal and external, good firmness and shelf life. Poland is the first European producer and exporter of blueberries.

Over the years, due to the temperate climate of Romania favourable to the growth and development of fruit species, many fruit growers have selected particularly high quality local varieties enjoying appreciation both in the country and abroad (Cociu, 1977). The activity of breeding the assortment of highbush blueberry, carried out at the **Research Institute for Fruit Growing, Pitești-Mărăcineni** began in 1982 (Mladin P. et al., 2012) still has the objectives: increased adaptability on low acid soils (with pH up to 6), extending the harvest season, and improving the quality parameters of the fruit (weight, firmness, high sugar content, thick pruin layer). As a research methodology, the selection of germplasm genitors is used. The directed hybridization was mainly used, base on the rich genofond collected in Pitești and containing of varieties, species and native wild forms or introduced from abroad (Mladin P. et al., 2007).

The wide genetic diversity and the large volume of work made it possible after 1990, for a number of 9 cultivars ('Simultan', 'Pastel', 'Lax', 'Delicia', 'Prod', 'Azur', 'Augusta', 'Vital' and 'Safir') to be registered and introduced in the Official Catalogue of Cultivated Plants in Romania.

CONCLUSIONS

Blueberry is one of the most popular berries in North America, rich in many valuable compounds.

By breeding the existing blueberry assortment in Romania, the aim is to obtain new cultivars with special qualities of fruits, with high production capacity, with resistance to diseases and pests, as well as with the ability to adapt to less acid soils.

The breeding programme will continue in close relative with current market requirements (big fruit, extension of the ripening season). All in the field of hybrids and in comparative crops and microculture, contain a rich and diverse genetic material, in different stages of evaluation of the breeding process, as a new source for the continuation of this process.

Worldwide blueberry production shows an upward trend. If in 2009 a production of 314,895 tons was registered, in 2018 the production doubled reaching up to 682,790 tons, and the area harvested registered in 2008 was 74,912 ha and in 2018 the surface registered was 109,270 ha.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ASSESSMENT OF THE IMPACT OF CONVENTIONAL AND ORGANIC FERTILIZATION ON THE DRYING PROCESS AND THE QUALITY CHARACTERISTICS OF 'STANLEY' PLUM FRUIT

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Abstract

During the period 2016-2017, in order to improve the soil fertility and the quality of the fruit production in the RIMSA - Troyan, a nourishing conventional and organic fertilizer was applied to the plum plantation with 'Stanley' cultivar. The impact of the applied fertilizers and the drying process on the quality characteristics of the plums was determined. It was found that plums in the organic chicken manure fertilization variant had the highest sensory evaluation and high quantitative values on the colour indicators, such as brightness, red and yellow colour tone. After the drying process, it was found through a heat pump dryer that the applied temperature regime $t = 43 \pm 2°C$ and $\varphi = 10\%$, had an impact on the quantitative values of fruit colour. In the case of dried fruits from bio fertilization, the red and yellow components of the colour increased, and for all other fruits from the variants, including the control, they decreased significantly. Dried fruits from conventional fertilization had the highest sensory score, while dried fruits from bio fertilization had the lowest.

Key words: *plums, fertilization, heat pump drying, quality characteristics, colour parameters.*

INTRODUCTION

Nowadays, effective management of soil fertility is needed to maintain the standards for quality fruit production (Staneva & Gospodinova, 2018). This requires updating or changing fertilization technologies (Pešaković et al., 2020). Fertilization is one of the main agrotechnical events in the cultivation of fruit plants (Georgiev et al., 2019), but before the selection and application of fertilizers it is necessary to determine the current agrochemical status of the plantation (Hristova, 2017; 2018). By establishing the impact of different fertilization rates on the growth characteristics and nutritional status of plants (Akova et al., 2019), from different fertilization variants (organic and conventional) on the biological activity and antioxidant potential of fresh and dried fruits (Hristova et al., 2020) achieved the possibility of growing and storing quality products that meet the requirements of the final user (Petrescu et al., 2020). Sensory indicators such as appearance, colour, texture, taste and aroma are used to characterize fruit quality, consumer preferences and consumption (Bruhn, 1991; Costa, 2011; Pathare, 2013). In turn, sensory evaluation is an opportunity to qualify high-quality products, with an increased guarantee of acceptance by consumers (Calín-Sánchez et al., 2020).

Colour is one of the most important sensory qualities when choosing for fresh fruits consumation (Silva et al., 2019) and dried products (Krokida & Maroulis, 2000). It results from the availability of natural pigments (chlorophylls, carotenoids, anthocyanins, flavonoids and betalains), which change continuously during fruit ripening (Barrett et al., 2010).

Colour changes can also occur during storage or processing of fruit products (Montefiori, 2005; Krokida, 2001). The reason for this is the activation and course of a number of biochemical processes, such as the degradation of the pigment composition (Roshanak et al., 2016), enzymatic and non-enzymatic reactions (Maskan, 2001; Terefe, 2014), which depend mainly on light, temperature and treatment (Lancaster, 1997; Bonazzi, 2011). Therefore, the colour parameters can be used as an indicator of the quality of fruit that has undergone heat treatment (Demirhan & Ozbek, 2009).

The process of fruit drying is widespread in the processing of raw materials in Bulgaria. Less well known is the process of drying with a heat pump dryer.

Thermodynamically, heat pumps are identical to refrigeration machines. The difference is that the heat obtained is used for the process. Their energy saving effect is based on the fact that the heat of the environment is used. When drying on a heat pump principle, the cold is used to condense the evaporated moisture from the dried product. Drying takes place at temperatures up to 45°C, which preserves the high quality and native properties of the product. The process takes place in a closed cycle, using the same air and eliminating additional microbial contamination from outside air.

The main objective of the present study is to monitor the effect of the applied conventional and organic fertilizers on the quality and colour characteristics of fresh and dried plums of 'Stanley' cultivar.

MATERIALS AND METHODS

Raw materials

Plum trees are grown in RIMSA-Troyan, and the applied variants of foliar and soil nourishing conventional and organic fertilization are from two consecutive years (2016-2017). The analyzed fresh and dried plums of 'Stanley' cultivar are from the 2017 harvest.

'Stanley' cultivar characteristics

'Stanley' is the most common American plum cultivar grown worldwide (Okie & Ramming, 1999). It was obtained by crossing 'd'Agen' and 'Grand Duke' cultivars at the New York Agricultural Experimental Station Geneva, USA. Due to its tolerance to *Plum pox virus,* since 1985, it is the main cultivar for Bulgaria (Djouvinov & Vitanova, 2002), occupying 80% of the plum plantations in the country (Georgieva & Serbezova, 2018). The cultivar is self-fertile and a good pollinator.

In many cases, it is used as a standard in comparative testing of culture genotypes. The fruits ripen in late August - early September. They are large, back ovoid, asymmetrical. The skin is healthy, thin, dark purple-blue with abundant wax. The flesh is greenish-yellow, dense, medium juicy, odorless, with good taste. The fruits are transport stable, with universal application. They are used for fresh and dried consumption or processed into various products (brandy, compotes, jams, etc.) (Djouvinov et al., 2012).

Experimental setup

The improvement of soil fertility in 'Stanley' plum plantation with nourishing fertilizer was applied in an experimental setup of four options.

Fertilization variants

I variant - Biofertilization **- including fertilizers, such as:** Agriful (soil) - 5 l/da, Tecamin Flower (foliar) - 0.3%, Teknokel Amino Ca (foliar) - 0.4%.

II variant - Conventional fertilization - Yara Mila Complex (soil) - 0.500 kg/tree, Yara Vita Frutrel (leaf) - 0.500 ml/da, Yara Vita Universal Bio (leaf) - 0.500 ml/da, ammonium nitrate - 0.220 gr/tree.

III variant - Organic fertilization (granulated chicken manure- 0.500 kg/tree).

IV variant - Control (without fertilization).

Composition of fertilizers

Agriful: Total humic extract-306 g/l; Fulvic acid - 306 g/l; Nitrogen (N) - 55 g/l; Phosphorus (P_2O_5) - 13 g/l; Potassium (KjO) - 13 g/l; Total organic matter - 551g/l; pH - 4.7.

Tecamin Flower: Seaweed Extract - 51g/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.

Teknokel Amino Ca: 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.

YaraMila Complex: Nitrogen (N) - 12%; 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%.

YaraVita Frutrel - 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.

YaraVita Universal Bio - Nitrogen (N) - 100 g/l; Phosphorus (P_2O_5) - 40 g/l; Potassium (K_2O) - 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.

Granulated chicken manure Vita Organic: Nitrogen (N) - 1.2%; Phosphorus (P) - 1.99%; Potassium (K) - 2.5%; Calcium (Ca) - 10.85%; Magnesium (Mg) - 0.75%; Zinc (Zn) - 350 mg/kg; Copper (Cu) - 50 mg/kg; Manganese (Mn) - 443 mg/kg; Iron (Fe) - 3450 mg/kg.

Fertilizer application periods

Agriful - applied five times from the beginning of vegetation over a period of 15-20 days.

Tecamin Flower - imported twice. Applied before blossoming and during the formation of a fruit-set.

Teknokel Amino Ca - imported twice. Applied after blossoming and a month before harvesting.

YaraMila Complex - imported once in the intra row spacing.

Ammonium nitrate - implied once in 2017;

YaraVita Frutrel - four-fold application. First application in the phase of winter buds, then in phase of white button, after that during the formation of fruit-set and finally a month before the harvest.

YaraVita Universal Bio - three-fold application. Applied before and after blossoming and after harvest.

Granulation of chicken manure - one application in 2016.

Drying

'Stanley' plums from different fertilization variants were compared to the control and used for the study.

The technological processes describing the drying process are: receiving, weighing, sorting, washing, separating the stones, cutting, drying and storage.

Fruit drying was performed in the laboratory of the Food Technologies Department at FRDI-Plovdiv on a heat pump stand for drying. The process takes place in a thin layer with a transversely oriented air flow relative to the product layer at a speed of 5-7 $m.s^{-1}$, with an initial temperature of 45°C and circulating air with an initial humidity of 8%. During the drying process, the mass of the sample was measured for the first two hours every 10 minutes, then every 30 minutes. The condition for the end of the experiment is drying to moisture balance. The dried fruit samples were packed in paper bags and stored at room temperature in the absence of light until the day of analysis.

Sensory analysis

Sensory evaluation of fresh and dried fruits from different fertilization variants was performed, based on the use of a 5 - point evaluation scale with a step of 0.25 on the indicators: appearance; colour; consistency/ hardness/; fruity taste and aroma.

Colour measurements

The colour characteristics of fresh and dried fruits from the fertilization variants were measured at the laboratory of Food Research and Development Institute - Plovdiv.

Gardner colour scale - laboratory apparatus "GOLORGRAD2000" was used, BYK-GARDNER INC. USA.

The samples from the analyzed variants of fresh and dried fruits were ground on a laboratory wolf МРІЯ–2М with a diameter of the openings of the grid 4 mm. The indicators were reported according to the CIELab system. During the measurement, the colour coordinates L, a and b were taken: L - colour brightness (L = 0 black and L = 100 white); + a - red colour; -a- green colour; + b - yellow colour - b - blue colour.

The colour tone value or the dominant wavelength is represented by the a/b ratio.

The colour saturation (C) was determined by the formula $\sqrt{a^2 + b^2}$

The colour differences were calculated by the following formulas:

$\Delta L = L - L_0$,

$\Delta a = a - a_0$

$\Delta b = b - b_0$

$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$

where for fresh fruits L_0, a_0 and b_0 are the control values, and L, a and b are the measured values of the fruits from the fertilization variants.

In the case of dried fruits, the colour difference was calculated by the same formulas, but L_0, a_0 and b_0 are the values of the fresh fruits from the fertilization variants, and L, a and b are the measured values of the dried fruit from the variants.

Statistical processing of the samples was performed by triplicate replications, the data were presented as averages and processed with the ANOVA program.

RESULTS AND DISCUSSIONS

Drying

The convective drying process is one of the most common methods for drying fruits, vegetables and medicinal plants in the food industry (Chua et al., 2019), transferring both heat and mass (Ertekin & Yaldiz, 2004; Nunez-Vega, 2012).

According to Kandić et al. (2017) the nature of the heat flow and the mass transfer process can be most fully described on the basis of experimental data through the drying curves. The change in moisture content as a function of time at constant temperature and air velocity for drying plums of the Stanley variety is shown in Figure 1.

$$U = f(\tau) \qquad (1)$$

where: U is the moisture content (kg H_2O/kg dry matter) and τ- drying time (min).

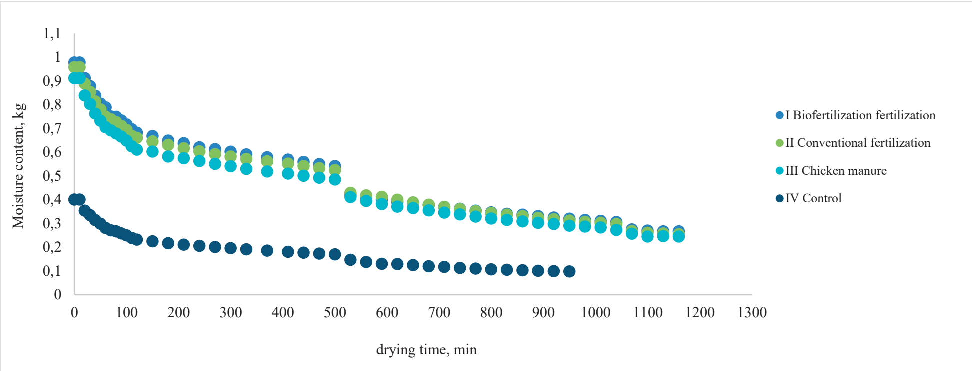

Figure 1. Drying curves of plums

The drying speed curves, could be seen by the drying curves through their graphical differentiation, defined by the dependence:

$$dU/d\tau = f(U) \qquad (2)$$

The drying rate in the first period is described by the equation:

$$-\frac{dU}{d\tau} = N = \text{const} \qquad (3)$$

where N is the drying rate during the first period (min^{-1}).

In the process of fruit drying in the variants and the control, a period of constant speed and a period of decreasing drying speed was observed, periods described by Kurmanov et al. (2015). Identical to Abasi et al. (2009), a continuous decrease of the speed of drying with increasing drying time, respectively reduced the moisture content in the product.

The values of the kinetic constants obtained during the second period for the control fruits and the bio-fertilization fruits are in high correlation with the experimental data ($R^2 > 0.75$) (Table 1).

Table 1. Speeds (N, min^{-1}) and coefficients (K, min^{-1}) of drying of plums, at different variants of fertilization

Variants	N, min^{-1}	R^2	K, min^{-1}	R^2
I. Biofertilization fertilization	4.21	0.94	0.04	0.78
II. Conventional fertilization	4.61	0.85	0.06	0.32
III. Chicken manure	4.83	0.82	0.10	0.57
IV. Control	3.54	0.98	0.05	0.83

Sensory analysis

Sensory continuity of food has been extremely important for people from an early age (Bourne, 2002). It characterizes its physicochemical quality and is a leading indicator in consumer choice (Kitzberger et al., 2017).

Figure 2 presents a sensory profile of fresh 'Stanley' cultivar plums, in different variants of fertilization and control. Sensory indicators were analyzed: appearance, colour, texture, taste and aroma.

With a maximum assessment of appearance 5 are the fruits of control, bio-fertilization and organic chicken manure. The fruits from the conventional fertilization had a good grade of 4.55 ± 0.29.

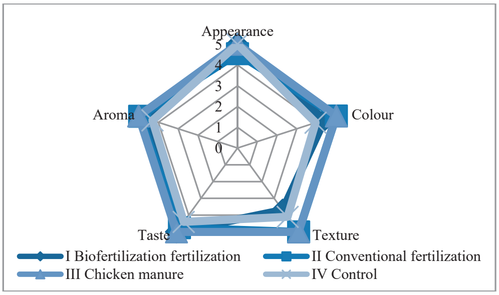

Figure 2. Sensory profile of fresh plums, with different fertilization variants

In terms of colour, the fruits of the fertilization variants had higher scores than the fruits of the control. The tasters gave the maximum marks for colour 5 to the fruits from the conventional fertilization with organic chicken manure. The consistency of the fruits from the bio-fertilization had the lowest marks 3.7 ± 0.37 in comparison with the fruits from the control 4.1 ± 0.25 and the other two variants 5. In terms of taste and aroma, the lowest marks were given to the fruits from the control in comparison with the fruits treated with bio-fertilizers and conventional fertilization. The analysis of the obtained average evaluations from the sensory indicators shows that the fruits from the variants of fertilization with organic chicken manure were maximally evaluated by the tasters in terms of appearance, colour, texture, taste and fruity aroma, confirming the cultivar character-ristics described by Dzhuvinov et al. (2012).

The applied agrotechniques had an impact on the sensory characteristics of the fruits from the fertilization variants (conventionally and biofertilization) ($P < 0.05$).

Sensory analysis was also performed on the dried plums from the control and from the fertilization variants (Figure 3).

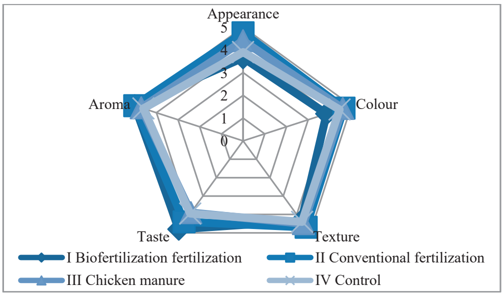

Figure 3. Sensory profile of dried plums, with different fertilization variants

Impressive is the lower assessment of dried fruits compared to fresh ones of the studied variants.

Assessments of the appearance of dried fruit significantly decreased after the applied drying regime.

Dried fruits from organic fertilization had the most significant differences compared to fresh fruits and the lowest scores on indicators of appearance, colour and texture compared to dried fruits of the control and the other two variants of fertilization. Very good ratings for the appearance of 4.75 ± 0.22, colour 4.65 ± 0.37 and consistency 4.7 ± 0.1 were given for dried fruits from conventional fertilization. In terms of taste and aroma, the dried fruits of the control and those fertilizers with organic chicken manure had equally lower scores compared to the dried fruits from organic fertilization and conventional fertilization ($P > 0.05$). The drying method and the applied agrotechniques had a significant impact on the sensory characteristics of the fruits of 'Stanley' ($P <0.05$).

For dried fruits from all variants of fertilization and control, a negative linear dependence was found, with an average coefficient $R^2 = 0.65$ between the sugar-acid coefficient of the fruit and the taste evaluation given by the tasters. (Figure 4). No such dependence was found in fresh plums.

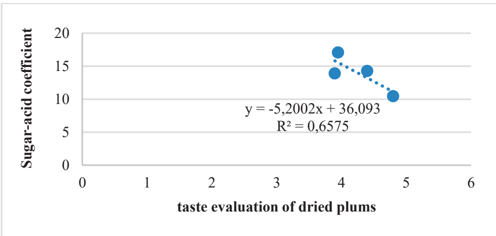

Figure 4. Regression dependence between the sugar-acid coefficient of fresh and the taste evaluation of dried plums

Colour characteristics

According to Pathare et al. (2013), by measuring the color characteristics it is possible to pre-model the nutritional qualities of fresh and processed foods.

The CIELab colour coordinates of fresh plums presented in figure 5 show that the brightness of the fruits had the highest values in the variant of organic chicken manure fertilization (L = 30.88). The results of the other fruits treated with biofertilizers and conventional fertilization were statistically indistinguishable from the control fruits (P > 0.05). With high quantitative values of red (a = 7.68) and yellow colour tone (b = 19.33), colour saturation (C = 20.8), and colour differences (ΔL = 2.83; Δa = 1.99; Δb = 11.9) were the fruits of third variant, with applied chicken manure. The applied agrotechnics (P <0.05) had an impact on the values of the studied indicators.

The red colour tone (a) in the fruits of the other fertilization variants and those of the control did not have statistically significant differences (P > 0.05). The applied fertilizers do not affect the values of the measured indicator (P > 0.05). This also applies to the quality indicator colour tone (a/b) (P > 0.05).

The yellow colour tone (b) and the colour saturation (C) were the lowest quantitatively in the control fruits (b = 7.42; C = 9.35). There were no significant differences in the studied colour parameters in the fruits of the variants of bio and conventional fertilization (P > 0.05). The applied agrotechniques in these two variants did not affect the yellow colour tone and colour saturation.

The change in colour characteristics (L, a, b) in turn can lead to a significant change in the value of the total colour difference (ΔE). According to Adekunte et al. (2010), ΔE can be used to classify differences in visual colour, which can be classified as very different (ΔE > 3), different (1.5 < ΔE < 3) and with a small difference (1.5 < ΔE).

In the present study, insignificant colour differences were found in fruits from the variants of conventional fertilization and bio-fertilization in comparison with the control. The colour difference of fruits from the studied variants of fertilization was influenced only by the organic chicken manure (ΔE = 12.4) (P < 0.05).

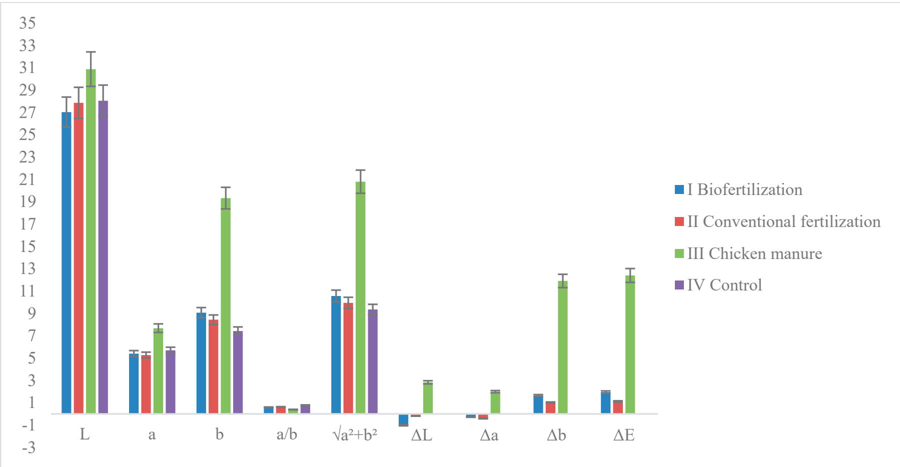

Figure 5. CIELab values and colour indices of fresh plums of ‘Stanley’ cultivar, according to the fertilization and control variants

After the drying process, the fruits significantly changed their qualitative and quantitative values of color (P < 0.05) (Figure 6).

The dried fruits from bio-fertilization had the highest values according to the studied colour parameters (L = 28.3; a = 7.89; b = 12.55; C = 14.82; ΔE = 13.98) (Figure 6).

Nowacka et al. (2017) state that if ΔE is higher than 2, then the observer will see a clear and visible colour difference between fresh and processed fruits. This statement confirms that the drying process had a significant impact on the studied indicators of fruits from the fertilization variants.

In both variants, fresh and dried fruits, the tendency to dehydrate fruits from the control variant with the lowest values according to the studied colour indicators was preserved (L = 19.99; b = 1.98; C = 3.80).

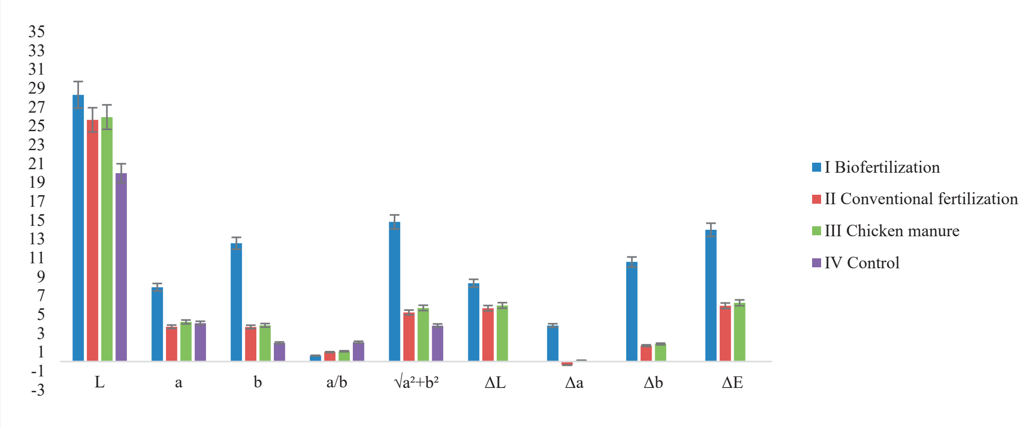

Figure 6. CIELab values and colour indices of dried plums of 'Stanley' cultivar, according to the fertilization and control variants

With average statistically indistinguishable values were the studied colour characteristics red, yellow colour tone, colour tone in dried fruits from conventional fertilization and chicken manure ($P > 0.05$). The applied drying mode did not affect the measured parameters ($P > 0.05$).

The colour difference in the brightness of the dried fruits in the control and the generalized colour difference in the dried fruits treated with organic chicken manure ($\Delta E = 16.63$) ($P < 0.05$) were significant.

The most stable colour was registered in dried fruits treated with biofertilizers, followed by dried fruits from conventional fertilization.

CONCLUSIONS

It was found that fertilization variants affect the quality of fresh fruit. Fruits in the organic chicken manure fertilizer were distinguished with the highest sensory assessment, formed by the indicators, such as appearance, colour, consistency, taste and aroma, and high quantitative values of the colour indicators, such as brightness, red and yellow colour tone, intensity of colour and total colour difference.

It was found that plums drying, by means of a heat pump dryer had an impact on their quantitative values of colour. In the dried fruits of the organic fertilizer variant, the red and yellow components of the colour increased, and in the other variants, including the control, they decreased significantly. The dried fruits from the conventional fertilization were evaluated with the highest sensory evaluation, and with the lowest in the organic fertilization.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

'Z1 VITROPLANT' - VALUABLE ROOTSTOCK FOR KIWIFRUIT CULTIVARS - GRAFTING RESULTS

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Abstract

Grafting of kiwifruit cultivars is more and more a necessity due to some soil born problems as high calcium and pH, plants die-off or "Kiwi Moria" etc. A solution can be the use of 'Z1 Vitroplant' rootstock (A. deliciosa x A. arguta hybrid obtained at Vitroplant Italia) that shows good resistance to cold, ferric chlorosis, low permeable soil and water scarcity. 'Hayward' and 'Bruno' cultivars, and some new valuable hybrid genotypes, obtained through intra and interspecific crosses, were used for grafting. The 'Z1' rootstock was planted in 10 liters pots and grafted in April, in a cold greenhouse, where the temperature varied between 20-24°C. Scions were taken from mother plants in January and preserved in dormancy at 2-3°C. Whip and tongue grafting was applied and Flexiband was used as wrapping material. To reduce water loss and oxidation, Arborinn special wax was used. Grafting success rate, rootstock, woody scion and main scion shoot diameters, total scion shoots length, total number and average shoots length, were analysed at 4 months after grafting. The results showed significant differences between cultivars for most of the analysed parameters.

Key words*: Actinidia sp., growth, propagation, whip and tongue.*

INTRODUCTION

Kiwifruit (*Actinidia* Lindl.) is a recently developed horticultural crop, with a very short history of cultivation (Sui et al., 2013). According to the latest revision, *Actinidia* genus belongs to the *Actinidiaceae* family and has over 75 species and about 125 known taxa worldwide (Huang & Ferguson, 2007).

Even the centre of origin is China, most of the *Actinidia* species are widely distributed in Asia (Huang & Ferguson, 2007). As Cui (1993) and Stirk (2005) mentioned, *Actinidia* species are found in different climates and geographical environments, from India to Japan and from Siberia to Indonesia.

Huang (2016) and Zhang et al. (2010) reported that the most common kiwifruit species are *A. deliciosa* and *A. chinensis* and the current commercial cultivation is almost entirely based on these ones. Lesser extent, *A. arguta* commercial potential started to be recognised, in colder regions, in the early 20th century (Ferguson & Huang, 2007).

Kiwi is a very appreciated fruit due to its nutritional properties, high vitamin C content, as well as its taste and flavour (Biao et al., 2018; Yang et al., 2010; Young et al., 1995). Also kiwifruit is a rich source of vitamin E, vitamin K, vitamin B complex, carotenoids, choline, minerals (Na, K, Ca, Mg, Mn, Fe, Cu, Zn), dietary fiber etc. (Çeliket et al., 2006a; Ferguson, 1999; Jesion et al., 2013; Kim et al., 2010; Mohammed et al., 2017).

The *Actinidiaceae* is a family of woody, deciduous and perennial vigorous vines (Ferguson, 1984; Stirk, 2005).

As most of the fruit trees, kiwifruit plant can be propagated by seedlings or by asexual methods, such as grafting, semi-hardwood or hard wood cuttings, and also tissue culture (Kumar & Sharma, 2002; Lawes, 1992; Peticilă et al., 2012a; Sale, 1985; Stănică et al., 2003a).

Because of its dioeciously nature, propagation from seed of kiwifruit for commercial plantations, are not recommended, the plant sex being unknown until flowers are produced (Sedaghathoor & Noie, 2016). Also, the seedlings fruiting starts later compared to the vegetative multiplied plants.

Sexual propagation is mostly used to produce rootstock seedlings for grafting (Irshad et al., 2014). According with Hartmann et al. (2011), Stănică et al. (1995), Stănică (2004a) and Tanimoto (1994), the common methods of kiwifruit propagation are grafting, cuttings and micropropagation.

Nevertheless, previous researches have shown that kiwifruit cuttings are characterized by a very low intrinsic rooting ability. This is why, particular techniques as, bench heating, temperature control, fog or mist, as well as rhyzogenetic substance treatments are always required in order to obtain satisfactory results (Alam et al., 2007; Babar et al., 2018; Biasi et al., 1990; Dumitrașcu et al., 2003; Ono et al., 2000; Peticilă et al., 2015; Peticilă et al., 2016; Stănică et al., 2003b; Zenginbal & Özcan, 2014). Root formation was strongly influenced by species, variety and rooting time (Kumar & Sharma, 2002).

Regarding *in vitro* kiwifruit propagation, according with Akbaş et al. (2007), Famiani et al. (1997), Ferradini et al. (1996), Irshad et al. (2014), Kumar & Sharma (2002), Ono et al. (2003), Peticilă et al. (2012b), Stănică & Armeanu (2004b) and Stănică et al. (2005), culture medium, genotypes and time of inoculation are very important for propagation rate.

Grafting is a vegetative method of propagation, often used in horticulture (Stănică et al., 2003a) with major applicability: introduction of cultivars with high biological characteristics, resistant or tolerant to pests and diseases, tolerant to abiotic stress factors, reduction of soil borne problems, improvement of water and nutrients absorption etc. (Çürük et al., 2009; Doltu et al., 2017; King et al., 2010; Lee, 1994; Lee et al., 2010; Rivero et al., 2003; Webster, 1995).

According to Hartmann & Kester (1975), the origin of grafting can be traced back to ancient times, the Chinese being familiar with the art of trees grafting at least as early as 1000 B.C. Throughout the time a large number of scientific research are reported in the literature concerning grafting in different horticultural crops: vegetables (Doltu & Bogoescu, 2014; Doltu et al., 2017; Ergun & Aktas, 2018; Lee, 1994; Oda, 1995; Rouphael et al., 2010; Sakata et al., 2007), flowers (Fang et al., 2009; Weinard & Dorner, 1927; Zhang et al., 2013), ornamental plants (Hinesley & Frampton, 2002; Jayawickrama et al., 1991; Melnyk & Meyerowitz, 2015; Roberto & Colombo, 2020; Tarroux & DesRochers, 2011), wine grapes (Cimpoi et al., 2020), fruit trees (Asănică & Tudor, 2011; Asănică et al., 2013; Bărăscu et al., 2018; Hoza et al., 2020; Stănică, 2019; Tabacu et al., 2020; Vercammen et al., 2007).

The most common types of grafting often used in fruit nurseries and orchards are represented in Figure 1: a) twin cleft whip grafting or tongue grafting; b) bud grafting (chip-budding or T-budding); c) notch grafting; d) cleft grafting.

a) b) c) d)

Source: DUE BUOI Agriculture

Figure 1. Common grafting types in fruit nurseries and orchards

For kiwifruit, the main used grafting methods are top grafting (tongue and cleft grafting), chip-budding or T-budding, and also side grafting (Luh & Wang, 1984; Sedaghathoor & Noie, 2016; Zenginbal et al., 2006a; Zenginbal et al., 2006b).

Top grafting is one of techniques frequently used in cultivars replacement and improvement (Huang, 2006; Liang et al., 2011; Liu & Wang, 2006).

Important contributions to the study of the *Actinidia* graft budding were made also by Çelik et al. (2006b), Gustafson & Morrissey (2003) and Zenginbal et al. (2006b).

Corresponding to several study, whip and tongue grafting is a suitable method for asexual propagation of kiwifruit (Mohammadi & Abdi, 1993; Pandey, 2019; Zenginbal et al., 2006a).

The grafting success can be affected by several factors such as temperature, humidity, scion variety, rootstock, grafting time, wrapping materials, grafting methods, pests and diseases etc. (Hamdi et al., 2007; Pandey et al., 2019; Tanimoto, 1994).

Few studies have been conducted regarding the behaviour of different rootstocks grafted with kiwifruit cultivars (Sedaghathoor & Noie, 2016; Zuccherelli, 1979). In the recent years, the use of wild species of *Actinidia* as rootstocks has been also evaluated (Liang et al., 2011; Sedaghathoor & Noie, 2016). Kiwifruit rootstocks can be propagated by seedling or rooted cuttings (Anderson & Lawes, 1980; Mohammadi & Abdi Senehkouhi, 1993; Pandey et al., 2019; Sedaghathoor & Noie, 2016; Zenginbal et al., 2006b).

Özcan (2000) and Sedaghathoor & Noie (2016) mentioned in their research that seedlings have high vigor and long roots than cuttings.

Different species of *Actinidia*, including *A. chrysantha*, *A. eriantha*, *A. globosa, A. hemsleyana*, *A. kolomikta, A. macrosperma*, *A. polygama*, *A. rufa* were used as rootstocks in combination with various scions cultivars (Clearwater et al., 2004, 2006, 2007; Liang et al., 2011; Wang et al., 1994). Also, some cultivars of *A. arguta*, *A. chinensis* and *A. deliciosa* ('Matua', 'Bruno', 'Hayward') obtained by seedlings or rooted cutting, were used as rootstock in grafting propagation (Çelik et al., 2006a; Sedaghathoor & Noie, 2016; Zenginbal et al., 2006b).

In Romania, kiwifruit research and culture started in 1993 (Peticilă et al., 2002; Stănică & Cepoiu, 1996; Stănică, 2009; Zuccherelli, 1994). The most important studies were conducted in a common Romanian-Italian kiwifruit breeding program, initiated at the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Stănică & Zuccherelli, 2007; Stănică & Zuccherelli, 2009).

Taking into consideration that grafting of kiwifruit cultivars is more and more a necessity due to some soil born problems as high calcium and pH, plants die-off or "Kiwi Moria" etc., this study can provide some solutions by using a resistant rootstock as 'Z1' showed to be.

MATERIALS AND METHODS

Experimental Site

The study was conducted in 2020, at the Faculty of Horticulture cold greenhouse, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania.

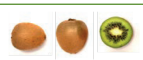
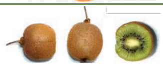
According to Asănică & Tudor (2011), the climate in the experimental area is typically temperate-continental, with cold winter and warm summer. Springs registered significant variations between day and night amplitude, and autumns distinguished by moderate thermal and slow transition to winter (Tudor et al., 2014).

Plant material

'Hayward' and 'Bruno' cultivars and eight Romanian intra and interspecific *Actinidia* hybrids, were grafted. The scions trial used for this study is presented in Table 1.

Scions were taken in early January from vigorous productive plants, grown in the Experimental Field of the Faculty of Horticulture, Bucharest. The shoots were preserved in dormancy until the grafting moment, at 2-3°C, wrapped in plastic film.

Table 1. Scions cultivars and hybrids description

Variety/Hybrid		Species
Hayward (♀)		*A. deliciosa*
Bruno (♀)		*A. deliciosa*
R0P9 (♂)		*A. chinensis* intraspecific hybrid
R0P10 (♀)		*A. chinensis* intraspecific hybrid
R0P13 (♀)		*A. deliciosa* x *A. chinensis* interspecific hybrid
R1P1 (♀)		*A. deliciosa* x *A. chinensis* interspecific hybrid
R1P8 (♀)		*A. deliciosa* x *A. chinensis* interspecific hybrid
R1P9 (♀)		*A. deliciosa* x *A. chinensis* interspecific hybrid
R1P12 (♀)		*A. deliciosa* x *A. chinensis* interspecific hybrid
R9P20 (Vip Red) (♀)		*A. arguta*

One-year-old 'Z1 Vitroplant' rootstocks, *in vitro* propagated, were planted in 10 liters pots, and grafted in April, in a cold greenhouse, where the temperature varied between 20-24°C. 'Z1 Vitroplant' (Vip Zedone ®) rootstock is a hybrid of *A. deliciosa* ('P1') and *A. arguta* ('Gemma'), obtained at Vitroplant, that shows good resistance to cold, ferric chlorosis, low permeable soil and water scarcity (Zuccherelli, 1994). The plants have medium vegetative vigor and good affinity with the *Actinidia deliciosa* and *A. chinensis* genotypes. According Vitroplant Italia, 'Z1 Vitroplant' is resistant to PSA and is currently in an advanced experimentation for the evaluation of its resistance to the so-called "Kiwi die-off" ("Kiwi Moria"), with satisfactory results.

Grafting

Twenty-four hours before grafting, the scions were placed with the base in water for hydration. The rootstock plants were also watered. Whip and tongue grafting was applied (Figure 2) and Flexiband was used as wrapping material. To reduce water loss and oxidation, Arborinn special wax was applied.

Cultural operations such as irrigation, weeding and removal of suckers below the grafting point followed at regular intervals. Four months after grafting (middle August), the anticipated shoots on the main scion shoot, were pinched at 20-25 cm. Flexiband has been removed to avoid strangulation of grafting point (generally, it is naturally degraded by UV in open field).

Figure 2. Kiwifruit whip and tongue grafting phases

Data collection

Data on sprouting were recorded after bud burst, while the grafting success percentage and the other observations and measured traits were recorded four months after grafting.

The number of days from grafting to the first bud burst was recorded. Starting with the 20th day after grafting, the percentage of the sprouting plants was calculated, every 10 days.

Grafting success rate was calculated following formula and was express by percentage:

$$\text{Graft success rate} = \frac{\text{Number of sprouted graft}}{\text{Total grafted plants}} \times 100$$

Average diameters of rootstock, woody scion and main scion shoot were measured with an electronic calliper and expressed in millimetres (mm).

Main scion was measured in centimetres and the average length was calculated. The length and the total number of anticipated shoots were also recorded.

Statistical analysis

Data statistical analyses were performed with Excel (MS Office).

RESULTS AND DISCUSSIONS

Callus along the union point began to occur in about two weeks after grafting.

Scions sprouting percentage at different days after grafting

The dynamic of plants sprouting percentage between 20 and 60 days after grafting, recorded every 10 days, is presented in Figure 3.

At the 20th day after grafting, all genotypes had sprouted plants.

After 30 days, most of the scion cultivars recorded over 50.0% sprouted plants, excepting 'Hayward' (30.0%) and R1P12 (40.0%).

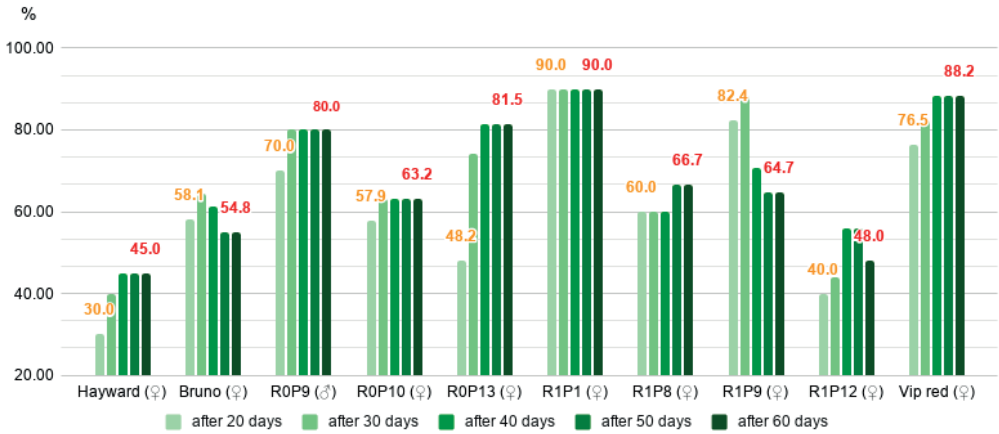


Figure 3. Dynamic of kiwi plants sprouting (%) after grafting

Figure 4. Scion shoot growth of R1P1 genotype (30 days after grafting)

At 60 days after grafting, the minimum percentage of scion sprouting was recorded at 'Hayward' (45%) and the maximum, at R1P1 (90%).

The sprouting percentage increased constantly till the 60th day after grafting, for almost cultivars. 'Bruno' and R1P9, registered a decreasing tendency from 58.1% to 54.8%, and from 82.4% to 64.7% respectively due to some plants lost.

R1P1 registered the highest sprouting percentage (90.0%) after 20 days.

According Bose et al. (2019), the variation of cultivars sprouting is due to genetic differences in translocation of food reserves and change in the cambial activity.

Figure 5. Grafting success rate (%)

Grafting success rate

The observation regarding kiwifruit grafting success rate on 'Z1' rootstock, are represented in Figure 5. The highest percentage of survived grafts was obtained at R1P1 genotype (90.00%), followed by Vip Red (88.24%), R0P13 (81.48%) and R0P9 (80%), while the lowest, was registered at Hayward (45.00%). Only Hayward (45.00%) and R1P12 (48.00%) registered lower values of grafting success rate than 50%. The effect of different scion variety on grafting success rate was significantly different at four months after grafting.

Hartmann et al. (2007) reported that genetic factors had a significant effect on grafting success. The ability of two kinds of plant to form a successful graft union is largely based on their natural relationship (Sharma, 2002).

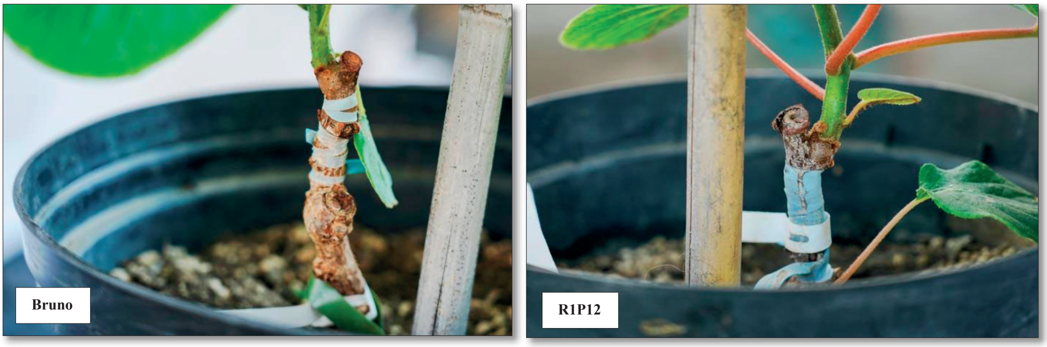


Figure 6. Grafting point union and main scion shoot details, after 4 months, for 'Bruno' and R1P12

Rootstock and woody scion diameters

Rootstock and woody scion diameters at the grafting point have quite similar values (Figure 7). The differences between rootstock and scion shoots diameters varied from 0.13 mm at 'Bruno' to 1.52 mm at R1P1.

Only in the case of the R1P12 genotype, the average diameter of the scions was thicker than the rootstock one.

Plants grafted vigour and total vegetative growth

In Figure 8, can be observed how the grafted plants on 'Z1 Vitroplant' looked at 90 days after grafting. Main scion shoot diameter (mm) and length (cm), total number and length of primary laterals shoots, total number and length of secondary laterals shoots, were presented in Table 2.

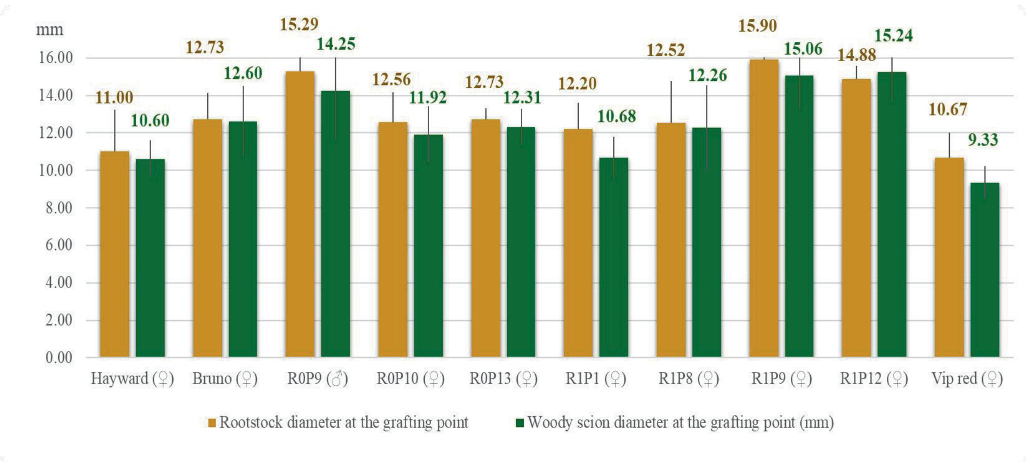


Figure 7. Rootstock and woody scion shoot diameters at the grafting point

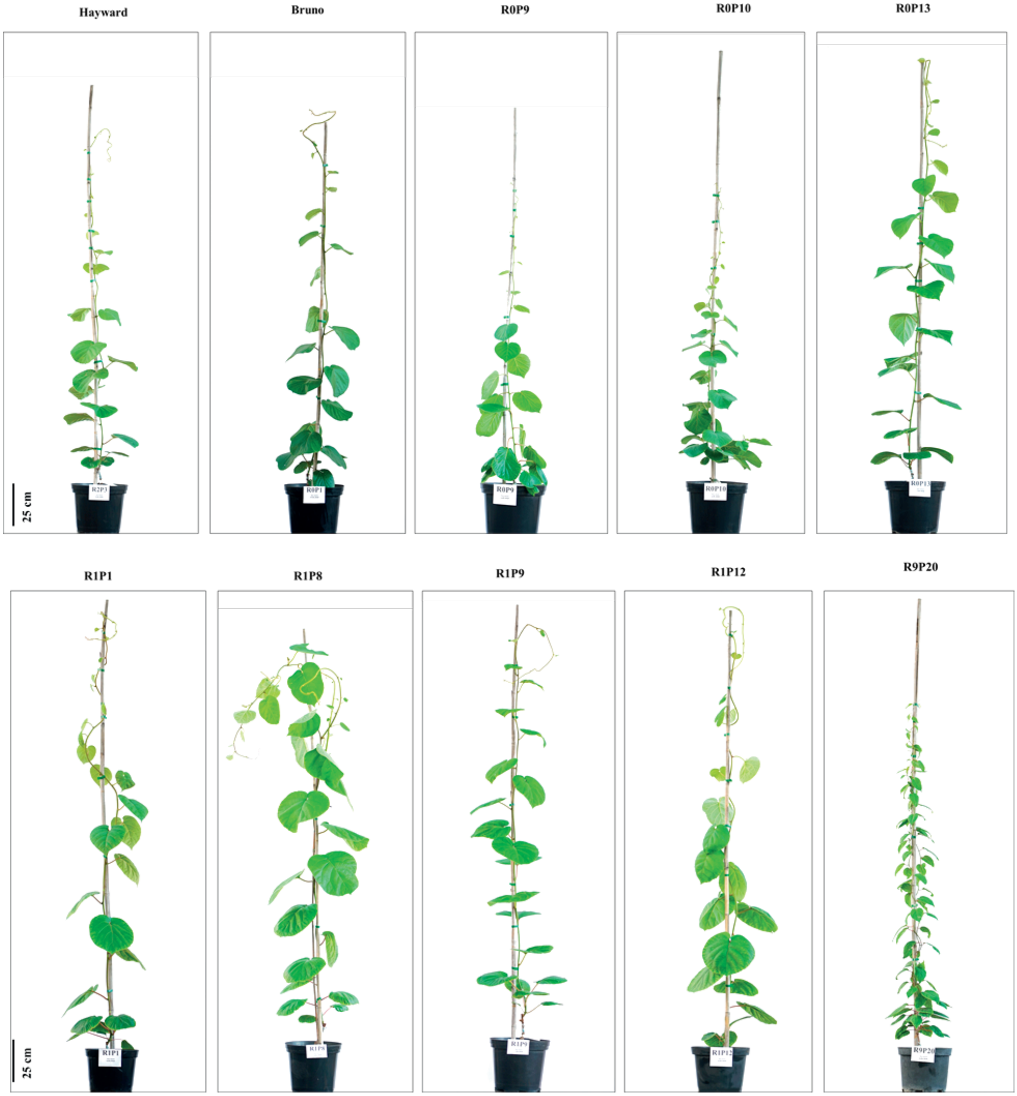


Figure 8. Grafted plants on 'Z1 Vitroplant' at 90 days after grafting

Main scion shoot diameter varied from 6.15 mm for 'Vip Red' to over 10 mm at R0P13, R1P9, R0P9 and R1P12.

R0P9 recorded the highest value of main scion average shoot length - 387.20 cm. The lowest value was registered at 'Hayward' 144.83 cm. High values were measured also at R0P13 (275.40 cm), Bruno (253.43 cm), R1P12 (246.50 cm) and R1P1 (231.80 cm). All genotypes presented anticipated lateral shoots. The most vigorous genotypes, with the highest number of anticipated shoots are 'Vip Red' (9.50), R0P13 (5.40), 'Bruno' (5.14), R1P9 (5.00) and R0P10 (5.00). The lowest number was registered at 'Hayward' (2.17).

Few plants from R1P12, R1P8, R1P1 and R0P10 genotypes, formed second anticipated lateral shoots. The longest values of the second anticipated shoots were registrated at R0P10 (60.00 cm) and R1P8 (47.50 cm).

Table 2. Kiwi plants vigour and total vegetative growth

Cultivar/Hybrid	Main scion shoot diameter (mm)	Main scion shoot length (cm)	Total anticipated shoots (no)	Anticipated lateral shoots length (cm)	Total second anticipated shoots (no)	Second anticipated shoots length (cm)
Hayward (♀)	8.65±0.66*	144.83	2.17	48.46	0	-
Bruno (♀)	9.34±0.65*	253.43	**5.14**	59.69	0	-
R0P9 (♂)	**10.62**±0.88*	**387.20**	3.33	**67.00**	0	-
R0P10 (♀)	9.44±2.71*	185.40	**5.00**	55.20	0.20	**60.00**
R0P13 (♀)	**10.97**±1.81*	275.40	**5.40**	50.19	0	-
R1P1 (♀)	9.66±0.95*	231.80	3.00	60.82	0.20	38.00
R1P8 (♀)	9.25±1.65*	198.50	3.44	**79.39**	**0.33**	**47.50**
R1P9 (♀)	**10.72**±1.21*	228.20	**5.00**	33.16	0	-
R1P12 (♀)	**10.41**±1.15*	246.50	3.50	**71.81**	**0.67**	23.50
Vip Red (♀)	6.15±0.35*	222.23	**9.50**	45.47	0	-

* Standard deviation

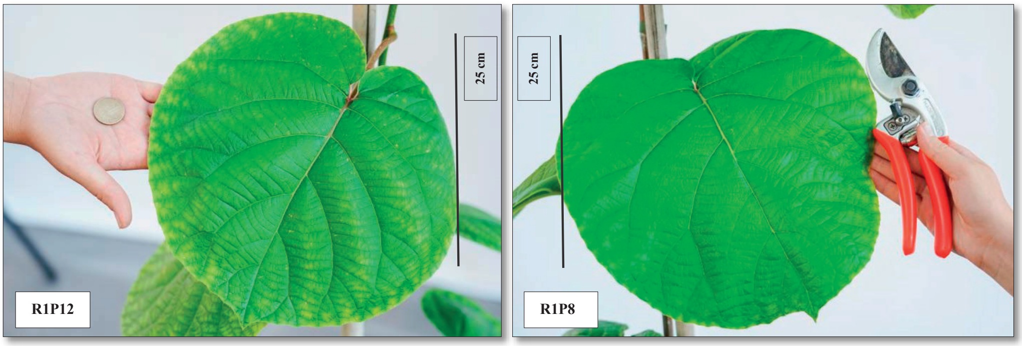


Figure 9. R1P12 and R1R8 kiwifruit hybrids leaf extension at 90 days after grafting

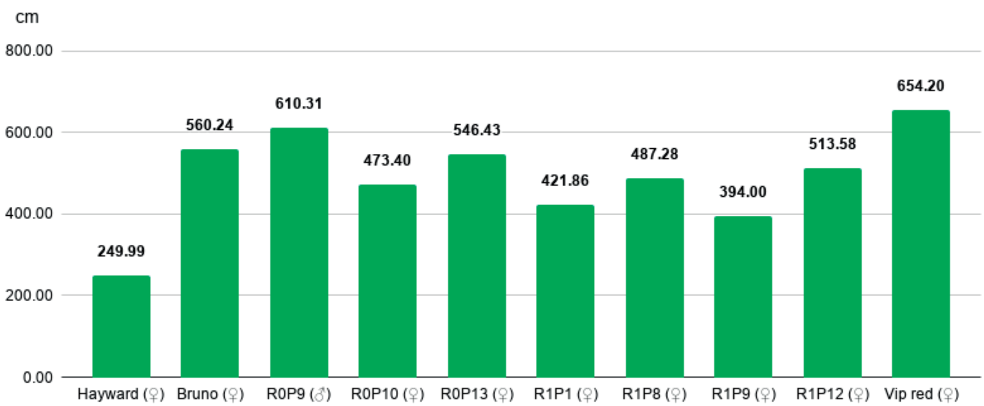


Figure 10. Total vegetative growth (cm) of kiwifruit scions at four months after grafting

Figure 11. R1P12 main scion and anticipated laterals shoots (90 days after grafting)

The average of total vegetative growth, calculated at four months after grafting, are represented by the average length of main scion shoot and the sum of the average length of anticipated shoots (Figure 10).

'Vip Red' and R0P9 registered the highest value of total vegetative growth (654.20 cm and 610.31 cm, respectively).

'Vip Red' formed also the biggest number of anticipated shoots.

The lowest total vegetative growth was registered at 'Hayward' (249.99 cm).

CONCLUSIONS

The results showed significant differences between cultivars and genotypes for most of the analysed parameters. Based on the observations and measurements we can conclude that, most of the chosen kiwifruit genotypes grafted on 'Z1 Vitroplant' showed good results. R1P1 registered the highest grafting success rate (90%) and showed good results regarding the growth vigour. The highest values of the total vegetative growth were registered by 'Vip Red' (654.20 cm) and R0P9 (610.31 cm).

'Z1 Vitroplant' rootstock showed good grafting compatibility with all tested kiwifruit cultivars and hybrid genotypes. Grafted plants will be tested in the experimental orchard for productivity and other field resistances.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE BEHAVIOUR IN THE CONTROLLED POLLINATION PROCESS OF SOME ABORIGINAL APPLE VARIETIES

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Abstract

This article presents the study on the behaviour of a local apple variety 'Măr dulce' in the process of pollination with two valuable varieties that are found in the European assortment. It was studied the behavior in the pollination process of two hybrid combinations C1 - 'Măr dulce' x 'Orion' and C2 - 'Măr dulce' x 'Bistrițean' where the maternal parent is the old apple variety 'Măr dulce' and the paternal parent is represeted by pollen from the apple varieties 'Orion' and 'Bistrițean'.This study provides a novelty for a future selection of elite genotypes for the production of hybrids with an increased resistance to Venturia inaequalis.The studies were conducted in the spring of 2019 and the controlled pollination method was used, which involves a number of steps as: pollen harvesting, pollen maturation, castration of the maternal parent, pollination when the ovarian exudate appears on the stigma and isolation of pollinated branches. After these steps, the number of linked flowers, the number of maturated hybrid fruits and the growth of hybrid fruits resulting from C1 and C2 hybrid combinations were studied.

Key words: *apple, pollination, scab, Venturia inaequalis.*

INTRODUCTION

Globally and locally, one of the diseases that causes the greatest material damage in apple crops is scab (Mitre et al., 2001; Oroian et al., 2006; Babuc, 2012; Le Camp B. et al., 2019).
The scab, caused by *Venturia inaequalis*, has become one of the most important and difficult to control diseases. Due to the need for very good planning of the application of fungicides with apple phenophases, but also due to the difficulty of eradication, fungicides are relatively ineffective, or to be effective requires a very large number of sprays which are contraindicated both for the consumer and from the point of view of very high costs.
The best way to fight scab remains in the hands of breeders and is dependent on the development of new apple species that are resistant to scab (Mihăescu, 1977; Brown, 2003; Ion et al., 2011).
In order to achieve good apple hybrids that present a good resistance to apple scab is required to perform different tests and to use several methods, the method that was used in this study is the controlled pollination (CP).
Controlled pollination is an essential method in apple breeding programs, it is used especially to make high quality seeds that quickly form the desired genotypes (Băncilă, 2003; Sestraș, 2004; Sestraș et al., 2008; Asănică and Hoza, 2013).

MATERIALS AND METHODS

In this study was used the controlled pollination method. Some of the most important steps regarding the working method that we used in the pollination process are:
• choosing parents;
• establishing cross combinations;
• pollen harvesting;
• pollen maturation;
• castration of the maternal parent;
• pollination when the ovarian exudate appears on the stigma.
The pollination works took place in April -May 2019 in an apple orchad located in Mihaești, Valcea County, Romania.
Two-hybrid apple combinations were studied, where paternal parents (pollen) are represented by two valuable varieties from the European assortment, the varieties 'Orion' and 'Bistrițean' and as a maternal parent was used the old apple variety 'Măr dulce' which proved

to be a variety with a special genetic resistance to diseases and pests.
The pollen from varieties taken in the study was harvested and maturated in the spring of 2018 and has been stored in the refrigerator at a maximum temperature of 4ºC.
On the chosen flower of the apple variety, the stamens were removed and then pollination was performed with exogenous pollen from the paternal parent chosen for hybridization.
Then we isolated the branch fragment with the pollinated flowers in a paper bag so that only the pollen from the paternal parent proposed in the pollination scheme participates in the fertilization process.

Figure 1. A. Stamen harvesting, B. Pollen maturation

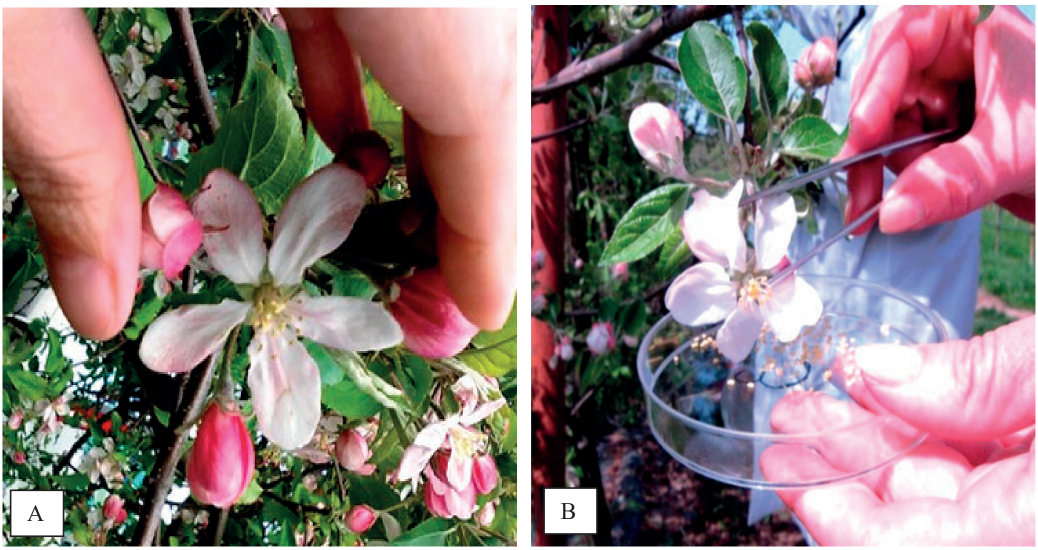

Figure 2. A. Choosing the flowers for pollen harvesting, B. Harvesting the pollen

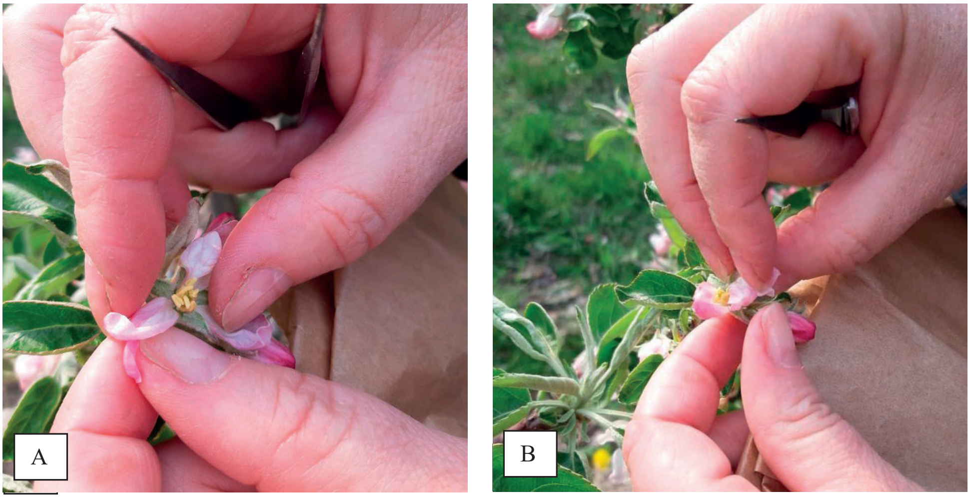

Figure 3. A. Choosing and opening the flower, B. Castration of the maternal parent

Table 1. Scheme of sexual hybridization ♀ x ♂

No. combination	Hybrid cross combination	No. of pollinated flowers
C1	'Măr dulce' x 'Orion'	172
C2	'Măr dulce' x 'Bistrițean'	196

As seen in Table 1 the following hybrid cross combinations were performed:
- hybrid combination C1 - 'Măr dulce' x 'Orion';
- hybrid combination C2 - 'Măr dulce' x 'Bistrițean'.

For hybrid combination C1 were pollinated 172 flowers and for hybrid combination C2 were pollinated 196 flowers.


Figure 4. Isolated branches with pollinated flowers

RESULTS AND DISCUSSIONS

Table 2. Cross combinations, fruit set

No. combination	No. of pollinated flowers	Fruit set (number; %)
C1	172	172; 100%
C2	190	190; 97%

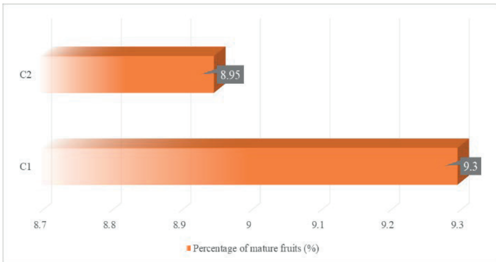

Figure 5. Percentage of matured fruits (%)

From Figure 5 we notice that at the hybrid combination C1 a percentage of 9.3% was achieved, representing a total of 16 matured fruits. For the C2 hybrid combination was achieved a percentage of 8.95% representing 17 matured fruits. The present results could be the effects of several factors, such as the authenticity of the genotypes analyzed (of varieties and offspring) or the method applied.

Use in the process of breeding some indigenous varieties of apple, well adapted to the Romanian climate can be an interesting principle regarding the induction of natural genetic resistance to *Venturia inaequalis*.

Figure 6 shows the growth of the fruits of the hybrid combination C1 - ‘Măr dulce’ x ‘Orion’. It is found that the hybrid fruits had a faster growth in the first period studied, they increased 2.2 cm between 15-22.04.2019, after this date the growth rate started to slightly decrease to 1.8, this increase was recorded at 7 days between 22.04.2019 - 06.05.2019. In the time interval 06-13.05.2019, there was the smallest increase of hybrid fruits (0.4 cm). From these, it results that the average growth rate at 7 days is 1.5 cm.

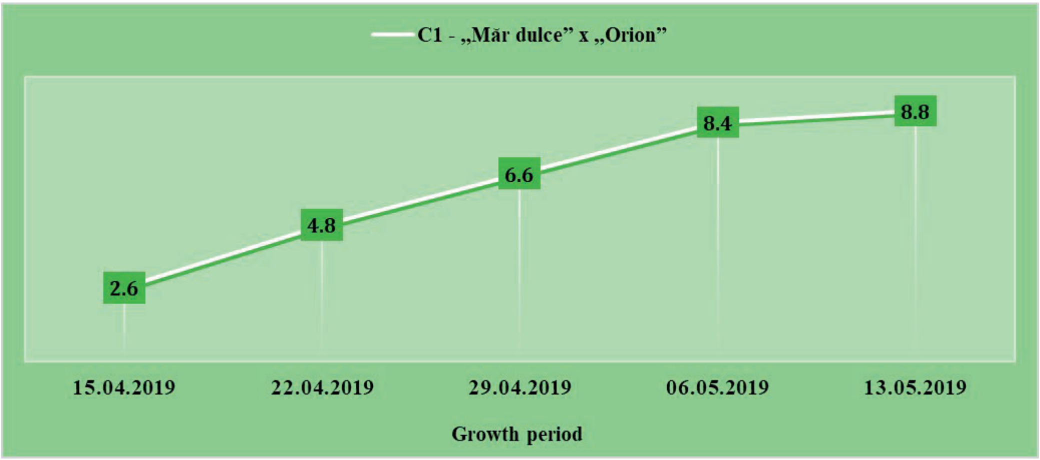

Figure 6. Growth rate of Hybrid Combination C1 - ‘Măr dulce’ x ‘Orion’ (cm)

The results in figure 7 clearly show a slow growth of hybrid fruits in the C2 hybrid combination (‘Măr dulce’ x ‘Bistrițean’). In the first period studied, the strongest growth is recorded, being 1.9 cm, the smallest increase is 0.6 cm and it was recorded twice between 29.04.2019 - 13.05.2019.

Undoubtedly, the study of varieties with very good resistance to scab in pollination processes is important, but the revaluation and reconsideration of an old local variety such as ‘Măr dulce’ it can form a starting point in improving new stable and durable characters, better adapted to the conditions in our country.

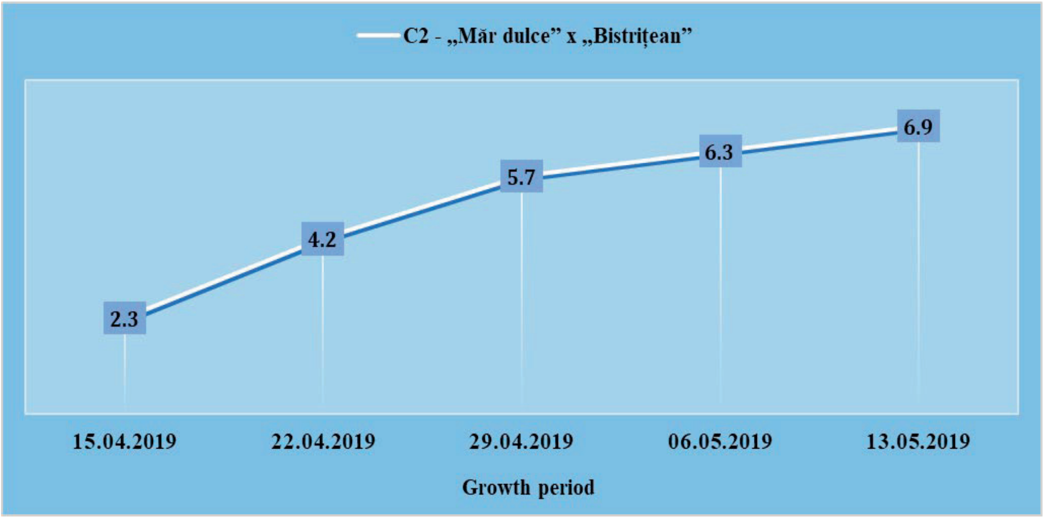

Figure 7. Growth rate of Hybrid Combination C1 - 'Măr dulce' x 'Bistrițean' (cm)

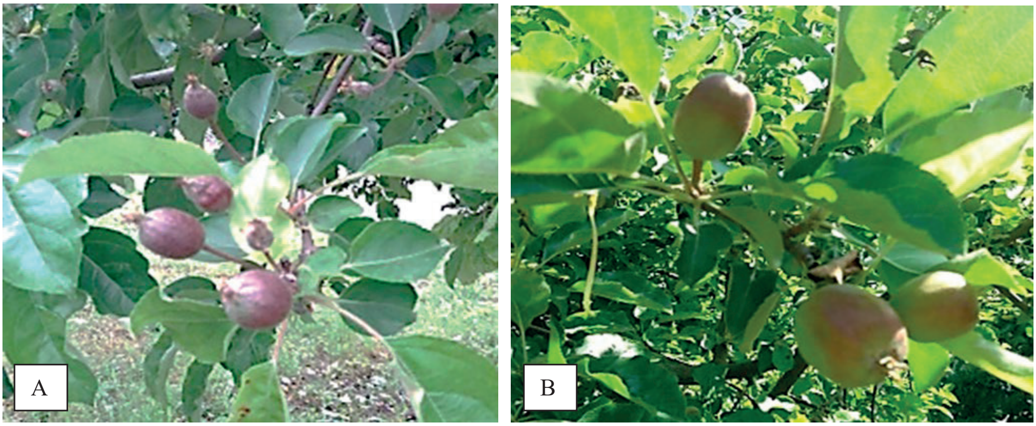

Figure 8. A. Hybrid fruits of the C1 hybrid combination, B. Hybrid fruits of the C2 hybrid combination

CONCLUSIONS

The results obtained in 2019 come with more information by exploring a regional apple population 'Măr dulce', it is very well adapted to the pedoclimatic conditions in Romania.

The conclusions emphasized that:

- in the combination 'Măr dulce' x 'Orion' from the total of 172 pollinated flowers, a 100% success was obtained in terms of fruit setting;
- as regards the maturing of the fruits in the cross combination 'Măr dulce' x 'Orion' 9.3 % of the hybrid fruits were matured, and to the cross combination 'Măr dulce' x 'Bistrițean' 8.95 % of the hybrid fruits were matured.

After studying the growth rate at 7 days, it was noticed the following:

- hybrid combination 'Măr dulce' x 'Orion' recorded a higher increase than the hybrid combination 'Măr dulce' x 'Bistrițean';
- the largest increase was recorded twice, the fruits grew 2.2 cm between 15-22.04.2019 at hybrid combination 'Măr dulce' x 'Orion';
- the smallest increase was recorded on 13.05.2019, being 0.4 cm in the hybrid combination 'Măr dulce' x 'Orion';
- to the hybrid combination 'Măr dulce' x 'Bistrițean' the largest increase in fruit was recorded in the first period studied, the fruits increasing by 1.9 cm.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MOLECULAR MARKERS USAGES IN CULTIVATED FRUIT TREES FROM *ROSACEAE* FAMILY

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Abstract

Most fruit tree species cultivated in Romania, such as apple, pear, plum, peach, apricot, cherry, as well as some berry species such as blackberry, raspberry and strawberry belong to the Rosaceae family. As most of these species are woody perennials, the traditional methods of creating new varieties are taking much longer time than in the case of annual species. New methods and tools based on the recent discoveries in molecular genetics are being developed in order to shorten the time needed to create new commercial varieties. Molecular markers, specific DNA regions linked to genes responsible for various traits (color, shape, taste, resistance/tolerance to biotic and abiotic stresses, etc.) are some of the tools used in genotype-assisted breeding programs. This review sums up the main results of studies on molecular markers regarding cultivated fruit tree species of Rosaceae family with commercial importance.

***Key words**: molecular markers, Rosaceae, Malus, Prunus, molecular breeding.*

INTRODUCTION

Rosaceae family consists of 91 genera and 2,950 species (Christenhusz and Byng, 2016). Most cultivated fruit species from Romania belong to this family. These species are important not only in alimentation, but also as ornamentals.

Recent sequencing techniques and genomes sequencing brought up new data that can be used for a variety of purposes, such as identification and characterization of genes responsible for agronomically important traits, genotyping by sequencing, marker assisted selection, identification of molecular markers linked to the traits of interest (Soundararajan et al., 2019). Molecular markers such as Single Nucleotide Polymorphism (SNP), Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Cleaved Amplified Polymorphic Sequence (CAPS), Sequence-Related Amplified Polymorphism (SRAP), Sequence Characterized Amplified Region (SCAR), can be used as tools for genetic and physical mapping of genomes, the identification of genes controlling various processes and phenotypes (trait association), genetic diversity and evolutionary analyses, and in marker-assisted breeding for crop improvement.

Single Sequence Repeats (SSRs) are molecular markers that have been used extensively for genotyping commercial crops, as they are multiallelic, highly polymorphic, they have high discriminatory potential and good reproducibility, and can detect polyploidy (Nybom and Lācis, 2021). Within the *Rosaceae* family, multiple SSR markers have been developed for most cultivated species. To improve comparison among different studies, European Cooperative Programme for Plant Genetic Resources (ECPGR; www.ecpgr.cgiar.org) published recommended SSR loci sets for apple, pear, cherry and plum (Nybom and Lācis, 2021). SSR markers may be used to discriminate between different genotypes of the same species and frequently those of closely related species, so they are used frequently in variability studies, fingerprinting and map construction. Mnejja et al. (2010) studied 145 *Rosaceae* SSR primer pairs (genomic and EST-derived) for transferability in nine *Rosaceae* species (almond, peach, apricot, Japanese plum, European plum, cherry, apple, pear, and strawberry), ending up with 32 polymorphic SSRs for all Prunus species studied and proposed a set of 12 SSRs as an "universal *Prunus* set". Fan et al. (2013) developed a set of SSR markers from pear that could be used in other Rosaceae species, their transferability varying from 12% (cherry) to 58% (apple). Heterologous markers designed from a species may be used in PCR amplification to amplify SSRs in a second related species phenomenon known ask transferability of SSR information.

Single Nucleotide Polymorphisms (SNPs) are the most abundant molecular markers present in a genome, some of their advantageous features being that they are relatively stable during evolution and they have a low mutation rate (Patel et al., 2015). SNPs in Rosaceae species were identified in various projects involving Sanger sequencing to sequence EST collections, direct sequencing of PCR products amplified from genomic regions of interest, through the utilization of the cleaved amplified polymorphic site approach where suitable restriction enzymes were available, high-throughput deep sequencing of both genomic DNA and mRNA, and genotyping by sequencing (Longhi et al., 2014).

In traditional breeding programs, the length of juvenile phase (length of time for seedling to produce the first flowers) affects the breeding cycle, specifically in respect to fruit traits evaluation, and consequently the selection of the best hybrids and cultivars. For instance, in *Prunus* species, the juvenile phase lasts 1-2 years for peach, 2-3 years for Japanese plums, and 3-5 years for sweet cherries (Carrasco et al., 2013). Plant domestication began roughly around 11,050 BC. Early farmers aimed for improvement of morphological traits, such as seed or fruit size and plant structure, or of physiological ones, such as the timing of germination and fruit ripening. However, domestication came with unintended consequences, such as more deleterious mutations as compared to wild types and lack of genetic diversity (Hunter, 2018). Study of plant domestication in the context of the new discoveries in the fields of genetics and genomics could lead toward new approaches in plant breeding. Molecular breeding adds genetic and genomic analysis to assist traditional breeding, not only reducing the breeding time, but also aiding in the selection of plants possessing traits of interest linked to specific molecular markers.

The present study aims to review the use of molecular markers in the past two decades in connection with the main species of fruit trees from *Rosaceae* family: apple, pear, quince, plum, almond, apricot, peach and cherry.

Apple

Cultivated apple, *Malus x domestica* Borkh., is an interspecific hybrid, with the main progenitors being *Malus sieversii* M. Roem. and/or *Malus sylvestris* L. (Brown et al., 2012). RFLP markers have been employed to study the chloroplast DNA variation in *M. sylvestris, M. sieversii* and *M. domestica* with the purpose of determining the phylogenetic relationship between the two species (Coart et al., 2006), the results of the study showing a closer relationship between *M. domestica* and *M. sylvestris* as opposed to *M. sievestrii* and *M. domestica*. In the same study, nuclear SSRs have been used to discriminate among wild, cultivated and hybridized genotypes. Koopman et al. (2007) used multilocus microsatellite haplotype-sharing as a tool to study introgression for cultivated (*M. domestica*) into wild apple (*M. sylvestris*) as well as gene flow among the remnant populations of *M. sylvestris*, demonstrating the usefulness of the method in obtaining general genetic information on population structure and population differentiation. Later, Cornille et al. (2012) used SSR markers to study the contribution of various *Malus* species to the genome of cultivated apple, *M. domestica* and determined that the two major contributors to domestic apple genome are *M. sylvestris* and *M. sieversii*.

Several economically important traits in apple have been studied using molecular markers, such as resistances to apple scab resistance, powdery mildew, and to the fire blight, red foliage, red fruit color, russeting, columnar growth.

Venturia inequalis is the most important fungal pathogen that affects cultivated apple. Consequently, multiple studies have been published, trying to unravel the genetics behind apple scab resistance. Patocchi et al. (2004) identified a qualitative apple scab resistant gene, *Vr2*, in the accession GMAL 2473, and developed three AFLP and one RAPD molecular markers associated to this gene. Gigax et al. (2004) developed RAPD, SCAR and SSR molecular markers associated to apple scab resistance gene *Vbj* from crab apple *Malus baccata* Jackii. Huaracha et al. (2004) used AFLP derived SCARs to narrow down the region of *Vf* locus for scab resistance. Another scab resistence gene is *Vh8*, discovered in *M. sieversii* by Bus et al. (2005). SCAR and SSR markers helped in demonstrating that *Vh8* locus differs but it is linked with the locus containing the *Vh2* gene from the F2 descendant of *Malus pumila* progenitor R12740-7A. Scab-resistance gene *Vb* was mapped on the linkage group 12 using SSR markers (Erdin et al., 2006). Lastly, Broggini et al. (2009) identified six *HcrVf2* paralogs in *M. domestica.* SSR markers were developed from BAC clones and used to map the six *Vf2* paralogs.

Podosphaera leucotricha is another fungal pathogen that affects apple, causing powdery mildew disease. Stankiewicz et al. (2002) used SCAR markers linked to the apple powdery mildew resistant gene *Pl2* in apple breeding for disease resistance. James et al. (2004) identified additional molecular markers (AFLP, RAPD and SSR) linked to a different mildew resistance gene, *Pl-d*.

Fire blight is a disease affecting apple, pears and several other *Rosaceae* species, caused by the bacterium *Erwinia amylovora*. Several types of molecular markers linked to fire blight have been developed to be used in future breeding programs. Khan et al. (2007) developed RAPD, SCAR and SSR markers linked to the QTL for fire blight resistance present in linkage group 7. Gardiner et al. (2012) mapped both SSR and SNP markers associated to QTLs for fire blight resistance in *Malus x robusta*. Recently, Emeriewen et al. (2020) used SNPs and SSRs to construct a dense genetic map of a *Malus fusca* C.K.Schneid. accession with resistance to fire blight disease.

Russeting, manifested by cuticle cracking followed by the replacement of the epidermis by a corky layer that protects the fruit surface from water loss and pathogens in apple is a disorder controlled genetically. Falginella et al. (2015), mapped the *Ru_RGT* locus from linkage group 12, using SNP and SSR markers, as a putative gene that controls this disorder.

Fresh apples can cause food allergies related to birch pollen. Gao et al (2005) used SNAP and SSR markers to map *Mal d 1 (P R10)* gene family, that encode an apple allergen, in *M. domestica*. From the eighteen genes belonging to this family, sixteen were found to be grouped into two clusters, one cluster with seven genes located on linkage group 13, and the second cluster with nine gene located on linkage group 16. One additional gene was mapped on linkage group 6, whereas another gene remained unmapped.

Color is one of the most studied traits in plant species. Chagne et al. (2007) reported that *MdMYB10* present in the *Rni* locus is encoding a transcription factor, a putative gene controlling red foliage and red coloration of apple fruit core. The authors located both SNPs and SSRs within the anthocyanin biosynthetic and regulatory genes, which may be responsible for red flesh phenotypes. Zhang et al. (2014) used 2 allele-specific DNA markers, (*A* and *MdMYB1* alleles) in order to discriminate between cultivars with different skin colors (red vs non-red skin color). El-Sharkawy et al. (2015) used SSRs to perform genetic fingerprinting in order to determine the relationship between red-colored parent 'Kidd's D-8' (KID) and the 'sport' apple mutant Blondee (BLO) (somatic mutation), a rare anthocyanin-deficient yellow-skin variety.

Columnar growth of apple trees is a useful trait for varieties used in high density planting. Yi-Ke et al. (2004) identified a RAPD marker in apple closely linked to *Co* gene responsible for columnar/non columnar trait.

Kumar et al. (2013) used genome wide SNP arrays to evaluate systematically the relative contribution of various genomic regions to several quality-related traits (fruit firmness, titratable acidity, red-flesh coverage) as well as contributions to some physiological disorders (internal flesh browning, bitter pit, fruit spitting).

Recent advances in sequencing techniques decreased both the price and time needed for whole genomes to be sequenced. Apple genome was the tenth sequenced whole genome after *Arabidopsis*, rice, poplar, grape, papaya, sorghum, cucumber, maize, and soybean. To date, there are three apple whole genomes published (Peace et al., 2019). Once enough of a genome is sequenced, a plethora of data is open to be used for a wide array of studies. Among these, fishing for putative molecular markers to be used for a variety of purposes is a priority for many researchers. Chagne et al. (2008) identified over 70,000 putative SNPs using *in silico* data, and 93 molecular markers containing 210 coding SNPs were mapped and could be used in further research. In a study made on ESTs from domestic apple cultivars Royal Gala, Pinkie, Pacific Rose, and dwarfing rootstock M9, Newcomb et al. (2006) identified multiple SSR and SNP markers. AG repeats where most common (88% of dinucleotides repeats), followed by AT repeats (7.6%), and AC repeats (4%). GC repeats were exceedingly rare (0.05%). Among sequences containing a dinucleotide repeat with more than 100 bp of flanking DNA, 83% contained SSRs in the putative 5' UTR, 2% in the putative coding region, and 15% in the putative 3' UTR. In addition, more than 18,000 biallelic SNPs were identified. A high-density genetic map was constructed from a Jonathan and Golden Delicious cross to be used for QTL analysis and SNP marker development (Sun et al, 2015). Liu et al (2016) used SSR and SRAP markers to construct a molecular genetic linkage map of *M. sieversii*.

SSR markers have been used to study the eupolyploidization and aneuploidization in *Malus* seedlings following diploid crosses (Considine et al., 2012), the results of the study indicating that aneuploidy exceeded eupolyploidy. Following the study, the authors also proposed a protocol for accelerating apple triploid breeding program using co-dominant markers. Han et al. (2011) developed and used SSR markers to construct an apple SSR-based genetic linkage map, which was used to demonstrate the presence of segmental and genome-wide duplications in apple genome, providing new insights in the complex polyploidy origin of apple.

Molecular markers could also be used for the identification of duplicates in collections as well as for correctly naming misidentified plants, thus reducing the size of collections without reducing the genetic variation (Harris et al., 2002). RAPD markers have been used to differentiate between cultivars, to analyze the maternal and paternal contribution to pedigrees, or to assess the variation between varieties, and SSR markers appears to be valuable for varietal genotyping and pedigree analysis, as well as anchor markers in genome mapping (Harris et al., 2002). Iannaccone et al. (2007) used a combination of RAPD and SSR markers analysis and flow cytometry to discriminate between all the clones of a valuable apple cultivar analyzed (Annurca), and to offer a method for efficient management for valuable germplasm preservation. Liu et al. (2014) used SSR markers to develop cultivar identification diagram (CID) strategy to identify apple cultivars and varieties easily with several pairs of SSR primers. Muranty et al. (2020) used whole genome SNP data to identify the relationships between more than 1400 old apple cultivars and reconstruct pedigrees, bringing new understanding off empirical selection and providing data for future breeding and selection.

Molecular markers are particularly useful in determining the genetic diversity of a certain population/collection. They have been employed worldwide for this purpose in multiple studies. Zhang et al. (2007) used SSR markers to analyze the genetic structure of *M. sieversii* population from Xinjiang, China. Omasheva et al. (2015) analyzed the genetic diversity of five populations of wild apple (*M. sieversii*) from Zailiysky Alatau using seven SSR markers. Yun et al. (2015) used 14 SSR markers to assess the genetic diversity within an apple collection (South Korea). Pérez-Romero et al. (2015) analyzed 29 domestic Apple accessions from Andalusia (Southern Spain) using 12 SSR markers. Öz et al. (2020) used 16 SSR markers to analyze 94 Eastern Anatolian apple accessions and found them different genetically from Anatolian apple accessions.

Finally, molecular markers can bring to light information of the far past of humankind. Genotyping with SSR primers specific for *Malus* confirmed that the mummified seeds found in amphorae in the cellar of a 1st century

BC Roman villa on Elba Island are apple seeds, and comparison with wild and modern domesticated seeds revealed that most archaeological seeds (from three amphorae) showed only 17% correlation with contemporary *Malus* accession. One amphora however contained seeds with genetic and morphological correlations with living *Malus sylvestris*. The results of this study brought new data on Roman economy and culture on Elba Island from the 2nd to the 1st century BC (Milanesi et al., 2016).

Pear

In pear (*Pyrus* sp.), molecular markers have been used to map various traits: fruit storage potential with CAPS markers (Itai et al., 2003), pear scab resistance with RAPD and AFLP markers (Terakami et al., 2006), black spot disease with SSRs (Terakami et al., 2007), self-incompatibility with CAPS markers (Moriya et al., 2007), length of pedicel, single fruit weight, soluble solid content, transverse diameter, vertical diameter, calyx status, flesh colour, juice content, number of seeds, skin colour, and skin smoothness with SNPs and SSRs (Wu et al., 2014), rootstock-induced dwarfing and precocity with SSRs (Knäbel et al., 2015), postzygotic hybrid necrosis with SSRs (Montanari et al., 2016), fruit quality traits (firmness, crispness, juiciness, sweetness, sourness, flavour intensity, fruit scuffing, shape, russet, fruit weight) with SNPs (Kumar et al., 2019).

Study of genetic variability is another objective for which molecular markers are being used. Genetic variability in South Korean cultivars (*Pyrus pyrifolia* Nak. and *Pyrus communis* L.) was assessed with RAPD and SCAR markers (Lee et al., 2004). Chinese cultivars assessed with RAPD (Lin et al., 2011) and SSRs (Xue et al., 2018). Belarus pear cultivars (Urbanovich et al., 2011) and Russian cultivars belonging to *Pyrus ussuriensis* Maxim., *Pyrus bretschneideri* Rehder., *Pyrus pyraster* L., *Pyrus elaegrifolia* Pall. and *P. communis* were assessed with SSRs (Yakovin et al., 2011). Jiang et al. (2015) developed 24 RBIP markers to assess the genetic variability in more than 100 *Pyrus* accessions from Eurasia. Li et al. (2015) investigated fruit transcriptome through massively parallel sequencing, identifying more than 30,000 SNPs and more than 7,000 putative SSR markers with the potential to be used in linkage map construction and marker-assisted breeding programs.

Quince

Not many studies are done to date regarding quinces (*Cydonia* sp.). However, ISSR markers associated with fruit traits: fire blight susceptibility, yield, mean fruit weight, citric acid content, soluble solid content, and fruit drop, have been developed by Ganopoulos et al. (2011), to be used for breeding purposes. Also, genetic diversity of Iranian quinces (Azad et al., 2013) and that of Turkish cultivars (Yüksel et al., 2013) were assessed with SSRs.

Prunus sp.

Genus *Prunus* consists of over 200 species of tree and shrubs, and it consists of five subgenera: *Amygdalus* (peaches and almonds), *Cerasus* (cherries), *Prunus* (plums), *Laurocerasus* (evergreen laurel-cherries), and *Padus* (deciduous bird-cherries), according to Rehder (1940).

AFLP markers were used to assess the genetic structure and differentiation among *Prunus* germplasm accessions belonging to seven cultivated species from subgenera *Prunus, Amygdalus* and *Cerasus* (Aradhya et al., 2004). Data acquired from the study could be used for genetic resources conservation and management.

European plum, *Prunus domestica* L., is a hexaploid species (2n = 48). A study of worldwide plum germplasm using sequence-based SNP markers confirmed that *P. domestica* originated as an interspecific hybrid of the diploid species *Prunus cerasifera* Ehrh. (cherry plum) and the tetraploid species *Prunus spinosa* L. (sloe), which in turn may be an interspecific hybrid of *P. cerasifera* and an unknown Eurasian plum species (Zhebentyayeva et al., 2019).

Genetic diversity studies on plums were performed in different reagions of the world. RAPD and ISSR markers were used to assess the genetic diversity and relationships among cultivated and wilt Tunisian plums (Ben Tamarzizt et al., 2015). In Romania, plum accessions from Râmnicu-Vâlcea Fruit Research Station, belonging to *P. domestica* and *Prunus insititia* L. were analyzed using nine SSR markers in order to categorize and genetically characterize them (Pop et al., 2018). In a study with the purpose of characterizing phenotypically and genotypically plum accessions from Tunisia, SSR markers in combination with *S*-allele intron markers were used to discriminate between eighteen diploid accessions, belonging to *Prunus salicina* Lindl. and *P. cerasifera*, and five polyploid accessions, belonging to *P. spinosa* and *P. insititia* (Baraket et al., 2019). ECPGR recommends the use of nine SSR loci as a standard set for genotyping European plum accessions, as the polymorphism in the loci is enough to differentiate between plum accessions in spite of problems caused by hexaploidy in both *P. domestica* and *P. insititia* (Nybom et al., 2020). The nine SSR loci were used to differentiate between 165 plum accessions, demonstrating a major dichotomy between *P. insititia*- and *P. domestica*-related cultivars.

As fruit set and yield depend largely on pollination, Meland et al. (2020) used SSR markers to determine the plum pollinizers' success rate in Norwegian orchards and identify pollen donors for each embryo, in order to reveal the level of self-pollination of the self-fertile cultivars and to assess individual pollinizer effectiveness, and lastly offering a valuable guideline to plum producers.

Almond

Domestication is one of the turning points in the establishments of human societies in Neolithic age (Delplancke et al., 2013). Nuclear and chloroplast SSRs were used to study the origin and dissemination of cultivated almond, *Prunus dulcis* Mill. (Zeinalabedini et al., 2010), proving that cultivated almond was disseminated from Asia to the Eastern Mediterranean basin and then to the Western Mediterranean basin, and subsequently to the New World. Delplancke et al. (2012) used a combination of nuclear and chloroplast SSR molecular markers to study the gene flow among wild (*Prunus orientalis* Mill.) and domesticated almond (*P. dulcis*) species, and they detected a high genetic diversity in both cultivated and wild almond. Almond tree domestication in the Mediterranean basin was studied using the same combination of SSR molecular markers, and both types of markers detected a single domestication event in the Eastern side of the Mediterranean basin (Delplancke et al., 2013).

ISSR and RAPD markers were employed to demonstrate the genetic stability of the almond plantlets obtained by micropropagation (Martins et al., 2004).

Genetic diversity of almonds was assessed in multiple studies. Portuguese almond cultivars and several wild almond (*Prunus webbii* Vierh.) plants was evaluated using ISSR and RAPD markers (Martins et al., 2003). Xu et al. (2004) used EST-SSRs to study genomic diversity of Chinese and Mediterranean region almond cultivars. Xie et al. (2006) used EST-SSR and genomic SSR markers to study the genetic diversity of Chinese almond cultivars. Gouta et al (2010) studied the genetic diversity of Tunisian almond varieties in comparison to European and American varieties using SSR markers, bringing new data that could be used in future breeding programs, as well as delivering genetic tools in the management of the genetic resources.

Going beyond genetic diversity studies, Wu et al. (2009) identified twelve SNPs-anchored genes using high resolution melting (HRM) technique, including abiotic stress-responsive genes, allergy and detoxification-related genes. SSR markers were used to construct a linkage map and identify QTLs associated with kernel chemical composition to be used in creating varieties with increased kernel quality (i Forcada et al., 2012). Lastly, Goonetilleke et al. (2018) used genotyping by sequencing technique to discover and map SNPs in two almond cultivars, developing more than 300 map pairs, with the potential to be used in the almond genetic analysis and genetic improvement.

Apricot

Apricot domestication and diffusion in the Mediterranean basin were studied by Bourguiba et al. (2012) using SSR markers. Following the study, more than 200 apricot accessions were grouped into 3 genetic clusters: Irano-Caucasian, North Mediterranean and South Mediterranean. In addition, two main routes of apricot diffusion from the Irano-Caucasian gene pool into the Mediterranean basin were detected: North Mediterranean and Southwest Mediterranean. Using SSR markers on 271 cultivated samples and 306 wild apricots across Eurasia, Liu et al. (2019) studied the apricot domestication, gene flow and species divergence. Apparently at least three domestication events gave rise to European, Southern Central Asian and Chinese cultivated apricots.

Genetic diversity of apricot was assessed in Turkey using SSR markers (Akpinar et al., 2010), in Tunisia with AFLP markers (Krichen et al., 2012), in China with SSR and ISSR markers (Liu et al., 2015), and fluorescent AFLP markers (Yuan et al., 2007), in Italy using AFLP markers (Ricciardi et al., 2002), and in Siberian apricots (*Prunus sibirica* L.) using nuclear SSRs (Wang et al., 2014). European, Irano-Caucasian, Chinese and Central Asian apricots were assessed with SSR molecular markers (Zhebentyayeva et al., 2003).

Regarding the linkage of markers to important traits, Olukolu et al. (2009) reported the construction of male and female high-density maps, as well as 12 putative chilling requirement (CR) QTLs using SSR and AFLP markers. Soriano et al (2005) developed AFLP-RGA (resistance gene analogues) markers as a means for marker-assisted selection and map-based cloning of R-genes in apricot.

Following de novo transcriptome analysis of Siberian apricots (*P. sibirica*), Dong et al. (2014) identified more than 7000 putative SSRs, most of them being dinucleotides (66%) and trinucleotides (31%), that will be extremely useful for future studies of breeding, genetic diversity and gene excavation about this species.

Peach

Genetic diversity of peach, *Prunus persica* Batsch, was assessed in Chinese peach cultivars using SSRs (Li et al., 2008).

Molecular markers have been used to map various traits in peach: flat fruit shape with SSR markers (Picanol et al, 2013; Lopez-Girona et al., 2017), fruit quality and chilling injury with SNPs (Martinez-Garcia et al., 2013), fruit acidity with SSRs and SNPs (Eduardo et al., 2014), slow ripening fruit with SSRs (Eduardo et al., 2015), fruit flesh color, fruit skin pubescence, fruit shape, sub-acid fruit, stone adhesion-flesh texture, resistance to green peach aphid using SNPs (Lambert et al, 2016), brachytic dwarfing with SSRs and SNPs (Cantin et al., 2018), stony hard with SSRs (Cirilli et al., 2018) and SNPs (Jiao et al., 2015), fruit maturity with SNPs (Elsadr et al., 2019), ever-growing with AFLP markers (Wang et al., 2002). Chou et al. (2020) developed a set of SNP markers linked to CR trait using the HRM technique. Shi et al. (2020) constructed a high-density SNP linkage map detecting 40 QTLs linked to 10 fruit-related traits, including fruit weight, fruit diameter, percentage of red skin color, eating quality, fruit flavor, red in flesh, red around pit, adherence to pit, fruit development period and fruit fiber content.

Yu et al. (2012) and Han et al. (2014) developed cultivar identification diagrams (CIDs) based on RAPD markers for cultivar identification of Chinese peach cultivars for fruit consumption and for the identification of ornamental peach cultivars.

Cherry

Genetic diversity in cultivated (samples from different regions in Europe as well as from different breeding programs worldwide) and wild sweet cherry (samples from France) was assessed with SSR primers to determine the effect of domestication and breeding on the genomic diversity (Mariette et al., 2010). It appears that the domestication in the case of cherry led to a breeding-related genetic bottleneck that in turn resulted in a narrow genetic diversity. Koepke et al. (2012) used a 3'UTR sequencing to develop SNP markers to detect genetic variability in closely related sweet cherry genotypes. Hewit et al. (2016) used a combination of approaches (a gel-based approach, a reduced representation sequencing, a 6k cherry SNParray, and whole genome sequencing) to identify genome wide polymorphisms (SNPs) in closely related genotypes of sweet cherry.

Genetic diversity in cherries was assessed with SSR markers in German wild and cultivated sweet cherry (Schueler et al., 2003), Turkish wild sweet cherry (*Prunus avium* L.) (Ercisli et al., 2011; Oz et al., 2013), and Italian (Campania) cultivars (Muccillo et al., 2019). Ukrainian sweet cherry cultivars were assessed using ISSR markers (Ivanovych et al., 2017).

CONCLUSIONS

Molecular markers are several of the tools used in genotype-assisted breeding programs. This review strives to bring together how molecular markers have been employed in fruit tree species of *Rosaceae* family with commercial importance.

ACKNOWLEDGEMENTS

This research work is supported by the Romanian Ministry of Agriculture and Rural Development (MADR-Bucharest), under the agricultural research and development program 2019-2022, ADER 7.2.6 project.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON FRUIT QUALITY OF SEVERAL APPLE CULTIVARS FROM DIFFERENT LOCATIONS IN THE AREA OF WESTERN ROMANIA

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Abstract

Known since ancient times, the apple remains one of the most appreciated fruits of temperate climate, contributing to a good functionality of the human body through its complex chemical composition, being recommended in the prophylaxis or control of many diseases, such as: liver, cardiovascular, renal, insomnia, rheumatic diseases et al. In Banat, Șiria area in Arad County, is among the areas with tradition in the cultivation of this fruit species. This paper, part of a larger research conducted in a doctoral thesis, aimed at a comparative study of the fruit quality on apple cultivars found in the private orchards of several families in Șiria area and cultivars purchased from various supermarkets in Arad and Lipova. The paper presents results on the fruits quality in three cosmopolitan cultivars: Starkrimson, Florina and Golden delicious, which could be purchased both from private orchards and from various stores. For example, in terms of the size and appearance of the fruit studied in the Starkrimsom cultivar, it was found that the fruit from three locations had higher values than those from the store; the content in mineral substances (g/100 g product) varied between 0.144 for the fruits in the store and 0.662. respectively 0.900 for the fruits from the same two locations; the sugar content had quite close values between samples, however, one of the private locations was highlighted (15.3°Brix); the total polyphenol content exceeded the value in the supermarket in four of the five locations.

The fruit weight of Florina cultivar apples was higher than those from the supermarket, with one exception. The content in minerals and sugars exceeded in two locations the fruits from the store; while the polyphenols exceeded in all five private orchards the value of the fruits purchased from the store (1491.7 ppm compared to 650.9 ppm). In the Golden delicious cultivar, the appearance and fruit weight were superior in the case of those in the supermarket, but the content in mineral substances and in the sugars was higher for the local fruits than for those in the store. The polyphenols accumulated in the fruit exceeded in three of the five locations the value of those from the fruits purchased in the store.

Key words: *Malus domestica*, organic system, supermarket, biometry, chemical composition.

INTRODUCTION

The global apple production in 2017 was 83.1 mil. t, China producing 50% of the total. Europe, including Turkey produced approx. 17% of the total. Therefore, the apple production is divided as follows: China 41.4 mil. t, USA 5.2 mil. t, Turkey 3.0 mil. t, Poland 2.4 mil. t, India 2.3 mil. t, Iran 2.1 mil. t, Italy 1.9 mil. t (FAOSTAT, 2017).

The apples, are most frequently consumed as fresh fruits, being well known as a source of carbohydrates, vitamins, minerals, fibres, pectines and certain classes of polyphenols, all of these contributing to the health improvement (Boyer and Liu, 2004).

Phytochemical concentrations vary greatly between different cultivars. The level of some chemical compounds varies during maturation of the fruits in response to available light, stage of fruit development and to some types of fertilization (Boyer and Liu, 2004; Iordănescu, 2012).

The fruits content in chemical compounds, respectively the nutritional value, depends on genotype, tissue structure, fruit maturation, pedoclimatic conditions and the culture technology used but also on harvesting and storing conditions (De Jager A., de Putter H., 1999; Drogoudi, 2011; Stănică, 2008; Stopar et al., 2002).

R. Veberic and F. Stampar (2005) considered that the fruits of organically grown apples, which are exposed to higher stress due to pests and diseases, have higher contents of phenolic compounds as well as different composition of sugars and organic acids compared to the apples of integrated production. The content of phenolic compound is highly dependent on the apple cultivar and various cultivation technologies (Mikulic-Petkovsek M., Slatnar A., Stampar F. & Veberic, 2010).

Apples cultivars are often organically cultivated and contain high levels of nutrients and phenolic compounds. Antioxidant activity of apples differs between cultivars, and was positively associated with the level of total phenolic content (Mikulic-Petkovsek, Slatnar, Stampar & Veberic, 2010; Mikulic-Petkovsek, Stampar & Veberic, 2007).

The consumption of fresh fruits or juice, food pastes, jellies, jams assure the vitamins for a better life. Câmpeanu et al. (2009) considered that apples are a part in all food diets and its therapeutic value is well known for different illnesses (determines the absorption of gastric secretions, the elimination of toxins, has diuretic effect, etc.).

MATERIALS AND METHODS

The biologic material studied represents three applecultivars: Starkrimson, Florina and Golden delicious, which can be found in both rural orchards (in this case, Șiria village, Arad county) and on the supermarket shelves (city of Arad).

The apple samples were harvested from the orchards of five families from Șiria (15 fruits/cultivar/orchard); the same cultivars were bought from the supermarkets in Arad (average samples of 15 fruits/cultivar).

It has to be mentioned that, in the private orchards in Șiria the simplest culture technology was used, people opting for fruits obtained in an organic system. From discussions with the owners, we found out that the only interventions on the trees were pruning and winter treatments, most of which were done with $CuSO_4$. The soil maintenance system is total grass covered, with mowing and optional use of mulch as natural fertilizer.

It is clear that, the fruits from the supermarket are obtained by using a differential culture technology which implies, apart from pruning, the usage of pest and disease repelling treatments and fertilization with different products.

From all cultivars samples for analysis (dry substance, ash, sugars, polyphenols, pH, acidity), were collected, weightings, measurements and photographies were made, in order to compare the apples from the rural areas, obtained organically, with the ones from the supermarkets, obtained by conventional cropping.

The fruit biometry implied the measuring of the big diameter and the height and then weighing with the precision scales. The biometric data served for calculating the fruit size index and registering the size categories. Determination of moisture content (SR 878/1996) was based on the principle of mass loss from the product subjected to the heating operation at $130 \pm 2^{\circ}C$.

The determination of the content of mineral substances (SR 878/1996) consisted in the incineration of the product at a temperature of $900 \pm 25^{\circ}C$ in the calcination furnace with air circulation, until the organic substances are completely burned.

Soluble dry matter was determined refractometrically, the results obtained being indicated on the Brix scale.

The fruit acidity was found by determining the pH using the pH meter 315i / SET.

The determination of the total polyphenols content with an UV-VIZ spectrophotometerwas performed by the Folin-Ciocâlteu method.

For some parameters, the results were statistically processed using the analysis of variance and for other indicators, the results were presented graphically.

RESULTS AND DISCUSSIONS

Biometriy of apple fruits of different origins

Concerning the fruit big diameter in Starkrimson cultivar (Table 1) it was found that, there are no major differences between the fruits purchased from different locations. However, the Șiria 5 location stands out, where the value of the diameter is significantly positive compared to the average, but also the Șiria 4 location, where the value although above average was not statistically assured. The fruit height (Table 2) exceeded the average value and the fruits in the supermarket in 3 locations (Șiria 5, Șiria 4 and Șiria 3). In the first case, the difference was significantly positive compared to the average, while in the other two cases, there were no differences.

Table 1. The fruit big diameter in Starkrimson cultivar from different locations

Provenance	The big diameter of fruit (cm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	6.76	95.08	-0.35	-
Siria2	6.96	97.89	-0.15	-
Siria3	6.70	94.23	-0.41	-
Siria4	7.50	105.49	0.39	-
Siria5	7.96	111.95	0.85	X
Supermarket	6.80	95.64	-0.31	-
Experiment average	7.11	100.00	0.00	control
DL5% = 0.8 cm DL1% = 1.08 cm DL0.1% = 1.44 cm				

Table 2. The fruit height in Starkrimson cultivar from different locations

Provenance	The height of fruit (cm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	5.90	90.96	-0.59	-
Siria2	6.13	94.50	-0.36	-
Siria3	6.63	102.21	0.14	-
Siria4	6.70	103.29	0.21	-
Siria5	7.33	113.00	0.84	X
Supermarket	6.26	96.51	-0.23	-
Experiment average	6.49	100.00	0.00	control
DL5% = 0.75 cm DL1% = 1.01 cm DL0.1% = 1.34 cm				

The biometric data (Tables 3-4) taken in the case of the Florina cultivar showed less variations in the three measured indicators, statistically there were no differences between apples with different origins.

Table 3. The fruit big diameter in Florina cultivar from different locations

Provenance	The height of fruit (cm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	6.31	99.68	-0.02	-
Siria2	5.87	92.73	-0.46	-
Siria3	6.63	104.74	0.30	-
Siria4	6.30	99.53	-0.03	-
Siria5	6.36	100.47	0.03	-
Supermarket	6.56	103.63	0.23	-
Experiment average	6.33	100.00	0.00	control
DL5% = 0.82 cm DL1% = 1.11 cm DL0.1% = 1.48 cm				

Table 4. The fruit height in Florina cultivar from different locations

Provenance	The height of fruit (cm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	6.31	99.68	-0.02	-
Siria2	5.87	92.73	-0.46	-
Siria3	6.63	104.74	0.30	-
Siria4	6.30	99.53	-0.03	-
Siria5	6.36	100.47	0.03	-
Supermarket	6.56	103.63	0.23	-
Experiment average	6.33	100.00	0.00	control
DL5% = 0.82 cm DL1% = 1.11 cm DL0.1% = 1.48 cm				

In the case of Golden delicious cultivar (Tables 5-6), the only fruits that exceeded the value of those in the supermarket were those from Șiria 4 location. Moreover, some had values below average (exp. those from Șiria 5 location) that allowed statistical assurance of results, being very significantly negative for both indicators.

Table 5. The fruit big diameter in Golden delicious cultivar from different locations

Provenance	The big diameter of fruit (cm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	6.66	102.94	0.19	-
Siria2	6.56	101.39	0.09	-
Siria3	5.76	89.03	-0.71	-
Siria4	7.40	114.37	0.93	X
Siria5	5.13	79.29	-1.34	000
Supermarket	7.33	113.29	0.86	X
Experiment average	6.47	100.00	0.00	control
DL5% = 0.73 cm DL1% = 0.99 cm DL0.1% = 1.32 cm				

Table 6. The fruit height in Golden delicious cultivar from different location

Provenance	The height of fruit (cm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	5.86	102.99	0.17	-
Siria2	5.86	102.99	0.17	-
Siria3	5.16	90.69	-0.53	-
Siria4	6.40	112.48	0.71	-
Siria5	4.26	74.87	-1.43	000
Supermarket	6.63	116.52	0.94	-
Experiment average	5.69	100.00	0.00	control
DL5% = 0.74 cm DL1% = 1.00 cm DL0.1% = 1.33 cm				

Concerning fruit size (Figures 1-3), reporting was made in accordance with the data from the specialized literature (Cosmulescu and Baciu, 2003; Mitre, 2005). In the Starkrimson cultivar, the fruits can be included in the group of large ones in the case of Şiria 4 and 5 locations and in the group of medium ones in the case of those from the rest of the locations.

For the Florina cultivar, the fruits from the location of Şiria 3 can be considered large,the ones from the locations of Şiria 1, Şiria 4, Şiria 5 and the supermarket were above medium size and the ones from the location Şiria 2 of medium size.

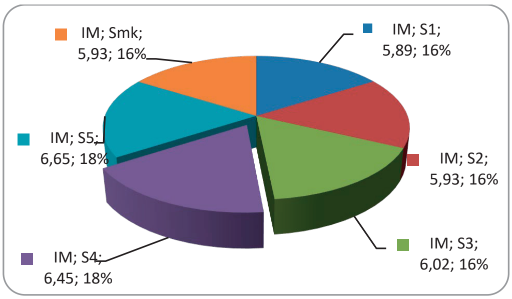

Figure 1. Fruits index size in Starkrimson cultivar

For the Golden delicious cultivar, the fruits above the average in size are those from the location of Şiria 4 and the supermarket, medium-sized fruits are those from the location of Şiria 1 and Şiria 2 and smaller are those from locations 3 and 5.

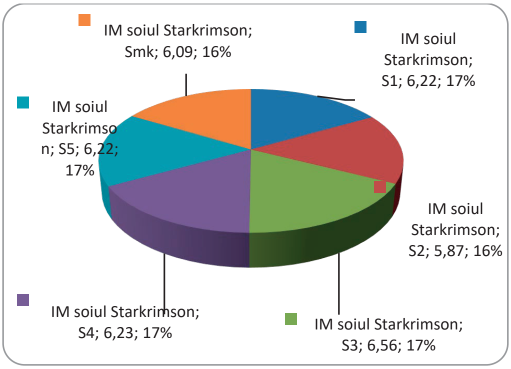

Figure 2. Fruit index size in Florina cultivar

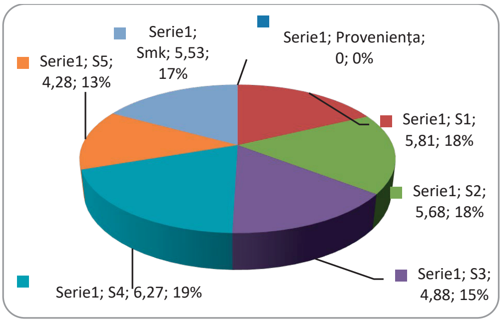

Figure 3. Fruit index size of in Golden delicious cultivar

Table 7. Fruit weight in Starkrimson cultivar from different locations

Provenance	Fruit weight (g)	Relative value (%)	Difference towards the control value	Significance
Siria 1	141.00	85.83	-23.29	000
Siria2	140.30	85.40	-23.99	000
Siria3	137.30	83.57	-26.99	000
Siria4	191.30	116.44	27.01	XXX
Siria5	224.60	136.71	60.31	XXX
Supermarket	151.30	92.10	-12.99	000
Experiment average	164.29	100.00	0.00	control
DL5% = 5.42 g DL1% = 7.32 g DL0.1% = 9.75 g				

The weight of the fruit in the Starkrimson cultivar was between 137.30 g for apples from the Șiria 3 location and 224.60 g for those from the Șiria 5 location.

The fruits from the Șiria 5 and 4 locations exceeded the average value, which were very significantly positive compared to the control and had values below the rest of the fruits, in all cases the difference compared to the controls being very significant negative. As a result, apples from the two locations (S5 and S4) bore very large fruits, the data agreeing with those in the literature (Zakia et al., 2018).

The weight of the fruit in the Florina cultivar was higher in apples from the supermarket, with one exception (Șiria 5).

Table 8. Fruit weight in Florina cultivar from different locations

Provenance	Fruit weight (g)	Relative value (%)	Difference towards the control value	Significance
Siria 1	168.57	105.95	9.47	XXX
Siria2	141.00	88.63	-18.10	000
Siria3	147.70	92.84	-11.40	000
Siria4	145.00	91.14	-14.10	000
Siria5	179.00	112.51	19.90	XXX
Supermarket	173.33	108.95	14.23	XXX
Experiment average	159.10	100.00	0.00	control
DL5% = 5.17 g DL1% = 6.99 g DL0.1% = 9.31 g				

Table 9. Fruit weight in Golden delicious cultivar from different locations

Provenance	Fruit weight (g)	Relative value (%)	Difference towards the control value	Significance
Siria 1	130.00	102.87	3.63	-
Siria2	138.00	109.20	11.63	XX
Siria3	95.30	75.41	-31.07	000
Siria4	161.60	127.88	35.23	XXX
Siria5	61.66	48.79	-64.71	000
Supermarket	171.66	135.84	45.29	XXX
Experiment average	126.37	100.00	0.00	control
DL5% = 6.76 g DL1% = 9.14 g DL0.1% = 12.18 g				

In the Golden delicious cultivar, the appearance and mass of the fruit were superior to those in the supermarket (171.66 g), however, the fruits from the locations of Șiria 4 and Șiria 2 were quite large, being very significantly positive and distinctly significantly positive compared tothe control.

The chemical composition of apples in cultivars of different origins

In Starkrimson cultivar, the humidity of the fruits varied between 81.04%, respectively 85.09% in the case of the fruits from Șiria 4 and 5 locations, both being very significant negative, going up to 85.87%, respectively 85.51% in the fruits from Șiria 1 and 2 locations, both being very significant positive compared to the control.

The apples mineral content varied between 0.144 ppm for fruits from the supermarket (very significant negative) and 0.900 ppm for fruits from the of Șiria 4 location (very significant positive).

A high content of mineral substances was also noticed in the apples from the Șiria 5 location, which were very positive compared to the control, while a lower content of mineral substances was noticed in the apples from the Șiria 2 location - very significant negative.

Table 10. Fruit moisture content in Starkrimson cultivar from different locations

Provenance	The moisture content (%)	Relative value (%)	Difference towards the control value	Significance
Siria 1	85.87	103.24	2.69	XXX
Siria2	82.19	98.81	-0.99	0
Siria3	85.51	102.81	2.33	XXX
Siria4	81.04	97.43	-2.14	000
Siria5	81.09	97.49	-2.09	000
Supermarket	82.57	99.27	-0.61	-
Experiment average	83.18	100.00	0.00	control
DL5% = 0.84% DL1% = 1.13% DL0.1% = 1.51%				

Table 11. Fruit mineral content in Starkrimson cultivar from different locations

Provenance	Mineral content (ppm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	0.321	76.73	-0.10	00
Siria2	0.159	38.01	-0.26	000
Siria3	0.326	77.93	-0.09	0
Siria4	0.900	215.14	0.48	XXX
Siria5	0.662	158.25	0.24	XXX
Supermarket	0.144	34.42	-0.27	000
Experiment average	0.420	100.00	0.00	control
DL5% = 0.08ppm DL1% = 0.10 ppm DL0.1% = 0.14ppm				

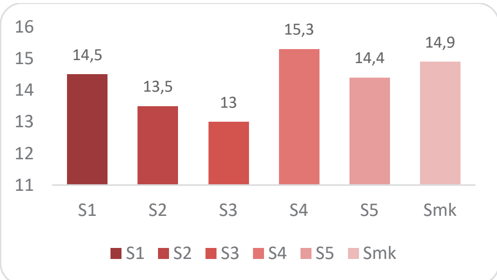

Figure 4. Total soluble solid content (°Brix) of Starkrimson fruits

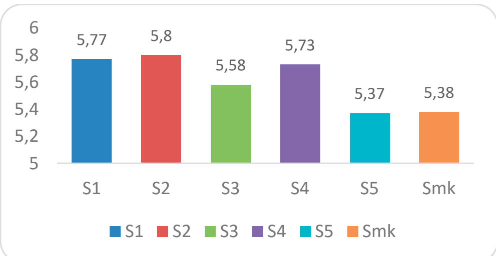

Figure 5. Fruits acidity (pH) in Starkrimson cultivar

The lowest sugar content of Starkrimson cultivar was recorded in fruits from the location of Șiria 3 (13.0°Brix) and the highest in those from Șiria 4 location (15.3°Brix). The apples from the supermarket also had a fairly high content, occupying the second position in terms of the sugar content of the fruit (14.9°Brix). The results obtained agree with those cited in the literature (Zakia, 2019).

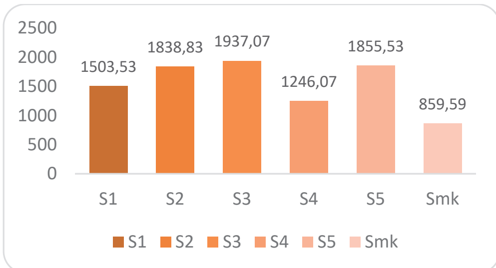

Figure 6. Fruits total polifenols content (ppm) in Starkrimson cultivar

The content in total polyphenols exceeded in all five locations the value of those in the supermarket and exceeds many of the values quoted by the literature (Otakar Rop et al., 2011). Khanizadeh *et al.*2007 considers that fruits antioxidant composition of varies among cultivars and genetics plays a significant role.

Table 12. Fruit moisture content in Florina cultivar from different locations

Provenance	The moisture content(%)	Relative value (%)	Difference towards the control value	Significance
Siria 1	87.80	101.96	1.69	XX
Siria2	91.12	105.82	5.01	XXX
Siria3	87.25	101.32	1.14	X
Siria4	85.42	99.20	-0.69	-
Siria5	82.43	95.73	-3.68	000
Supermarket	82.67	96.01	-3.44	000
Experiment average	86.11	100.00	0.00	control
DL5% = 1.10%DL1% = 1.49%DL0.1% = 1.98%				

The fruits moisture content in Florina cultivar varied between 82.43% for those from the Șiria 5 location, respectively for those purchased from the supermarket (82.67%), both being very significantly negative compared to the average and 91.12% for those from the Șiria 2 location, which is very significantly positive compared to the control.

The fruits mineral content of Florina cultivar varied between 0.160 ppm for those from Șiria 2 location and 0.327 ppm for those from Șiria 1 location. The fruits from two family plantations and the supermarket had a high content of mineral substances being distinctly significantly positive and respectively significantly positive, from the other three locations. The content of mineral substances was below the value of the control, being distinctly significantly negative.

Table 13. Fruit mineral content in Florina cultivar from different locations

Provenance	Mineral content (ppm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	0.323	135.71	0.09	XX
Siria2	0.160	67.23	-0.08	00
Siria3	0.163	68.49	-0.08	00
Siria4	0.161	67.65	-0.08	00
Siria5	0.317	133.19	0.08	XX
Supermarket	0.306	128.57	0.07	X
Experiment average	0.238	100.00	0.00	control
DL5% = 0.06ppm DL1% = 0.08ppm DL0.1% = 0.11ppm				

The fruits mineral content of Florina cultivar varied between 0.160 ppm for those from the Șiria 2 location and 0.327 ppm for those from the Șiria 1.

The fruits from two family plantations and the supermarket had a high content of mineral substances being distinctly significantly positive and respectively significantly positive, from the other three locations. The content of mineral substances was below the value of the control, being distinct significantly negative.

In the fruits of Florina cultivar, the content in mineral substances and sugars exceeded in two locations, the fruits coming from the store; while the polyphenols exceeded in all 5 private orchards the value of the fruits purchased from the store (eg. 1491.7 ppm to 650.9 ppm). The data obtained is in accordance with those in literature (Persic, 2017; Ticha, 2014; Iordănescu, 2012)

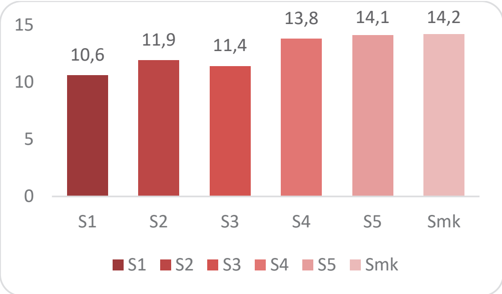

Figure 7. Total soluble solid content (°Brix) of Florina fruits

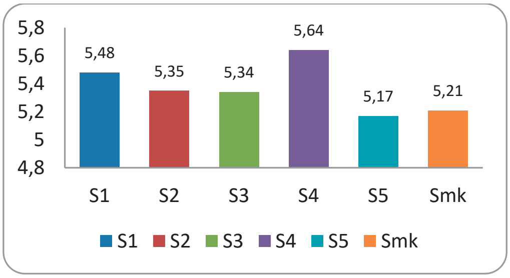

Figure 8. Fruits acidity (pH) in Florina cultivar

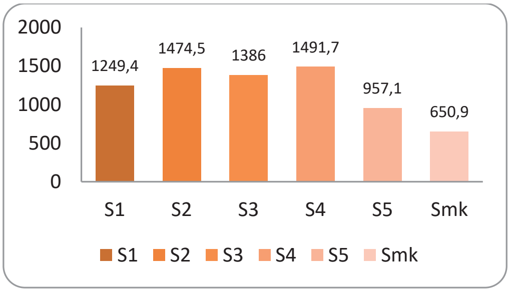

Figure 9. Total polifenols content (ppm) of fruits in Florina cultivar

The total polyphenol content exceeded the value of those in the supermarket, in all five locations. Compared to the data from the literature, they are above the quoted values, for example Otakar Rop et al. (2011) and Khanizadeh et al. (2007) considered that the phytochemical content of the fruits not only increases their quality, but it also, has a major impact on shelf life and susceptibility to diseases.

The moisture content of Golden delicious cultivar had quite close values in the experience, except for the fruits from the supermarket - very significantly negative and at the opposite pole, those from the Șiria 3 location - distinctly significantly positive. In all cases, the values obtained exceed to some extent those in the literature (Câmpeanu G. et al., 2009).

The highest content of mineral substances in *Golden delicious* fruits was recorded in the samples from Șiria 1 location and supermarket, both being distinctly significantly positive. The lowest mineral content was recorded from Șiria 3 fruits and 5, in both cases, the difference from the control being distinctly significantly negative.

Table 14. Fruit moisture content in Golden delicious cultivar from different locations

Provenance	The moisture content (%)	Relative value (%)	Difference towards the control value	Significance
Siria 1	85.54	101.10	0.93	-
Siria2	83.40	98.57	-1.21	0
Siria3	86.42	102.14	1.81	XX
Siria4	85.05	100.52	0.44	-
Siria5	84.79	100.21	0.18	-
Supermarket	82.51	97.51	-2.10	000
Experiment average	84.61	100.00	0.00	control
DL5% = 1.15% DL1% = 1.55%DL0.1% = 2.07%				

Table 15. Fruit mineral content in Golden delicious cultivar from different locations

Provenance	Mineral content (ppm)	Relative value (%)	Difference towards the control value	Significance
Siria 1	0.324	133.06	0.08	XX
Siria2	0.322	130.58	0.07	X
Siria3	0.162	66.12	-0.08	00
Siria4	0.172	70.66	-0.07	0
Siria5	0.164	67.49	-0.08	00
Supermarket	0.324	132.78	0.08	XX
Experiment average	0.240	100.00	0.00	control
DL5% = 0.06ppm DL1% = 0.08ppm DL0.1% = 0.11ppm				

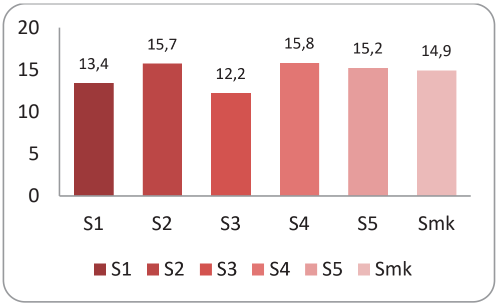

Figure 10. Total soluble solid content (ºBrix) of Golden delicious fruits

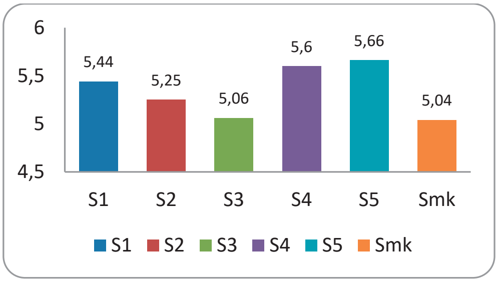

Figure 11. Fruits acidity (pH) in Golden delicious cultivar

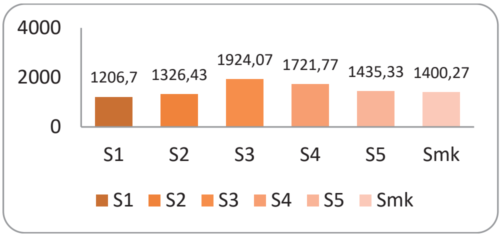

Figure 12. Total polifenols content (ppm) of Golden delicious fruits

The sugar content in the fruits of the *Golden delicious* cultivar exceeded the value of 15.0 ºBrix in those coming from the locations of Șiria 4, 2 but also from the supermarket, the lowest content being registered in the fruits coming from the location Șiria 12.2 ºBrix.

It should be noted that the fruits of the *Golden delicious* cultivar have accumulated high amounts of sugars in the locations Șiria 4, 2 and 5, values that exceed the data cited in the literature (Campeanu, 2009; Pleić, 2015; Persic, 2017; Asif, 2004)), in the other cases the results agreeing with other authors (Ticha, 2014; Šic Žlabur J. et al., 2013).

The acidity of the fruits had quite close values, the lower limits being registered in the fruits coming from the supermarket and from the location of Șiria 3, while the highest values were registered in Șiria 5 and Șiria 4 locations. Overall, the fruits acidity recorded higher values compared to those cited in the literature (Šic Žlabur J. et al., 2013).

Apples content in total polyphenols had the highest value from the Șiria 3 location (1924.07 ppm), followed by those from the Șiria 4 and 5 locations (1721.77 ppm, respectively 1435.33 ppm), all the others having lower values.

Compared to the data in the literature, the concentration in polyphenols was higher (Boyer, 2004, Chang Y., 2012, Pleić, 2015), fruits from private orchards accumulating larger quantities, thus having a stronger antioxidant action.

CONCLUSIONS

The study aimed the comparison of some physical and chemical features of three apple cultivars of different origin, starting from the fact that the fruits coming from the commercial orchards in which a proper crop technology is applied, have superior qualities in comparison to those coming from private orchards, which tend to produce fruits in an organic system, with minimal technological interventions.

Surprisingly, in some cases the aspect of the organicaly produced fruits was close to those bought from the supermarket. Therefore, Starkrimson and Florina fruits produced in Șiria 5, 4 and 3, overpassed in size the ones from the supermarket. In the case of Golden delicious cultivar, the values were closer, although the fruits from the Șiria 4, 1 and 2 locations overpassed the ones from the supermarket.

Regarding the chemical composition of Starkrimson cultivar, the ones form Șiria 4 accumulated the largest sugars and minerals content. The polyphenols content was higher in all the cultivars from the private orchards. In case of Florina apples, the minerals content was high in Șiria 1 and 3 locations, but the sugars content was below the supermarket samples. Golden delicious cultivar in Șiria 1 had the same minerals content as the supermarket apples, in the other cases, being somehow lower, but the sugars content exceeded the supermarket apples in three out of five locations. The total polyphenol composition had closer values, with the apples from S 3, S 4 and S 5 standing out.

All in all, the Șiria area proved that it has a pedoclimatic potential that allows, even in organic system, the production of high-quality apples that can compete with the fruits coming from the supermarket and produced in a conventional cropping system.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GROWING OF APPLE GENOTYPES WITH GENETIC RESISTANCE TO DISEASES - AN EFFICIENT METHOD TO MITIGATE PESTICIDE POLLUTION IN THE VOINEȘTI APPLE GROWING AREA

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Abstract

The aim of this study is to provide information on genetically diseases-resistant varieties of apple inbred at Voinești station and their behaviour under reduced number of phytosanitary treatments. The evolution of two harmful pathogens attack (apple scab and powdery mildew) on two common varieties, nine Romanian varieties and three foreign varieties was monitored in untreated control samples, samples treated following specific scheme and samples with no specific fungicides. The degree of attack and the effectiveness of the treatments were calculated. Assessments have shown that the classic varieties Ionathan and Golden Delicious had a high sensitivity to diseases in the climatic conditions of 2020. Romanian apple varieties proved total resistance to scab (100%) and very low susceptibility to powdery mildew (0.26-4.54%) so that a limited number of fungicidal treatments are needed. Their yields were at high level of quality and quantity, ranging from 24 to 36.7 tons/ha. The result of the growing of genetically diseases-resistant varieties is that pollution is mitigated and increased fruit production is achieved.

Key words*: apple diseases, effectiveness, resistance, treatments, yield.*

INTRODUCTION

Fruit growing, considering the diversity of species cultivated almost all over the world, is an important branch of agriculture by reason of its occupied area, ecological plasticity, and the nutritional importance of fruits. At the same time, it ensures the development of related economic fields (production of pesticides and fertilizers, manufacture of agricultural machinery and tools, employment and maintenance in rural areas, fruit trade, etc.). Practiced at high technological parameters, it can provide significant income to farmers. The apple (*Malus domestica* Borkh) is one of the oldest fruit species and ancient Roman and Greek historians have offered extensive descriptions (Jaloba et al., 2019). Apples have an energy value that sometimes reaches and exceeds 85 calories per 100 g (Chira & Pasca, 2004), and their content in aromatic and nutritious compounds gives them both nutritional and therapeutic value. Its high ecological plasticity, adaptability to different climatic conditions make it possible to grow apples from the plains to the mountain areas on any continent, except the Arctic. Due to this fact and the role played in human nutrition, the apple provides, along with bananas and citrus fruits, 2/3 of world fruit production (Popescu et al., 1982). The apple is very well adapted to the temperate climate whose extremes endures much better than other fruit tree species (Hoza, 2000). Soil and climatic conditions in Romania are very favourable for obtaining high quality apple productions as apple ranks second after plum, both in terms of production and area (Ghena & Braniste, 2003). A significant number of pathogens may cause large qualitative and quantitative losses in apple orchards and warehouses (Cociu et al., 1999). In the Voinești area, the most harmful are scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*), which makes it mandatory to apply phytosanitary treatments. The reason is the long-lasting monoculture and the climatic conditions specific to the area, too, which makes it possible the existence of a significant amount of inoculum in the area (Jaloba et al., 2019). The cost of the treatments is very high and represents approximately 50% of the maintenance costs and their non-application can lead to the loss of the yield both quantitatively and qualitatively (Chira & Pasca, 2004). On the other hand, the damages generated by the attack of pathogens and pests represent over 50% of the harvest, sometimes when no treatments are applied, the production being completely compromised (Ghena & Braniste, 2003). At the same time, chemical treatments are absolutely necessary (Baicu & Sesan, 1996), especially for sensitive varieties and in wetlands. For example, in the UK, current control of scab and mildew of apple requires the routine application of fungicides at 7-14-day intervals to achieve the blemish-free fruit required by the market. Such practices are generally effective, but with increased public concern about pesticides and rising costs to the grower, they are now less acceptable (Berrie & Xu, 2003).

The **scab** caused by the pathogen *V. inaequalis* is found in all areas where there are apple orchards (personally I saw apple attacked by scab in the extreme southeast of the country, too, in Lipnita village, CT county). Scab is important in areas of rain-fed agriculture throughout the world and must be controlled to produce marketable fruit on susceptible varieties (Aylor, 1998). The pathogen infects all the green organs of the tree, leaves, shoots, fruits. On both sides of the leaf surface appear small, gray spots with radial development (Gheorghies & Geaman, 2003). The attack causes exfoliation of shoots and branches and changes in tissues, which at strong infections, endanger the life of the tree itself (Dulugeac, 2011). The attacked fruits have gray-olive spots on which the tissue turns brown and cracks (Tomsa & Tomsa, 2003). Economic losses can be compounded by secondary rotting pathogens that enter these wounds and further destroy the apples (Aylor, 1998). The commercial aspect is damaged, the fruits become unfit for sale. The disease is favored by high atmospheric humidity, heavy rains, steam-saturated atmosphere and a temperature of 18°C, at which ascospores produce infections in 9-18 days, after an incubation period of 8 days. The primary infection is favored by high humidity that persists for 30 hours at 6°C (12 hours at 11°C or 9 hours at 25°C) (Gheorghies & Geaman, 2003). Secondary infections occur if plant parts are moistened for 4 to 18 hours at temperatures between 15 and 25°C (optimally at 19-20°C) (Baicu & Sesan, 1996).

V. inaequalis overwinters predominantly as pseudothecia (sexual fruiting bodies) that develop in apple leaf litter following a phase of saprobic growth after leaf abscission. Some overwintering may also occur as conidial pustules on shoots and bud scales without the involvement of the teleomorph (Becker et al., 1992; Holb et al., 2006; Holb, 2006). Infection is initiated in spring and early summer by ascospores (sexual spores) that are released by rainfall from pseudothecia. This release is timed to coincide with host budburst and leaf unfurling (Szkolnik, 1969; Brook, 1976; MacHardy & Gadoury, 1986.). Infection risk is greatest early in the growing season when leaves and fruit are young and at their most susceptible (Bowen et al., 2011).

Powdery mildew is also a disease found in all fruit growing areas and can cause significant damage to susceptible varieties (Ionathan, James Grives, Ionared). Its importance results from the fact that it can start very early, from the bud burst and can manifest throughout the growing season (Gheorghies & Geaman 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.When young leaves are infected, they tend to increase in length but not in width, to be stunted, and to become folded longitudinally (Anderson, 1956). Infected leaves initially show white lesions on the adaxial surface and chlorotic patches on the abaxial surface. Infected leaves tend to crinkle and curl, turn brown and drop prematurely (Turechek et al., 2005). Severely infected terminals have shortened internodes, and are covered with a silver-gray mycelium. The grayish white fungal growth turns brown, any many dark brown fruiting bodies appear (Hickey & Yoder, 1990; Gheorghies & Geaman, 2003). The attack on young fruits, caused by secondary infection, can lead to their drying and falling, but they often result in the appearance of a brown network of suberified tissue, very obvious after the fruit has developed (Gheorghies & Geaman, 2003). Infected plants are characterized by reduced photosynthesis and transpiration, resulting in suboptimal carbohydrate assimilation and reduced growth (Ellis et al., 1981). In nureseries, the fungus can spread to all developing leaves and cause severe stunting of vegetative terminal shoot (Hickey & Yoder, 1990). Mycelium overwintering in dormant buds normally initiates primary infection on young leaves, which produce inoculum in the form of conidia for the secondary cycles (Urbanietz & Duneman, 2005). The fungus typically overwinters in vegetative buds. In the early spring, the fungus resumes growth, and spores from infected shoots can initiate secondary infections (Turechek et al., 2005). Secondary infections may occur on newly forming flower buds, which will remain dormant until the following spring. Since these buds will be diseased when they open, severe infection can eliminate the crop the following season (Marine et al., 2010). The fungus survives the winter in buds, making it difficult to control during the early spring development of apple trees. First sprays against powdery mildew can only be effective after bud burst when budscales open and overwintered mildew mycelia become available for fungicides (Holb, 2014). 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).

MATERIALS AND METHODS

The aim of this paper was to monitor the behaviour towards the main diseases of some apple varieties created at the Research and Development Station for Fruit Growing Voineşti in comparison with two well-known classic varieties, as well as their yield.

The research was carried out between March and October 2020. 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 soil 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. Two classic varieties were tested (Ionathan and Golden Delicious, 17-year-old orchards), apple varieties with genetic resistance to diseases created at S.C.D.P. Voineşti, between 2004 - 2009 (Real, Revidar, Remar, Voinicel, Cezar, Iris, Inedit, Valery, Redix) and 3 varieties from abroad (Rubinola, Goldrush, Topaz). The trial was conducted using the randomized complete block method in four repetitions. Each plot had 5 trees. For the classic varieties (Ionathan and Golden Delicious) taken as a control, four untreated plots were included, and for the rest of the orchard a scheme of 14 treatments with fungicides, insecticides and foliar fertilizers was applied, as shown in table 1. Varieties with genetic resistance have not been treated with fungicides for scab and powdery mildew. Assessments made 10 to 12 days after each treatment focused on the evolution of the pathogens *V. inaequalis* (scab) and *P. leucotricha* (powdery mildew).

Thus, a comparative study was made between the apple varieties with genetic resistance to diseases created at SCDP Voinești and the apple varieties from abroad, tested in the orchard, in conditions of reduced number of treatments, cultivated in high density plantations, regarding the reaction to the attack of the two pathogens, in order to identify the optimal assortment for this region in the conditions of a technology as clean as possible.

50 leaves per tree and 30 fruits per three trees in the middle of each plot were assessed. Assessments were made on the frequency (F %) and the intensity (I %) 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 fungicide 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-9 software. At harvest time, the yield/tree was also recorded by weighing all the fruits per tree. The application equipment was a mist blower Solo 423 atomizer. The planting distance for Ionathan 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. For the varieties with genetic resistance to diseases, the trees were planted at a distance of 3.5 x 1 m (2,857 trees/ha), being grafted on M9 rootstock, with the shape of a spindle crown. In 2020, the trees have reached the age of 11 years.

Table 1. Schedule of phytosanitary treatments performed on apples to prevent and control diseases and pests (Mix tank 1500 l/ha)

No. crt.	Stage (Season)	Disease or/and pest	Recommended commercial products and rates			
			Sensitive varieties		Resistant varieties	
			Products	Conc. %	Products	Conc. %
0	1	2	3	4	5	6
1	Bud swelling	San José scale, moths eggs, aphids, mites, etc.	Chemol 90 EL	1.5	Chemol 90 EL	1.5
2	Bud break	Apple blossom weevil Powdery mildew	Novadim Progress Kumulus DF (Polisulf tip MIF)	0.1 0.1 (0.2)		
3	Leaves unfolded	Fireblight Scab, powdery mildew Mites	Bouillie Bordelaise WDG (Topsin 70 WDG + Merpan 80) Novadim progres	0.5 (0.07, 0.15) 0.1		
4	Beginning of flowering: about 10% of flowers open	Scab, powdery mildew Fireblight Leafminers, defoliators, aphids	Chorus 50 + (Score 250 EC) Merpan 80 WDG Aliette 80 WG Insegar 25 WG Foliar fertilizer	0.05 (0.02) 0.15 0.3 0.03 0.2	Aliette 80 WG Insegar 25 WG Foliar	0.3 0.03 0.2
5	Flowers fading: majority of petals fallen	Scab, powdery mildew Leafminers, defoliators	Score 250 EC + Dithane M 45 Mavrik 2 F Foliar NPK	0.02 0.2 0.05 0.5	Mavrik 2 F Foliar NPK	0.05 0.5
6	Fruit size up to 10 mm	Scab, powdery mildew Leafminers, defoliators, aphids	Sercadis (Embrelia) Merpan 80 WDG Actara 25 WG	0.015 (0.1) 0.15 0.01	Actara 25 WG	0.01
7	Fruit diameter up to 40 mm	Identical to treatment 6 + Codling moth	Luna Experience 400 SC+ Dithane M 45 Novadim Progress Foliar - Calcibor	0.05 0.2 0.1 0.2	Novadim Progress Foliar - Calcibor	0.1 0.2
8	Fruit about half final size	Identical to treatment 6 + Codling moth	Sercadis Delan Pro Actara 25 WG	0.015 0.16 0.01		
9-10	Fruit about 70% final size	San José scale Scab, powdery mildew Apple woolly aphid	Luna Experience 400 SC + Dithane M 45 Mospilan 20 SG	0.05 0.2 0.025	Mospilan 20 SG	0.025
11	Fruit about 90% final size	Scab, powdery mildew San José scale Mites	Topsin 70 WDG Dithane M 45 Proteus OD 110	0.1 0.2 0.05		
12-13	Beginning of ripening	Scab, powdery mildew Codling moth (G_2)	Topsin 70 WDG Merpan 80 WDG Nurelle D 500 EC	0.1 0.15 0.1	Nurelle D 500 EC	0.1
14	Advanced ripening	Scab, powdery mildew Storage diseases Codling moth, mites Reticulated tortrix	Bellis Mospilan 20 SG Foliar Calcitek	0.05 0.025 0.2	Mospilan 20 SG Foliar Calcitek	0.025 0.2
	TOTAL TREATMENTS		14		8	

- Sensitive varieties: Amount of pesticides = 101.6 kg (l)
- Resistant varieties: Amount of pesticides = 49.4 kg (l) Represents 48.6%

The soil maintenance system includes strips between trees within a row clean of weeds alternating with temporarily grassed strips (1 - 3 years) between rows of trees that have been maintained by 3 passes with the lawn mower during the growing season. During the vegetative rest, the autumn plowing was carried out at a depth of 20 cm. The pruning was carried out in early spring, before the beginning of the vegetation.

RESULTS AND DISCUSSIONS

Weather conditions

In the Voinești area, the average annual (normal) temperature is 8.8°C, specific to the hill areas, but optimal for trees growing, especially for apple. The amount of average multiannual precipitation is 782 mm, but in recent decades there has been a rainfall deficit in August-September, when the requirements for fruit growing are increasing, requiring irrigation of trees. The vegetation period covers about 180 days, from April 1-10 to October 1-10.

In the research year 2020, the weather conditions were quite atypical compared to the average of the region. The weather data are summarized in Table 3. It is noted that in May, June and July were recorded the largest amounts of precipitation (61.3 mm, 119.6 mm and 77.2 mm) and that April was very dry (11.2 mm).

Behaviour of apple varieties against the attack of scab andpowdery mildew

The reaction of apple varieties in the agro-climatic conditions of 2020 was different, especially in terms of the attack of powdery mildew.

The values of temperature and humidity facilitated the spread of the two diseases and the fact that only 8 treaments were applied to the control sample, mainly with insecticides, determined the massive existence of the infection.

Thus, on the leaves, the Ionathan variety registered a degree of attack of 50.85% for scab (which makes it medium resistant to weakly resistant) and 64% for powdery mildew (sensitive). The Golden Delicious variety proved to be very sensitive to rape (degree of attack 63.25%) and quite sensitive to powdery mildew (degree of attack 25.38%).

The scab attack (Figure 1) occured from spring to autumn through isolated or confluent spots on both sides of the leaves, having a gray color and indefinite edges. In their center could be seen a central point, darker coloured, developed subcutically. After a while, the spots became olive-green or brown, due to the appearance of the fruiting bodies of the fungus (Severin et al., 2001). The symptoms on the fruit were in the form of spots similar to those on the leaves, circular, brownish-black, with a diameter of 5-10 mm. Young, intensely attacked fruits were also noted, which remained underdeveloped and cracked near the spots.

Figure 1. Scab on Ionathan leaves (original)

Table 2. Behaviour of apple varieties studied against the attack of scab and powdery mildew on leaves

No. crt	Variety	Degree of atack (%) Scab	Degree of atack (%) P. mildew
I. Control varieties			
1.	Ionathan	50.85b	64a
2.	Golden Delicious	63.25a	25.38b
II. Varieties inbred at SCDP Voinești			
1	Real	0.0c	4.54c
2	Revidar	0.0c	0.0e
3	Remar	0.0c	2.16cde
4	Voinicel	0.0c	0.6de
5	Cezar	0.0c	0.0e
6	Iris	0.0c	4.2c
7	Inedit	0.0c	0.72de
8	Valery	0.0c	2.36cde
9	Redix	0.0c	0.36de
III. Varieties from abroad			
1	Rubinola	0.0c	2.8cde
2	Goldrush	0.0c	0.6de
3	Topaz	0.0c	3.6cd
LSD P = .05		3.592	2.053
Standard Deviation		2.511	1.436

The attack of powdery mildew produced the first symptoms on the young leaves, which in the strongly attacked varieties were covered by white powder, then yellowish, formed by the mycelium and conidiophores with conidias of the pathogen. Over time, the leaves remained small, narrow, thickened and twisted towards the upper face, taking on the appearance of a little boat (Figure 2).

Figure 2. Powdery mildew on Ionathan leaves (original)

Many of them became breakable and fell early. On the fruit, the attack generated the appearance of a brown network of suberified tissue, very visible when harvesting the fruit. The results are shown in Tables 2 and 4.

Assessing the behaviour of genetically resistant varieties to diseases against powdery mildew, it was found the manifestation of a slight sensitivity differentiated from variety to variety. The values of the degree of attack are insignificant compared to the attack recorded on the variety Ionathan (64%), known as standard of sensitivity to powdery mildew.

Table 3. Precipitation and temperature during 2020 growing vegetation season

	Month							
	Apr	May	June	July	Aug.	Sept	Oct	
Periods	The growing season 2020: Precipitation (mm) for 10-day periods							Sum
1-10	0	29.7	21.1	22.5	0	49.5	43.8	166.6
11-20	11.4	0.6	91.4	54.7	14.1	0	9.1	181.3
21-30	1	31	7.1	0	0.2	2.7	5	47
Sum	12.4	61.3	119.6	77.2	14.3	52.2	57.9	394.9
	The growing season 2020: Mean min. air (°C) for 10-day periods							Mean
1-10	-0.2	7.2	10.7	16.5	17.4	13.9	12.4	11.12
11-20	2.6	11.4	15.6	13.3	15.3	12.3	6.6	11.01
21-30	3.3	7.8	14.9	16.7	15.6	10.2	6.3	10.68
Mean	1.9	8.8	13.73	15.5	16.1	12.3	8.43	10.93
	The growing season 2020: Mean max. air (°C) for 10-day periods							Mean
1-10	17.7	22.4	25.9	31	32.5	30.1	24.1	26.24
11-20	21.2	25.6	27.5	29.47	30.8	28.3	20.2	26.14
21-30	22.1	20.8	29.8	32.6	33.5	26	17.5	26.04
Mean	20.33	22.93	27.73	31.00	32.27	28.13	20.60	26.14

On fruits, the DA% values in scab were between 12.3% in Ionathan and 15.49% in Golden Delicious. In powdery mildew, DA% in the control varieties had values of 20.6% in Ionathan and 7.14% in Golden Delicious. These values made the fruit look damaged, crusty and rough, with no commercial value. They are also susceptible to be attacked by other pathogens such as moniliosis (Chitulescu & Cristea, 2017a; 2017b; Cristea et al., 2017) and do not resist during storage.

Under these conditions, in all studied varieties, the scab infection was non-existent, they showed maximum resistance on both leaves and fruits (Figure 3).

In contrast, in the case of *P. leucotricha* infection, some varieties showed some sensitivity to leaf attack (Real 4.54%, Remar 2.16%, Iris 4.2%, Valery 2.36%, Rubinola 2.8%, Topaz 3.6%). This requires the need for treatments against this disease at critical stages. Regarding the treated sample in which the treatment scheme was applied in 14 phenophases (Table 1) to the two varieties, Ionathan and Golden Delicious, a good efficacy was found in controlling the 2 pathogens. The negative consequence was the increase of production cost due to major inputs and the intensification of pesticide pollution. The results are summarized in Tables 5 and 6.

The influence of genotype and treatments on production. The production potential of the apple varieties studied is expressed by the production achieved per unit area, recorded in 2020 (Table 7). The yield of the studied varieties had good and very good values, the production obtained per hectare being different, clearly superior in quantity and quality to that of the classic varieties without antifungal treatments.

Tabel 4. Behaviour of apple varieties studied against the attack of scab and powdery mildew on fruits

No. crt	Variety	Degree of atack (%)	
		Scab	P. mildew
I. Control varieties			
1.	Ionathan	12.3b	20.6a
2.	Golden Delicious	15.49a	7.14b
II. Varieties inbred at SCDP Voineşti			
1	Real	0c	1.56c
2	Revidar	0c	0c
3	Remar	0c	0.76c
4	Voinicel	0c	0c
5	Cezar	0c	0c
6	Iris	0c	1.38c
7	Inedit	0c	0.26c
8	Valery	0c	0.81c
9	Redix	0c	0c
III. Varieties from abroad			
1	Rubinola	0c	0.92c
2	Goldrush	0c	0c
3	Topaz	0c	1.18c

Standard Deviation 1.235 1.413
LSD P = .05 0.864 0.988

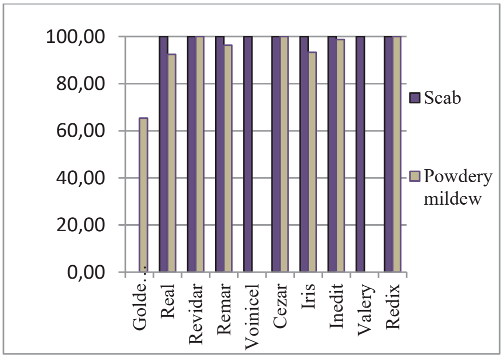

Figure 3. Effectiveness% of growing varieties with genetic resistance to scab and powdery mildew on leaves

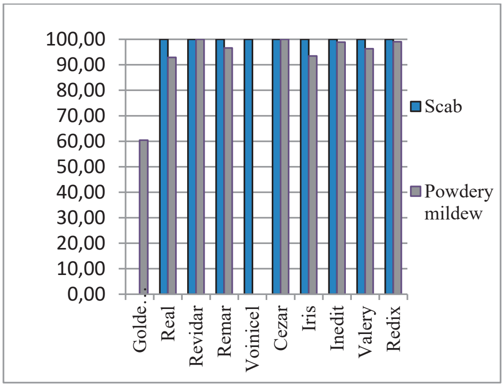

Figure 4. Effectiveness% of growing varieties with genetic resistance to scab and powdery mildew on fruits

Table 5. Behaviour of treated control apple varieties to the attack of scab and powdery mildew on leaves

No. crt	Variety	Degree of attack (%) Leaves	
		Scab	P. mildew
Untreated			
1.	Ionathan	50.85	64
2.	Golden Delicious	63.25	25.3
Treated			
1	Ionathan	5.59	15.51
2	Golden Delicious	16.99	2.14

Table 6. Behaviour of treated control apple varieties to the attack of scab and powdery mildew on fruits

No. crt	Variety	Degree of attack (%) Fruits	
		Scab	Scab
Untreated			
1.	Ionathan	12.3	20.6
2.	Golden Delicious	15.49	7.14
Treated			
1	Ionathan	2.18	3.24
2	Golden Delicious	3.69	0

In the 2020 study year, the production of the varieties created at SCDP Voineşti was between 24 t/ha for the Redix variety and 36.7 t/ha for the Valery variety. Productions of over 30 t/ha were also made by Real (31.5 t/ha), Remar (34.7 t/ha), Cezar (35.6 t/ha) and Iris (36.5 t/ha) varieties. For varieties of foreign origin, productions were made for all varieties over 30 t/ha, culminating in 39.7 t/ha (Goldrush).

The classic varieties where no fungicides were applied to control rape and powdery mildew used as a control had low and poor quality yields (14.3 and 12.4 t/ha), which differed significantly from the others (Table 7).

Even if there is research on the application of non-chemicals in controlling plant pathogens (Cristea et al., 2017; Ichim et al., 2017) the application of fungicides is the main interven-tion in stopping the attack of dangerous pathogens on plants.

In the Voineşti orchards, in the years with excessive rains in May and June, the non-application of one or two treatments against scab to sensitive varieties compromises the harvest in proportion of 50-70%, especially in terms of fruit quality (Petre et al., 2006).

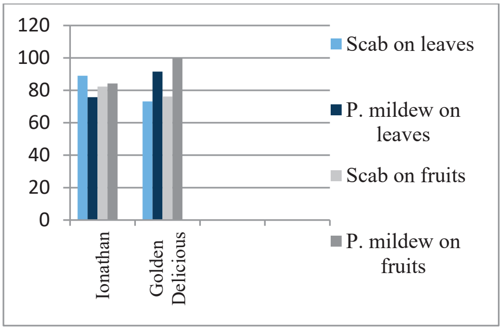

Figure 5. Effectiveness% of treatments applied to control varieties in 2020

Figure 6. Experimental orchard in Voinești

Tabel 7. Production of varieties with genetic resistance to diseases compared to untreated controls

No. crt.	Variety/ rootstock	Yield potential (t/ha)	Diference (%)
I. Control varieties - Untreated			
1	Ionathan	14.3	15.3
2	Golden Delicious	12.4	0
II. Varieties created at SCDP Voineşti			
1	Redix / M.9	24.0	93.5
2	Iris / M.9	36.5	194.3
3	Real / M.9	31.5	154
4	Remar / M.9	34.7	179.8
5	Inedit / M.9	29.9	141.1
6	Voinicel / M.9	25.4	104.8
7	Valery / M.9	36.7	195.9
8	Cezar / M.9	35.6	187
9	Revidar / M.9	26	109.6
III. Varieties from abroad			
1	Rubinola / M.9	31.7	155.6
2	Goldrush / M.9	39.7	220.2
3	Topaz / M.9	33.6	170.9

Regarding the grouping of fruits in quality classes for each apple variety studied, for fruits Extra + Ist category, the best performing varieties were the varieties Real (94%), Cezar (93%), Remar (92%), Valery (91%) and Redix (90%). Furthermore, a number of 5 varieties, respectively: Redix, Real, Remar, Valery and Cezar, created at SCDP Voinești, offered fruits in the extra category, with a percentage of over 70%.

The apple varieties from abroad, Rubinola and Topaz, also registered excellent results, having over 70% fruits in the extra category and 86%, respectively 85% cumulated in the extra + Ist categories (table 8). The lowest percentage of fruits in the extra category was registered by the varieties Iris (62%), Revidar (63%), Inedit (65%) and Goldrush (60%), which indicates that these varieties may have a more suitable destination, much for industrialization.

Table 8. Grouping of fruits in quality classes for the apple varieties studied

Nr. crt	Variety	Quality classes (%)			
		Extra	Ist	IInd	Extra + Ist
I. Varieties created at SCDP Voineşti					
1	Redix	72	18	10	90
2	Iris	62	13	25	75
3	Real	75	19	6	94
4	Remar	74	18	8	92
5	Inedit	65	13	22	78
6	Voinicel	68	13	19	81
7	Valery	74	17	9	91
8	Cezar	76	17	7	93
9	Revidar	63	17	20	80
II. Varieties from abroad					
1	Rubinola	71	15	14	86
2	Goldrush	60	15	25	75
3	Topaz	70	15	15	85

Varieties Iris (25%), Inedit (22%) and Goldrush (25%) had the highest percentage of low quality fruits, but their productions were satisfactory.

CONCLUSIONS

In 2020, all apple varieties known to have genetic resistance to diseases, grown in a high density system, showed a very good resistance to scab (*V. inaequalis*) and a high degree of resistance to powdery mildew (*P. leucotricha*) with insignificant degree of attack values, between 0.26 and 4.54%.

On the other hand, the classic varieties Ionathan and Golden Delicious, in the untreated samples, proved high susceptibility to diseases. At scab, on the leaf, the degree of attack% was 50.85 and 63.25%, respectively, and at powdery mildew 64, respectively 25.3%.

It is therefore necessary to apply a large number of phytosanitary treatments to reduce the negative effects on the production and integrity of orchard trees, applied to the warning at the optimal stages and with appropriate pesticides.

Depending on the treatments applied to the sensitive apple varieties, in number of 14, there was a consumption of pesticides of 101.6 kg per hectare, compared to 8 for the varieties with disease resistance in which the amount of pesticides was only 49.4 kg, representing 48.6%.

The treatment scheme applied to sensitive varieties failed, under the climatic conditions of 2020, to control the two pathogens completely, resulting in an efficiency on leaves of 89% (scab) and 75.77% (powdery mildew) for the variety Ionathan, respectively of 73.14% (scab) and 91.55% (powdery mildew), for the Golden Delicious variety.

In terms of fruit production, varieties with genetic resistance to diseases to which no treatment for scab and powdery mildew were applied had a high productive yield, clearly superior in quantity and quality to that offered by the untreated classic varieties.

Apple varieties with genetic resistance to diseases, grown in a high density system, offer beneficial economic results for producers, environmental protection and commercial products with low pesticide residues, fit for human consumption, marketable and increasingly demanded in the distribution network.

ACKNOWLEDGEMENTS

Thanks to IMRVA Doctoral School within University of Agronomic Sciences and Veterinary Medicine of Bucharest and to Research and Development Station for Fruit Growing Voineşti for their support.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDY OF BLOSSOM FREEZING IN PEACHES BY MATHEMATICAL AND STATISTICAL METHODS

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Abstract

Fourteen varieties of peaches were included in the present study. The degree of blossom freezing (%) in two consecutive years 2019-2020 was studied. For this purpose, hierarchical cluster analysis and single-factor analysis of variance and Duncan's test were applied to assess the differences. It was found that the studied varieties can be grouped in four clusters according to similarity in the degree of frost in 2019 and in 2020. It was proved that in 2019 with the highest percentage of frost was 'El. 19-78' (98.33 %), followed by 'Ferlino', but with minor damage (50.67%). The varieties 'Evmolpia', 'Filina', 'Flavia', 'Redhaven' and 'El. 19-77' had no frozen blossoms. According to the degree of frost in 2020, the varieties formed three clusters, with 'Evmolpia' in a separate cluster, as a variety with minimal effects of low temperatures (59%). In 2020, the percentage of freezing of blossoms increased in most varieties: 'Ferlino' (92%), 'Puldin' (99%), 'Filina' (96%), 'Flavia' (92%), 'Maycrest' (98.67%), 'Redhaven' (99.67%), 'El. 7-59' (92%), 'El. 4-22' (94.67%), 'El. 91-23' (96.23%), and 'El. 19-78' (93%).

***Key words**: peaches, frost, clusters, dispersion analysis.*

INTRODUCTION

Peach is a heat-loving fruit plant (Petrov & Grigorov, 1981). The thermal regime affects the development of the peach tree during the different periods of vegetation and winter dormancy. During the winter dormancy, the peach tree satisfies its needs for cold. In order to pass the same period, the individual peach varieties require a different amount of cold with temperatures lower than 7°C. The peach tree is often exposed to extremely low temperatures. The flower buds are the most sensitive, followed by the core, wood, cambium and leaf buds.

In order for the peach tree to be stable at the same low temperature, it depends on several factors - hereditary varietal characteristics, degree of nutrient supply, degree of hardening, nature of the cold - gradually or sharply lowering the temperature, its duration of action. After satisfying the need for cold and the warming of weather, resistance begins to decline. It may rise again in the presence of constant negative temperatures. When the positive temperatures increase, the process of hardening is very fast, due to which, the cold resistance of the flower buds drops sharply. This happens most often in late February and March, when the development of flower buds begins.

As a result of a sharp cold snap on March 29, 2015, in the USA, Chen et al. (2016) proved that spring temperature drops have a critical impact on peach yields and led to serious complications in fruit growing. The effects of peach freezing and crop reduction as a direct consequence of this was analyzed. The presence of significant correlations between the tolerance to temperature anomalies and yields was proven.

Reig et al. (2013) examined frost-damaged blossoms in fifty-six varieties of peaches and nectarines obtained from different breeding programs over a period of two years. The tolerance of the varieties to low temperatures and the susceptibility of their pistils to damage as a result of frost were analyzed.

Miranda et al. (2013) claimed that the phenological evolution of peach can be adequately simulated by a simple sequential statistical model that involves cooling by a dynamic method, and forced heat accumulation, through increasing sums of degrees. They conducted a study and found that there is good accuracy for all varieties and stages of the test, and the studied parameters of the model make physiological sense. The results suggested that this method can be used to accurately assess the phenological evolution of these species in a wide range of climatic situations. This made it suitable for assessing the impact of climate change on crop phenology and for assessing the risk associated with climate and extremely low spring temperatures.

Szalay et al. (2009) study on the frost resistance of flower buds found that different parts of trees have very different levels of frost resistance and this resistance also varies considerably over time. During dormancy, flower buds were most sensitive to frost. The authors conducted a study in three consecutive winters with very diverse weather conditions. The freezing processes of overwintering organs were determined by environmental factors and mainly by temperature. Thus, in the same place, the degree of development of flower buds and the development of frost resistance might differ from year to year.

It is important to know the cold tolerance of peach varieties during the various phenological stages of flowering. Szalay et al. (2018) conducted an experiment for artificial freezing in a climate chamber in five selected years between 2007 and 2016. They determined the frost tolerance of the generative organs of three varieties of peaches at different phenological stages of flowering.

Their results showed that LT 50 values of the studied peach varieties in the phase of swollen buds on average for five years were between -6.8°C and -11.2°C depending on the variety, and with the progress of the phenological phases the cold resistance of the generative organs decreases. At the end of flowering, LT 50 values varied between -1.7°C and -4.1°C. This study showed that trees with delayed development were more susceptible to damage of flower organs by cold.

Szalay et al (1999) conducted a study on the artificial frost tolerance of peach varieties and found that during dormancy flower buds are the most sensitive organs of trees to frost. Their aim was to determine the frost tolerance of six varieties of peaches during dormancy. The buds were examined in artificial freezing tests and LT50 values were determined for each variety as well a sampling date. The authors determine that the frost tolerance of flower buds is highest in mid-December, regardless of the variety. The dynamics of the varieties were similar to each other.

In 1998, due to the mild weather in January, the frost tolerance of flower buds decreased rapidly and the LT50 reached -15°C by mid-February. In 1999, the decrease in the level of frost tolerance of flower buds was significantly lower due to the cold weather. LT50 values reached -15°C only in the first half of March.

Mokreva et al. (2001) studied the resistance of plants to environmental conditions by analysis of variance.

The aim of the present study was to examine the impact of low ambient temperatures on peach orchards.

MATERIALS AND METHODS

The experiment was conducted in the experimental base of the Institute of Fruit Growing - Plovdiv. The plants were planted in 2014 and refined on a peach seedbed. The trees were formed by the free-growing crown system planted at a distance of 5 m between rows and 3m in a row. They were grown under the system of black fallow and conventional plant protection. The subject of the study were eight varieties of dessert peaches 'Ferlino', 'Laskava', 'Evmolpia', 'Puldin', 'Filina', 'Flavia', 'Maycrest', 'Redhaven' and six selected hybrids, observing their phenological development. Experimental data on the degree of freezing of blossoms (%) of each variety in the period 2019-2020 were reported. Values were obtained on the basis of 300 examined blossoms, divided into three replications (one hundred plants), taken randomly from all parts of the tree crowns. 'Flavia' was created at the Institute of Fruit Growing - Plovdiv, Bulgaria. Its fruits ripened very early, June 15-20 and had an average weight of 140-150 g. They have good taste.

'Maycrest' was created in the United States. The fruits ripened on June 20-25. They were medium to large 150-160 g, and had a very good sensor profile.

'Filina' was established at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripened 3-4 days after those of the 'Maycrest' standard.

They are very large with an average weight of 170 g. They have very good taste, surpassing ‘Maycrest’ in terms of fruit weight.
‘Redhaven’ was created in Michigan USA. The fruits ripened in late July - early August. They were large with an average weight of 160 g. The fruit flesh had very good taste. The variety was resistant to winter frosts and tolerates lower temperatures relatively well during flowering.
‘Puldin’ was established at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripened in early August 2-3 days after the ‘Redhaven’ standard. They have excellent taste and an average weight of 180 g.
‘Laskava’ was established at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripened on August 5-10. They were 250-300 g., red, with excellent taste. The variety had a high degree of resistance to *Sphaerotheca pannosa* var. *persicae* and good resistance to *Taphrina deformans.*
‘Laskino’ was established at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripened in late August, they had an average weight of 210-240 g. It had high resistance to *Sphaerotheca pannosa* var. *persicae* and good resistance to *Taphrina deformans.*
‘Ferlino’ was introduced by Italy. The fruits ripened in late August. They were large with an average weight of 220-250 g. It had very good taste.
‘Evmolpia’ was created at the Institute of Fruit Growing - Plovdiv, Bulgaria. Its fruits ripened on September 10. They were very large 210-220 g. with a delicate texture, very juicy. The variety had a pronounced resistance to *Taphrina deformans.*
‘El. 7-59’ was established at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripened after August 20. They had very good taste. They had an average weight of 240-260 g.
‘El. 4-22’ was established at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripen in late August. The average fruit weight is 130-160 g.
‘El. 91-23’ was created at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripened in late August. The average weight of the fruit was 180-210 g. It has a yellow-red color and bright yellow fruit flesh.
‘El. 19-77’ was established at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripened on June 20-30 and had an average weight of 130-160 g. The skin was orange-red in color and the taste was very good.
‘El. 19-78’ was created at the Institute of Fruit Growing - Plovdiv, Bulgaria. The fruits ripened on June 20-30 and had an average weight of 120-150 g. The color was orange-red and the taste was very good.
Peach varieties were grouped through hierarchical cluster analysis by the method of intergroup binding and the quadratic Euclidean distance as a measure of similarity. A comparative assessment of the degree of freezing of the different varieties for each year of study was performed by single-factor analysis of variance and Duncan's test to assess the differences at a significance level of $\alpha \leq 0.05$. Graphic images visualizing the change of frozen plants during the two years of the study were constructed.
The reported negative temperatures in 2019 (Table 1) were from March. On March 4, the first negative temperature was reported -0.8°C for three hours and the lowest measured -1.9°C. The next measured temperature was also the lowest for the month -2.3°C and average -1.3°C with a duration of four hours. These first negative temperatures did not significantly affect the flower buds because their development had not begun yet.

Table 1. Minimum, average daily temperatures and duration of action for 2019 and 2020

Year	Data	Average negative temperature	Lowest negative temperature	Duration of the period (hours)
2019	4.3.2019	-0.8	-1.9	3
	5.3.2019	-1.3	-2.3	4
	25.3.2019	-0.2	-0.2	3
	29.3.2019	-0.8	-1.7	5
2020	16.3.2020	-1.2	-2.6	6
	17.3.2020	-2.3	-4.9	10
	18.3.2020	-1.2	-2.4	6
	19.3.2020	-0.9	-1.6	6

The third measured negative temperature was March 25, respectively -0.2°C with a duration of three hours. At that time, the ‘Ferlino’ variety was in the full flowering phase BBCH63-65, and the ‘Laskava’, ‘Puldin’ and ‘Redhaven’ varieties were in the BBCH67 petal fall phase. All other varieties and elites were located between the two phenological phases.

The last measured negative temperature for 2019 was on March 29 with an average negative temperature of -0.8°C for five hours and the lowest reported -1.7°C. On this date, only ‘Elite 4-22’ was in the phenological phase of petal fall BBCH 67. All others were between the same phenological phase and the formation of BBCH69.

In 2020, the first negative temperature was recorded on 16 March, with the lowest being -2.6°C and the average -1.2°C for six hours. At that time, the ‘Laskino’ variety was in the phenological phase BBCH57 of the beginning of the display of petals. The variety ‘Evmolpia’ and ‘Elite 7-59’ were in the next phase of development BBCH59 - mass display of petals. ‘Ferlino’ and ‘Puldin’ were at the beginning of flowering BBCH61-62, and the ‘Filina’ variety was in full flowering BBCH 63-65.

The next reported temperature was also the lowest: -4.9 °C with an average temperature of -2.3°C for ten hours on March 17. At this time, the ‘Redhaven’ variety was at the beginning of flowering BBCH 61-62. Variety ‘Flavia’ and ‘Elite 91-23’, ‘Elite 19-78’ were in full flowering BBCH63-65.

The third reported negative temperature was on March 18 with an average value of -1.2°C with a duration of action of six hours and the lowest reported -2.4°C. This date coincides with the beginning of flowering BBCH61-62 of ‘Lascava’ and ‘Maycrest’ and ‘Elite 7-59’ and full flowering BBCH63-65 of ‘Puldin’. The last negative temperature was reported on March 19, which was also the lowest -1.6°C with an average value of -0.9°C for six hours. Only the ‘Redhaven’ variety is in the full flowering phase of BBCH 63-65. Due to the faster phenological development in 2020, the cause damage was greater.

The IBM Statistics SPSS 24 statistical software product (Landau & Everitt, 2004) was used for the mathematical data processing, as well as the tools provided by MicroSoft Excel.

RESULTS AND DISCUSSIONS

The phenological development of peach varieties and elites in 2019 (Table 2) and 2020 (Table 3) varies due to the difference in temperatures. In 2019, the development of flower buds began in March and in 2020 it began in February. Negative temperatures in 2019 do not cause serious damage, as they do not coincide with the sensitive phases of development and were not too low. In the next year the reported temperature was significantly lower and with a longer effect. It coincided with the development of fruit buds, and therefore the damage was greater.

To establish similarities in the degree of frost in 2019 and 2020 between the studied peach varieties, a hierarchical cluster analysis was applied. The result of the performed clustering was presented by a dendrogram in Figure 1.

Table 2. Phenological development of peach varieties and elites for 2019

Name	A	B	C	D	E	F	G	H	I	J
Ferlino	8.03	12.03	16.03	18.03	20.03	25.03	28.03	4.04	27.07	28.08
Lascava	6.03	10.03	14.03	16.03	19.03	23.03	25.03	3.04	30.07	9.08
Evmolpia	7.03	11.03	15.03	17.03	20.03	24.03	26.03	4.04	4.09	16.09
Puldin	6.03	10.03	14.03	16.03	19.03	23.03	25.03	3.04	15.07	29.07
Filina	6.03	10.03	14.03	16.03	18.03	22.03	24.03	2.04	29.05	18.06
Flavia	4.03	8.03	12.03	14.03	17.03	21.03	23.03	1.04	29.05	19.06
Maycrest	6.03	10.03	14.03	16.03	18.03	22.03	24.03	2.04	29.05	18.06
Lascino	8.03	12.03	16.03	19.03	22.03	26.03	30.03	5.04	27.07	26.08
Redhaven	6.03	10.03	14.03	16.03	19.03	23.03	25.03	2.04	3.07	23.07
El. 7-59	7.03	11.03	15.03	18.03	20.03	24.03	27.03	3.04	29.07	26.08
El. 4-22	7.03	10.03	15.03	19.03	22.03	26.03	29.03	6.04	2.08	4.09
El. 91-23	7.03	11.03	15.03	18.03	21.03	24.03	27.03	3.04	26.07	25.08
El. 19-77	6.03	11.03	15.03	17.03	20.03	24.03	26.03	1.04	28.05	29.06
El. 19-78	6.03	10.03	14.03	16.03	18.03	22.03	24.03	2.04	20.06	1.07

Table 3. Phenological development of peach varieties and elites for 2020

Name	A	B	C	D	E	F	G	H	I	J
Ferlino	18.02	7.03	9.03	12.03	16.03	21.03	28.03	6.04	1.08	25.08
Lascava	16.02	4.03	6.03	14.03	18.03	23.03	31.03	7.04	13.07	5.08
Evmolpia	19.02	9.03	14.03	16.03	20.03	30.03	6.04	15.04	14.08	15.09
Puldin	15.02	7.03	9.03	13.03	16.03	18.03	27.03	7.04	8.06	31.07
Filina	16.02	4.03	9.03	12.03	14.03	16.03	24.03	30.03	5.06	20.06
Flavia	16.02	3.03	10.03	13.03	15.03	17.03	25.03	31.03	5.06	19.06
Maycrest	18.02	3.03	12.03	14.03	18.03	25.03	30.03	8.04	10.06	22.06
Lascino	18.02	10.03	16.03	20.03	27.03	7.04	14.04	19.04	31.07	31,08
Redhaven	17.02	10.03	13.03	15.03	17.03	19.03	26.03	7.04	10.07	30.07
El. 7-59	17.02	8.03	12.03	16.03	18.03	22.03	28.03	8.04	10.07	24,08
El. 4-22	15.02	4.03	7.03	11.03	13.03	16.03	28.03	6.04	28.07	30.08
El. 91-23	16.02	2.03	6.03	11.03	13.03	17.03	23.03	29.03	02.08	27.08
El. 19-77	16.02	3.03	7.03	12.03	14.03	16.03	26.03	31.03	5.06	20.06
El. 19-78	16.02	4.03	7.03	12.03	14.03	17.03	27.03	31.03	7.06	24.06

Legend for Table 2 and Table 3: A-Swelling of the buds BBCH-51, B-Greening of the tip BBCH-54, C-Start of petal display BBCH-57, D-Mass display of petals BBCH-59, E-Start of flowering BBCH- 61-62, E-Full flowering BBCH-63-65, G-Falling of petals BBCH-67, H-Formation of knot BBCH-69, I-Beginning of pigmentation BBCH-85, J-Full maturity BBCH- 89.

The studied varieties were grouped into four clusters. The first consisted of El. 4-22, El. 91-23, El. 7-59, as well as Maycrest, Lascava, Filina, Redhaven and Flavia, which showed higher resistance to low temperatures in 2019 and were more unstable in 2020 The second cluster included Laskino, Elite 19-77 and Evmolpia, which in both years of study were relatively resistant to cold. Ferlino and Puldin form a third group due to the high degree of sensitivity to low temperatures in both 2019 and 2020.

Elite 19-78 formed a separate cluster, joining the others at the maximum Euclidean distance, due to the high degree of frost in both years of study.

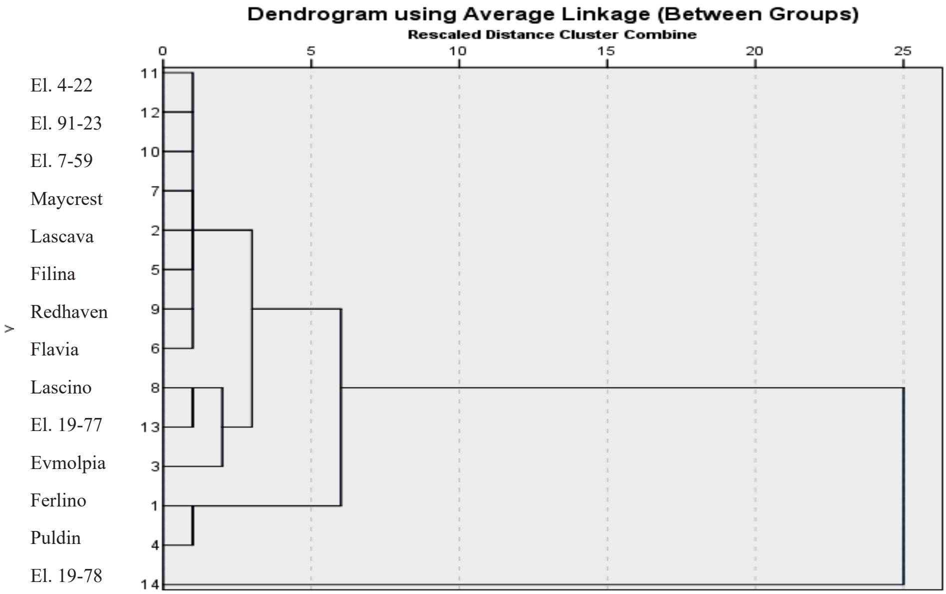

Figure 1. Grouping of peach varieties according to the degree of frost during the period 2019-2020

As a result of the applied single-factor analysis of variance on peaches, it was found that in 2019 ‘Elite 19-78’ had the highest percentage of frost (98.33%), followed by ‘Ferlino’, but much more resistant to low temperatures (50.67%) (Table 4). It has been proven that ‘Evmolpia’, ‘Filina’, ‘Flavia’, ‘Redhaven’ and ‘Elite 19-77’ do not have frozen blossoms. The

results in 2020 were significantly different. The most resistant to low temperatures varieties in 2019 were found to be among the most affected in 2020 (Figure 2). These were ‘Ferlino’ (92%), ‘Puldin’ (99%), ‘Filina’ (96%), ‘Flavia’ (92%), ‘Maycrest’ (98.67%), ‘Redhaven’ (99.67%), ‘Elite 7-59’ (92%) , ‘Elite 4-22’ (94.67%), ‘Elite 91-23’ (96.33%), ‘Elite 19-78’ (93%).
From the graphic image of Figure 2 it followed that in all varieties, except Elite 19-78, a significant increase in the percentage of frozen blossoms were demonstrated. Only with the specified elite the tendency is to decrease, but the same stands out as the most susceptible to the negative influences of low temperatures, which makes it unattractive for selection activities. Damage from negative temperatures on blossoms and pistil were presented in Figure 3. Blossom thinning and fruit thinning to moderate crop densities can influence the cold tolerance of peach flower buds in late winter (Byers & Marini, 1994). Temperatures of −30°C or lower may kill the tender fruit trees and −25°C may damage the buds in winter, although late spring frosts during bloom reduce production more frequently (Brown & Blackburn, 1987).

Table 4. Comparative assessment and analysis of variance of peach varieties according to the degree of frost and Duncan's test for evaluation of differences, a, b, c,… - degrees of significance of differences at level $\alpha \leq 0.05$

Name	Degree of frost - 2019 (%)	Degree of frost - 2020 (%)
Ferlino	50.67^{b}	92.00^{bc}
Lascava	12.33^{d}	88.00^{c}
Evmolpia	0.00^{f}	59.00^{e}
Puldin	36.33^{c}	99.00^{ab}
Filina	0.00^{f}	96.33^{ab}
Flavia	0.00^{f}	92.00^{bc}
Maycrest	13.00^{d}	98.67^{ab}
Lascino	9.00^{e}	74.67^{d}
Redhaven	0.00^{f}	99.67^{a}
El. 7-59	13.67^{d}	92.00^{bc}
El. 4-22	15.33^{d}	94.67^{abc}
El. 91-23	14.67^{d}	96.33^{ab}
El. 19-77	0.00^{f}	77.00^{d}
El. 19-78	98.33^{a}	93.00^{bc}
SS	28927.81	5212.40
Df	13	13
MS	2225.216	400.954
F-test	584.119	26.730
Sig.	0.000	0.000

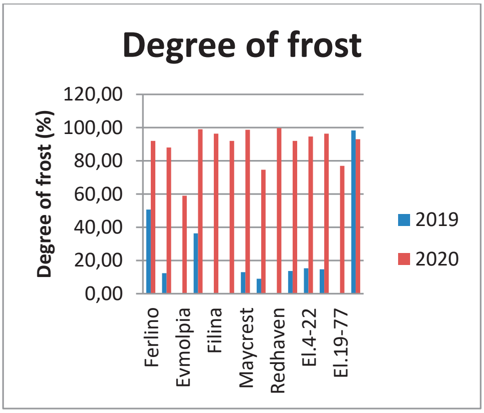

Figure 2. Change in the degree of frost in 2019 and 2020 in peaches


Figure 3. Damage from negative temperatures on blossoms and pistil

CONCLUSIONS

In 2019, ‘Evmolpia’, ‘Filina’, ‘Flavia’, ‘Redhaven’ and ‘Elite 19-77’ were the most cold-resistant varieties, and ‘Elite 19-78’ was the most sensitive one. In 2020 the blossoms of ‘Ferlino’, ‘Puldin’, ‘Filina’, ‘Flavia’, ‘Maycrest’, ‘Redhaven’, ‘El. 7-59’, ‘El. 4-22’, ‘El. 91-23’, ‘El. 19-78’ were sensitive to frost, and they have an increase in the percentage of frost compared to the previous year. The phenological phase of development had a significant impact on frost during the negative spring temperatures.

ACKNOWLEDGEMENTS

This research work was carried out with the financial support of the Agricultural University-Plovdiv.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MANAGEMENT AND CONTROL OF *RESSELIELLA THEOBALDI* (DIPTERA: CECIDOMYIIDAE) IN BIOLOGICAL RASPBERRY PRODUCTION

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Abstract

This study was carried out in a raspberry plantation (2017-2019) in the experimental field of the Institute of Agriculture - Kyustendil, Bulgaria. The purpose of the study was to investigate the effect of organic fertilizer application on the larvae density of Resseliella theobaldi (Barnes) and population management, using biological plant protection products. Our results indicate that the use of organic fertilizers has a negative effect on the survival of R. theobaldi larvae but has no effect on the larval endoparasitoid Aprostocetus epicharmus (Walker) in the cultivars 'Willamette' and 'Lyulin'. The interrelationship between R. theobaldi and A. epicharmus in organic raspberry production has also been studied. The assessed rate of parasitism by A. epicharmus (24.94-36.11%). is considered to be enough to reduce the population of R. theobaldi in cv. 'Lyulin' effectively without chemical control. Two treatments with bioinsecticides have been carried out for the protection of cv. 'Willamette' due to a significantly lower rate of parasitism in this cultivar (11.68-20.05%). The most effective insecticides for the control of adults of R. theobaldi have been identified as follows: Pyrethrum FS EC - conc. 0.05% and NeemAzal®-T/S - conc. 0.3%.

***Key words**: organic fertilizers, biological control, raspberry cane midge.*

INTRODUCTION

In many European countries (Germany, Poland, United Kingdom, other Nordic Southeast Europe countries) raspberry production is very important for the agricultural sector (Wollbold & Behr, 2020). According to statistics, in the last 15 years the number of organic raspberry producers in Bulgaria and the interest in organically grown fruits has significantly increased (Bioregister, 2021).

It is known that the organic fertilization system can influence and change the cane characteristics, productivity and berry quality, including the fruit aroma, of red raspberry (Xiu-yan, 2011; Stojanov et al., 2019; Estrada-Beltran et al., 2020). However, the fertilization system could lead to changes in pest management practices (MacConnell et al., 2001; Altieri & Nicholls, 2003; Barzman et al., 2015) which has not yet been studied in detail. According to the results of earlier study by Tsolova and Koleva (2019), it was proved that fertilizing with organic fertilizers Humustim-100, Haemosim bio N5 + Haemofol H4 and Biohumax® has a reductive effect on the survival of the *Agrilus aurichalceus* population and does not affect the population of the larval endoparasitoid *Trastichus heeringi* in the cultivars 'Willamette' and 'Lyulin'.

The raspberry cane midge, *Resseliella theobaldi* (Barnes, 1927) (Diptera: Cecidomyiidae) is a major pest of raspberry in Europe (Mitchell et al., 2018). By feeding and developing under the rind of canes, the larvae cause direct damage to the plants.

Moreover, in the wounds, several fungal pathogens can occur, which together with the pest cause a syndrome known as midge blight (Pitcher & Webb, 1952).

Control of *R. theobaldi* is difficult because larvae are protected under the epidermis of first-year canes (Nilsson, 2008). Effective insecticide treatments are appropriate against the first generation, at the peak of adult emergence, because adults of the further generations are on the wing during the flowering and harvest of raspberry (Sipos, 2012). Timing of insecticides application and the population density are very important to

consider in raspberry cane midge management not only in the conventional, but also in organic production.
The objective of this study was to evaluate the larvae density of *R. theobaldi* in different variants of organic fertilization and the effect of the application of botanical insecticides for control.

MATERIALS AND METHODS

This study was carried out during 2017-2019 at the Institute of Agriculture in Kyustendil (N 42°81'68.18", E 23°22'48.50"), Bulgaria. The organic raspberry plantation was established in 2010 with 'Willamette' and 'Lyulin' cultivars. The research was conducted as part of a project entitled 'Development of technology for organic raspberry production', which includes research on the stem insect pests of raspberry (*Rubus idaeus* L.) in organic production of raspberries. The investigations of three intrastem pests: rose stem girdler (*Agrilus aurichalceus* Redtenbacher 1849) raspberry cane midge (*R. theobaldi*) raspberry gall midge (*Lasioptera rubi* (Schrank, 1803)) were performed according to a standardized methodology described by Tsolova & Koleva (2019). The meteorological characteristics of the study region based on the derived data since 2015–2017 were mostly in line with previous studies, although an increase in rainfall was observed (Tsolova & Koleva, 2019). Also, during the research period no significant deviations from the meteorological conditions specific to the region were observed. Usually, the flowering period of cv. 'Willamette' was over 20 days, and in the cv. 'Lyulin' it was over 60 days. The BBCH-scale was used to identify the phenological development stages of each cultivar. The experimental plants were grown by using authorized fertilizers and plant protection products according to Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products regarding organic production, labelling and control. The liquid organic fertilizers Humustim-100, Haemosim bio N5, Haemofol H4 and Biohumax® were tested, applied three times by foliar treatment to the plants during inflorescence emergence (BBCH 51-57), the beginning of flowering (BBCH 60-61), and the formation of the development of fruit (BBCH 71-73), in following four fertilisation variants: V0 (untreated); V1 (Humustim - 1 L ha^{-1}); V2 (Haemosim bio N5 - 5 L ha^{-1} + Haemofol H4 - 4 L ha^{-1}) and V3 (Biohumax® - 10 L ha^{-1}). The rows in between were maintained by mowing and mulching. The experiment was arranged by block method with four variants and four replicates each. Drip irrigation was applied at 80% evapotranspiration.
The level of infestation by *R. theobaldi* was determined 1 time a month (from June to end of September). The test canes (10 canes in the case of each fertilisation variant and treatment) were collected by cutting to the soil surface and processed in laboratory conditions. The canes were cut lengthwise and all larvae were removed, divided into groups: live and parasitized.
Under field conditions in cv. 'Willamette', two treatments with botanical insecticides pyrethrum and azadirachtin were carried out: pyrethrum in three concentrations 0.05, 0.06, and 0.08% and azadirachtin in 0.25, 0.30 and 0.35%; in four replications (4×5 m^2), with an area of 20 m^2 for each variant. The applications were targeted against the adults of the second generation. The timing of treatment was determined by standard pheromone white delta traps. The first treatment was carried out during the peak of flight activity, whereas the second after 7 days. The survival data on adult's population were transformed into percent mortality by Abbott (1925) and Henderson and Tilton (1955). The results were statistically evaluated by dispersion analysis (ANOVA).

RESULTS AND DISCUSSIONS

The survival of the population of *R. theobaldi* larvae in cv. 'Willamette' and cv. 'Lyulin' during 2017-2019 and between fertilization variants is shown in Figure 1 and 2.
The highest survival rate of larvae in cv. 'Willamette was characterized in 2019 (84 larvae/fertilization variant or 39.25% in variant Haemosim bio N5 + Haemofol H4/V2) and the lowest in 2017 (10 larvae/fertilisation variant or 13.25% in variant with Humustim/V1).

In the parasitism by the larvae of *Aprostocetus epicharmus* (Hymenoptera: Eulophidae), the opposite trend was observed from total 11.68% (2019) to 21.15% (2017).

Though the highest value of parasitism between variants 11 larvae/variant or 7.89% was reported for fertilization with Biohumax® (V3) during 2019.

It is notable that during three years of the study the population of live larvae in the cultivar 'Lyulin' had the total highest values (99–55.56%) when fertilized with Biohumax® (V3), while the total lowest values (2–1.04%) when fertilized with Humustim (V1) (Figure 2). During the three years of the study, the number of live larvae in the cultivar 'Lyulin' was the highest in 2018 and the lowest in 2019 (Figure 2).

The infestation of the larvae of the raspberry cane midge by *A. epicharmus* was as follows: total 27.07% (2017); 24.94% (2018) and 36.11% (2019). The carried out partial studies on the relationship of *R. theobaldi* to *A. epicharmus* showed that the obtained values of parasitism in the range of over 35% were sufficient to regulate and maintain the raspberry cane midge population under threshold of economic harm.

The high rate of parasitism could lead to a reduction of the number of insecticide treatments during the growing season for the cultivar 'Willamette', our data and observations were not enough to make even preliminary recommendations. Population monitoring show *A. epicharmus* have at least three generations for a year under average continental climatic conditions. The parasitoid conspicuously emerges in late May and can be characterized by the same number of generations as its host, the raspberry cane midge (Stoyanov, 1963). According to Vétek et al. (2006), the primocane splitting during the vegetation periods influenced the population dynamics of the host and the parasitoid. It also concerned the interrelationship between the raspberry cane midge and their parasitoid *A. epicharmus.*

The statistical processing of the obtained data was performed by two-factor analysis of variance (ANOVA) for the effect of the impact of organic fertilizer variants on the *R. theobaldi* larvae in the cultivars 'Willamette' and 'Lyulin' in 2017-2019 (Tables 1, 2).

Table 1. Two-factor dispersion analysis (ANOVA) of the effect of the fertilization variants on the density of *R. theobaldi* larvae in cv. 'Willamette' in 2017-2019

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Fertilization	200.9167	3	66.97222	2.833137	0.128516	4.757063
Years	112.1667	2	56.08333	2.372503	0.174114	5.143253
Error	141.8333	6	23.63889			
Total	454.9167	11				

The two factors fertilization and research period had no influence on the number of *R. theobaldi* larvae in cultivar Willamette during 2017–2019. The reported low population of raspberry cane midge probably depends on adult's treatments and the degree of parasitism by the endoparasitoid *A. epicharmus*.

Table 2. Two-factor dispersion analysis (ANOVA) of the effect of the fertilization variants on the density of *R. theobaldi* larvae in cultivar 'Lyulin' in 2017-2019

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Fertilization	300.9167	3	100.3056	0.381189	0.770534	4.757063
Years	5181.167	2	2590.583	9.844928	0.01274	5.143253
Error	1578.833	6	263.1389			
Total	7060.917	11				

The research period also affected the larvae of *R. theobaldi* in the cultivar 'Lyulin' during 2017-2019. Differences between fertilization variants were significant between the years. There were no significant differences between the variants for a given year. Further research should be focused on examining the long-term effects of the use of tested organic fertilizers in direct relation to the biochemical parameters of the soil in organic raspberry production.

Based on the data on density of *R. theobaldi* larvae, treatments with the biopesticides Pyrethrum FS EC® and NeemAzal® T/S were carried out in 2018 and 2019. The test results of botanical insecticides against adults of the second generation of raspberry cane midge in field conditions were characterized by low values, which ranged from 60.6% to 69.8%. Double treatment against second generation showed very good efficacy of Pyrethrum FS

EC® (0.05%), which ranged from 90.1% to 90.8%. Similar results were obtained with NeemAzal® T/C, where the efficiency was in the range of 90.4-92.3% for the study years (Figure 3). Mohamedova (2017) also reports for lowest number of larvae in raspberry canes was observed after application of NeemAzal® T/C and *B. subtilis* in raspberry fields near Samokov, Bulgaria. Both products demonstrated highest efficacy, when the number of larvae per splits was 67.1-82.5% for NeemAzal® T/C, and 75.1-81.2% for *B. subtilis* lower compared with the control.

The results show that the organic production of raspberry poses risks associated with the occurrence of certain biotic stress factors which could cause damage of the production. The control against cane midge is very important in the biological management of raspberry in many raspberry growing regions.

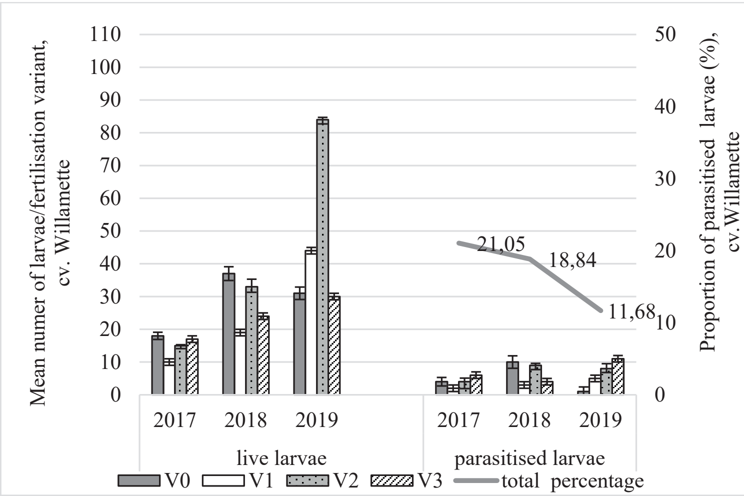

Figure 1. Number of *R. theobaldi* larvae by different fertilization treatments in cultivar 'Willamette'

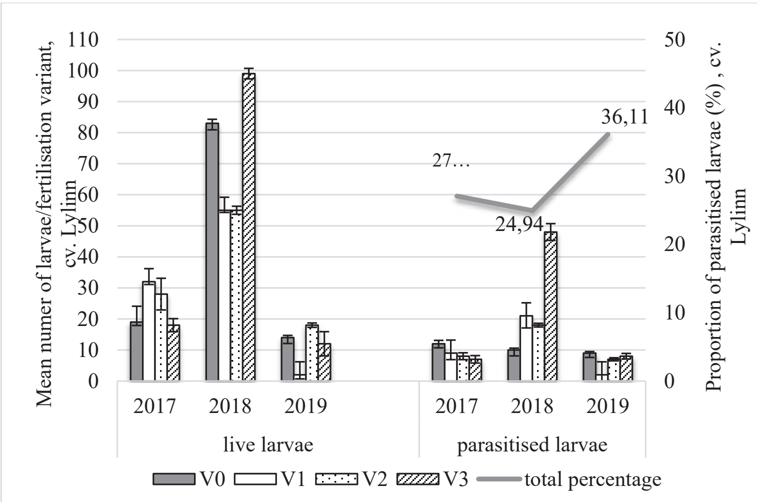

Figure 2. Number of *R. theobaldi* larvae by different fertilization treatments in cultivar 'Lyulin'

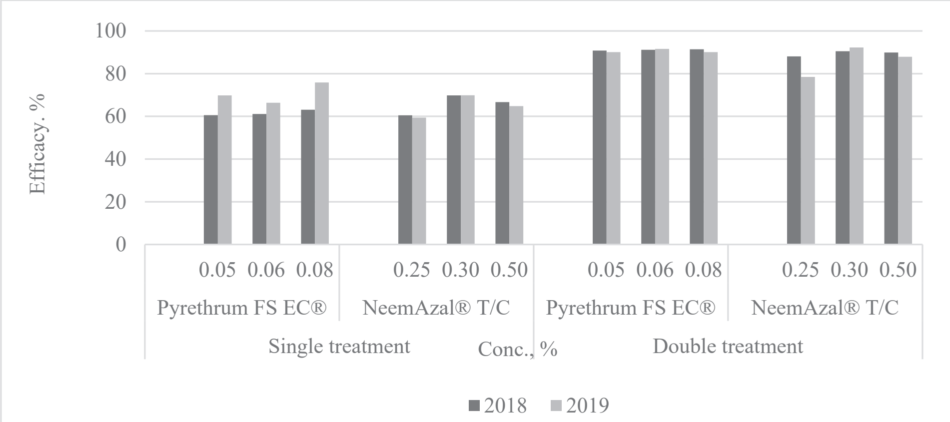

Figure 3. Efficacy of botanical insecticides against *R. theobaldi* adults under field conditions in 2017-2019

CONCLUSIONS

The duration of fertilization with various organic fertilizers does not had a direct influence on over-wintering stock of *R. theobaldi* larvae in the cultivars ′Willamette′ and ′Lyulin′.
A higher degree of parasitism from the endoparasitoid *A. epicharmus* was observed in the larvae of the cultivar ′Lyulin′ (36.11%) than in the cultivar ′Willamette′(21.05%).
The double treatment against adults of the second-generation raspberry cane midge with Pyrethrum FS EC® - 0.05% and NeemAzal® T/S - 0.3% showed very good efficacy up to 90.8% resp. 92.3%.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

BASIC PHYSICO-CHEMICAL PARAMETERS OF THE FRUITS OF SOME PLUM CULTIVARS, RELATED TO THEIR SUITABILITY FOR FRESH CONSUMPTION AND PROCESSING

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Abstract

The study was conducted in the period 2019-2020 in plum plantations of RIMSA Troyan. The pomological and physico-chemical parameters of widespread and newly studied plum cultivars with different directions of use were studied. It was found that cv. 'Strinava', 'Stanley', 'Jojo' and 'Elena' have a dry matter of over 19%, and in 'Stanley' and 'Jojo' the total sugars are also at most 11.5-12.5%. 'Hanita' cv has the highest content of titratable acidity (1.0%), all others are <1.00%. The large-fruited cultivars with an early ripening period 'C. Naibolya', 'C. Lepotitsa', 'Tuleu timpuriu', as well as the later ripening 'Jojo' have a well-balanced taste, glucoacidimetric coefficient of about 20, which makes them suitable for fresh consumption. 'Kyustendilska', 'Elena', 'Gabrovska', 'Valevka' cultivars have low fruit weight, the late ripening period allows them to accumulate a high content of dry matter, which makes them suitable for industrial processing and distillation.

Key words: *plum, pomological and physicochemical parameters, fruit qualities.*

INTRODUCTION

Plums (*Prunus domestica* L.) have long been of interest in the human diet, as food for fresh consumption, dried or processed. The modern consumers are becoming more and more demanding to the quality of fruits, so they seek information about the sensory characteristics and chemical composition of the widespread old cultivars and the new recently introduced plum cultivars.

Trends in the world selection of new commercial plum cultivars are focused on the large fruit size, the dark color of their skin and their resistance or tolerance to Plum pox virus. In many cases, however, the chemical and sensory characteristics do not improve. Such cultivars are attractive to producers, but not acceptable to consumers (Bozhkova, 2014).

In human nutrition, plums are valued as a rich energy source with high protective, dietary and therapeutic value. Fresh fruits are low in calories and relatively high in nutritional value. They can make a significant contribution to human nutrition due to their richness in antioxidants. Plums are also a major natural source of phytochemicals such as flavonoids, phenols, anthocyanins, etc., which have been shown to have antioxidant capacity and can help protect cells against oxidative damage caused by free radicals (Ertekin et al., 2006; Voca et al., 2009; Božović et al., 2017).

Usenik et al. (2014) examined the maturity at harvest, which determines the quality, potential shelf life and acceptance of fruits by consumers, who evaluate plum fruits by their color and taste. The qualities of four plum cultivars ('Haganta', 'Jojo', 'Stanley' and 'Toptaste') were measured, showing high variability in pomological characteristics among different cultivars and vegetation seasons, especially at the ripening stage when the fruit quality changes. Plums become delicious when the skin is completely colored and the fruit flesh color is changed from green to the characteristic of the cultivar. Skin color is one of the most important criteria for ripening of stone fruit, but it is not suitable for determining their ripeness, as many genotypes develop pigmentation at the beginning of their growth (Usenik et al., 2008).

The introduction of new cultivars improves the technological characteristics of plum production, which increases yields, productivity and fruits have a more attractive appearance, size and balanced nutritional composition. Markuszewski and Kopytowski (2013) believe that most late ripening plum cultivars have higher dry matter and sugar content than early ripening cultivars. The resistance of new cultivars to sharka (Plum pox virus) is also important, as these cultivars are more tolerant.

The structure of the assortment of plum cultivars is created depending on: the production capacity and the environmental impact on the genotypes, the quality of the fruits and the trends in the use (Botu, 2012).

The objective was to study the main physico-chemical parameters of fruits of some plum cultivars and their suitability for use in different trends.

MATERIALS AND METHODS

The study was conducted in the period 2019-2020 in RIMSA Troyan. The climatic conditions for the foothills are characterized by moderately cool winters and dry but not very hot summers. The altitude is 380 m, the terrain is inclined from 5 to 8°.

The trees are grown using standard plum technology, keeping the soil surface grassy, without irrigation, without additional fertilization, without plant protection.

The following indicators were registered:

1. Ripening period of fruits
2. Biometric (physical) characteristics of fruit:

- Fruit weight (g);
- Stone weight (g);
- Height (mm);
- Diameter (mm);
- Stalk length (mm);

3. Chemical composition of fresh plum fruit;

- Dry matter (DM) according to (refractometer) Re (%);
- Determination of sugars (total, invert and sucrose) and acid, according to the method of Schoorl (Donchev et al., 2001),
- Tanning substances according to the method of Levental (Donchev et al., 2000),
- Anthocyanins (mg/%) according to the method of Fuleki and Francis (1968),
- Total polyphenols (mg GAE/100 g FW) - according to Singleton and Rossi (1965)

The experimental data were subjected to statistical analysis by Fisher's single-factors ANOVA. The significance of differences between the mean values of the factors and the interaction means was determined by LSD test at significance levels of $P \leq 0.05$.

Fruits were determined at the laboratory of RIMSA Troyan.

RESULTS AND DISCUSSIONS

The studied cultivars cover the harvest period from the second ten days of July to the second half of September. The fruits of ‘Katinka’ cultivar became ripened first, and ‘Elena’ cultivar had the latest ripening fruit. Fruits of ‘Stanley’, in most cases reach harvest maturity at the end of August (Table 1).

Table 1. Harvest ripening stage for plum cultivars (2020)

Cultivars	Ripening stage period
Katinka	27.07
TuleuTimpuriu	29.07
Čačanska Najbolja	31.07
Tegera	3.08
Čačanska lepotica	5.08
Čačanska Rodna	14.08
Hanita	16.08
Strinava	16.08
Gabrovska	17.08
Mirabella de Nancy	25.08
Valevka	27.08
Stanley	2.09
Jojo	5.09
Elena	10.09
Kyustendilska	15.09

The following measurements were made in 2019: ‘Jojo’ had the largest fruit weight (87.13 g,), diameter (50.38/46.87 mm) and height 62.93 mm, followed by ‘Čačanska Najbolja’, respectively (73.32 g), diameter (45.38/48.98 mm) and height (56.14 mm). The lowest values were measured in: ‘Kjustendildka sinia sliva’ with 21.39 g, diameter 29.48/30.35 mm, height 40.42 mm, followed by ‘Gabrovska’ and ‘Elena’. The other cultivars ranged from 30.21 g for ‘Katinka’ to 43.68 g for ‘Stanley’ (Table 2).

Table 2. Biometric indicators of fruits by cultivars (2019-2020)

Cultivars	Fruit weight (g)	Stone weight (g)	Relative share of stone (%)	Height (mm)	Diameter (mm)	Fruit stalk length
			2019			
Katinka	34.09±2.13	1.38±0.26	0.25	43.37±0.99	34.42/36.27	12.15±1.57
T. Timpuriu	43.66±2.86	2.04±0.11	0.21	45.73±1.47	39.40/38.82	7.58±1.08
C. najbolja	73.31±8.06	2.38±0.22	0.31	57.10±1.44	45.38/48.98	15.86±1.43
Tegera	37.84±4.12	1.72±0.19	0.22	48.32±2.36	36.00/37.78	12.53±0.56
C. lepotica	57.47±5.62	2.08±0.19	0.28	48.83±2.00	37.30/40.29	13.18±2.37
C. rodna	33.271±3.44	1.38±0.08	0.24	45.53±2.65	33.10/36.64	16.94±1.28
Hanita	43.45±5.96	2.52±0.19	0.17	46.56±2.76	38.80/38.25	14.18±0.76
Strinava	38.27±2.01	1.24±0.11	0.31	46.55±1.26	36.71/37.70	15.63±1.06
Gabrovska	29.88±2.63	1.50±0.10	0.20	43.85±1.83	33.79/33.76	13.27±1.56
Mirabella de Nancy	11.93±1.31	1.00±0.14	0.12	28.21±1.30	26.73/26.74	12.16±0.93
Valevka	30.98±1.81	1.48±0.19	0.21	46.24±3.70	34.69/33.54	16.32±0.88
Stanley	50.45±5.68	2.46±0.21	0.21	51.78±2.55	38.04/37.12	18.03±1.01
Jojo	87.13±5.97	3.26±0.09	0.27	62.75±2.14	50.38/46.87	8.77±1.33
Elena	36.12±3.20	1.46±0.09	0.25	42.40±2.13	34.45/32.90	15.81±0.63
Kyustendilska	21.36±1.46	0.94±0.09	0.23	41.01±1.99	29.48/30.35	16.85±0.71
LSD 0.05	*3.75*	*2.20*		2.72		*1.56*
			2020			
Katinka	25. 00±3.36	0.84±0.15	0.30	39.81±3.10	31.44/32.48	11.04±1.22
T. Timpuriu	36.25±3.66	1.54±0.13	0.24	44.17±1.40	38.17/36.12	12.53±0.67
C. najbolja	48.44±5.61	2.70±0.12	0.18	48.26±2.12	41.82/40.90	13.31±1.24
Tegera	27.69±2.12	1.42±0.6	0.20	43.46±1.80	32.40/34.02	8.44±1.41
C. lepotica	38.01±1.88	1.68±0.13	0.23	42.64±1.00	36.51/38.37	11.18±2.14
C. rodna	31.83±2.35	1.38±0.08	0.23	44.55±1.25	33.69/36.49	13.62±1.40
Hanita	29.85±3.28	1.80±0.25	0.17	44.38±1.73	34.87/36.71	10.47±1.96
Strinava	32.08±2.62	1.22±0.25	0.26	44.49±1.51	34.70/34.83	12.65±1.78
Gabrovska	24.00±2.55	1.30±0.12	0.18	44.00±2.93	32.16/31.10	12.49±2.75
Mirabella de Nancy	12.10±1.78	0.46±0.05	0.26	27.31±0.54	25.72/26.12	10.67±1.60
Valevka	24.24±1.95	1.26±0.05	0.19	44.91±0.73	31.88/31.23	11.64±0.69
Stanley	37.35±3.15	2.18±0.16	0.17	49.61±2.75	36.43/36.38	17.62±1.52
Jojo	55.92±6.43	2.40±0.35	0.23	57.41±1.96	42.60/39.05	12.33±2.62
Elena	29.78±4.49	1.44±0.18	0.21	42.40±3.08	34.55/32.07	15.05±3.19
Kyustendilska	17.75±1.17	0.64±0.05	0.28	38.30±1.13	28.35/28.77	12.38±0.83
LSD 0.05	*3.01*	*0.22*		*2.50*		*2.30*

Ertekin et al. (2006) presented the following measurements for 'Stanley': average weight 36 g, average fruit length 48.25 mm, 33.24 mm and 31.32 mm, far smaller compared to our results.

According to the degree of separation of the stone from the fruit flesh, it is from separating to semi-separating. The fruit skin of the studied cultivars has a basic blue color with a shade of purple in 'Čačanska Rodna' and 'Katinka' (Minev et al., 2017).

Relative share of stone in the standard cultivar 'Stanley' in 2019 was 0.21, in other cultivars it varied from 0.17 ('Hanita') to 0.31 in 'Čačanska Najbolja' (Table 2). In 2020 the lowest value of the coefficient was in 'Hanita'

(0.17), and the highest in ‘Kyustendilska’ and ‘Katinka’ (0.28; 0.30), compared to ‘Stanley’ (0.17). During the study period, the highest fruit stone weight was reported in ‘Jojo’ cultivar (2.3-3.2 g *LSD 0.05* = 0.22).

The largest fruit stalk length was measured in ‘Stanley’ (18.03 mm) *LSD 0.05* = 1.56 (Table 2).

The dry matter content in the fresh fruits of the studied cultivars varied from 14% (‘Katinka’, 2019) to 23% (‘N. Mirabela’, 2019), as the standard cultivar ‘Stanley’ they were respectively 19.0% in 2019, 19.6% in 2020. In the group with the lowest dry matter content were ‘Tuleu timpuriu’, ‘Tegera’, ‘Hanita’, ‘Gabrovska’ (Table 3).

In 2019, the average monthly temperatures in July and August were around 20°C, in September 16.2°C and the precipitation was less than for the same period in 2020 (Figure 1). Thus, the late-ripening cultivars ‘Stanley’, ‘Jojo’, ‘Elena’ for this year had 11.45-12.95% content of total sugars. The organic acids in these cultivars were 0.35%, which determined a very high glucoacidimetric index (32.71-37.00).

At the end of August 2020 (28-31.08) and the beginning of September (1-5.09), a very high temperature amplitude was reported between the minimum and maximum value of T (°C), around 20.0-22.5°C. This is extremely conducive to the accumulation of more total sugars (8.7%-10.9%) in the fruits of cultivars ripening in this period (late ripening - ‘Valevka’, ‘Stanley’, ‘Jojo’, ‘Elena’).

An exception is ‘Kyustendilska’, where the content of total sugars was low for both years (6.7%-7.7%) due to the cultivar susceptibility to PPV. In the earlier ripening cultivars, this indicator had lower values, especially 'Čačanska lepotica' (5.85%), ‘Tuleu timpuriu’ (6.00%).

The lowest glucoacidimetric coefficient for the studied plum cultivars was registered in ‘Hanita’ in 2019 (6.87) and 'Čačanska lepotica' in 2020 (7.31), and the highest (37.00) for ‘Elena’ cultivar in 2019, as the values of the coefficient by years differed depending on the abiotic factors (temperature and precipitation) (Figure 1).

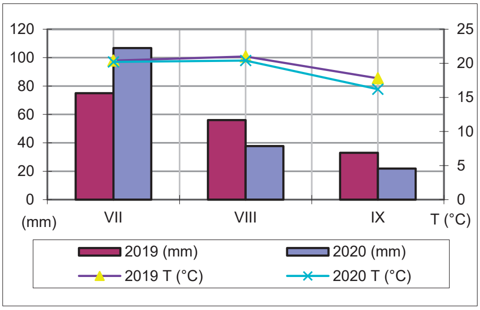

Figure 1. Climatic conditions (2019-2020)

The closest to the optimal value of glucoacidimetric coefficient (according to Stanchev et al. (1968) were ‘Katinka’ 22.75 (2019) and Čačanska Najbolja (2020) with 17.69, formed by 14.15% total sugars and 0.80% acids, for ‘Stanley’ cultivar in 2020 was 16.27 (Table 3).

The organic acids of the fruits largely determine their taste. In general, the studied cultivars had a low acid content, ranging from 0.35% (‘Elena’, 2019) to 1.07% (‘Hanita’, 2020). Voca et al. (2009) report the acid content in the range of 0.40% and 0.69% in the plum cultivars ‘Top’, ‘Bistritsa’ and ‘Elena’, i.e. the data are comparable with the results obtained in our study for other cultivars grown in different climatic conditions.

Tannins and dyeing substances varied widely depending on the cultivar characteristics, the exposure of the slope (direction to sunlight), the intensity of fruit bearing (fruit load).

The polyphenols in the second year of the study were much higher compared to 2019. In Bulgaria they vary from 119.09 (mg/g) in ‘Mirabelle de Nancy’ to 595.34 (mg/g) in ‘Strinava’. Voca et al. (2009) reported a total phenol content ranging from 157.70 mg in ‘Elena’ to 344.10 mg in ‘Bistritsa’, expressed as gallic acid equivalents (GAE), based on fresh weight. Under our conditions, ‘Elena’ cultivar in 2020 had 341.48 (mg/g) polyphenols (Table 3).

The present study determined high correlation among the content of dry matter, total and inverted sugars (r = 0.837) (Figure 2), which confirms the findings of Dzhuvinov et al. (2012) (r = 0.852).

Table 3. Chemical analysis of fresh plums (2019-2020)

	Soluble Solids (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Acids (%)	Glucoacidi-metric index	Tanning Substances (%)	Total polyphenols (mg/g)	Anthocyanins (mg/%)
					2019				
Katinka	14.00	11.60	5.35	5.94	0.51	22.75	0.15	27.78±0.12	33.71
T.Timpuriu	14.50	9.20	5.20	3.80	0.76	12.11	0.09	31.90±0.10	12.74
C.najbolja	17.00	8.20	4.05	3.94	0.76	10.79	0.13	89.57±0.12	15.32
Tegera	15.60	10.05	6.00	3.85	0.63	15.95	0.15	25.49±0.18	19.03
C.lepotica	18.50	8.90	8.90	-	0.63	14.13	0.16	73.19±0.27	39.84
C.rodna	18.00	10.40	6.35	3.85	0.63	16.51	0.11	38.83±0.30	21.13
Hanita	15.30	8.70	7.85	0.81	1.01	8.61	0.20	113.27±0.16	12.10
Strinava	19.50	8.70	7.35	1.28	0.50	17.40	0.13	40.40±0.46	21.61
Gabrovska	16.00	7.00	2.70	4.09	0.57	12.28	0.13	52.50±0.18	23.71
Mirabella de Nancy	23.00	5.35	2.55	2.66	0.38	14.08	0.13	9.37±0.05	5.97
Valevka	17.00	8.20	3.85	4.13	0.76	10.79	0.18	21.88±0.33	44.19
Stanley	19.60	11.45	8.20	3.09	0.35	32.71	0.17	11.09±0.06	9.19
Jojo	19.50	12.60	8.55	3.85	0.38	33.16	0.08	11.97±0.18	5.00
Elena	19.60	12.95	5.20	7.36	0.35	37.00	0.19	69.05±0.05	13.77
Kyustendilska	16.00	6.65	3.20	3.28	0.63	10.56	0.19	13.31±0.05	9.84
					2020				
Katinka	15.00	7.70	6.00	1.62	0.60	12.83	0.19	412.31±1.41	19.84
T. Timpuriu	13.50	6.00	2.40	3.42	0.80	7.50	0.06	349.97±2.83	13.71
C. najbolja	13.50	14.15	9.05	4.85	0.80	17.69	0.10	163.74±0.71	4.68
Tegera	15.00	7.35	4.50	2.71	0.80	9.19	0.10	25.49±0.61	4.03
C. lepotica	15.60	5.85	4.05	1.71	0.80	7.31	0.10	173.18±0.71	3.06
C.rodna	21.50	9.55	5.70	3.66	0.60	15.92	0.17	621.73±0.72	15.32
Hanita	16.00	7.35	5.00	2.23	1.07	**6.87**	0.08	350.78±0.64	8.55
Strinava	21.00	6.00	3.85	2.04	0.67	8.96	0.23	595.34±0.33	14.03
Gabrovska	16.50	7.85	2.85	3.80	0.67	11.72	0.19	413.22±0.18	11.77
Mirabella de Nancy	19.00	7.70	5.35	2.32	0.74	10.41	0.13	119.09±0.06	4.52
Valevka	16.70	9.20	5.70	3.33	0.87	10.57	0.19	228.49±0.37	5.32
Stanley	19.00	10.90	5.38	5.27	0.67	16.27	0.17	281.96±0.04	3.71
Jojo	19.50	8.70	5.35	3.18	0.67	12.99	0.17	465.41±0.21	3.39
Elena	19.00	8.05	5.35	2.57	0.67	12.01	0.15	341.48±1.05	13.39
Kyustendilska	18.90	7.70	7.70	-	0.80	9.63	0.17	280.82±0.14	17.58

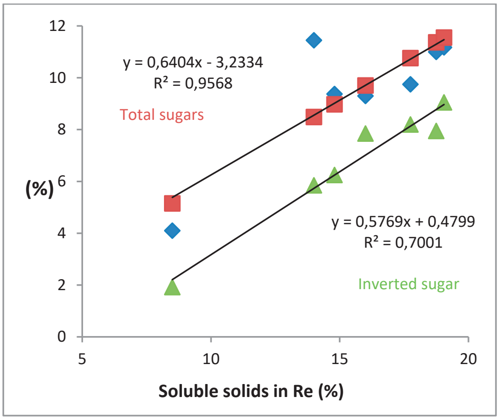

Figure 2. Correlation dependence, among soluble solids content, total and invert sugars

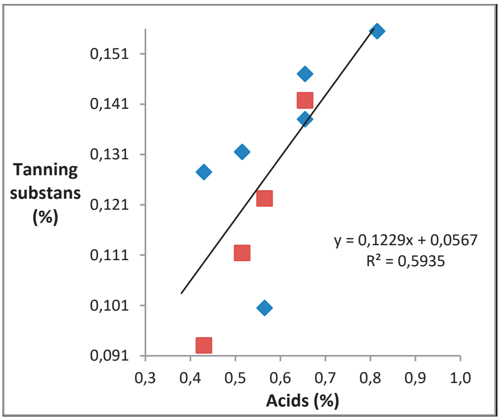

Figure 3. Correlation dependence, between the content of acids and tannins

The same dependence was found between the content of organic acids and tannins by the function y = 0.1229x + 0.0567, where $R^2 = 0.5935$ (Figure 3).

Based on the ripening period, biometric indicators and chemical composition of fruit we have the reason to distribute the studied cultivars in 3 trends.

Fresh consumption, due to early and late ripening, attractive appearance - early ripening ‘Katinka’, ‘Tuleu timpuriu’, ‘Čačanska Najbolja’, ‘Tegera’, 'Čačanska lepotica' and late ripening cultivars, such as ‘Jojo’ and ‘Elena’.

For drying: based on the high content of dry matter, dark blue skin and averaged-sized fruit for this group are suitable cultivars, such as: ‘Gabrovska’, ‘Valevka’, ‘Stanley’, ‘Čačanska Rodna’ and the standard of taste and drying ‘Kyustendilska’.

For processing. due to completely detachable stone and balanced taste ‘Gabrovska’, ‘Valevka’, ‘Kyustendilska’ are suitable for processing into jam, marmalades, etc., as well as for distillation, incl. ‘Stanley’, ‘Strinava’, ‘Hanita’.

CONCLUSIONS

The studied cultivars cover the harvest period from the second ten days of July to the second half of September.

The largest fruit weight was registered in ‘Jojo’ (87.13 g), diameter (50.38/46.87 mm) and height 62.93 mm, followed by ‘Chachanska najbolja’, respectively (73.32 g), diameter (45.38/48.98 mm) and height (56.14 mm). The lowest values were measured in: ‘Kjustendildka sinia sliva’ 21.39 g, followed by ‘Gabrovska’ and ‘Elena’.

The lowest dry matter content in fresh fruits was registered in ‘Katinka’ with 14% (2019), and the highest in ‘Mirabelle du Nancy’ with 23% (2019), in the standard cultivar ‘Stanley’ it was respectively 19.0% in 2019; 19.6% in 2020.

The lowest glucoacidimetric index for the studied plum cultivars was registered in ‘Hanita’ in 2019 (6.87) and 'Čačanska lepotica' in 2020 (7.31), and the highest (37.00) in ‘Elena’ in 2019, as the values of the index over the years differed depending on the abiotic factors.

An exceptional correlation dependence was found among the content of dry matter, total and inverted sugars, as well as the content of organic acids and tannins.

Based on the studied indicators, the plum cultivars are grouped in 3 technological trends for use.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IMPACT OF THE IRRIGATION OF THE APRICOT TREES ON THE ORGANOLEPTIC FRUITS QUALITY

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Abstract

The research purpose was to establish the most efficient/suitable strategy for apricot irrigation, in order to obtain high fruits quality, with great aspect, firmness and taste. The apricot trees of 'Orizont' Romanian cultivar were irrigated in three variants: fully irrigated - FI (V_1 = non hydric stress) according to the irrigation needs (100% of ET), low irrigation LI (V_2 = partial hydric stress) irrigated with half of water the amount (50% of ETc) and non-irrigated trees, representing the control NI (V_3 = hydric stress). After the harvest, the fruits were stored in different storage options: at 20-22℃ (room temperature), 10-12°C (refrigerant conditions) and at 3-5℃ (cold storage), with and without modified atmosphere for different periods of time. At the harvest moment and after that, a couple of determinations were made, as concern the organoleptic quality of the fruits (aspect, firmness and taste). The results revealed that the organoleptic quality of the apricots at the time of the harvest and its evolution during storage, varied according to the irrigation schedule. Thus, the highest values of the organoleptic parameters were obtained at the non-irrigated variant, followed by the low irrigated variant.

Key words: *aspect, Prunus armeniaca, firmness, irrigation, storage, taste.*

INTRODUCTION

Lately, the Romanian consumers increasingly prefer to consume fruits of Romanian cultivars. At the same time, their demands for the quality of the purchased products have increased, determining the producers to reconsider not only the fruits quantity of, but also their quality. The sensory quality is the one that promotes the goods, because it is the first one with which the consumer meets.
The apricot (*Prunus armeniaca* L.) is a thermophile fruit tree species, highly appreciated in Romania, but its favorability area is relatively restricted in our country due to the climate. It gets suitable growing conditions in the region of Dobrogea where the winter temperatures are not too low, and, also, proper soil conditions (mainly fertile black soil, called chernozem). As regard the water shortage that can be solved by applying irrigation measures (Septar, 2017).
Nowadays, in the global warming up context, in arid and semi-arid regions, the irrigation is necessary for fruit trees growing, but the optimal irrigation application requires large amounts of water. Thus, water saving is a major objective. An alternative, is the irrigation with low amount of water (Fereres & Soriano, 2007; Hoffman, 1990) which requires a moderate reduction of water amount during the critical stages of trees development, while keeping on a satisfactory quantity and quality of fruit production. Low irrigation leads to water saving without short-term yields loss (Naor, 2006), while the entire fruit yield can be reduced due the long-term cumulative effects on trees (Intrigliolo & Castel, 2005).
Perez-Pastor (2007) studied the effect of different irrigation strategies on the fruit quality of apricot at the harvesting time and during the storage at 1°C and found that low irrigation was demonstrated to be commercially favorable for maintaining fruit quality, saving, at the

same time, considerable amounts of water. The apricot fruits are an excellent source of nutrients and they are appreciated for their special flavor. However, high rates of ripening and susceptibility to mechanical injury and diseases, limit their shelf life. Being a climacteric fruit, the ethylene can regulate the ripening of apricot fruits. The application of different pre and post-harvest treatments, such as harvesting at optimum maturity, maintenance of cold chain, selection of proper packaging and storage atmospheres, determines/influences the post-harvest behavior of fruits (Muzzaffar et al., 2018).

For delaying the ripening and maintaining the quality of harvested fruits, quick cooling and low-temperature storage are recommended (Alexe, 2012; Moise, 2018). However, if apricots are kept at low temperatures for longer periods, chilling injury occurs in the fruits, which is manifested in the form of various symptoms. Therefore, postharvest technology of apricots aims to reduce fruit losses, as well as optimizing their quality through the postharvest chain.

The objective of this study was to evaluate the effect of the irrigation schedule on the sensory quality of 'Orizont' apricots at harvesting time and at 7, 15, respectively 30 days after the harvest, in different storage conditions.

MATERIALS AND METHODS

The research was carried out in years 2017 and 2018 at the Research and Development Institute for Processing and Marketing of the Horticultural Products "Horting" Bucharest and at the University of Agronomic Sciences and Veterinary Medicine of Bucharest on the Romanian apricot cultivar 'Orizont' (Figures 1 and 2), cultivated in experimental plots at the Constanța Fruit Growing Research and Development Station.

Figure 1. 'Orizont' apricots on orchard

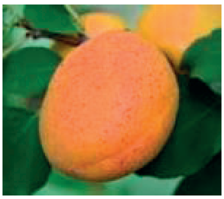

Figure 2. Detail of 'Orizont' apricot fruit

A two-level factorial experience was organized setting the irrigation schedule and the post-harvest storage conditions.

The experimental variants were:

-V_1: non hydric stress - the irrigation regime consisted of a fully irrigated (FI) treatment according to the irrigation needs (100% of ETc = ETo x Kc, Penman-Monteith method);

-V_2: partial hydric stress (LI) - low irrigation variant; the trees received just half of the water amount provided at V_1 (50% of ETc);

-V_3: hydric stress - the trees were non-irrigated (NI), representing the control variant

After harvest, the fruits were stored in different storage conditions: at 20-22ºC (room temperature), 10-12°C (refrigeration) and at 3-5ºC (cold storage), with and without modified atmosphere, for different periods of time.

The experimental plots consisted of three rows of apricot tress, with the central row containing three trees for measurements and observations. Plant available soil water capacity was measured using Watermark resistance blocks which were installed at four levels of depth: 20 cm, 40 cm, 60 cm and 80 cm, at 150 cm distance from the trunk, with two repetitions for each tree.

Relating the fruit organoleptic evaluation, seven persons observed, tasted and evaluated the organoleptic quality (aspect, firmness, taste) of the apricot fruits.

In order to determine the post-harvest factors involved in promoting the organoleptic quality, the apricot fruits were stored under different technological conditions, as follows:

- room temperature: warm conditions (T = 24-27°C, RH = 69-71%), for 7 days;
- fridge storage: refrigerant conditions (T = 10-12°C, RH = 75-78%), for 15 days;
- cold storage: low temperature conditions (T = 3-5°C, RH = 82-86%), for 30 days;
- cold + modified atmosphere (MA) storage, in airtight packages: the composition of the atmosphere inside was modified, be reducing the amount of O_2 and increasing the amount of CO_2 - storage in modified atmosphere - MA (T= 3-5°C, RH = 92-96%), for 30 and 45 days;

After storage, the fruits were re-examined in order to establish the effect of the technologic measures, especially as regard the irrigation schedule and the storage conditions on the post-harvest quality and apricot fruits shelf life.

The oorganoleptic quality assessment was done by performing a sensory testing which represents a method of fruit assessing using a grading scale from 1 to 100. Tasting sheets had three evaluation criteria (aspect, texture, taste). Each criteria of evaluation had a different. percentage in the general scoring, according to their importance: aspect - 15%, firmness - 35% and taste - 50%. Depending on the score, five quality classes were defined, as follows:

Grading scale	**Score**
Very Good	80-100
Good	60-79
Satisfactory	40-59
Unsatisfactory	20-39

RESULTS AND DISCUSSIONS

The results of the organoleptic quality (Table 1) reveal the fact that at the harvesting time, at all irrigation variants, the apricot fruits obtained “very good” grade. Notable is the close range of the variants.

Table 1. The organoleptic evaluation of apricots at the harvesting time

Criteria for assessment	Variant		
	V_1	V_2	V_3
Aspect	12.60	12.40	11.40
Firmness	28.00	28.60	29,00
Taste	42.00	44.00	46.00
Total	82.60	85.00	86.40
Grading scale	very good	very good	very good

The variant V_3 (non irrigated trees – control variant) got the highest score (86.40), due to the higher values of taste and firmness, while V_1 (non-stressed - fully irrigated) got the lowest score (82.60).
The results of sensory analysis of the apricot fruits stored in warm conditions for seven days are presented in Table 2.

Table 2. The organoleptic evaluation of apricot fruits after room temperature storage for 7 days

Criteria for assessment	Variant		
	V_1	V_2	V_3
Aspect	10.20	10.80	11.40
Firmness	23.80	23.80	25.20
Taste	36.00	36.00	38.00
Total	70.00	70.60	74.60
Grading scale	good	good	good

It can be noticed that the total score decreased to all irrigation variants, due mainly to the taste depreciation, so it got only "good" grade.
Also, during warm storage, the apricot fruits rapidly lost their firmness due to the fast ripening. Thus, V_3 (non-irrigated - the control variant) obtained the highest score (74.60).
After 15 days of fridge storage (T = 10-12°C), the organoleptic properties of the apricot fruits remained in good parameters only at V_3 which received “very good” grade (Table 3).

Table 3. The organoleptic evaluation of apricots after fridge storage for 15 days

Criteria for assessment	Variant		
	V_1	V_2	V_3
Aspect	11.40	12.00	13.40
Firmness	25.40	24.60	27.80
Taste	34.00	38.00	40.00
Total	70.80	74.60	81.20
Grading scale	good	good	very good

V_1 and V2 (partial hydric stress) obtained “good” grade, with 70.80 and, respectively, 74.60 score.
After the cold storage (T = 3-5°C) for 30 days, the apricots of V2 and V3 maintained their commercial aspect and good firmness and obtained a high score and “very good” grade (Table 4).

Table 4. The organoleptic evaluation of apricots fruits after cold storage for 30 days

Criteria for assessment	Variant		
	V_1	V_2	V_3
Aspect	9.00	12.60	13.40
Firmness	26.40	27.20	29.40
Taste	38.00	40.40	41.00
Total	73.40	80.20	83.80
Grading scale	good	very good	very good

V3, with 83.80 score is again in on top. V1, with 73.40 score obtained “good” grade.
After storage under conditions of modified atmosphere (T = 3-5°C) for 30 days, the apricot fruits continued to maintain their firmness and pleasant aspect, but their taste slightly decreased and finally it obtained the grade “very good”, for all variants, with score at the lower limit of this quality class (Table 5).

Table 5. The organoleptic evaluation of apricots fruits after cold+ M.A. storage for 30 days

Criteria for assessment	Variant		
	V1	V2	V3
Aspect	12.20	12.60	12.80
Firmness	28.60	28.00	29.40
Taste	39.20	40.40	40.20
Total	80.00	81.00	82.40
Grading scale	very good	very good	very good

After storage under conditions of modified atmosphere for 45 days, although the apricot fruits maintained their commercial aspect, they lost their firmness and decreased the quality of their taste. For this reason, V_2 and V_3 obtained "good" grade, while V_1 with 38.80-42.00 score obtained "satisfactory" grade (Table 6).

Table 6. The organoleptic evaluation of apricots fruits after cold+ M.A. storage for 45 days

Criteria for assessment	Variant		
	V1	V2	V3
Aspect	12.00	12.00	12.80
Firmness	18.80	19.20	20.00
Taste	8.00	10.60	9.20
Total	38.80	41.80	42.00
Grading scale	satisfactory	good	good

In fact, in this case, the irrigation schedule had not an important influence on the sensory quality. The only factor with direct impact on the organoleptic quality of the apricot fruits was the storage time and its characteristics. In some cases, the apricots stored in MA conditions displayed certain physiological impairments, manifested through deterioration of the pulp around the stone. This reveals the fact that the period in which the fruits had the capacity to maintain their quality was overdue, meaning that the storage time in an atmosphere enriched with carbon dioxide was too long.

Following the evolution of the sensory quality during storage using different technological methods of each irrigation variant (Table 7), it is found that there are differences between the variants in terms of score and grade obtained.

Table 7. The grades obtained by the apricot fruits at the harvesting time and after storage

Moment of determination	Grading scale		
	Variant		
	V_1	V_2	V_3
At harvest	very good	very good	very good
After warm storage	good	good	good
After fridge storage	good	good	very good
After cold storage	good	very good	very good
After MA storage-30 days	very good	very good	very good
After MA storage-45 days	unsatisfactory	satifactory	satisfactory

Although at the harvesting time, all fruits obtained a high score and "very good" grade, later their evolution was different.

Thus, the fruits of V_1 received less than 80 score and the "good" grade at room temperature, fridge and cold storage and less than 40 score and "unsatisfactory" grade at modified atmosphere storage for 45 days (Figure 3).

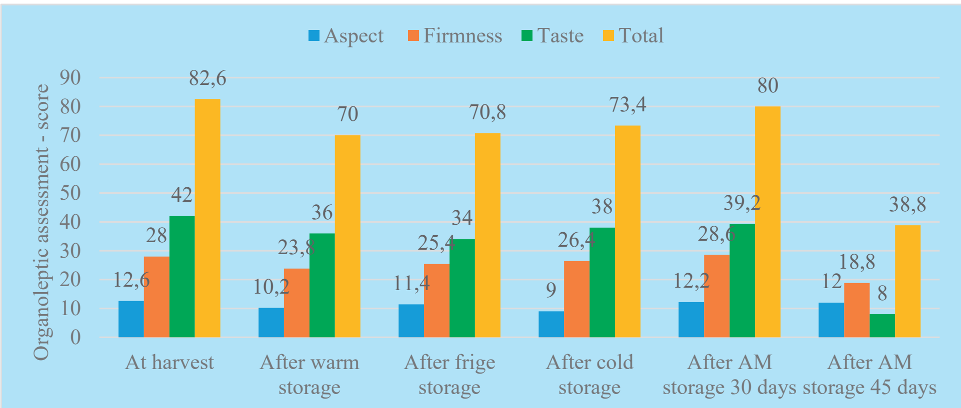

Figure 3. Organoleptic evaluation of V_1 apricot fruits at harvesting time and after storage, using different technological conditions

The fruits of V2 obtained a higher score, compared to V1, receiving the “very good” grade at cold storage without and with modified atmosphere for 30 days, “good” grade for room temperature and fridge storage and “satisfactory” grade at modified atmosphere storage for 45 days (Figure 4).

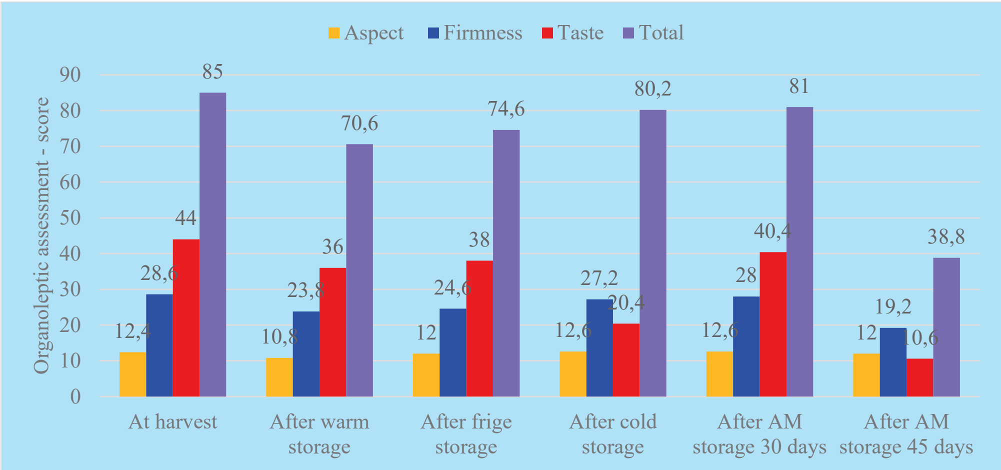

Figure 4. Organoleptic evaluation of apricots from V2 at harvesting time and after storage, using different technological conditions

The highest score was received by the V3 apricot fruits, including all technological storage options. V3 has got “good” grade at room temperature storage for 7 days, “satisfactory” at modified atmosphere storage for 45 days and “very good” for keeping them in the other technological conditions (Figure 5).

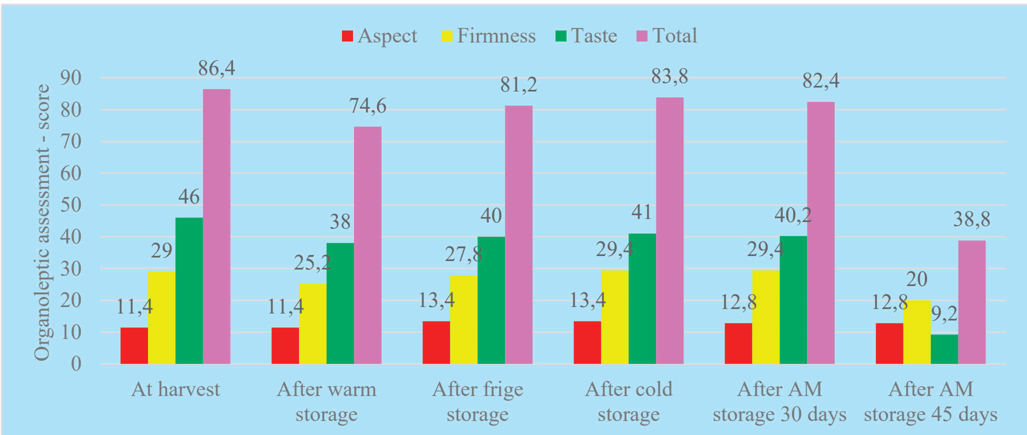

Figure 5. Organoleptic evaluation of V_3 apricots fruits at harvesting time and after storage, using different technological conditions

CONCLUSIONS

The ‘Orizont’ apricot cultivar is appreciated for the organoleptic characteristics of its fruits. This fact is clearly shown in the obtained score (the highest from all three variants) at the moment of harvest and at further determinations.

For getting best fruit sensory values, the apricot trees do not need extra water amount supplied by full or partial irrigation. This aspect is reflected in the results obtained at non irrigated trees where a real hydric stress existed. The intermediary values were obtained at low irrigated trees where a partial hydric stress existed.

The evolution of the sensory quality of apricot fruits during storage depends on irrigation schedule and storage environmental conditions, especially the temperature and gaseous composition.

The fruits harvested from the apricot trees irrigated with large amount of water (V_1 = non-stressed - fully irrigated) lost easier their organoleptic quality.

The best method to promote the organoleptic quality is the storage at cold + modified atmosphere (T = 3-5°C), taking into account the storage period and the score obtained at the end of it.

A low irrigation schedule represents a trade advantage by promoting the sensory quality and shelf life of the apricot fruits and by saving considerable amounts of water, as well.

As regard the global warming up process, a huge challenge for plant cultivation is represented by water shortages but a solution, not only in arid or semi - arid areas, is the wise irrigation schedule by supplying lower amounts of water and using species and cultivars resistant to the hydric stress.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRELIMINARY RESULTS REGARDING THE BEHAVIOR OF SOME FIG GENOTYPES IN BUCHAREST AREA CONDITIONS

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Abstract

Fig (Ficus carica L.) is a Mediterranean fruit specie that have been introduced from centuries in our country by Greeks, Romans, Ottomans and probably, Armenians colonists. Even it is a thermophile plant, fig trees are spread all around the country, several genotypes being found. Recently, a certain interest on fig cultivation raised, and there is a request of valuable cultivars and respectively, planting material. At the Faculty of Horticulture in București, an important collection of fig genotypes found in Romania and introduced from Italy and Iraq, was started in 2015 and since then their evaluation is in process. The present paper presents the selection process of 25 fig genotypes, with interesting pomological characteristics, fruits measurements being performed in accordance with IPGRI descriptors. Fruit biochemical analyses on soluble solids, glucose and fructose were realized. The results showed that some of local and foreign genotypes produced no fruits or had very small fruits. All the analyzed parameters were influenced by the genotype. The plants that produced good results concerning fruit quality, size, taste and cracking resistance, will be further studied in order to observe their productivity and resistance to biotic and abiotic stresses.

Key words: *earliness, Ficus carica, fruits, soluble solids, vigour.*

INTRODUCTION

Fig is a member of Moraceae family and includes over 1000 genotypes, of which, over 600 were studied by the American horticulturist Condit (1947).

It is considered that the fig was cultivated by humans before cereals (Kislev, 2006) being an important plant in the Mediterranean area, next to vines and olive trees.

We find it mentioned in various mythological legends and songs (Sinha, 2003), it appears in the Bible and the Quran (Tomescu, 2014) and the translation of the name Dyonisos, the Greek god of wine, means "the friend of figs" (Minonne et al., 2011).

The origin of the fig is not known, but the Swedish botanist De Candolle states that, it is Caria, Syria and the Mediterranean Basin.

He supports his hypothesis by the fact that in these areas we frequently find wild specimens, a criterion that is not sufficient to ensure the certainty of the plant's origin (Minonne et al., 2011).

In Romania, it is not known who brought the fig. The south of the country was occupied or transited by Greeks, Romans, Ottomans, Armenians, peoples on whose territory fig trees grew. Greek settlers in the Dobrogea area had in the 1st century BC. Exchange of goods and food with Greece, offering cereals, honey, fish and receiving fruits and vegetables in exchange along other products (Kaufman, 2006). Under the influence of the Ottomans, in the nineteenth century, Romanians included figs in the daily diet (Giurescu, 1966) and in Bucharest there was a fig tree in almost all people's yards (Stănică, 2017).

The fig tree grows as a bush with 4-7 stems, or as a tree (Mediterranean Basin) (Vidaud, 1997). It is heat sensitive, the trunk freezes at temperatures bellow -21°C and the annual branches at -15ºC.

To ripen the fruit needs temperatures of 20 - 21ºC, does not tolerate water excess in the soil (Chira, 2009) and due to its root system, the fig is the species in the plant world that makes the best use of water (Vidaud, 1997). In areas favourable to fig fruiting, such as the Mediterranean Basin, 40-80-100 kg/plant are obtained (Accorsi e Beldi, 2011), while in our country 10-12-15 kg/plant (Hoza, 2001). It is a robust Mediterranean tree and if it is well cultivated it requires little or no phytosanitary treatments (Jullien and Jullien, 2011).

Fig is a unisexual dioecious plant (Evreinoff, 1947). The fruit is a sicone, a false fruit composed of many achenes (Hoza, 2001; Hoza, 2003).

Fresh fruits contain between 13 - 25% carbohydrates, significant amounts of Ca, Zn, Fe, K, vitamin B1, vitamin. B2, B6, C, Pp, fiber (Hoza, 2000; Gherman, 2013).

Fruits and leaves have nutraceutical properties, being recommended in alleviating diseases such as: anaemia, physical asthenia, intestinal inflammation, senescence (Gherman, 2013), diabetes (Wojdilo et al., 2016), cancer (Wang et al., 2008; Menichini et al., 2012).

The vast majority of cultivated species have undergone breeding programs, which has not happened in the case of figs, which is why this plant now has great genetic variability. This is a great advantage for research in terms of germplasm collection.

In Romania, the fig is found especially in people's yards, a situation in which genotypes have different synonyms or are unknown. This fact determined the need to identify the plants, propagate and study them in fruit tree collections. The first study of this kind was conducted at the Faculty of Horticulture in Bucharest, and the results were published in the paper Preliminary Characterization of some Fig Biotypes in Romania (Ahmad et al., 2017).

Pomological characterizations or identification of fig genotypes were also performed in Morocco (Khadari et al., 2008), Tunisia (Aljiane et Ferchichi, 2008), Italy (Resta et al., 2008), Albania (Koka, 2008), California (Stover and Aradhya, 2008), Lebanon (Chalak et al., 2008), Canary Islands (Gil et al., 2008).

MATERIALS AND METHODS

The natural framework of the areas where the reseach was carried out is the Experimental field of the Faculty of Horticulture, Bucharest. It is located in the Northen part of the capital, geographical coordinates 44°28’10” latitude N and 26°04’00” longitude E, at 78 m altitude. Bucharest borders with: Câmpia Titu – Gheorghița at N, Câmpia Bărăganul Mostiștei at E and SE, Ialomița River at NE, Argeș Valley at V and SV. The climate is temperate-continental, with cold winters and hot and dry summers.

The annual average temperature is 10.5ºC, the annual average of precipitations sums up to 550-600 mm.

The vegetal material is represented by the fig trees, on their own roots, come through cuttings from the figs grown in the country (Romania) and abroad (Italy and Irak). The cuttings were rooted in the vegetation house of the Faculty of Horticulture Bucharest.

The following genotypes were identified and selected for study: Stork, 1 Mai, Piața Obor, Dr. Constantinescu from Bucharest; Smochin negru and Ploiesti nr.1 from Ploiești; Brazi from Brazi, PH; Fântânele from FântânelePH; Negoiești 01 from Negoiești, PH; Viscool from Mărăcineni, AG; Galben mare from Braniștea, GR; Oli Timișoara from Timisoara, TM; Săvârșin from Săvârșin, AR; Rot negru from Svinița, CS; Melo nero, Bifera bianco, Awitato, Bianco Etna, Natawa, Bianco Sicilia, Passulana nera, Cilento nero, Fiorone Etna, Fig primizia from Italy; Irak 1 from Kerkut, Irak.

These fig genotypes were analysed and described in accordance with IPGRI descriptors. We made determinations and measurements, such as: harvest period, tree vigour, fruit size (weight, length, diameter), neck, stalk, ostiole, skin and flesh (colour, aspect, texture, etc.). Biochemical analyses, such as: soluble solids, glucose and fructose, completed the morphological and pomological description and were performed in the Researcher Centre for Study of Food Quality and Agricultural Products, USAMV Bucharest. The period analysed in this study was 2020.

RESULTS AND DISSCUSIONS

For the studied genotypes, the second harvest was analysed, and the results of the measurements showed a high phenotypic variability and different harvesting periods.

The most early ripening genotypes were: Galben mare, Brazi and Viscool, all of them had the first harvest in the second decade of August (Tabel 1).

Table 1. Harvest schedule

Genotype	Harvest schedule
Galben mare	Decade II August – Decade III October
Stork	Decade III August – Decade III October
Brazi	Decade II August – Decade III October
Viscool	Decade II August – Decade III October
Rot negru	Decade III August – Decade III October
Fântânele PH	Decade III August – Decade III October
Passulana nera	Decade III August – Decade III October
Dr. Constantinescu	Decade III August– Decade III October
Cilento nero	Decade I September – Decade III October
Awitato	Decade I September – Decade III October
1 Mai	Decade I September – Decade III October
Smochin negru	Decade I September– Decade III October
Savârşin	Decade I September– Decade III October
Fig Primizia	Decade I September– Decade III October
Negoieşti 01	Decade I September– Decade III October
Piaţa Obor	Decade I September– Decade III October
Ploieşti 1	Decade I September – Decade III October
Oli Timişoara	Decade I September– Decade III October
Bianco Etna	Decade III September– Decade III October

The fruit ostiole opening was between 4.97 mm, Cilento nero; 4.94 mm, 1 Mai; 4.94 mm Oli Timişoara; 3.87, Dr. Constantinescu; 3.57 mm, Viscool (Figure 1).
The weight of the fruits is between: Săvârşin - 54.11 g, Ploiesti nr.1 - 51.36 g, and Smochin negru - 18.83 g, Negoieşti 01-17.16 g (Figure 2).
The amount of soluble solids was between 20.83°Bx in Awitato; 19.53°Bx in Passulana nera and 14.6°Bx Fântânele PH; 13.9°Bx Negoieşti 01genotypes; glucose 22.96%, fructose 22.61% in Awitato; glucose 21.5%, fructose 21.63% in Passulana nera and glucose 15.98%, fructose 15.72% in Fig Primizia; glucose 15.28%, fructose 15.29% in Negoieşti 01 (Figure 3).

The comparison of the growth vigour was performed (Figure 4).

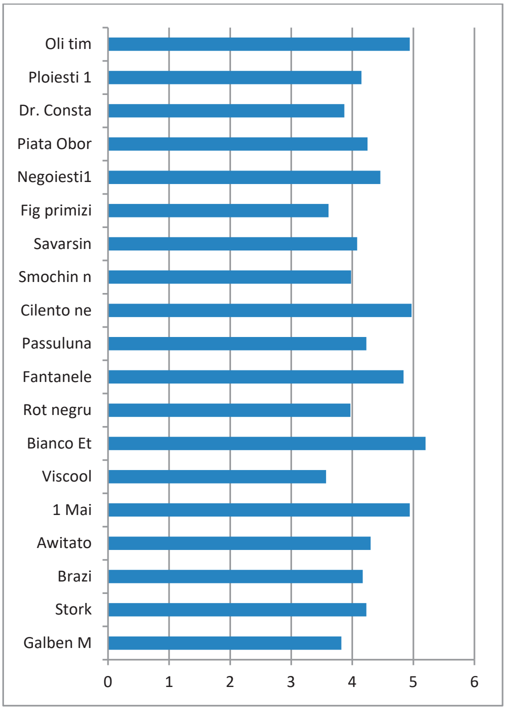

Figure 1. Fruit ostiole opening

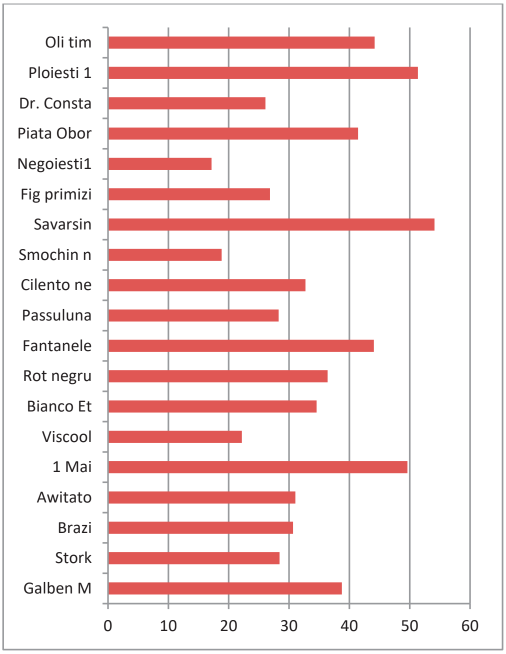

Figure 2. Average fruit weight (g)

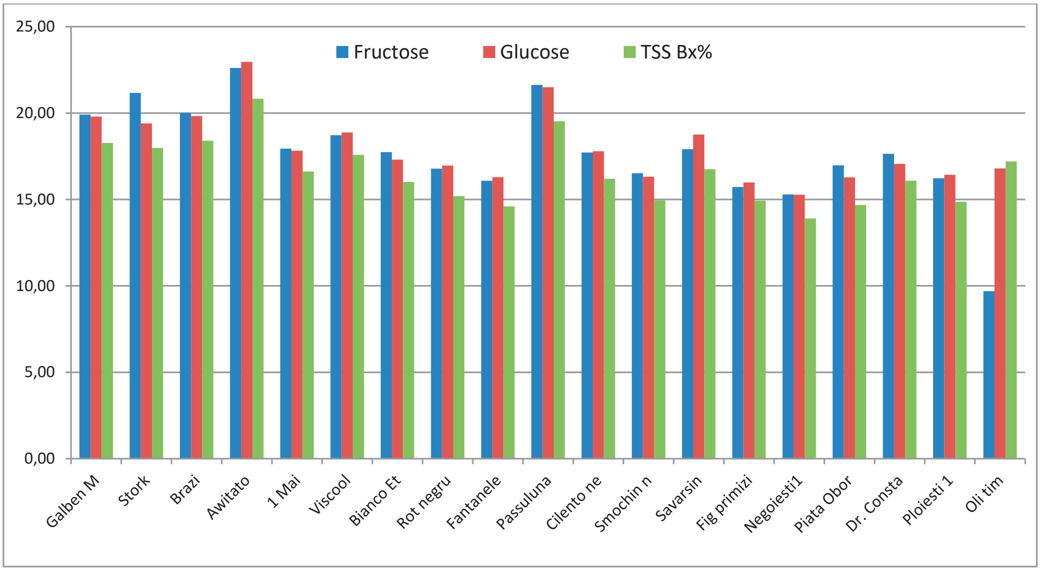

Figure 3. Fruit biochemical determinations (%)

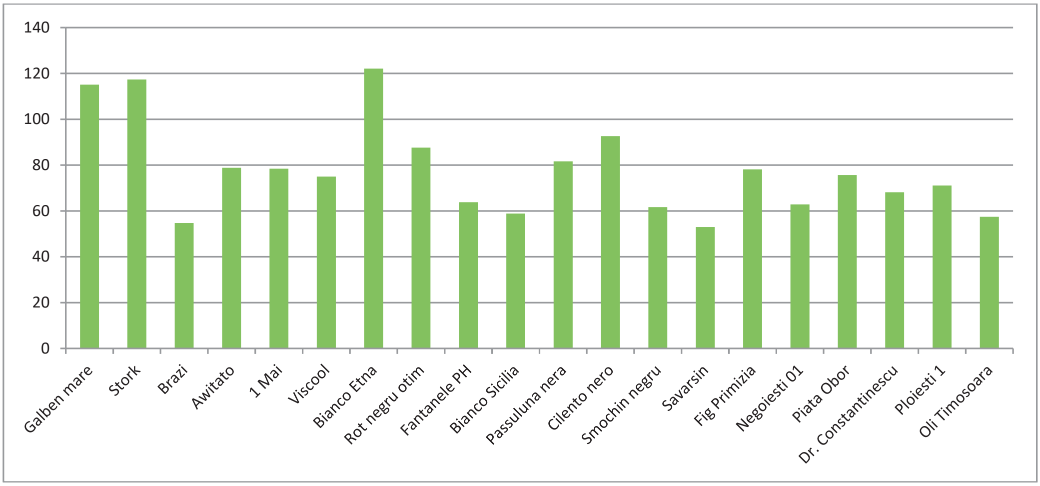

Figure 4. Fig average shoot length (cm)

Pomological fruit description is made in accordance with IPGRI descriptors (International Plant Genetic Resources Institute) and it is presented below (Table 1a and 1b).

Table 1a. Fig fruit pomological characteristics

Genotype	1. Fruit width	2. Fruit length	3. Fruit shape; index (width/ length)	4. Fruit shape according to the location of the maximum width	5. Fruit shape near the stalk	6. Fruit symmetry according to the vertical axis	7. Uniformity of fruit size	10. Fruit neck length (mm)	12. Shape of the fruit stalk	17. Ostiole colour	18. Resistance to ostiole-end cracks	21. Ease of peeling
Galben mare	42.07	52.03	oblong	pyriform	acut	yes	yes	12.26	long slender	pink	susceptible	no
Stork	38.47	52.73	oblong	short pyriform	acut	no	yes	18.94	short slender	pink	intermed.	no
Brazi	37.73	43.59	oblong	short pyriform	acut/ round	yes	yes	8.85	long slender	white	intermed.	no
Awitato	35.54	43.68	oblong	pyriform	acut	slightly asymmetric	yes	4.84	long slender	white	intermed.	yes
1 Mai	42.64	50.47	oblong	pyriform	flat	slightly asymmetric	yes	no	long slender	pink	intermed.	no
Viscool	33.67	39.47	oblong	short pyriform	acut	yes	yes	7.28	long slender	brown	intermed.	no
Bianco Etna	37.83	45.1	oblong	short pyriform	acut	yes	yes	no	long slender	white	susceptible	no
Rot negru	42.95	42.76	oblate	short pyriform	acut	yes	yes	7.79	long slender	red	resistant	no
Fântânele Ph	42.85	45.16	oblong	pyriform	round	yes	yes	no	long slender	pink	intermed.	no
Passulana nera	35.05	36.53	globose	short pyriform	round	yes	yes	no	long slender	pink	intermed.	no
Cilento nero	38.3	38.8	globose	short pyriform	round	yes	yes	no	long slender	brown	intermed.	no
Smochin negru	31.22	31.36	globose	short pyriform	round	yes	yes	5.14	short slender	pink	intermed.	no
Săvârșin	43.23	44.43	globose	pyriform	flat	slighly asymmetric	yes	no	long slender	pink	intermed.	no
Fig Primizia	35.35	40.97	oblong	pyriform	acut	yes	yes	no	long slender	white	resistant	no
Negoiești 1	34.87	28.67	oblate	short pyriform	acut	yes	yes	no	short slender	white-pink	resistant	no
Piața Obor	41.26	42.21	globose	pyriform	round	slightly asymmetric	yes	no	short thick	red	susceptible	no
Dr. Constantinescu	35.43	42.07	oblong	short pyriform	acut	yes	yes	7.43	long slender	white	intermed.	no
Ploiesti 1	44.12	53.26	oblong	pyriform	round long	yes	yes	no	short thick	pink	intermed.	no
Oli Timișoara	40.79	55.55	oblong	pyriform	flat	yes	yes	no	long medium	red	resistant	no

Table 1b. Fig fruit pomological characteristics

Genotype	22. Fruit ribs	23. Fruit skin cracks	24. Resistance to cracks	27. Fruit skin ground colour	28. Shape and colour of the overcolour	29. Fruit lenticels quantity	30. Fruit lenticels colour	31. Fruit lenticels size	33. Pulp internal colour	34. Pulp texture	35. Pulp flavour
Galben mare	Intermed..	minute	susceptible	green-yellow	yellow	no			pink-amber	coarse	medium sweet
Stork	slightly	minute	intermed.	light-green	brown-green	no			red	coarse	medium sweet
Brazi	slightly	no	susceptible	light - green	yelloe	no			pink	medium	medium sweet
Awitato	intermed.	no	resistant	yellow	yellow	no			amber	medium	very swee
1 Mai	no	minute	intermed.	yellow-green	brown-green	scarce	white	medium	pink	medium	sweet
Viscool	intermed.	longitudin	intermed.	brown - green	brown	scarce	white	medium	red	coarse	medium sweet
Bianco Etna	no	no	susceptible	light-green	yellow	no			amber	fine	very swee
Rot negru	intermed.	minute	intermed.	light-green	brown-green	scarce	white	small	red	coarse	medium sweet
Fântânele Ph	no	longitudn	intermed.	light-green	brown-green	scarce	white	medium	pink	fine	sweet
Passulana nera	intermed.	no	resistant	green light	purple-green	intermed.	white	medium	red	medium	very swee
Cilento nero	yes	no	resistant	green light	purple-green	scarce	white	small	pink	medium	very swee
Smochin negru	yes	minute	intermed.	yellow-green	brown-green	scarce	white	small	red	coarse	medium sweet

Săvârșin	yes	minute	resistant	light green	brown-green	sarce	white	small	amber	fine-med	medium sweet
Fig Primizia	yes	minute	intermed.	yellow	yellow	no			amber	fine	very swee
Negoiești 1	no	no	resistant	yellow-green	brown-green	intermed.	white	medium	pink	coarse	medium sweet
Piața Obor	no	minute	intermed.	light-green	light-green	no			amber-pink	fine-med	medium sweet
Dr. Constantinescu	intermed.	minute	intermed.	light-green	yellow-green	no			amber	medium	sweet
Ploiesti 1	no	intermed.	intermed.	yellow-green	brown-green	scarce	white	medium	amber	fine	sweet
Oli Timișoara	no	minute	intermed.	light green	brown-green	no			pink	fine-med	medium sweet

CONCLUSIONS

The earliest genotypes were the Romanian genotypes, such as: Brazi, Galben mare and Viscool, all of them had the first crop in the second decade of August.

In order of the vigor growth, the smallest were: Săvârșin, Brazi, Oli Timișoara (Romanian genotypes) and the biggest: Bianco Etna (Italian genotype), Stork and Galben mare (Romanian genotypes).

The biggest fruits were registered at the following genotypes: Săvârșin – 54.11 g, Ploiesti nr.1 - 51.36 g, 1 Mai – 49.63 g, Oli Timișoara – 44.18 g, Fântânele 44.6 g, Piața Obor – 41.45 g.

The ostiole opening is an important criterion for the fruit quality. Viscool and Fig Primizia genotypes have the smallest ostiole opening.

The highest amount of sugar (soluble solids, glucose, fructose) was found in Awitato and Passulana Nera genotypes.

At the end of this year's studies, 5 genotypes with foreign origin were eliminated from the culture.

Melo nero, Fiorone Etna – Italian genotypes and Irak 1 – Irakian genotype, did not develop fruits.

Bifera bianca – Italian genotype, has produced a significant quantity of fruits, but in early September, when the fruits were in the size of an olive, they all fell down.

Natawa – italian genotype, has produced a significant quantity of fruits which once reached at the consumption maturity are very small in olives size.

We will keep on monitoring the Romanian originated fig trees which have had a good evolution.

The plants of Italian origin, such as Awitato, Bianco Etna, Passulana nera, Cilento nero which have had a good evolution, with a very tasty fruits and good cracking resistance will be monitored in the coming years.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE RESPONSE OF SEMI-HARDWOOD CUTTINGS OF 'FORTIVAL' ROOTSTOCK TO THE ACTION OF SOME ROOTING BIOSTIMULATORS

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Abstract

This study was conducted to investigate the response of the plum rootstock 'Fortival' to the action of rooting biostimulators, using semi-hardwood cuttings. The experience was located in a solarium equipped with artificial mist installation. The cuttings were treated with different hormones (IBA, NAA and Radistim no.2) and reported to control. The results show that among the biostimulators applied to 'Fortival' rootstock cuttings, Radistim no. 2 had a superior influence on the development of the root system, compared to both the control and the other used biostimulators, determining the highest rooting percentage, the highest number of roots, the highest average length of roots/cutting, the highest average length of the rooted segment and the largest volume of roots. The F-Test Two-Sample for Variances shows that the variance of the average length of roots/cutting is higher in the case of chemically treated cuttings, regardless of the used biostimulator, which shows the favorable response of 'Fortival' rootstock to the application of rooting biostimulators used in the study.

***Key words**: plum rootstock, semi-hardwood cuttings, rooting biostimulators.*

INTRODUCTION

The discovery of new technologies, simple and modern, through which to obtain large quantities of fruit planting material, in a short time and with low costs, represents a necessity for the development of romanian fruit growing. An effective method of obtaining fruit seedlings is to root the cuttings to obtain uniform and quality rootstocks (Markovski et al., 2015). Green pruning requires a more laborious harvesting and shaping technique, as well as installations to ensure the microclimate needed to root the rootstock (Sumedrea & Sumedrea, 2011). "Green" pruning made in good conditions, succeeds in virtually all fruit species, because rhizogenesis occurs more easily in young tissues, leaves ensuring both a longer trophic autonomy to rooting and hormonal equipment, especially auxins, which migrates basipetally and facilitates rooting (Drăgănescu, 1998). The rootstock improves plant vigor, prolongs the vegetation period (Lee et al., 2010), productivity and fruit quality (Tsaballa et al., 2013), prolongs fruit quality after harvest (Zhao et al., 2011), increases tolerance to low and high temperatures (Li et al., 2016), reduces stress caused by salinity and heavy metals (Penella et al., 2016), increases flood resistance (Bhatt et al., 2015), manages resistance to soil pathogens (Arwiyanto et al., 2015), manages nematode resistance (Lee et al., 2010), controls weeds and plant propagation (Fuentes et al., 2014). Rootstocks have an important role in modernizing plum culture (Achim et al., 2015). In current literature there are many studies, in different species, that approaches the rooting process, *in vivo*, but also the influence of growth regulators on this process. Botu et al. (2002) undertook a study on new selections of plum rootstocks with low vigor and high propagation capacity and reported a high propagation capacity through cuttings, with variation limits between 64-72% rooted cuttings. Based on these considerations, the aim of the paper is to evaluate the response of semi-hardwood cuttings 'Fortival' rootstock to the action of rooting biostimulators.

MATERIALS AND METHODS

The 'Fortival' rootstock was created by the Vâlcea Research-Development Station for Fruit Growing. The material was harvested from the mother plantation producer of cutting shoots. At the time of harvest, the mother plants were in good condition and did not have significant diseases and pests. The shoots were harvested at the end of June and the experiment was placed at Pitești - Mărăcineni Research-Development Institute, in plastic green houses provided with artificial mist. The cuttings were made from annual branches, the length of the cuttings being 25 cm and 6-7 leaves/cutting were left at the top. The cuttings were planted 0-4 mm with the base in river washed sand substrate, its height being 15 cm. The distance between rows was 8 cm and 5 cm between cuttings per row. The conditions in the plastic green houses with artificial mist were of 80-90% humidity in the air and the temperature of about 30˚C. It is important that until root formation, a film of water is permanently maintained on the leaves during the day. After the roots have formed, the watering interval has been increased only to keep the soil moist. The experimental variants were the following: V1 (CT) - without stimulation; V2 - chemical stimulation (Indole-3-butyric acid, IBA 2000 ppm, solution); V3 - chemical stimulation (1-Naphthylacetic acid, NAA 2000 ppm, solution); V4 - chemical stimulation (Radistim no.2, powder). In the NAA and IBA stimulators, the cuttings were kept for 4-5 seconds and in Radistim biostimulator, the cuttings were passed through powder up to 1-2 cm, after having previously been passed through water. For each variant, a number of 100 cuttings were made with a total of 400 rootstocks. At the end of November, the following determinations were made on the rooted cuttings: the number of roots/cutting; root length/cutting (cm); length of the rooted segment/cutting (cm); root volume (cm^3), number of anticipated shoots and length of anticipated shoots (cm). The data was statistically processed using Microsot Excel, Data Analysis and F-Test Two-Sample for Variances to test the variance of the average root length/ cutting depending on the applied biostimulator compared to the control. In order to achieve the distribution of cuttings according to the number of roots and the average length of roots/cutting for each biostimulator, the histogram was used and in order to highlight the connection between different characteristics of the root system and different growth characteristics, correlations were used.

RESULTS AND DISCUSSIONS

The use of rootstocks is an important tool used by horticulturists to improve crop adaptation to certain stresses, especially those related to ecological plasticity (Mondragón-Valero et al., 2017). Rootstocks can change the ecological adaptability of varieties, so an accurate characterization of them is essential to obtain high quality crops (Mondragón-Valero et al., 2019). Table 1 shows the characteristics of the root system of the 'Fortival' rootstock, depending on the biostimulator applied exogenous on the cuttings. The highest percentage of rooted cuttings (98%) was obtained when applying Radistim no. 2. By applying NAA 2000 ppm a rooting percentage of 77% was obtained and by applying IBA 2000 ppm a rooting percentage of 64% was obtained. Control cuttings, not chemically stimulated, recorded a rooting percentage of 90%, which may explain the dependence of the rooting capacity on the genotype and the quality of the propagating material. Johnson et al. (2020) claim that in *Prunus* the rooting capacity is genotype dependent. Also Mutu et al. (2020) support the influence of genotype on rooting capacity. Ancu et al. (2008) showed that the percentage of rooting of herbaceous cuttings treated with Radistim 2, varied between 82.83% and 95.96% in the analyzed plum rootstock selections. Markovski et al. (2015) reported a rooting rate of rootstocks for plum and peach of 31.3%. Edizer & Demirel (2012) reported a rooting percentage of herbaceous cuttings treated with 3000 ppm IBA of 90.00% at 'St. Julien', 'Marianna GF 8-1' and 'SL-64' rootstocks. On the other hand, 86.67% rooting percentage was obtained at the 'Garnem' clonal rootstock at 4000 ppm IBA concentration. Markovski et al. (2015) recorded at 'St. Julian Orleans' cuttings a negative influence of auxin treatments on the rooting percentage. Regarding the number of roots/

cutting, the highest average value was also obtained for cuttings stimulated with Radistim no. 2 (21.41). Stimulation of cuttings with NAA 2000 ppm determined a number of 11.17 roots/cutting and stimulation of cuttings with IBA 2000 ppm determined a number of 9.69 roots/cutting. Chemically unstimulated cuttings showed the lowest number of roots/cutting, respectively 9.14, which shows that biostimulators determine the development of rootstocks root system. The highest average number of roots/cutting obtained by Markovski et al. (2015) was 13.2 cm and the influence of IBA auxin 2% was crucial in the formation of large number of roots, respectively 27.3 cm while auxin NAA did not have a positive influence on root formation compared to the control. Also, the best results regarding the average length of roots/cutting (6.16 cm) were obtained for cuttings treated with Radistim no. 2, followed by cuttings treated with NAA 2000 ppm (6 cm). The lowest average root length/cutting (5.62 cm) was obtained for cuttings treated with IBA 2000 ppm while chemically untreated cuttings recorded an average root length/cutting even lower, 5.20 cm. The results obtained by Szecskó et al. (2006) showed a relatively weak link between rooting and physiological factors. Rooting biostimulators have also led to an increase in the rooted segment. Its average value varied depending on the biostimulator. For cuttings treated with Radistim no. 2, the average length of the rooted segment was 2.13 cm, for those treated with NAA 2000 ppm it was 1.48 cm, for those treated with IBA 2000 pppm it was 1.18 cm and for those not chemically treated 1.18 cm. Also, the largest volume of roots/cutting (4.26 cm^3) was obtained for cuttings treated with Radistim no. 2. It is found that among the biostimulators applied to the cuttings of 'Fortival' rootstock, Radistim no. 2 had a superior influence on the development of the root system, compared to both the control and the other used biostimulators. Johnson et al. (2020) analyzed the influence of K-IBA (potassium salt of IBA) on herbaceous cuttings on *Prunus* genotypes; in plum cuttings the highest number of roots was obtained at 2000-4000 mg/L K-IBA, and rooting was different in *Prunus* depending on the genotype. Shoots and roots are autotrophic and heterotrophic organs of plants with different physiological functions, with different metabolism, which respond differently to environmental changes. Plants have complex regulatory mechanisms that coordinate physiological activity, growth and development (Hibberd & Quick, 2002). Based on this idea, the percentage of cuttings that showed early shoots, the number of early shoots/cutting and the average length of early shoots/cutting were analyzed and the results are shown in Table 2. It can be seen that the cuttings that recorded the highest number of roots, the largest average length of the root/cutting, the largest average length of the rooted segment and the largest volume of roots/cutting, meaning those stimulated with Radistim no. 2, recorded the lowest percentage of anticipated shoots (56.12%). The explanation lies in the fact that nutrients are allocated differently to optimize the efficiency of their use. Plants under different available resources allocate differently the available resources for shoots and roots to optimize the efficiency of their use. The results obtained by Gargallo-Garriga et al. (2014) provide clear evidence that plants have a high capacity to modulate and vary the nutrient allocation and relative activities of different metabolic pathways for biomass production in both shoots and roots. Table 3 shows the correlations established between the characteristics of the root system and the anticipated shoots depending on the used biostimulator. Significantly positive correlations were established between the length of the rooted segment and the number of roots/cutting, regardless of the applied biostimulator to the cuttings ($r = 0.65$ for cuttings stimulated with Radistim no. 2; $r = 0.50$ for cuttings stimulated with IBA and $r = 0.31$ for cuttings stimulated with NAA). Also, significant positive correlations were established between the volume of roots and the number of roots/cutting ($r = 0.82$; $r = 0.66$; $r = 0.48$), between the volume of roots and the length of the rooted segment, between the number of roots/cutting and their average length. Negative correlations were established between the number of anticipated shoots and the average length of the root/cutting, between the average number of roots/cutting and the length of the rooted segment. These can be explained by the fact that the anticipated shoots negatively influence the development of the root system, using the nutrients needed by the rootstock for rooting. To test the variance of the average length of roots/cutting according to the applied biostimulator compared to the control, the F-Test Two-Sample for Variances test was applied and the results are shown in Table 4. It can be seen that in the case of all three biostimulators applied to cuttings compared to control cuttings, not chemically stimulated, the statistical value of F is higher than Fcrit, so the null hypothesis that the two variables, meaning chemically treated and untreated cuttings, would have equal variances, is rejected, so the variances of the two analyzed areas are uneven, which shows that there are variations in the average length of the roots/cutting depending on the biostimulator applied, compared to the control cuttings. It can also be seen that the variance of the average length of roots/cutting is higher in the case of cuttings treated with biostimulators compared to those not chemically treated. The P value is higher than the specified alpha level of 0.05, so the probability of obtaining an F greater than F critical is between 0.18 and 0.38, which strengthens the rejection of the null hypothesis that the two variables would be equal. In conclusion, the variance of the average root length/cutting is higher in the case of chemically treated cuttings, regardless of the used biostimulator, which shows the favorable response of semi-hardwood cuttings 'Fortival' rootstock to the application of rooting biostimulators used in the study.

Table 1. Characteristics of the root system of ‘Fortival’ rootstock depending on the growth stimulator

Characteristics	Descriptive statistics	Radistim	IBA	NAA	Control
Rooting	%	98%	64%	77%	90%
No. of roots/ cutting	Mean±SD	21,41±11.33	9.69±4.47	11.17±5.85	9.14±6.41
	CV%	52.94	46.20	52.36	70.06
Average root length/cuting (cm)	Mean±SD	6.16±1.99	5.62±1.92	6.00±2.08	5.20±2.15
	CV%	32.30	34.25	34.57	41.33
Length of rooted segment (cm)	Mean±SD	2.13±1.35	1.19±0.99	1.48±1.02	1.18±1.21
	CV%	62.97	82.97	69.03	103.24
Roots volume/cutting (cm^3)	Mean±SD	4.26±2.96	1.31±0.77	2.06±1.66	1.61±1.24
	CV%	69.76	58.98	80.25	77.11

Table 2. Characteristics of the anticipated shoots of ‘Fortival’ rootstock depending on the growth stimulator

Characteristics	Descriptive statistics	Radistim	IBA	NAA	Control
Cuttings with anticipated shoots	%	56.12%	67.19	74.02	64.44%
Number of anticipated shoots	Mean ±SD	2.15±1.13	1.77±0.87	2.67±1.33	2.38±1.82
	CV%	52.63	49.13	49.87	49.69
Average length of anticipated shoots/cutting (cm)	Mean±SD	8.72±5.39	6.15±4.69	5.80±4.00	6.48±4.31
	CV%	61.76	76.16	69.03	66.57

Table 3. Correlations between the growth characteristics of ‘Fortival’ rootstock cuttings depending on the chemical stimulator

	L				N				LS				V				l			
	RAD	NAA	IBA	CT	RAD	NAA	IBA	CT	RAD	NAA	IBA	CT	RAD	NAA	IBA	CT	RAD	NAA	IBA	CT
L	1	1	1	1																
N	0.30	0.43	0.32	0.29	1	1	1	1												
LS	0.22	0.19	0.26	0.28	0.65	0.31	0.50	0.47	1	1	1	1								
V	0.47	0.37	0.23	0.42	0.82	0.66	0.48	0.78	0.61	0.38	0.36	0.49	1	1	1	1				
l	-0.17	-0.08	0.13	-0.15	0.19	0.14	0.08	0.03	0.13	0.11	0.40	0.01	0.16	0.05	-0.01	-0.01	1	1	1	1
n*	0.27	0.09	-0.03	-0.12	-0.02	0.00	-0.02	-0.03	0.16	-0.15	-0.19	-0.27	0.10	0.16	0.03	-0.09	-0.37	-0.25	-0.28	-0.33

L= Average root length/cutting (cm); N= Number of roots/cutting; LS= Length of the rooted segment (cm); V= Roots volume/cutting; l= Average length of the anticipated shoots/cutting; CT= control

Table 4. Variation of the average length of roots/cutting depending on the chemical stimulator (F-Test Two-Sample for Variances) (cm)

	Radistim 2	Control	NAA	Control	IBA	Control
	Variable 1	*Variable 2*	*Variable 1*	*Variable 2*	*Variable 1*	*Variable 2*
Mean	6.16	5.20	6.00	5.20	5.62	5.20
Variance	3.99	4.61	4.31	4.61	3.71	4.61
Observations	98	90	77	90	64	90
df	97	89	76	89	63	89
F	0.87		0.93		0.80	
P(F<=f) one-tail	0.24		0.38		0.18	
F Critical one-tail	0.71		0.69		0.68	

CONCLUSIONS

It was found that among the biostimulators applied to the cuttings of 'Fortival' rootstock, Radistim no. 2 had a superior influence on the development of the root system, compared to both the control and the other biostimulators used, determining the highest percentage of rooting, the highest number of roots, the highest average length of roots/cutting, the highest average length of the rooted segment and the largest volume of roots.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Pitești - Mărăcineni Research-Development Institute.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE ADAPTATION CAPACITY OF THE NEW APRICOT VARIETIES IN SOUTHERN CLIMATE CONDITIONS OF REPUBLIC OF MOLDOVA

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Abstract

The experimental plot is placed in fruit-growing orchard of 'Agroparc Management' Ltd., during the 2020 year. The object of the research was the trees of the apricot varieties of 'Wonder Cot', 'Spring Blush', 'Magic Cot', 'Lilly Cot', 'Pinkcot', 'Perle Cot', 'Orange Red', 'Sweet Cot', 'Big Red', 'Kyoto', 'Faralia' and 'Farbaly', grafted on the 'Mirobalan 29C' rootstock. Planting distance was 5.0 x 3.0 m. The planting of apricot trees was carried out in spring of 2015. During the researches was studied the period of beginning of flowering and the ripening of fruit harvesting phases, the period between these two phases on different varieties of apricots, morphological parameters of fruits and kernels, shape index and redistribution of fruits in various quality of classes. It has been established that the biological particularities of the variety influence on the period of beginning of the flowering and the ripening of the apricot fruits, the period between these two phases, the morphological parameters, the shape index and the redistribution of the fruits in different quality classes.

Key words: *apricot, blooming, ripening, medium weight, shape index, quality.*

INTRODUCTION

Apricots are in high demand among consumers and therefore apricot is one of the main fruit species grown in the world (Chira et al., 2005; Cociu et al., 1993). The largest production of apricots in the world comes from Asian countries and those in the Mediterranean basin (Balan et al. 2008; Cociu, 1993).

The Republic of Moldova is located in a temperate zone, where there is a great potential to cultivate many fruit species as a result of a great variety of soil and environmental conditions.

Apricot is one of them with an annual production that varies in the last 4-5 years from 9.50 to 21.50 thousand tons (Peşteanu et al., 2018).

Large apricot areas in the Republic of Moldova are located in the southern and central part of the country, but due to climate change in the last 10-15 years; apricot cultivation is planted on large areas in the northern area (Peşteanu et al., 2018; Pîntea, 2018).

However, the main factors limiting the wider spread of apricot cultivation among fruit growers is: the disease of the generative organs of low temperatures at the end of the rest period and late spring (Abbas et al., 2016; Cociu et al., 1993), premature wilting of trees (apoplexy), infection with viruses (Balan et al., 2008; Cociu et al., 1993), the absence of a wide assortment of modern varieties and rootstocks that would allow to intensify the culture, to obtain productions high, constant and competitive (Balan et al., 2008; Cociu et al., 1993; Maria & Sosna, 2006; Peşteanu et al., 2018).

The primary task of fruit growers is to replace the old varieties, less corresponding to the requirements in force, with new ones, more suitable for the conditions of our country, more productive and already known by consumers in the markets we want to penetrate.

This replacement may be possible based on the introduction of new modern varieties from the global range, which based on testing over 4-5 years on their adaptation to climatic, edaphic, biotic, and abiotic and agrotechnical conditions in various areas of the country recommended in production. (Cociu et al., 1993; Milatovic et al., 2016; Pîntea, 2019).

Among the varieties cultivated with apricots in the Republic of Moldova, there is an acute lack of valuable varieties with maturity from very early to very late.

This would allow completing the assortment of varieties, ensuring a harvesting conveyor, the consumption of these fresh fruits and their

industrial capitalization for a period of 50-60 days or even more (Negru, 2018; Pîntea, 2019).
Apricots being mostly intended for fresh consumption, they must meet certain requirements submitted by consumers, which are very difficult to achieve due to biological and technological features of the species (Cociu et al., 1993; Peşteanu et al., 2018).
In order to satisfy the consumer's necessities regarding the quality of the fruits and to penetrate new markets, the organoleptic apricots must be homogeneous in size, with specific color to the variety, but priority is given to varieties where 60-80% of the fruit surface is red, medium weight, pulp firm, dry detachment, relatively small pips, attractive appearance and valuable nutritional biochemical composition (Balan et al., 2008; Cociu et al., 1993; Milatovic et al., 2013; Pîntea, 2019; Souty et al., 1990).
The practical argumentation of some phenophases of fruit development in apricots, the morphological characteristic and quality indices of fruits in various apricot varieties were the main objectives of the research in question.

MATERIALS AND METHODS

The research was carried out during the year 2020 by setting up an experience in field conditions at the company SRL "Agroparc Management". The orchard was planted in the spring of 2015, with trees "Certificate" category twigs produced in the "Battistini Vivai" fruit nursery, Italy.
The object of research was the trees of apricot varieties introduced from the world selection (Cot International, Escande, International Plant Selection, etc.), which, due to a preliminary theoretical study showed to be prospective for the Republic of Moldova as: 'Wonder Cot', 'Spring Blush', 'Magic Cot', 'Lilly Cot', 'Pinkcot', 'Perle Cot', 'Orange Red', 'Sweet Cot', 'Big Red', 'Kyoto', 'Faralia' and 'Farbaly'. The 'Kyoto' variety, which has the same maturation period as the native 'Nadejda' apricot variety, was taken as a control variant. The 'Mirobalan 29C' biotype was used as the rootstock. The trees were guided according to the usual vessel crown system. Planting distance is 5.0 x 3.0 m.
Methodological principles and approved methods in genetic improvement and the study of fruit species were used for the research.
The study of biological, phenological and production properties was carried out based on observations, determinations and analyses regarding the development of apricot trees and fruits.
Observations on the onset of early-flowering phenophases and harvest maturity were made taking into account the apricot landmark stages described by Baggiolini (1952).
The average weight of the fruits and kernels was calculated by the weighing method, and the height, small and large diameter of the fruits by the measuring method. The shape of the fruits was expressed on the basis of the shape index, which was the correlation between the height of the fruit and the large diameter of the fruit.
The quality of apricots was determined by the method of measuring the large diameter in the equatorial area of the fruits. Apricots with a diameter of 30-35 mm are assigned to quality category I and II fruits, or are marked with the letter C, and those with a diameter greater than 35 mm to the extra quality category. Apricots of extra quality category are divided into the following classes: B - diameter 35-40 mm; A - diameter 40-45 mm; 2A - diameter 45-50 mm; 3A - diameter 50-55 mm and 4A - diameter 55 mm and larger.
The average value of the index under study was calculated at the main morphological parameters.

RESULTS AND DISCUSSIONS

The requirements of apricot against heat are very dynamic and therefore each development phase has a certain optimum temperature and travel time.
The data in Table 1 show that flowering in the early maturing varieties 'Wonder Cot' and 'Magic Cot' began on March 9. On March 11, the 'Perle Cot' variety began to bloom, on March 12, the 'Pinkcot' variety. One day later, the flowering of 'Spring Blush' and 'Sweet Cot' varieties started. Next, in the next row, the 'Lilly Cot' variety - March 14, the 'Orange Red' variety - March 16, the 'Big Red' variety - March 17, the 'Faralia' variety - March 18,

the ‘Farbaly’ variety - March 19 and the Kyoto variety - March 20, blossomed. Basically, during 12 days, the phenophase started flowering in the studied varieties.

The study performed on the degree of flowering, we record that in the varieties studied with different precocity, 50% of the flowers were flowering at different stages. The flowering rate of 50% of flowers in the crown of apricot trees in the ‘Wonder Cot’ and ‘Magic Cot’ varieties started on March 11, in the ‘Pinkcot’ and ‘Perle Cot’ varieties on March 13, and in the ‘Spring Blush’ and ‘Sweet Cot’ varieties on of March 15. Next, in the next row, the ‘Lilly Cot’ variety - March 17, the ‘Orange Red’ variety - March 18, the ‘Big Red’ variety - March 19, the ‘Faralia’ variety - March 19, the ‘Farbaly’ variety - March 20 and the ‘Kyoto’ variety - March 21.

Table 1. The influence of the biological particularities of apricot varieties on the triggering of the flowering phases of the trees in the southern part of the country

Variety	The start of the beginning of the flowering phase of the trees			
	The start of flowering	Flowering 50%	Full flowering	Falling petals
Wonder Cot	09.03	11.03	13.03	19.03
Spring Blush	13.03	15.03	17.03	21.03
Magic Cot	09.03	11.03	13.03	18.03
Pinkcot	12.03	13.03	15.03	19.03
Perle Cot	11.03	13.03	15.03	21.03
Orange Red	16.03	18.03	20.03	24.03
Sweet Cot	13.03	15.03	19.03	23.03
Lilly Cot	14.03	17.03	20.03	23.03
Big Red	17.03	19.03	21.03	25.03
Kioto (c)	20.03	21.03	23.03	26.03
Faralia	18.03	19.03	21.03	25.03
Farbaly	19.03	20.03	22.03	26.03

That is, the period between the beginning of flowering and the 50% flowering phenophase lasted about 2-3 days depending on the biological characteristics of the variety and the air temperature in that period.

The end of flowering is considered when 100% of the flowers in the multiannual wood and annual branches in the first wave of growth have fully flowered. For the varieties studied, the end period coincided with 13.03-23.03, which lasted 11 days.

The duration between the phenophase from the beginning of flowering and full flowering (100%) in the apricot varieties studied was from 4 to 7 days depending on the biological characteristics of the variety. In the case of varieties with early ripening this period was 5-7 days, but for varieties with late fruit ripening period of 4 days.

Depending on when 100% flowering was recorded, the varieties studied can be placed in the following sequence: ‘Wonder Cot’ variety - March 13, ‘Spring Blush’ variety - March 17, ‘Magic Cot’ variety - March 13, ‘Lilly Cot’ variety - March 20, variety ‘Pinkcot’ - March 15, ‘Perle Cot’ variety - March 15, ‘Orange Red’ variety - March 20, ‘Sweet Cot’ variety - March 19, ‘Big Red’ variety - March 21, ‘Faralia’ variety - March 21, ‘Farbaly’ variety - March 22 and ‘Kyoto’ variety - March 23 .

The fall of the petals invokes the period when the fruits have just been formed and it is necessary not to allow their condition to be affected by the abiotic and biotic factors. They are quite sensitive to various biotic and abiotic hazards. The obtained results show that the fall of the petals in the apricot varieties studied in the southern area took place from March 18 to March 26, starting with the varieties with earlier age (‘Wonder Cot’, ‘Spring Blush’, ‘Magic Cot’, ‘Lilly Cot’, ‘Pinkcot’), continuing with those with medium maturation (‘Perle Cot’, ‘Orange Red’, ‘Sweet Cot’, ‘Big Red’) and ending with late maturing varieties (‘Faralia’, ‘Farbaly’, ‘Kyoto’).

Further studying the sequence of fruits ripening of apricot varieties studied in the southern part of the country during the research, we notice that large deviations from the harvest period that each variety possesses has not been recorded.

The study carried out on the period of fruits set in the apricot varieties studied shows that this phenophase began on average 88 days after flowering with the ‘Spring Blush’ variety and ended after the 129-day period with the ‘Farbaly’ variety. If we compare the ripening period of apricot fruits with the ‘Kyoto’ variety, considered as a control, we record that all the varieties studied can be divided into 4 groups (Figure 1).

The group 'Wonder Cot' and 'Spring Blush' are attributed to the group of extra-early ripening varieties, whose fruits harvesting started 12-14 days earlier compared to the control variety. The group of varieties with early maturation is assigned the varieties 'Magic Cot', 'Pinkcot', 'Sweet Cot', 'Lilly Cot' and 'Orange Red', whose difference in terms of triggering the harvest period compared to the control variant was 6-8 days. The varieties with medium maturation include the varieties 'Perle Cot', 'Sweet Cot', 'Big Red' and 'Kyoto' (0-4 days), and the group of late maturing varieties 'Faralia' and 'Farbaly' varieties, which recorded a retention of the beginning of the harvest period compared to the variant witness with 16 and 27 days, respectively.

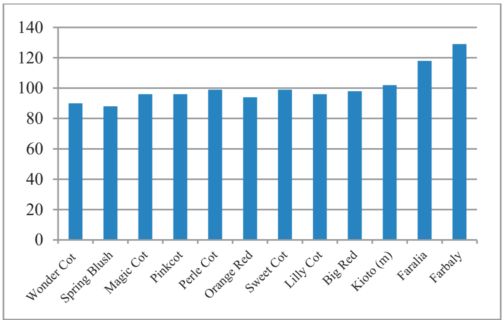

Figure 1. The influence of the biological particularities of apricot varieties on the period from the beginning of the flowering of the trees to the ripening of the fruits harvest

The size and shape of the fruits influence the market value and are important indicators in the study of varieties, because they are directly related to the quality of the product obtained, productivity in sorting and packaging of fruits.
The study carried out in 2020, in the apricot plantation shows that the biological particularities of the varieties influenced the average weight of the fruits. After average weight, the apricot varieties studied can be divided into the following groups. To the group of very small varieties, weighing between 20-30 g, is assigned the 'Big Red' variety, which has borne fruits in anticipated branches and for the future in order to obtain competitive productions it will be necessary to regulate the fruits load from the tree crown by manual thinning. 'Lilly Cot' and 'Wonder Cot' varieties are assigned to the group of those with small fruits whose average weight is 31-40 g. To the group of varieties with medium fruits are placed the varieties 'Spring Blush', 'Orange Red', 'Kyoto' and 'Farbaly', whose average weight of the fruits was 41-50 g. The varieties 'Sweet Cot' and 'Faralia', according to the average weight of the fruits belong to the group of varieties with large fruits (50-60 g), and the variety 'Magic Cot' and 'Pinkcot' to the group of varieties with very large fruits, whose value was greater than 60 g (Table 2).

Table 2. Morphological parameters of apricot fruits according to the biological particularities of the variety

Variety	The average weight, g	Height, mm	Large diameter, mm	Small diameter, mm	The shape index
Wonder Cot	38.4	46.0	40.5	38.7	114
Spring Blush	47.3	44.2	43.2	41.8	1.02
Magic Cot	61.7	50.9	46.2	45.3	1.10
Lilly Cot	32.8	41.0	38.2	33.3	1.05
Pinkcot	62.4	49.3	47.3	44.9	1.04
Perle Cot	51.6	46.2	46.0	41.7	1.00
Orange Red	44.7	47.2	46.0	40.5	1.03
Sweet Cot	50.8	47.9	46.6	41.0	1.03
Big Red	38.6	38.9	37.6	34.7	1.03
Kioto (c)	49.3	45.5	45.6	43.1	1.00
Faralia	56.4	48.9	47.6	42.5	1.04
Farbaly	46.7	47.0	44.7	34.7	1.06
Average	47.6	46.1	44.1	40.2	1.04

The shape of the fruit is in strict accordance with the biological characteristics of the variety and can be from spherical, ovate to ellipsoidal. Deviations from the fruit form indicate an incomplete development due to water insufficiency during fruit development (Mitrea V. 2007; Piagnani M. C., 2013).
The height and large diameter of the apricots are the characteristics that define the shape of the fruit. Differences in fruits height are genetic in nature. The lowest fruit heights were recorded in the 'Big Red' variety (38.9 mm), and the highest in the 'Faralia', 'Pinkcot' and 'Magic Cot' varieties, where the index under study was 48.9; 49.3 and 50.9 mm, respectively. In general, all apricot varieties studied can be divided according to the height of the fruits into 3 groups. That is, varieties with a fruit height of up to 40 mm are assigned

the ‘Big Red’ variety, varieties with a height of 40-50 mm where most of the studied varieties include ‘Wonder Cot’, ‘Spring Blush’, ‘Lilly Cot’, ‘Pinkcot’, ‘Perle Cot’, ‘Orange Red’, ‘Big Red’, ‘Kyoto’, ‘Faralia’ and ‘Farbaly’. The ‘Magic Cot’ variety recorded values greater than 50 mm.

The large diameter and small diameter of the varieties studied were influenced by the biological characteristics of the variety. If, for example, values of more than 40 mm were recorded for most apricot varieties after large diameter, then ‘Lilly Cot’ and ‘Big Red’ varieties had a diameter between 37.6-38.2 mm. According to the values of the small diameter we register diametrically opposite correlations. In this case only for the varieties ‘Spring Blush’, ‘Magic Cot’, ‘Pinkcot’, ‘Perle Cot’, ‘Orange Red’, ‘Sweet Cot’, ‘Kyoto’ and ‘Faralia’ the diameter of apricot fruits had values greater than 40 mm, and for varieties taken ‘Wonder Cot’, ‘Lilly Cot’, ‘Big Red’ and ‘Farbaly’ index in the study recorded values between 33.3-38.7 mm.

The study performed on the shape of the fruit through the shape index, it is highlighted that in all the studied varieties values higher than 1.0 were registered. If in the varieties ‘Spring’ ‘Blush’, ‘Lilly Cot’, ‘Pinkcot’, ‘Perle Cot’, ‘Orange Red’, ‘Sweet Cot’, ‘Big Red’, ‘Kyoto’, ‘Faralia’ and ‘Farbaly’ the shape index varied from 1.00 to 1.06, which highlights the spherical shape of the fruit. In the ‘Wonder Cot’ and ‘Magic Cot’ varieties, this index registered values of 1.1-1.26, meaning the fruits had an elongated spherical and cordiform shape.

The stone/fruit ratio and their morphological parameters are important indicators in the study of varieties, because they are directly related to the quality of the product obtained and how the consumer will perceive the importance of the variety.

The biological particularities of the varieties also influenced the share of the pit in the fruit (Table 3). According to the weight of the pit in the fruit, the apricot varieties studied can be divided into the following groups. ‘Wonder Cot’ (3.0%), ‘Magic Cot’ (4.4%), ‘Perle Cot’ (4.2%) and ‘Kyoto’ (4%) are assigned to the group of varieties with a weight of fruit pit 3.0-5.0%. The varieties ‘Spring Blush’, ‘Lilly Cot’, ‘Pinkcot’, ‘Orange Red’, ‘Sweet Cot’, ‘Big Red’ and ‘Faralia’, the share of seed in the fruit varied from 5.1 to 7.0% and for the varieties mentioned above it was 5.9; 5.7; 6.0; 5.1; 6.5 and 5.7, respectively. The ‘Farbaly’ variety (8.9%) is placed in the group of varieties with a share of seed in the fruit higher than 7.0%.

The heights, large and small diameter of the apricot pit are hereditary characteristics of the variety. All apricot varieties according to the height of the pit studied can be divided into 3 groups. That is, varieties with a seed height of 20-25 mm, varieties with a height of 25.1-30 mm and varieties with values greater than 30 mm. The lowest pit heights were recorded for the ‘Kyoto’ (21.1 mm), ‘Lilly Cot’ (23.1 mm) and ‘Big Red’ (24.3 mm) varieties, and the highest for the ‘Magic Cot’ (30.5 mm), ‘Farbaly’ (31.2 mm) and ‘Faralia’ (33.4 mm) varieties. The varieties ‘Wonder Cot’, ‘Spring Blush’, ‘Pinkcot’, ‘Perle Cot’, ‘Orange Red’ and ‘Sweet Cot’ recorded average values between 25.3-27.5 mm.

Table 3. The morphological parameters of apricot kernels according to the biological particularities of the variety

Variety	The share of pit in fruit, %	Heigh t, mm	Large diameter, mm	Small diameter, mm	The shape index
Wonder Cot	3.0	266	18.9	9.4	1.40
Spring Blush	5.9	25..3	21.4	13.5	1.18
Magic Cot	4.4	30.5	21.6	11.0	1.41
Lilly Cot	5.7	23.1	18.0	9,.	1.28
Pinkcot	5.2	27.5	21.8	11.6	1.26
Perle Cot	4.2	26.1	19.3	11.6	1.35
Orange Red	6.0	28.0	21.8	11.6	1.28
Sweet Cot	5.1	27.4	20.4	11.8	1.34
Big Red	6.5	24.3	17.8	10.9	1.36
Kioto (c)	4.4	21.1	19.3	11.2	1.10
Faralia	5.7	33.4	24.1	14.4	1.37
Farbaly	8.9	31.2	21.8	12.0	1.43
Average	-	27.0	20.5	11.6	1.31

The large diameter and small diameter of the seed in the varieties studied were influenced by the biological particularities of the variety. If, for example, values of 17-20 mm were recorded for the apricot varieties ‘Wonder Cot’ (18.9 mm), ‘Lilly Cot’ (18.0 mm), ‘Big Red’

(17.8 mm), after the large diameter of the stone, ‘Pearls Cot’ (19.3 mm) and ‘Kyoto’ (19.3 mm), then value greater than 23 mm was entered by the variety ‘Faralia’ (24.1 mm). The varieties ‘Spring Blush’, ‘Magic Cot’, ‘Pinkcot’, ‘Orange Red’, ‘Sweet Cot’ and ‘Farbaly’, according to the large diameter of the stone, recorded average values, ranging from 20.4 to 21.8 mm.

All apricot varieties according to the small diameter of the pit can be divided into 3 groups. That is, varieties with a small diameter of 8-10 mm, registered in the varieties ‘Wonder Cot’ (9.4 mm) and ‘Lilly Cot’ (9.8 mm), varieties with values of 10.1-12.0 mm to which it is assigned most varieties studied as: ‘Magic Cot’, ‘Pinkcot’, ‘Perle Cot’, ‘Orange Red’, ‘Sweet Cot’, ‘Big Red’, ‘Kyoto’ and ‘Farbaly’ with values of the studied index between 10.9-12.0 mm. In the third group can be attributed to the varieties ‘Spring Blush’ and ‘Faralia’ whose small core diameter was 13.5 and 14.4 mm, respectively.

In addition to the indicators studied previously, the biological particularities of the variety also influence the pit shape index. Lower pit shape index values were recorded for the ‘Kyoto’ (1.10) and ‘Spring Blush’ (1.18) varieties and higher for the ‘Wonder Cot’ (1.40), ‘Magic Cot’ (1.41) and ‘Farbaly’ varieties. (1.43). In the case of ‘Lilly Cot’, ‘Pinkcot’, ‘Perle Cot’, ‘Orange Red’, ‘Sweet Cot’, ‘Big Red’ and ‘Faralia’ varieties, the pit shape index registered average values, between 1.26-1.37.

The dimensions of the fruits are of special importance because depending on them, they are redistributed on different quality classes, on which then depends the selling price, so implicitly the economic efficiency. Particularly important is the diameter of the fruit in the equatorial plane, which is a very important quality element, which apart from hereditary influences is strongly conditioned by environmental and cultural factors (Table 4).

The carried out investigations show that from the studied varieties, fruits of quality category I and II (class C) are not registered, because due to the low temperatures in the spring, a large part of the harvest was compromised. In the varieties studied, fruits with a diameter greater than 35 mm were recorded in the ‘Lilly Cot’ (100.0%) and ‘Big Red’ (100.0%) varieties.

Table 4. The influence of biological characteristics of apricot varieties on fruit quality by diameter and weight, %

Variety	By diameter			
	B	A	2A	3A
Wonder Cot	-	100.0	-	-
Spring Blush	-	65.9	34,1	-
Magic Cot	-	30.6	69.4	-
Lilly Cot	34.5	65.5	-	-
Pinkcot	-	-	83.9	16.1
Perle Cot	-	34.2	65.8	-
Orange Red	-	16.1	83.9	-
Sweet Cot	-	48,2	51,8	-
Big Red	9.5	87.7	2.8	-
Kioto (c)	-	83.4	16.6	-
Faralia	-	7.0	80.2	12.8
Farbaly	-	100.0	-	-

In quality class A, a higher share of fruits belonged to the ‘Wonder Cot’ (100.0%) and ‘Farbaly’ (100.0%) varieties, average values to the varieties ‘Spring Blush’ (65.9%), ‘Kyoto’ (83.4%), sub-averages for the varieties ‘Sweet Cot’ (48.2%) and ‘Perle Cot’ (34.2%), and a lower share formed the varieties ‘Orange Red’ (16.1%) and ‘Faralia’ (7.0%).

Apricot fruits with a diameter of 45-50 mm are attributed to class 2A, and those with values of 50-55 mm to category 3A, which are the most requested among consumers. In quality class 2A, a higher share of fruits belonged to the varieties ‘Pinkcot’ (83.9%), ‘Orange Red’ (83.9%) and ‘Faralia’ (80.2%), and lower values, to the varieties ‘Kyoto’ (16.5%), ‘Spring Blush’ (34.1%). The varieties ‘Magic Cot’ (69.4%), ‘Perle Cot’ (65.8%) and ‘Sweet Cot’ (51.8%), registered average values in terms of quality of apricot fruits in the respective class.

Further research has shown that quality of 3A fruits has been obtained in a limited amount of fruits, only in the case of ‘Pinkcot’ (16.1%) and ‘Faralia’ (12.8%).

CONCLUSIONS

The flowering period of apricot in the spring of 2020 with different maturation period was staggered over 12 days, and in the northern part of 7 days.

The morphological parameters are a valuable tool in assessing fruits quality, which is valuable information for fruit growers, who need to pay more attention to technological elements in order for cultivated fruits to be successful among consumers.

Most of the studied apricot varieties have a spherical fruit shape and only in the 'Wonder Cot', 'Magic Cot', 'Faralia' and 'Farbaly' varieties the fruits had an elongated spherical and cordiform shape.

Of the varieties studied in the southern part of the country, a higher share of quality class A and 2A fruits was registered for the varieties 'Spring Blush', 'Magic Cot', 'Pinkcot', 'Big Red', 'Orange Red', 'Kyoto', 'Faralia' and 'Farbaly'.

ACKNOWLEDGEMENTS

This study was supported by the National Agency for Research and Development of the Republic of Moldova, project 20.80009.5107.04 "Adaptation of sustainable and ecological technologies for fruit production in terms of quantity and quality according to the integrity of the cropping system and climate change."

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SPRING FROST DAMAGES OF PLUM AND APRICOT CULTIVARS GROWN IN THE REGION OF PLOVDIV, BULGARIA

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Abstract

Frost injuries are one of the main limiting factors to fruit crops production. In the region of Plovdiv, Bulgaria the rising temperatures early in the spring force the development of flower buds and some fruit trees are often affected by the late spring frosts. Studying the cultivars under natural stress conditions provides a good basis for the selection of suitable ones for growing in each region. This study was conducted in plum and apricot collection orchards at the Fruit Growing Institute in Plovdiv. Spring frosts were observed in 2012, 2018 and 2019. In 2018, yields only from the apricot cultivars 'Goldrich', 'Perle Cot', 'Sweet Cot' and 'Harcot' were obtained. Of the 31 apricot samples examined in 2019, 21 were 100% injured and for 10, the damages ranged from 68% to 98%. In 2012, severe spring frost damages were observed for the plum cultivars 'Toptaste' - 51%, 'Topking' - 30% and 'Bellamira' - 20%. In 2019, the plum cultivars 'Jojo' and 'Pacific' showed as highly sensitive to spring frosts. The lowest damages were recorded for 'Tita' - 6%, followed by the standard 'Stanley' - 12%.

***Key words**: apricot, plum, spring frost, damages.*

INTRODUCTION

Winters and early springs are predicted to become warmer in temperate climates under continued global warming, which in turn is expected to promote earlier plant development (Vitasse et al., 2018). The Plovdiv region is situated in the South Central part of Bulgaria. The most commonly grown fruits are apples, plums and cherries. Plum is a traditional fruit crop for Plovdiv and the region is the second biggest producer of plum fruits in Bulgaria (SENTER, 2000). The South - Central Region represents 25.6 % of the total area occupied with plum trees, which is the first place among the six regions of the country (Agrostatistics, 2018). The region is not typical for apricots growing. Due to their short dormancy period apricots are among the first fruit species that start their development, especially in the last years when winters are getting warmer. Sustainable yields from these fruit trees could be obtained only in separate regions of the country, where the meteorological conditions are most suitable for their growth and development (Kazandjiev & Malasheva, 2019). Climate change and especially the variation of spring temperatures is reflected by changes in the timing of phenology which is dependent on a combination of internal (genetic) settings and environmental influences.

In the Plovdiv region, a considerable number of incidences of spring frosts occurred over the last decade - 2008, 2013, 2016, 2019 and 2020 (Malchev & Savchovska, 2020). Thus, late freezing injuries are a major limiting factor to both cultures production in the region (Bozhkova & Ivanova, 2001; Bozhkova & Zhivondov, 2004).

The effects of spring frosts on the reproductive organs of deciduous fruit trees are highly variable and depend on the characteristics of both the freezing stress and the plant material (Rodrigo, 2000).

One of the effective and long-lasting solutions is the development of genotypes with frost resistance as well as late flowering (Dumanoglu et al., 2019). Studying the cultivars under natural stress conditions provides a good basis for selecting suitable for growing in each region of the country and predicting the economic results of them. It is also important for breeders to choose proper parental cultivars as donors of this characteristic in the controlled hybridization schemes. This study aimed to select apricot and plum cultivars that exhibit resistance to spring frosts and are suitable for growing in the Plovdiv region.

MATERIALS AND METHODS

The study was conducted in plum and apricot collection orchards at the Fruit Growing Institute - Plovdiv. For determining the phenological stage of the cultivars BBCH scale was used (Meier, 2018). The frost damages of flowers and young fruitlets were recorded three days later. For each plum and apricot cultivar, 100 flowers or young fruitlets located in 3 different directions of the crown and up to 2.00 m high were studied. The results were expressed in the percentage of damaged flowers or fruitlets. Data were statistically processed by Duncan's multiple range test of the IBM SPSS statistics 26 software.

RESULTS AND DISCUSSIONS

In the Plovdiv region, late spring frosts that affected the flower buds of both plum and apricot cultures were recorded on 03.04. 2012 (-2.8^{o}C), 20.03.2018 (-2.5^{o}C) and on 29.03.2019 (-5.0^{o}C). Data for the minimum temperatures fluctuation in March and April, in 2012, 2018 and 2019 is shown in Figure 1. The impact of these critical temperatures lasted for more than two hours in the three years of observations.
During these weather conditions, the apricot and plum trees were in different phenological stages, which caused variation in the damages.
Severe frost injuries for the apricot cultivars planted in the collection orchard were observed in 2018 and 2019. On 20.03.2018, the apricot cultivars were in different phenophases to the principal growth stage "flowering". In an earlier stage of their development (BBCH 60) were the cultivars Augustin, Litoral, Selena, Sirena, Euxin, Perlecot and Veharda (Table 1). At that time the flowers of Sweetcot were fading (BBCH 67). The percentage of damaged flower buds for all cultivars ranged from 94% to 99% with statistically non-significant differences. No matter the earlier phenological stage, the Romanian cultivars also were severely injured. In this year the least injured was 'Goldrich' cv. - with only 1% damaged flowers.
In 2018, yields were obtained only from the cultivars 'Goldrich', 'Perle Cot', 'Sweet Cot' and 'Harcot'.
In 2019, the apricot cultivars were between the phenophases end of flowering and ovary growing - 69-71 BBCH, when the late spring frost occurred. Of the 31 genotypes examined this year, 21 were 100% damaged and 10 ranged from 68% to 98%. The lowest percentage of injured flowers was recorded for 'Tomcot' cv. - 68%, followed by 'Euxin', 'Sirena' 'Harcot', and 'Silistrenska ranna'. The lower damages of this cultivars are statistically significant compared to the others. All flowers and fruitlets of the studied apricot hybrids were injured and for them, the damages were 100%. In our other studies, as in this one, 'Harcot' has been found to be cold resistant and therefore it is recommended for growing in the Plovdiv region (Bozhkova et al., 2013). Hybrids obtained with this cultivar used as a parent in the cross did not show any kind of resistance at this stage. The fact that these trees are young and with a still undeveloped crown (hybrids with the abbreviation HH in Table 1) may also have an impact.

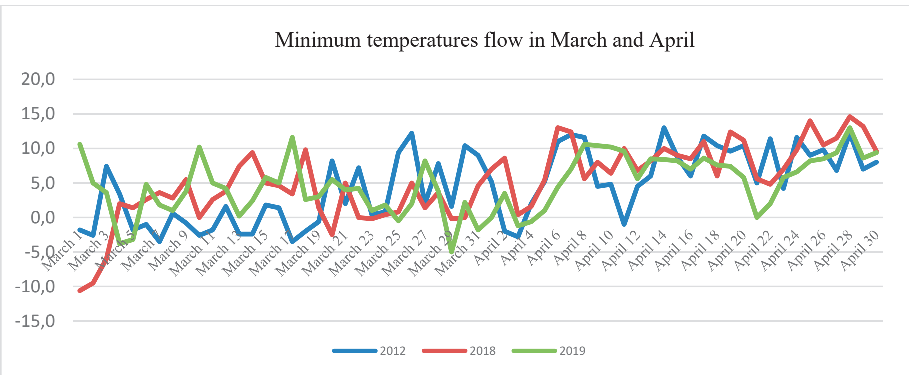

Figure 1. Minimum temperatures fluctuations for the observation period

Table 1. Spring frost damages of the apricot flowers (2018) and fruitlets (2019)

Cultivar/ Elite	BBCH code	Damages in 2018 (%)	BBCH code	Damages in 2019 (%)
Harcot	60	90 a**	69-71	82 bc
Sweetcot	67	90 a	69-71	90 ab
Augustin	60	99 a	69-71	100 a
Litoral	60	95 a	69-71	100 a
Selena	60	95 a	69-71	96 a
Euxin	60	95 a	69-71	74 cd
Sirena	60	95 a	69-71	84 bc
Perlecot	60	90 a	69-71	100 a
Flavorcot	65	95 a	69-71	94 a
Veharda	60	97 a	69-71	100 a
Tomcot	65	96 a	69-71	68 d
Jennycot	61	96 a	69-71	100 a
Bergecot	60	94 a	69-71	100 a
Lito	65	96 a	69-71	100 a
Goldrich	61	1 b	69-71	96 a
Sil. ranna	65	98 a	69-71	80 bc
Harlayne	65	94 a	69-71	98 a
HH 12-60	md*	md	69-71	100 a
HH 12-58	md	md	69-71	100 a
HH 9-1	md	md	69-71	100 a
HH 9-2	md	md	69-71	100 a
HH 12-53	md	md	69-71	100 a
HS 12-20	md	md	69-71	100 a
HS 12-19	md	md	69-71	100 a
HH 12-47	md	md	69-71	100 a
HS 12-16	md	md	69-71	100 a
HH 13-3	md	md	69-71	100 a
HH 14-34	md	md	69-71	100 a
HH 13-67	md	md	69-71	100 a
HH 13-51	md	md	69-71	100 a
HH 12-22	md	md	69-71	100 a

md* - missing data, due to the young age of the trees
** - different letters show a statistically significant difference in the same column

Figure 2. Frost injury in 2019

Studies on the effect of low winter temperatures showed that the cultivars of *P. domestica* species demonstrated good winter hardiness. Problems arise at the end of the winter and the beginning of spring, in case the temperatures drop down below -1°C (Bozhkova, 2013). Frost injuries of the plum cultivars in the Plovdiv region are not a frequent phenomenon and the damages usually are not as high percentage as for apricots.

Figure 3. Flowers of 'Stanley' (left) and 'Jojo' (right) on 01.04.2019

Table 2. Spring frost damages of plum cultivars

Cultivar	BBCH code	Damages in 2012 (%)	BBCH code	Damages in 2019 (%)
Jojo	62	16 cd**	65	100 a
Haganta	62	13 cd	65	50 cde
Top First	62	17 cd	65	34 fg
Top Hit Plus	62	5 e	65	52 cd
Bellamira	62	20 c	65	44 de
Topking	62	33 b	65	48 cde
Toptaste	62	51 a	65	26 gh
Topstar Plus	md*	md	65	32 gh
Roman	md	md	65	42 ef
Robelle	md	md	65	22 h
Tita	md	md	65	6 i
Yalomita	md	md	67	72 b
HL 20-30	md	md	67	100 a
Pitestean	md	md	65	24 h
Stanley	62	11 de	65	12 i
Pasific	61	20 c	65	100 a
Cacanska Najbolja	62	10 de	65	56 c
Sirma	md	md	67	100 a
Pagane	md	md	67	100 a

md* - missing data, due to the young age of the trees
** - different letters show a statistically significant difference in the same column

In 2012, during the spring frost, the plum cultivars were at beginning of the flowering phase (60-62 BBCH). A high rate of frost damage was recorded for the cultivars 'Toptaste' cultivar - 51%, 'Topking' - 30% and 'Bellamira', respectively 20% (Table 2). The lowest percentage of damaged flower buds was

observed for ‘Top Hit Plus’, ‘Cacanska najbolja’ and ‘Stanley’. For the standard cultivar, the frost damage rate was 11%. In 2018, the plum cultivars were not affected by the late spring frosts.
In 2019, during the frost, the plum cultivars were in full flowering phenophase and flowers fading (65-67 BBCH). The lowest damages were recorded in the ‘Tita’ cultivar - 6%, followed by the standard ‘Stanley’ - 12%. The differences in the calculated percentage of damages for these two cultivars and the others are statistically significant. Low freezing rates were also reported for the cultivars ‘Top First’, ‘Toptaste’, ‘Topstar Plus’, ‘Robelle’ and ‘Pitestean’. A hundred percent frost-damaged flowers were observed for ‘Jojo’, ‘Pacific’, the new cultivars of the Fruit Growing Institute – ‘Sirma’ and ‘Pagane’ and the Romanian plum hybrid HL 20-30. The ‘Jojo’ cultivar was in full bloom, while the other cultivars and hybrids were at the end of flowering when considered to be more sensitive. The data from the different years show great differences depending on the phenophases of development, and during the flowering period, the cold resistance decreases. In some cultivars, this decrease is gradual and in others drastic. Besides, other factors, such as the duration of the low temperatures, the relief, the health status of the trees, the yield of the previous year, and the nutrient reserve, have an impact on the frost sensitiveness of the cultivars. This allows us when grown under the same environmental conditions, to select frost resistant cultivars for growing and breeding purposes.

CONCLUSIONS

From several years of observations, it can be said that the apricot cultivars ‘Goldrich’, ‘Harcot’, ‘Perle Cot’ and ‘Sweet Cot’ exhibit some resistance to early spring frosts. The ‘Jojo’ plum cultivar is highly sensitive to spring frosts, as is ‘Pacific’. This year's data once again prove the good cold resistance of the standard ‘Stanley’.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

YIELD AND FRUIT CHEMICAL COMPOSITION OF SOME PLUM CULTIVARS AFFECTED BY DIFFERENT SOIL TYPES

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Abstract

Soil type and properties, soil fertilizers have influence on development of root system, on crop productivity and aromatic and texture features of fruits. The aim of the present study was to compare the effect induced by three different soils type on plum fruit quality and yield. The plum 'Vânăt românesc' and 'Stanley' varieties were studied in two experimental orchards Caransebes and Lugoj areas. The plum quality parameters and yield were analysed to assess the relationship between soil type and fruit quality. There was little difference in the pigment content of the varieties, but variety such as 'Vanat romanesc' had a higher anthocyanin content, cultivated in the soil type typical brown luvic while the anthocyanin content increase at 'Stanley' variety cultivated in brown luvic pseudogleyic. The titratable acidity was similar in the both cultivars from all soil type. Also, significant differences were found in fruit weight and dry matter between soil type for every cultivar studied. The results obtained suggest that brown luvic pseudogleyic soil have a special favorability for 'Vânăt românesc' cultivar but 'Stanley' cultivar is much more suitable for cultivation in erodisol iluvial clay anthropically covered.

Key words: *brown luvic soil, brown luvic pseudogleyic, fruit weight, pigments, soluble solids.*

INTRODUCTION

Plum (*Prunus domestica*) growing has a long tradition in Romania and this species is widely spread throughout country because of favourable pedo-climatic conditions this covering an area of 65,114 ha and having a production of 512,975 tons (Zamfirescu et al., 2019; Butac et al., 2015).

Nowadays, cultivated varieties are diverse depending on the environment, so different varieties have been adapted at different soil and climatic conditions. Selection of the appropriate cultivar for particular climatic and soil conditions is a factor of primary importance (Sarawathi et al., 1998) for improving fruit quality and yield. Therefore, some growing areas require a different selection of fruit cultivars than others.

Obtaining a high yield and good fruit quality is dependently by different factors such as climate (temperature, humidity, light) and soil characteristics and management practices (e.g., soil management, pruning, planting density, rootstocks, water management) (Reig et al., 2018; Zoppollo et al., 2011).

Soil texture influences soil water holding capacity (SWHC), and thus the availability of water for uptake by the roots in grapevine, which is a key factor for obtaining high quality grape (Lovisolo et al., 2016). In another study, a higher percentage of sand was positively correlated with increase of total soluble solids (TSS) and polyphenol concentration (De Santis et al., 2017).

It is well known that soil have an important impact on aromatic characteristic of fruits like grape (Royer et al., 2012). Also, studies of Cudur et al. (2014) supported that soil type influenced grape acidity and production but have insignificant effect on the sugar accumulation. The previous studies of Aruani et al. (2014) shows that soil have an hight impact on pear tree yield.

Several studies had shown that soil type exercised a considerable influence on grow characteristics, quality and minerals content of blueberries cultivars (Matsouka et al., 2017; Tasa et al., 2012; Ancu et al., 2010).

Little information has been available in the literature regarding to role of soil type on quality and yield of plums (Guerra and Casquero, 2009; Rato et al., 2008).
The objective of the present study is to compare the effect induced by three different soils (brown luvic typical and brown luvic pseudogleyic and erodisol iluvial clay anthropically covered) on yield and fruit quality of plums.

MATERIALS AND METHODS

Studied cultivars 'Vânăt românesc' and 'Stanley' have been collected from experimental plum orchards located at Caransebeș and Lugoj regions. Both cultivars resulted by three different soil: brown luvic typical (S1) and brown luvic pseudogleyic (S2) and erodisol iluvial clay anthropically covered (S3).
Soil chemical composition from 0-20 cm and 20-50 cm soil depth is presented in Table 1. Mineral composition and humus content of three soils type was performed according to the methods described by Borlan and Răuță, 1981. Atomic absorption spectrophotometry was used for Ca, Mg, Mn, Zn, Fe, Co and Cu content determination, while flame photometry was used for available P and K content determination. Nitrogen content were determined by Kjeldahl method. Soil pH was determined with pH meter.
Yield per tree (kg) of each cultivar was measured on five trees on three replications. A sample of randomly picked 15 fruits per cultivar was harvested at commercial maturity for determining of quality attributes. Fruit quality parameters were immediately assayed after harvest with specific analytical methods.
Soluble solids content (SSC) was assessed in juices of fruits using a thermo-compensated Atago hand-refractometer (model PR-101, ATAGO, Japan) expressed as Brix (Harril, 1998).
Titratable acidity (TA) was determined by titration of known volume of juice aliquot with 0.1N NaOH to an end point pH 8.1 using a pH Meter (Hanna Instruments, Italy) and the total acidity calculated and expressed as malic acid (Crisosto, 2008).
Assessment of ascorbic acid content was achieved by quantitative reduction of 2,6-diclor- phenol-indophenol and the excess of dye is spectrophotometrical determined at 500nm (AOAC, 1990). The results were expressed as mg/100g fresh weight.
Flesh firmness (kg/cm^2) was averaged from two measurements taken at the equator of each fruit, after removing a peel evaluated with a penetrometer (Model FT 327) fitted with a cylindrical 11.1mm diameter head (Bramlage, 1983).
External color (L*, a*, and b*) was measured on 10 fruit from each group with Hunter Lab colorimeter (Model MiniScan XE Plus) according to the method of Hunter and Harold, (1987). Measurements were conducted in CIE L*a*b* system. L* is a measure of lightness, where values range from completely opaque (0) to completely transparent (100), a* is a measure of redness (or - a* of greenness) and b* of yellowness (or - b* of blueness) on the hue circle. The hue angle, h°, describes the relative amounts of redness and yellowness where 0°/360° is defined for red/magenta, 90° for yellow, 180° for green and 270° for blue colour.
The content of total anthocyanins of the fruit juice was determined by pH differential method previously described by Giusti et al. (2002). Results were expressed as mg cyanidin-3 glucoside/100 g fresh tissue.
Total phenolics were determined using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965). Results were expressed as mg gallic acid equivalents (GAE) per 100g of fresh weight of edible part of fruit.

RESULTS AND DISCUSSIONS

Our results showed that soil played an important role in quality of plum affecting plum yield and fruit chemical composition.
The soil pH was increased and ranged from 4.75 to 6.92. S1 and S2 have a low total nitrogen content (0.07-0.11%) but S3 had a moderate content of total nitrogen (0.228%). The contents of CaO, in the soil was lower in the case of S2 and higher in S1 and S3. However, the Mg content was lowest in S3 soil and highest in S1 soil. These values shows that the soil S1 and S2 has good cation exchangeable capacity.
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Table1. Soil chemical properties at different soil depth

Soil	Depth (cm)	Humus %	NO_3^- %	P_2O_5 ppm	K_2O ppm	Fe ppm	Cu ppm	Mn ppm	Zn ppm	Co ppm	Ca^{2+} mequiv./ 100g	Mg^{2+} mequiv. /100g	pH H_2O
Brown luvic typical (S1)	0-20	0.64	0.070	2	60	1	6	46	1	0	44.1	26.1	5.2
	20-50	1.51	0.119	4	123	5	4	79	1	0	45.4	13.2	4.9
Brown luvic pseudogleyic (S2)	0-20	1.96	0.110	90	149	10	5	189	2	0	37.6	16.7	4.75
	20-50	0.59	0.062	3	80	3	4	52	1	0	39.7	16.9	5.25
Erodisol iluvial clay anthropically covered (S3)	0-20	2.65	0.170	34	236	16	3	21	1	2	70.4	12.9	6.40
	20-50	4.52	0.228	2	200	21	4	17	2	0	76.33	8.6	6.92

Yield significantly differs between soil type and cultivars studied (Table 2). A comparison of brown luvic typical (S1) with erodisol iluvial clay anthropically covered(S3) and brown luvic pseudogleyic (S2) revealed marked differences between them both in yield and fruit weight. As for effect of type of soil on the plum production the data obtained put S3 on the first place followed at a hight difference by S1 for 'Stanley' cultivar studied which supported the higher economically efficiency of the S3 through bigger yield. However, in case of 'Vânăt românesc' cultivar was highlighted positive influence of S2 on yield.

We notice from the data presented in Table 2 that the fruits size of both cultivars is not influenced by soil type. However, we can observe that the fruits of 'Stanley' variety have a larger size compared to 'Vânăt românesc' with small size.

In case of 'Vânăt românesc' cultivar higher fruit weight is on brown luvic pseudogleyic (S2) and for another two types of soil the fruit weight is similar. Significantly higher fruit weight was found in 'Stanley' cultivar for all soil type, emphasize brown luvic typical (S1) and the erodisol iluvial clay anthropically covered (S3). These results are in accordance with previous studies carried out with 'Rainha Claudia Verde' plum cultivar, who found that fruits were bigger in the Haplic Luvisol soil compared to VerticLuvisol (Rato et al., 2008). Results of Matsoukaa et al. (2017) showed the blueberry fruit dry weight did not differ significantly by soil type, but was significantly influenced by the interaction across all soil types and treatments. As previously reported by Guerra and Casquero (2009) fruit weight of 'Green Gage' plum cultivar was positively influenced by soil type. Generally, the effect of soil on the fruit weight and yield varied function of cultivars and pedo-climatic factors.

Table 2. Influence of soil type on growth parameters and yield of plum

Cultivar/soil	Fruit weight (g)	Yield per tree (kg)	Yield per ha (t)	Length (L) mm	Width (W) mm	Thickness (T) mm
Vânăt românesc (S1)	18±0.28	9±2.05	2.3±1.09	37±0.68	27±2.05	25±1.43
Vânăt românesc (S2)	20±0.16	10±2.31	2.7±1.05	38±0.75	28±1.61	27±1.58
Vânăt românesc (S3)	18±0.25	8±2.41	2±1.06	38±0.80	28±1.48	26±1.45
Stanley (S1)	50±0.47	24±1.80	6.8±0.87	51±1.60	38±2.86	36±1.75
Stanley (S2)	48±0.47	16±1.55	4.7±1.43	53±1.88	37±2.51	35±1.84
Stanley (S3)	51±0.51	29.3±1.98	8.4±0.96	52±1.44	39±2.88	37±1.66

Values are expressed as means ± SD for n = 3

Soluble solids content, titratable acidity, pH, soluble solids/titratable acidity (SSC/TA) ratio of plum studied are shown in Table 3. There were no significant differences for titratable acidity among cultivars for all soil type. Can be seen from the obtained results that the soluble solids content is lower in fruit of Stanley cultivar growing in the brown luvic pseudogleyic soil (S2) but for 'Vânăt românesc' the lower content of SSC is in fruit from erodisol iluvial clay anthropically covered (S3) soil type. The effect of soil type on the soluble solids was obvious in 'Stanley' than 'Vânăt românesc' cultivar. However, results of Rato et al. (2008), regarding soluble solid content and SSC: TA ratio of plum fruit at harvest have shown that these parameters were not affected by soil, which means that would not be useful to distinguish fruit quality from different soil at harvest. Similar results were obtained by Usenik et al., (2008) which concluded that cultivars have a significant influence on soluble solids but not soil.

Table 3. Influence of soil type on the chemical composition of plum fruit

Cultivar/soil	Firmness kg/cm^2	Dry matter g%	Titratable acidity (TA) g malic acid/ 100 g fw	Soluble solids content (SSC) °Brix	SSC/TA ratio	Ascorbic acid mg/100 g fw
Vânăt românesc (S1)	1.10±0.33	18.50±0.26	1.2±0.02	16.8±0.98	14	3.8±0.40
Vânăt românesc (S2)	1.52±0.30	23.15±0.16	1.3±0.01	17±1.42	13.07	4.5±0.24
Vânăt românesc (S3)	1.05±0.36	17.40±0.18	1.2±0.02	16±0.55	13.3	4.2±0.26
Stanley(S1)	2.98±0.22	22.10±0.61	1.4±0.01	20±1.08	14.28	4.6±0.11
Stanley(S2)	1.63±0.19	20.14±0.52	1.3±0.01	17.5±1.45	13.46	4.1±0.31
Stanley(S3)	3.01±0.28	26.30±0.74	1.5±0.02	23±1.15	15.33	5±0.15

Values are expressed as means ± SD for n=3

Plums from 'Vânăt românesc' cultivated in S3 soil type showed dry matter content slightly lower than those from S1 and S2 soil type. However, S2 soil type influence positively dry matter content of 'Vânăt românesc' plum fruit. S3 and S1 soil type positively affected dry matter content of Stanley cultivar (Table 3). Effect of soil type on firmness in Vânăt românesc cultivar was not observed, but fruits firmness of 'Stanley' cultivar was influenced positively by S1 and S3 soil type. This results are in accordance with previous work carried out in plum by Rato et al. (2008) whose observed differences between sites in firmness of plum fruits. Also, our results are consisted with those of Royer et al. (2003) who reported that apples coming from orchards from loam and sandy-clay soil were crunchier and firmer (highest values of hardness) than apples from clay soil.

Regarding fruit anthocyanins content, data presented in Table 4 show that this compound was similar for all soil type in case of 'Stanley' cultivar but the content of this compound was higher for S1 an S3 soil type for 'Vânăt românesc' cultivar. From the obtained results we can observe that the phenols content is higher in the fruits of the 'Stanley' variety cultivated in S2 and S3 soils. Brown luvic typical (S1) soil only affected phenols content of 'Vânăt românesc' cultivar, the other two types of soil (S2 and S3) do not affect the amount of phenols in the fruits of this variety. But some authors, Diaz- Mula et al. (2008) have argued that this compounds it is dependent on the variety and not on the pedoclimatic conditions.

Lightness did not show any significant difference with regards soil type and cultivar. Chromatic parameter a* of 'Stanley' fruit have great value for S2 and S3 soil which means that the fruit has a pronounced degree of red, but 'Vânăt românesc' have an intense degree of red only for S1 soil type. Chromatic parameter b* of both cultivars in all three soil types have negative value which means that the fruit has a pronounced degree of violet. Kim et al. (2012) showed that sugar content, color and weight of grapevine cultivars were great influence by soils with hardened layers.

Table 4 Influence of soil type on the pigments content and color of plum fruits

Cultivar /soil	Total phenols mg GAE/100 g fw	Antocyanins mg/100 g fw	Color parametrs				
			L^*	a^*	b^*	C^*	h°
Vânăt românesc (S1)	125.3±1.85	57.4±0.44	24.27	2.32	-0.60	2.37	345.27
Vânăt românesc (S2)	111.3±1.65	48.9±0.95	25.04	1.34	-0.10	1.35	355.78
Vânăt românesc (S3)	115.2±0.19	50.3±0.98	25.67	1.47	-0.11	1.47	355.58
Stanley(S1)	150.2±1.75	68.4±0.21	25	1.93	-0.25	1.94	352.68
Stanley(S2)	185.6±0.79	71.5±0.11	25.2	2.69	-0.29	2.70	340.10
Stanley(S3)	180.1±0.55	70.35±0.13	25.99	2.61	-0.89	2.76	341.24

Values are expressed as means ± SD for n = 3

CONCLUSIONS

The resulted revealed that the brown luvic pseudogleyic soil (S2) had better performance for cultivated of 'Vânăt românesc' cultivar than S1 soil in terms of the accumulation of dry matter, soluble solids and ascorbic acid.

Erodisol iluvial clay anthropically covered soil type had especially positively influence on yield and quality potential of 'Stanley' cultivar.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF THE EFFECT OF BREVIS PRODUCT ON THE CHEMICAL THINNING OF FRUITS IN THE PINK LADY APPLE PLANTATION

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Abstract

The study subject of the experience was Pink Lady apple variety, grafted on M26. The trees were trained as slender spindle system. The distance of plantation is 4.0 x 1.5 m. The aim was to determine the efficacy of different agents on the chemical thinning of apple fruits. To investigate thinning effect of apple fruits proposed to study the following variants: 1. Control – without treatment; 2. Geramid New, 1.3 l/ha; 3. Brevis, 1.1 kg/ha; 4. Brevis, 1.65 kg/ha; 5. Brevis, 2.2 kg/ha. The treatment with the product Geramid New was carried out by spraying, at the fall of 80% of the petals, + 2-3 days, and with Brevis et development stage 10-12 mm fruitlet diameter. During the research, it was studied the number of blossom clusters after thinning, mean fruit weight, yield, average fruit diameter and size classes based on their diameter. It was established that, the good effect of thinning was noticed after application of Brevis, 1.65 kg/ha.

Key words: *apple, fruit production, growth regulator, quality, thinning.*

INTRODUCTION

During the productive period apple varieties frequently produce an excessive number of fruitlets but this can be insufficient to guarantee fruit of good marketable size at harvest (Babuc et al., 2013; Balan & Vămăşescu, 2011; Cimpoieş, 2012; Peşteanu, 2013). Furthermore, an unwanted excessive number of fruit per tree during that period can reduce the growth and photosynthetic trees activity (Babuc, 2012; Peşteanu, 2013).

Because of this, trees require adequate thinning to reach marketable fruit sizes and regular yields by preventing alternate bearing (Maas & Meland, 2016; Peşteanu & Calestru, 2020).

The apple varieties have a high ability to setting a lot of fruit organs and can produce an excessive number of fruits (Brunner, 2016; Peşteanu, 2015).

In order to obtain high, constant and qualitative productions, it is intervened by crop loading in the apple plantation (Babuc et al., 2013; Vămăşescu, 2012). In mature orchards chemical fruit thinning is a major cultural practice (Bound, 2006; Dorigoni & Lezzer, 2007; Greene, 2014).

Plant growth regulators such as auxins have become essential in commercial apple production (Brunner, 2014; Peşteanu & Calestru, 2020; Stern, 2015).

One of the new chemical thinners is the triazinone herbicide metamitron, which at a low dosage reduces photosynthesis by blocking electron transport in photosystem and consequently enhances fruit drop (Basak, 2011; Botton et al., 2020; Dorigoni & Lezzer, 2007; Lafer, 2010; Stern, 2014).

Its application to apple trees can result in transient carbohydrate stress that may increase the sensitivity of the fruit to a chemical thinner application (McArtney et al., 2012).

The normal time for application of thinning chemicals in apples extends from bloom until 3 weeks after bloom, when the fruit reach a mean diameter of ≈16 mm (McArtney & Obermiller, 2012). During that period single metamitron treatments, regardless of application time, when the fruit diameter reached 6-9 mm or 12-15 mm, showed inconsistent action in the different experimental years.

In other thinning activity metamitron demonstrate positive results when applied to apple fruitlets 10 to 12 mm in diameter (Lafer,

2010) or even later at 20 mm (Brunner, 2014; Gabardo et al., 2017; McArtney et al., 2012).

Research conducted in Serbia (Radivojevic et al., 2020) has shown that a successful thinning effect was achieved in single metamitron treatments during the normal application time (5-15 mm in fruit diameter) at concentrations of 250 mg L^{-1} ('Gala') and 300 mg L^{-1} ('Golden Delicious') only under favorable weather conditions (minimum night temperature 10^0C, maximum daily temperature >25^0C).

If a single application is made, just a few hours after treating the leaves, the product reduces their capacity for photosynthesis by up to 50% depending on the dose administered. The wider inhibition of the photosynthesis process takes place gradually and maximum values are recorded from the fourth day after treatment, but the trees recover their complete photosynthetic activity at 25-45 days after the first application. (Gonzalez et al., 2015; Stern, 2015).

The objective of the present study was to evaluate the thinning efficacy of metamitron applied in single application in 'Pink Lady' apple plantation.

MATERIALS AND METHODS

The experiment was conducted on 'Pink Lady' apple variety, trees in the commercial plantation "Terra Vitis" Ltd, Burlacu village, Cahul district. The trees grafted on a M26 rootstock, were planted in 2013, in single rows at a distance of 4.0 × 1.5 m (1666 trees per hectare).

The trees were trained as a slender spindle system. The experiment was conducted during the period of 2018 year.

In accordance with the endowment growth regulators intended for the chemical thinning of the fruits, the following scheme of experiments was elaborated: 1. Without thinning (control); 2. Geramid New, 1.3 l/ha; 3. Brevis, 1.1 kg/ha; 4. Brevis, 1.65 kg/ha; 5. Brevis, 2.2 kg/ha.

The following commercial products containing a different active ingredient were applied "Geramid New" (L. Gobbi S.R.L., Italy) containing 44.8 g L^{-1} alpha-naphthylacetamide acid (NAD) as the active ingredient and "Brevis" (Adama Agan Ltd., Israel) containing 150 g kg^{-1} metamitron as the active ingredient.

In the first variant, no intervention was performed on the reproductive organs in the crown of the trees.

In the second variant, according to the scheme of experience, a single treatment was performed (30.04.2018) with the growth regulator Geramid New at a dose of 1.3 l/ha, when 80% of the petals fell plus 2-3 days, et development stage 4-5 mm fruitlet diameter.

In third, fourth and fifth variants, according to the experiment scheme, a single treatment were performed (06.05.2018) with the Brevis product, in dose 1.1; 1.65 and 2.2 kg/ha, respectively, et development stage 10.4 mm fruitlet diameter.

The amount of solution administered to a tree was 0.6 liters, based on the number of trees per unit area and the recommended amount of water of 1000 l/ha.

The experimental design was randomised block with four replicates, whereby every replication consisted of eight trees.

The research was performed in the field and laboratory conditions according to the accepted method of carrying out experiments on fruit crops with growth regulators.

During the research the following indices were studied: the number of blossom clusters after thinning and the share of fruits in the inflorescence.

The apples were harvested in beginning of November in a single pass-through and afterwards, based on their diameter, they were graded into six classes by hand calibrator at intervals of 5 mm (from 50 mm to >80 mm). Total yield per tree was calculated as a total of the mass of all classes. Average fruit weight (g) was obtained from a ratio of total yield and total number of harvested fruit.

The statistical processing of the main data was determined by the dispersion analysis method.

RESULTS AND DISCUSSIONS

The results presented in Table 1 show that the Geramid New growth regulator did not influence the number of inflorescences related to the 'Pink Lady' variety. In the case of treatment with the growth regulator Geramid New at a dose of 1.3 l/ha, the number of inflorescences related to the 'Pink Lady' trees

was 181 pcs/tree, ie a decrease of 1.7% less compared to the control variant.
In the case of variants where the treatment was performed with the Brevis growth regulator, these indicators registered essential deviations in comparison with the Geramid New variant in the 1.3 l/ha dose and the control variant.
Treatment with Brevis at a dose of 1.1 kg/ha decreased the index in the study by 44.9% compared to the control variant. Increasing the dose to 1.65 and 2.2 kg/ha of the studied product, respectively, decreased the number of tied inflorescences, constituting 101 and 41 pcs/tree, respectively.
In the control variant, where the thinning of the fruit organs was not performed at 29.9% of the inflorescences, one fruit was registered, at 26.6% three fruits, at 29.4% two fruits, 28.8% each three fruits, at 10.9% four fruits each and only within 1.0% of the inflorescences were five fruits each.

Table 1. Number of setting inflorescences (NSI) in the crown of 'Pink Lady' apple trees and the share of fruits in an inflorescence according to the growth regulators used to thin the fruit organs

Variants	NSI, pcs/tree	The share of fruits in an inflorescence, %				
		1 pcs	2 pcs	3 pcs	4 pcs	5 pcs
Without thinning, (control)	184	29.9	29.4	28.8	10.9	1.0
Geramid New, 1.3 l/ha	181	30.4	38.2	18.8	12.7	-
Brevis, 1.1 kg/ha	127	55.9	25.2	11.1	7.1	0.7
Brevis, 1.65 kg/ha	101	52.5	22.8	18.9	5.8	-
Brevis, 2.2 kg/ha	41	65.9	19.6	14.5	-	-

In the variant with the use of the growth regulator Geramid New in the dose of 1.3 l/ha, the index in the study did not increase the share of inflorescences with a smaller number of fruits, constituting 31.4%, 38.2%, 18.8% and respectively 12.7%.
Among the variants exposed to chemical thinning, an increase in the share with a higher number of fruits in one inflorescence was recorded when treating the trees with Brevis product at a dose of 2.2 kg/ha, where 65.8% of the inflorescences formed a fruit, at 19.6% two fruits each, and at 14.5% three fruits each.
In the case of treating the trees with the Brevis product in the dose of 1.1 and 1.65 kg/ha, large deviations regarding the location of the fruits in an inflorescence were not obvious. A more rational location of the fruits in an inflorescence of the 'Pink Lady' trees was registered in the version treated with Brevis at a dose of 1.65 kg/ha, where 52.5% of the fruits are one in the inflorescence, at 22.8% two fruits each, and at 18.9 and 5.8% three and four fruits, respectively.
The study performed on the number of fruits reported per 100 inflorescences (Figure 1), showed higher values in the control variant, without thinning, where the index in question constituted 227 pcs.
In the variants treated with the growth regulator Geramid New and Brevis, the mentioned index varied from 151 to 218 pieces per 100 inflorescences. In the variant treated with the Geramid New growth regulator at a dose of 1.3 l/ha, the studied index registered practically identical values with the control variant, where it constituted 218 pieces per 100 inflorescences.

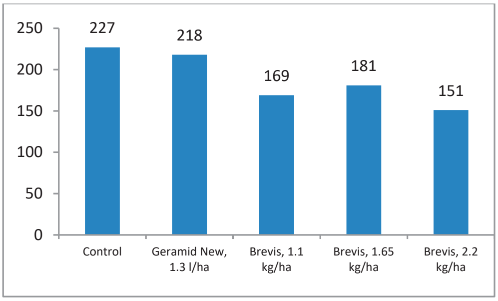

Figure 1. The influence of the growth regulators and the treatment dose on the number of fruits per 100 inflorescences, pcs

When thinned with Brevis 1.1 in the dose; 1.65 and 2.2 kg/ha, the number of fruits per 100 inflorescences was lower compared to the previous variants and amounted to 169; 181 and 151 pieces, respectively.
The number of fruits in the crown of the trees of the 'Pink Lady' variety is in turn influenced by the method of thinning and the dose administered during treatment.
The investigations carried out show that a higher number of fruits (Table 2) in the trees of the 'Pink Lady' variety were registered in the variant where the thinning was not performed - 411 pcs/tree. Next, an insignificant decrease in the number of fruits was observed in the variant

treated with the growth regulator Geramid New in the dose of 1.3 l/ha - 395 pcs/tree.

Treatment with Brevis at a dose of 1.1 kg/ha decreased the index in the study by 91.1% compared to the control variant. Increasing the dose to 1.65 and 2.2 kg/ha of the studied product, decreased the number of fruits formed in the trees, amounting to 183 and 62 pcs/tree, respectively.

If, in the control variant, without thinning, the average weight of a fruit in the 'Pink Lady' variety was 111 g, then in the variants with chemical thinning it ranged from 115 to 165 g. Lower weight of the fruits were recorded when treating the trees with Geramid New at a dose of 1.3 l/ha - 115 g, and the highest average fruit weight was achieved by treatments with Brevis at a dose of 2.2 kg/ha -165 g.

Table 2. The influence of the growth regulators on the fruits production in the crown of the apple trees of the 'Pink Lady' variety

Variants	The number of fruits, pcs/tree	Average weight, g	The fruits production	
			kg/tree	t/ha
Without thinning, (control)	411	111	45.62	76.00
Geramid New, 1.3 l/ha	395	115	45.42	75.67
Brevis, 1.1 kg/ha	215	131	28.16	46.91
Brevis, 1.65 kg/ha	183	138	25.25	42.07
Brevis, 2.2 kg/ha	62	165	10.23	17.04
LDS 5%	18.7	6.1	2.4	3.2

As the treatment dose decreases, the average weight of the fruits decreased too being in the variant treated with Brevis in the dose 1.1 kg/ha - 131 g, and in the variant Brevis in the dose 1.65 kg/ha - 138 g.

Higher fruit productions per tree and per unit area were registered in the control variant, without thinning where the mentioned indices constituted respectively 45.62 kg/tree and 76.00 t/ha. Similar values with the control variant were registered in the variant treated with Geramid New in the dose of 1.3 l/ha, where the fruit production registered, 45.42 kg/tree and, respectively, 75.67 t/ha. On the side of the respective variant, it can be observed that the treatment with NAD-based products of the trees of the Pink Lady variety did not influence as a growth regulator for crop loading.

In the case of the variant thinned with Brevis at a dose of 1.1 kg/ha, the productivity of a tree was 28.16 kg, and at one area unit 46.91 t/ha was obtained, or it was 61.8% compared to the control variant. Variants treated with the Brevis product in doses of 1.65 and 2.2 kg/ha, the fruit production constituted 46.91 and, respectively 42.07 t/ha.

In the study carried out on how the growth regulator influences the treatment dose on the number of Pink Lady fruits in one kilogram of apples, we register a more rational redistribution in the variants treated with the Brevis product, compared to the control variant, without treatment and the variant treated with Geramid New at a dose of 1.3 l/ha (Figure 2).

If, in the control variant and the one treated with the Geramid New product in the dose of 1.3 l/ha, we register a higher number of fruits within one kilogram of apples (9.01 and 8.7 pieces, respectively), then in the variants treated with Brevis, a decrease in the index under study was obtained.

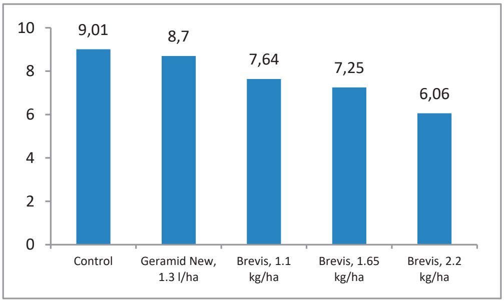

Figure 2. The influence of the growth regulators and the treatment dose on the number of fruits per one kg, pcs

If, in the variant treated with the Brevis growth regulator in the dose of 1.1 kg/ha, we register a value of 7.64 fruits/kg, then with the increase the dose to 1.65 and 2.2 kg/ha the number of fruits of the variety 'Pink Lady' within one kilogram of apples decreased, and amounted to 7.25 and 6.06 fruits/kg, respectively (Figure 3).

Figure 3. The influence of the growth regulators and the treatment dose on the quality of fruits from 'Pink Lady' variety

The study performed as it influences the growth regulator on the fruits quality of the 'Pink Lady' trees, we register a more rational redistribution in the variants treated with the Brevis product, compared to the control variant, without treatment and the variant treated with Geramid New in the dose 1.3 l/ha (Table 3).

Among the trees of the 'Pink Lady' variety, lower quality productions were obtained in the control variant, where the share of fruits with a diameter of less than 55 mm is 0.9%, with a diameter of 55-60 mm - 5.4%, with a diameter of 60-65 mm - 62.1%, with a diameter of 65-70 mm - 24.9% and only 6.7% have a diameter greater than 70 mm.

Table 3. The influence of the growth regulators on the redistribution of fruits according to their diameter in the apple trees of the 'Pink Lady' variety

Variants	The share of fruits (%) according to their diameter (mm)					
	50-55	55-60	60-65	65-70	70-75	75-80
Without thinning, (control)	0.9	5.4	62.1	24.9	6.7	-
Geramid New, 1.3 l/ha	0.3	4.6	55.0	36.1	4.0	-
Brevis, 1,1 kg/ha	-	2.6	27.9	23.3	32.9	13.3
Brevis, 1,65 kg/ha	-	4.0	9.9	26.0	41.3	18.8
Brevis, 2,2 kg/ha	-	0.7	3.6	13.5	37.9	44.3

In the variant treated with the product Geramid New, 1.3 l/ha, we register a redistribution of the fruits on the studied diameters as in the control variant with small deviations within the fruits with a diameter of 60-65 mm (55.0%) and diameter of 65-70 mm (36.1%). Thus, in the control variant, the share of fruits with a diameter greater than 65 mm was 31.6%, and in the Geramid New version, 1.3 l/ha - 40.1%. This proves once again that the NAD product cannot be recommended for the standardization of the fruits load in the 'Pink Lady' variety.

The use of the Brevis growth regulator in the regulation of the fruits load positively influenced the quality of the obtained fruits. If, for example, in the variant treated with the Brevis growth regulator at a dose of 1.1 kg/ha, we register a higher weight for fruits with a diameter of 60-70 mm, increasing the treatment dose of Brevis to 1.65 and 2.2 kg/ha, decreased the share of fruits with a diameter of 60-70 mm, but increased the index in question for fruits with a diameter of 70-80 mm (Figure 4).

Figure 4. The influence of treatment dose of Brevis product on the quality of fruits from 'Pink Lady' variety

More convincing results regarding the production ratio and the diameter of the fruits were registered during the researches at the 'Pink Lady' variety in the variant of using the Brevis growth regulator at a dose of 1.65 kg/ha. The average diameter of the fruits is an indicator that has a direct tangent with the average weight of the obtained production.

Lower values of the average diameter of the fruits in the trees of the 'Pink Lady' variety were registered in the control variant - 64.4 mm, where the trees were without thinning, and higher values were registered in the variant treated with the Brevis product in the dose 2.2 kg/ha - 73.3 mm. The variant treated with the Geramid New growth regulator at a dose of 1.3 l/ha registered practically identical values with the control variant, where it constituted 64.6 mm (Figure 5).

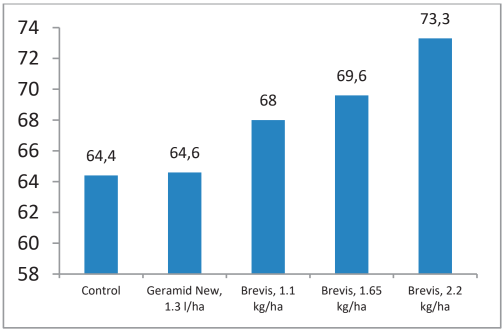

Figure 5. The influence of the growth regulators on the diameter of the 'Pink Lady' apple variety fruits, mm

In the variants treated with the Brevis product in the dose of 1.1 and 1.65 kg/ha, the average diameter of a fruit registered average values of the index under study, which constituted 68.0 and 69.6 mm, respectively.

CONCLUSIONS

The number of fruits, their average weight and the recorded production differ depending on the active ingredient taken in the study of crop loading and the dose of the product used to thin the fruit.

The active ingredient taken in the study to normalize the crop loading influenced the weight of fruits of different diameters.

In commercial industrial plantations for crop loading of apple fruits, the treatment of 'Pink Lady' trees to be carried out with the Brevis growth regulator at a dose of 1.65 kg/ha, et 10-12 mm fruitlet diameter.

The sky should be clear, the air temperature in the atmosphere should vary from +10 to 25^{0}C and without atmospheric precipitation during 2-3 days after treatment.

The 'Pink Lady' variety is not prone to fruit load regulation with the Geramid New growth regulator.

ACKNOWLEDGEMENTS

This study was supported by the National Agency for Research and Development of the Republic of Moldova, project 18.817.05.29A "Improvement of maintenance technologies for super-intensive sweet cherry and apple orchards, development of techniques of increasing fruit quality in Europe".

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFECTION OF *ERWINIA AMYLOVORA* ON DIFFERENT APPLE VARIETIES AND THE IMPACT ON FRUITS QUALITY

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Abstract

Fire Blight (FB) caused by Erwinia amylovora is the most feared bacterial disease in the genus Malus and other genera belonging to Rosaceae family, causing severe losses in orchards in favourable years, unmarketable fruits quality and even completely trees dieback, especially in highly susceptible genotypes. Currently E. amylovora is of quarantine concern in many countries all over the world and continue to conquer new territories. During 2018-year, six apple genotypes ('Golden Delicious', 'Idared', 'Gala', 'Jonagold', 'Fuji' and 'Jonathan'), within an orchard located in the proximity of Craiova city, Romania, have been assessed for their response to the infection to Erwinia amylovora and the impact of the pathogen attack on fruits yield and quality. The response of apple genotypes to the Fire Blight (FB) attack ranged from moderately resistant/susceptible ('Golden Delicious' and 'Fuji') to highly susceptible ('Idared', 'Gala', 'Jonagold' and 'Jonathan') depending on genetic background and environmental conditions. Fruits chemical parameters Total Soluble Solids (TSS), Total acidity (TA), Total Sugar (TS) were significantly affected by the pathogen attack in all apple varieties.

Key words: *apple, AUDPC, Erwinia amylovora, Fire Blight, fruits quality.*

INTRODUCTION

Originated in Central Asia, apple trees (*Rosaceae* family, genus *Malus*, species *Malus domestica* Borkh.) have been grown for thousands of years in Europe and Asia and are the most widely grown species in the genus *Malus* worldwide. Nowadays, aside other spontaneous and cultivated plants and trees in temperate regions, apples are in the first place among fruits in Eastern Europe, where they meet favourable climatic conditions for thousands of varieties, resulting in a range of colours, tastes, textures and chemical properties (Wilton, 2001; Blažek and Hlušičková, 2003; Sansavini et al., 2004; Rosculete and Rosculete, 2017; Bushal et al., 2018; Viškelis et al., 2019; Łysiak et al., 2020; Prundeanu et al., 2020; Răduțoiu, 2019; Răduțoiu, 2020; Răduțoiu and Cătuțoiu, 2020; Răduțoiu and Cosmulescu, 2020).

Considered to be a major functional food resource, apples are one of the most desirable fruits, being rich in vitamins, fibre, antioxidants, phytochemicals associated with human health benefits (Wolfe et al., 2003; Charde et al., 2011; Hyson, 2011; Ahmad et al., 2020; Bondonno et al., 2020; Li et al., 2020).

Rapid progress in breeding, in biotechnology in functional food and nutraceuticals has provided insight into disease resistance, genetic basis of food quality, improving favourable traits in various crops and products (Gardiner et al., 2007; Marić et al., 2010; Gardiner et al., 2012; Lusser et al., 2012; Broggini et al., 2014; Celton et al., 2014; Bonciu, 2020).

In 2019 world apple production has been reported as 87.236.221 tons (FAO 2019). Factors that contribute to increased apple demand include new varieties, population growth, products that meet healthy lifestyle, food diversification and rising incomes.

Despite their health benefits and costumer's preference for fresh fruits, the most important factor that limits apple cultivation worldwide is the necrogenic and highly infectious Gram-negative bacterium *Erwinia amylovora*, which develops the disease known as Fire Blight (FB) (Winslow et al., 1920).
The pathogen is considered quarantine pest on the "black" list of European and Mediterranean Plant Protection Organization (EPPO, http://www.eppo.org/QUARANTINE/quarantine.htm). Currently phytosanitary control and early eradication of any Fire Blight are the best measures to delay disease spread and avoid losses (Braun-Kiewnick et al., 2011).
Since its discovery in the USA in the late 1700s, despite the control measures adopted, Fire Blight has spread in more than 50 countries from America, Australia, Europe, Middle East, Africa and Asia recently (Denning, 1794; Jock et al., 2000; Bonn and Van der Zwet, 2000; Jock et al., 2013; Gagnidze et al., 2018; Zhao et al., 2019), affecting pear, apple, quince and other rosaceous plants. In Romania, Fire Blight symptoms were first observed in 1992 in the south region of the country, affecting apple, pear, quince and ornamental shrubs such as *Cotoneaster*, *Crataegus* and *Pyracantha* (Severin et al., 1999; Amzăr and Ivănescu, 2003).
Fire Blight affects blossoms, leaves, shoots, branches, causing serious fruits losses and even whole tree dieback, especially on sensitive genotypes (Kuflik et al.,2008; Braun-Kiewnick et al., 2011; Gaganidze et al., 2018). When fruits are not lost due to the pathogen attack, they have reduced size and quality (Đorđević et al., 2019; Emeriewen et al., 2019; Paraschivu et al., 2020).
The bacterium can be spread easily by vectors (wind, rain, insects, birds), but also by contaminated pruning tools and infected plant material.
Despite management, phytosanitary control, biological, chemical and cultural methods, the use of resistant genotypes remains one of the most efficient method to limit losses in fruit trees and other cultivated crops (Peil et al., 2009; Matei, 2011; Matei and Rosculete, 2011; Partal et al., 2013; Popa et al., 2013; Paraschivu et al., 2014; Partal et al.,2014; Calis et al., 2017; Hashman et al., 2017; Kellerhals et al., 2017; Marin et al., 2018; Cotuna et al., 2020a; Cotuna et al., 2020b; Partal and Paraschivu, 2020; Pompili et al., 2020; Tegtmeier et al., 2020).
The present study aimed the response of six apple varieties to the attack of the bacterium *Erwinia amylovora* under natural infection in terms of the relationship between weather conditions, varieties susceptibility to Fire Blight and the bacterium impact on apple fruits yield and quality.

MATERIALS AND METHODS

The focus of the experiment was to evaluate the response of ninety-six fruit trees including six apple genotypes (cv. 'Golden Delicious', 'Idared', 'Gala', 'Jonagold', 'Fuji' and 'Jonathan'') to the attack of the bacteria *Erwinia amylovora* assessed in natural conditions of infection.
The experiment was conducted during 2018 year to individual trees in a randomized compete block design in four replicate blocks (24 apple trees/block) within a private apple orchard established in 2008 year (3.5 m between rows x 3.5 m between trees on row) in the proximity of Craiova city, Dolj county, Romania. There was calculated the cumulative number of Fire Blight infections per each assessed apple tree.
For the pathogen isolation and identification have been taken samples of diseased young shoots, flower clusters, leaves and fruits with visible symptoms of Fire Blight (necrosis, wilting, bacterial ooze), taken after symptoms were visible for each assessed apple tree from all genotypes.
Isolation of the pathogen was made from fresh samples (symptomatic shoots, flowers, leaves, fruits) according to the EPPO protocol (EPPO, 2013). Detection of the bacterium was done using PCR assays and MALDI-TOF mass spectroscopy protocols (Sauer et al., 2008; Wensing et al., 2012). For all assessed apple trees were determined Frequency (F%) and Intensity (I%) of Fire Blight attack. These parameters were used to calculate Attack Degree (AD%) using the formula: AD% = (F% x I%)/100 (Cociu and Oprea, 1989). Then the AD% was used to assess disease severity at

each measurement and three consecutive AD values were used to calculate Area under Disease Progress Curve (AUDPC), which shows the evolution and disease quantity on each apple tree included in the trail, following the formula (Campbell and Madden, 1990):

$$\text{AUDPC} = \sum_{i=1}^{a}\left[\left\{\frac{Yi + Y(i+1)}{2}\right\} x(t(i+1) - ti)\right]$$

where, Yi = disease severity (AD%) at each measurement; ti = time in days of each measurement; n = number of Fire Blight (FB) assessments.

To estimate the response of apple genotypes to Fire Blight attack was used the scale 1 (no attack) to 9 (tree dead), corresponding to AD% classes.

The fruits yield for each assessed apple tree was calculated using the formula: number of fruits/tree x average weight of the fruit.

The juice from apple fruits with no visible symptoms (but taken from apple trees affected by Fire Blight) was extracted at ambient temperature (29°C ± 1°C) using a domestic juice extractor and was filtered. Total Soluble Solids (TSS) of filtered juice was done using a digital refractometer (WYT-J 0–32% Chong Qing, China) and reported as degrees Brix, which is equivalent in percentage (Wei and Wang, 2013; Dongare et al., 2014). Total soluble solids (TSS) values obtained from the digital refractometer have been adjusted using the factor 0.85 which means that sugars (TS) are 85% of TSS. Total sugars (TS) content was determined by Luff-Schoorl method and presented in percent (%).

Titratable Acidity (TA) was determined in a water extract of a weight amount of homogenized apple tissue using standard titration method, titrating to pH 8.1 with 0.1 NaOH (AOAC, 2019). The results of Titratable Acidity (TA) were expressed as g/100 g apple tissue calculated as malic acid at harvest. All analysis was carried out three times and the results are mean values. The experimental data were calculated and analysed, using MS Office Excel 2010 facilities.

RESULTS AND DISCUSSIONS

Since its discovery Fire Blight has been considered the most destructive disease of apple and pear fruit trees, being included recently in the top 10 plant pathogenic bacteria worldwide (Akhlaghi et al., 2021).

In Romania the disease has been spread in all regions of the country (mostly in the south and south east) with variable intensity.

Marin et al. (2018) showed that in the south of Romania the years 2016-2018 were very favourable for the Fire Blight attack on apples.

The inspections in the evaluated apple orchard have been performed periodically during the growing season in order to identify typical symptoms of fire blight, assuming an infection occurred.

Scouting of the disease has started for each apple genotype during blooming and continue in other three moments on leaves, shoots and fruits. Necrotic symptoms of Fire Blight have been observed on all apple genotypes assessed (Figure 1).

Figure 1. Fire Blight attack symptoms on apple (shoot blight symptoms and affected fruits found on diseased branches) (original photos Cotuna O. and Paraschivu M., 2018)

For scouting optimization and to predict the disease development, rainfalls and temperatures were taken into account.

Thus, climatic conditions of 2018 year favoured the infection with *Erwinia amylovora* and further Fire Blight development.

Humidity was determined by the amount of rain of 908.5 mm, comparatively with multiannual average rainfall of 585.4 mm, while the average temperature was 12.6°C comparatively with multiannual average temperature of 10.8°C (Figure 2).

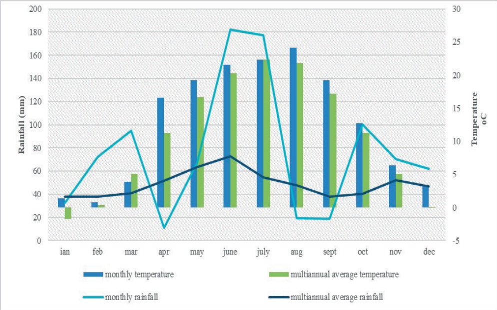

Figure 2. Weather conditions during the study period (2018 year)

These high humidity and warm temperature make young apple fruitlets to became water soaked and dull, covered with small droplets of bacterial ooze rich in polysaccharide, which creates a matrix that protects the pathogen on plant surfaces and attracts insects that disseminate the pathogen (Figure 3).

Figure 3. Apple fruitlets with creamy amber small droplets of bacterial ooze under humid conditions (original photos Cotuna O. and Paraschivu M., 2018)

The severity of the disease was noticed by Attack Degree (AD%) which was calculated for each scouting and introduced in the formula of AUDPC.

All apple genotypes correspond to different classes for their response to Fire Blight attack.

The response of tested apple genotypes to the fire blight attack ranged on a large scale of variability depending on the genotype resistance or sensitivity to disease and environmental conditions. Thus, appreciation scale indicates class 3 (‘Golden Delicious’), class 4 (‘Fuji’), class 5 (‘Jonagold’) class 6 (‘Idared’, ‘Gala’ and ‘Jonathan’) (Table 1).

Table 1. Attack appreciation scale to Fire Blight (*Erwinia amylovora*) (Sestras et al., 2008)

Class	Attack appreciation	Attack degree (AD%)
1	No attack	0
2	Very low attack	0.1-5.0
3	Low attack	5.1-10.0
4	Medium attack	10.1-20.0
5	Supra medium attack	20.1-40.0
6	Strong attack	40.1-60.0
7	Very strong attack	60.1-80.0
8	Extreme strong attack	80.1-99.9
9	Complete scorching (trees dead)	100

The susceptibility to Fire Blight of the six apple genotypes included in the study confirm previous research which emphasized that some cultivars may appear highly or moderately susceptible in one year and moderately resistant in the other (Lee et al., 2010; Kostick et al., 2019; Pompili et al., 2020).

The most susceptible genotypes to Fire Blight (FB) were ‘Jonathan’, ‘Idared’ and ‘’Gala’ which also proved the lowest yielding capacity under Fire Blight impact (Table 2).

The best response to Fire Blight was recorded by ‘Golden Delicious’ and ‘Fuji’.

Table 2. Classification of apple genotypes according with their resistance/susceptibility to Fire Blight (FB) attack and the impact on fruits yield (t/ha)

Apple Genotype	2018		
	Class*	AUDPC	Yield (t/ha)
Golden Delicious	3 MS/MR	176	22.4
Fuji	4 MS/MS	158	21.6
Jonagold	5 MS/MS	264	20.1
Idared	6 HS/MS	361	15.8
Gala	6 MS/HS	327	19.2
Jonathan	6 HS/HS	438	16.1

* MS = moderate susceptible; MR = moderate resistant; HS = high susceptible.

The value of determination coefficient (R^2 = 0.8757), for all apple genotypes assessed, indicated that up to 87% of variation in apple yield could be explained by AUDPC variability.

It was noticed a significant correlation between AUDPC values and fruits yield (r = -0.9358*). (Figure 4).

Yield loses due to Fire Blight in apples were also reported previously by different authors (Longstroth, 2001; Norelli et al., 2003; Zwet et al., 2012)

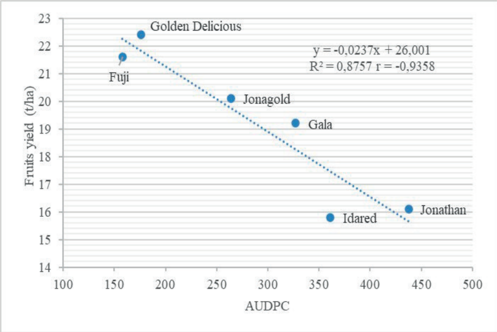

Figure 4. Relationship between Fire Blight AUDPC and apple fruits yield in 2018 year

The AUDPC had significant impact on the variation in Total Soluble Solids (TTS), Total Sugars (TS) and Titratable Acidity of apple fruits provided from the trees affected by Fire Blight. Thus, up to 82% of variation in TTS could be explained by AUDPC variability ($R^2 = 0.8209$), being recorded also significant correlation between TTS and AUDPC ($r = -0.9060$*) (Figure 5).

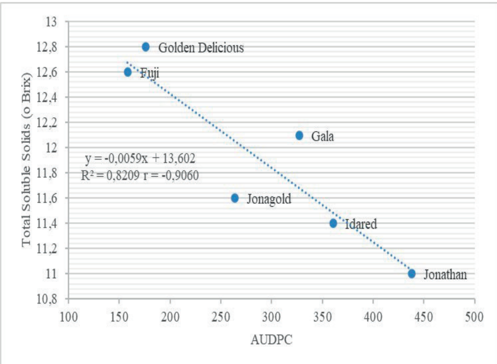

Figure 5. Relationship between Fire Blight AUDPC and Total Soluble Solids (TTS) in apple fruits in 2018 year

It was found that up to 78% of variation in Total Sugars (TS) content ($R^2 = 0.7826$) could be explained by AUDPC variability, being reported also significant correlation between TS and AUDPC ($r = -0.8847$*) (Figure 6).
Together with sugars the acidity has a profound effect on the perception of apple fruits quality and therefore a proper balance between these two quality parameters makes a desirable apple variety.

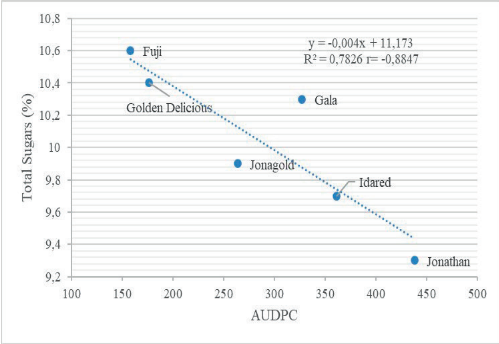

Figure 6. Relationship between Fire Blight AUDPC and Total Sugars (TS) in apple fruits in 2018 year

In apples the predominant acid is malic acid, representing 80-90% of total organic acids and contributes to the sourness of the fruits (Wu et al., 2007; Zhang et al., 2010).
The results from the experiment showed that the Fire Blight had a significant impact on Titratable Acidity (TA) in apples leading to increased values for all genotypes. Approximatively 71% of variation in Titratable Acidity ($R^2 = 0.7104$) could be explained by AUDPC variability and it was noticed also significant correlation between TS and AUDPC ($r = 0.8428$*) (Figure 7).

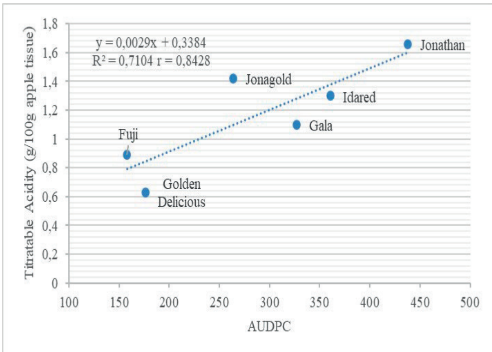

Figure 7. Relationship between Fire Blight AUDPC and Titratable Acidity (TA) in apple fruits in 2018 year

The apple fruits provided from the apple trees with the highest AUDPC values showed higher levels of acidity and lower levels of sugars, being noticed a significant correlation between these two quality parameters ($r = -0.8913$*) (Figure 8).

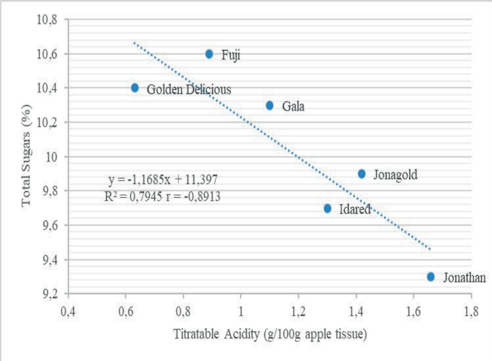

Figure 8. Relationship between Titratable Acidity (TA) and Total Sugars (TS) in apple fruits from the genotypes affected by Fire Blight in 2018 year

However, the results of the experiment show that Fire Blight is a serious concern for yield and fruits quality and even for trees live itself, while effective management of Fire Blight is complex and engage large resources. Therefore, Fire Blight management requires a combination of sanitation, cultural practices chemical or biological control and genetic resistance to keep the disease in check.

CONCLUSIONS

The present study was carried out to assess the response of six apple genotypes to the attack of Fire Blight (*Erwinia amylovora*) in natural infections and to evaluate the impact of the pathogen attack on yield and apple fruits quality during 2018 year. The response of apple genotypes to the Fire Blight (FB) attack ranged from moderately resistant/susceptible ('Golden Delicious' and 'Fuji') to highly susceptible ('Idared', 'Gala', 'Jonagold' and 'Jonathan') depending on genetic background and environmental conditions.

The values of Area under Disease Progress Curve (AUDPC) ranged from 176 to 438. The best behaviour to Fire Blight was recorded by 'Golden Delicious' and 'Fuji', while the lowest behaviour was recorded by 'Jonathan'. Fruits chemical parameters Total Soluble Solids (TSS), Total acidity (TA), Total Sugar (TS) were significantly affected by the pathogen attack in all apple varieties. The apple genotypes that recorded the highest AUDPC values had the highest acidity values and the lowest sugars content.

The monitoring of Fire Blight and severe quarantine measures may lead to the Fire Blight control especially in private gardens and wild *Malus* and *Pyrus* sp., which will optimize yields and fruits quality beside breeding programs.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

OBSERVATIONS ON THE STRUCTURE, DYNAMICS AND ABUNDANCE OF EXISTING ARTHROPODS IN WALNUT ORCHARDS

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Abstract

The research was carried out within SCDP Iaşi, in two lots with a different crop technology. The observations concerned the entire entomofauna of arthropods from a walnut orchard where a conventional cultivation technology was applied and in a walnut orchard where ecological methods of protection against diseases and pests were applied. The research was carried out during 2018-2019, when the method of soil traps Barber type was used, while the flight curve of the pest Cydia pomonella was followed with help of synthetic pheromones traps, AtraPom type. The observations were made throughout the vegetation period, from May to September, having as research material 4 walnut genotypes, in the seventh year after planting, being grafted on Juglans regia. In the case of the conventional variant, phytosanitary treatments with fungicides and insecticides were performed, and in the ecological walnut variant, no phytosanitary treatments were performed, pest control being performed using biotechnical means, using only traps with synthetic sex pheromones AtraPom. During this period, climatic factors were also analyzed, which influence the biology of useful and harmful species. Following the monitoring and determination of all species collected, data were recorded on the abundance and dynamics of existing fauna.

Key words: *soil traps, AtraPom, Cydia pomonella, dinamics.*

INTRODUCTION

Through a specific strategy adopted at the location of the orchards, for the selection of rootstocks and the appropriate assortment, fertilization works, soil works, formation and fruiting cuttings, etc. the aim is to ensure a certain production potential.

This production potential can be reduced by 20-30% or sometimes totally compromised due to the attack of diseases and pests. Therefore, the protection of these orchards is seriously and permanently required, in order to highlight their production potential at the highest level.

The permanence of walnut orchards on the same land, for several decades, make the fight against diseases and pests quite difficult, compared to annual agricultural plants, where crop rotation is a very effective means of control.

The large number of pests that attack the walnut plantations make the organization of their control occupy an important volume of the concerns of the fruit grower. Protecting the plantations at the level of current requirements, must not only save the harvest, but by combating this need to increase the percentage of perfectly healthy quality fruit.

Damages caused by pests are on the rise and this requires increased attention by specialists. By using classical control measures, especially chemical ones, it is not possible to prevent the occurrence of damage caused by these arthropods.

The application of pesticides that have increased toxicity on a large scale, produces a harmful effect on the environment, which causes pollution of ecosystems.

A serious consequence of the use of these classical methods of control is the loss of the biosphere's ability to self-regulate.

As a result, regarding the disadvantages of using classical control methods, the use of modern control methods should not be ignored, as they are based on natural control methods, but also ecological methods.

It is necessary that all control actions be applied in accordance with the new ecological concept, of integrated control, which consists in a system of regulation of pest populations, taking into account the specific flight and dynamics of pest and zoophagous species, using in harmoniously all control methods (agrophytotechnical, physical-mechanical, biological and chemical), in order to maintain

the density of pests or their attack, at a level that does not cause crop losses.
The aim of our research is based on the identification and centralization of the arthropod fauna existing in walnut plantations in order to establish as accurately as possible the useful and harmful fauna and following these determinations we will be able to establish the control method that can be used efficiently in these experimental lots.

MATERIALS AND METHODS

For the study of the arthropod fauna from the walnut orchard both in conventional system and in ecological system from Sîrca, Iași county, the specific collection method was practiced with the help of soil traps type Barber, and the obtained material serving for the study of the determination and centralization of the epigean fauna from the respective plantation, and the data referring to the frequency and abundance of the number of identified species, it gives us an appreciation of the degree of population in the ecosystems.
The method of collecting insects with Barber cups gives good results especially in areas where otherwise we cannot collect insects, and here we refer to the collection of insect species that move mainly at ground level or cannot be collected by the method of threading.
Petrous or epigean species that move on the ground (arachnids, beetles, gastropods or isopods) can also be collected. The trap consists of a container buried in the ground with an opening at its surface. As a collection liquid it is recommended to use a saturated salt solution, this solution thus allowing the visit of the trap once every 15-20 days. Collected specimens should be washed in clean water before being transferred to alcohol. The advantage of using the solution is that the specimens do not usually collapse when dry and are obviously not toxic to humans and other animals. (Tălmaciu, 2010; Tălmaciu, 2016)
The tracking of the flight curve of the species was performed with the help of pheromone traps, which was based on the principle of intensive attraction of males and their capture on the sticky support of the trap on which the pheromone bait was placed, plugs made of rubber or plastic, porous material which allowed the gradual diffusion of the pheromone. The supports are hung with clips or wires in the crown. This method is also used in biological control by mass capture and even disorientation of males by synthetic sex pheromones.

RESULTS AND DISCUSSIONS

From our observations following the experiments carried out by applying the Barber trap method to the walnut orchard in the ecological stationary (Table 1), 4274 specimens belonging to a number of 9 orders were captured at the 6 collections, namely: *Coleoptera*, *Orthoptera*, *Diptera*, *Hymenoptera, Homoptera, Heteroptera, Colembola* and *Isopoda*, all belonging to the class *Insecta*, and the order *Aranea* belonging to the class *Arachnida*. The best represented were the orders *Orthoptera* with 437 specimens; *Hymenopera* with 395 samples; Isoptera with 278 specimens; *Coleoptera*, with 263 specimens, Spider with 123 specimens, and *Heteroptera* with 117 specimens. The orders *Diptera* and *Homoptera* recorded less than one hundred specimens each. For the species of the order *Colembola*, it was not possible to calculate a total number of specimens because the biological material collected that belonged to this order in three of the six collections was represented by numerous colonies (Panin, 1951; Reitter, 1908)
Following the centralization of catches on each collection, we could see that the largest number of specimens was recorded at the first collection, the one on 22.05 where a total of 2218 specimens belonging to 21 species or taxa. The next two samplings recorded a number of specimens of 724 and 680, respectively, belonging to 208 and 34 species and taxa, respectively. The lowest number of collected specimens was registered at the fourth collection on 27.07.2019 and was 146. Following the collections on 07.08.2019, and on 27.08.2019 278 were collected respectively 228 specimens that belonged to 23 and 25 species and taxa, respectively.
The collection of biological material with the help of these two methods was done in the following data: 22.05.2019; 18.06.2019; 06.07.2019; 27.07.2019; 07.08.2019, 27.08.2019;

Table 1. The entomofauna from the ecological walnut orchard collected with the soil traps Barber method in the Sârca stationary in 2019

No.	Order	Specie/Taxa	No of harvesting						Total samples
			I	II	III	IV	V	VI	
1	Coleoptera	*Harpalus pubescens* Müll.	10	3	9	17			39
		Harpalus calceatus Duft.	11	1	5	10	2	9	38
		Harpalus distinguendus Duft.	11	1	12	2	3	3	32
		Harpalus tenebrosus Dej	3	16	9	2	8	1	38
		Harpalus aeneus F.		1	2	1	1	1	6
		Pterostichus cupreus L.	1		8	1			9
		Opatrum sabulosum L.	2		1		1		4
		Hister quadrimaculatus L.	4	1	4			2	11
		Dermestes laniarius Illig.	1	2			2	3	8
		Otiorhynchus kollari Gyll.	1		2			2	5
		Rhizophagus politus Hel.	1		1			1	3
		Pteryngium crenatum Gyll.	1		1				2
		Ceuthorynchus obsoletus Germ.		2					2
		Aphthona euphorbiae Sch.	-	2	4		1	1	8
		Agriotes ustulatus Schall.		1	3		1	1	6
		Otiorhynchus fuscipes O.		2	2		1		5
		Apion apricans Herbst		2					2
		Apion virens Herbst		1	1				2
		Quedius cinctus Payk		1					1
		Coccinella 7 punctata L.		2				3	5
		Amara aenea Deeg		1			1	1	3
		Metabletus truncatelus L.		1	4		1		6
		Cyaniris cianea F.		1			2		3
		Anthicus humeralis Geb.		2				1	3
		Anthicus floralis L.	1		1			1	3
		Chrysomela marginata L.		1					1
		Anisodactylus binotatus F.			1		1	4	6
		Nebria brevicollis F.			1		1		2
		Psylliodes affinis Payk			1			2	3
		Tachyusa constricta Erich			2				2
		Oxypora vittata Mär			1			1	
		Tachyporus abdominalis F.			2				2
		Longitarsus gracilis Kuts.			1`				1
		Pentodon idiota Herbst			1		1		2
2	Orthoptera	*Gryllus campestris* L.	306		40		69	22	437
3	Isoptera	*Armadillidium vulgare* L.	1605	511	450	-	121	101	2788
4	Diptera		17	25	11	6	2	18	79
5	Aranea		44	30	19	12	14	4	123
6	Hymenoptera	*Formicidae*	131	54	56	38	40	35	354
		Viespidae	3	31	4	2	-	1	41
7	Heteroptera		39	16	21	14	4	9	117
8	Colembola		22	colony	colony	40	colony	-	colony
9	Homoptera	*Cicadidae*	4	13	1	1	1	1	21
Total order and samples			2218	724	680	14 6	278	228	4223

In order to establish the structure and dynamics of the arthropod entomofauna as a result of applying the soil trap type Barber method to the walnut orchards in the conventional lot (Table 2), 1211 specimens belonging to a number of 10 orders were captured at the 6 collections, namely: *Coleoptera*, *Orthoptera*, *Diptera*, *Hymenoptera*, *Homoptera*, *Heteroptera*, *Lepidoptera*, *Colembola* and *Isopoda*, all belonging to the class *Insecta*, and the order *Aranea* belonging to the class *Arachnida*. The best represented were the orders of *Hymenopera* with 382 copies; Isoptera with 323 specimens; *Homoptera* with 126 specimens; *Coleoptera*, with 118 specimens, *Aranea* with 97 specimens, *Colembolla* with 70 specimens; *Diptera* with 43 specimens and *Heteroptera* with 30 specimens. The orders *Orthoptera* and *Lepidoptera* registered 9 and 3 specimens, respectively.

Table 2. The entomofauna from the conventional walnut orchard, collected with the soil traps Barber method in the Sârca stationary in 2019

No.	**Order**	**Name of species**	**No of harvesting**						**Total**
			I	II	III	IV	V	VI	**samples**
1	Coleoptera	*Amara crenata* Deeg		1		4		1	6
		Anthicus floralis L.			1	1		2	4
		Aphthona euphorbiae Sch.			4		1	1	6
		Apion virens Herbst			1				1
		Chrysomela marginata L.			1			2	3
		Coccinella 7 punctata L.			8			4	12
		Dermestes laniarius Illig.			1		2		3
		Harpalus aeneus F.			3	6		8	17
		Harpalus azureus F.		2	2		1		5
		Harpalus calceatus Duft.		4	1				5
		Harpalus pubescens Müll.		3	7	2			12
		Microlestes maurus Sturm				1		2	3
		Nebria brevicollis F.			1		1		2
		Opatrum sabulosum L.	1	1	21		1	3	27
		Ophonus sabulicola Panz				1	2		3
		Otiorhynchus ovatus L.	1						1
		Oxypora vittata Mär.	1	1					2
		Pterostichus nigrita L.	1						1
		Pentodom idiota Herbst			1		1		2
		Pteryngium crenatum Gyll.			2			1	3
2	Aranea		4	28	44	17	2	2	97
3	Colembola		23		47	-			70
4	Diptera		6	2	18	2	12	3	43
5	Orthoptera	*Gryllus campestris* L.		1	1	2		5	9
6	Heteroptera		7	10		11	2		30
7	Hymenoptera	*Formicidae*	195	43	39	40	32	16	365
		Viespidae	6	5	3	2	1		17
8	Homoptera	*Cicadelidae*	8		3				11
		Aphididae	22	73		19	11		125
9	Isoptera	*Armadillidium vulgare* L.	15	42	83	80	62	41	323
10	Lepidoptera			1	1	1			3
Total order and samples			290	217	293	189	131	91	1211

As a result of the collection of arthropod species from the conventional walnut orchard in 2019, we can see that the number of catches per harvest varied within fairly wide limits, so the largest number of specimens was recorded at the first collection, that of dated 22.05 where a total of 290 specimens belonging to 13 species or taxa were collected. The next two collections recorded a number of over 200 specimens belonging to over 20 species and taxa. At harvest no. 4, the total number of specimens was 189 and for this a number of 15 species and taxa were identified. Following the collections from 07.08.2019, 131 specimens were collected that belonged to 14 species and taxa. The lowest number of specimens collected was also recorded at the sixth collection on 27.08.2019 and was 91.

The tracking of the flight curve of the species *Cydia pomonella*, the main pest found in the walnut orchard was made using the method of capturing adults by using traps with synthetic pheromones of AtraPom type (Beşleagă, 2008) in the two experimental lots in the walnut orchard and then the cycle scheme was made evolution of the pest.

In the period 2018-2019 in Iasi, the average and maximum daily temperatures, but also the monthly temperatures were very favorable for the evolution of the pest. For example, in 2018 the temperature was very high, registering in May 32.4°C, and in 2019, in July 40.0°C.

Based on the research undertaken, it was concluded that the codling moth has two generations per year in the area where the studies were conducted (Table 3).

Table 3. Synthesis table of *Cydia pomonella* L. species in 2019

No.	Biological stage	*Date of first appearance*	Sum temp. effective (tn-to)	*Date of last appearance*	Sum temp. effective (tn-to)
1	Pupae	15.04	46.3	04.05	98.4
2	Adult	05.05	105.8	15.05	196.9
3	Egg	12.05	158.9	26.05	349.5
4	Larvae	22.05	285.1	07.06	514.3
5	Pupae	07.06	514.3	12.06	584.1
6	Adult	16.06	649.5	22.06	735.3
7	Egg	04.07	911.4	15.07	1053.8
8	Larvae	10.07	993.2	overwinter	

Regarding the evolutionary cycle, from the data obtained, it results that in 2019 the first adult appeared in the first decade of May at an effective temperature of 105.8°C. Butterflies generally take place on warm, windless evenings. Temperature has the greatest influence on flight. All these observations highlight the influence of climatic conditions, temperature and humidity factors, on the appearance and evolution of each stage of development of the codling moth.

CONCLUSIONS

Following the collection and determination of the collected insect species, it could be observed that both the number of collected specimens and the number of species or taxa determined by the soil traps method was significantly higher in the lot of organic walnuts than in the lot of conventional walnut orchards.

The collected entomofauna was represented by a very wide range of insects that can present as food both phytophagous and zoophagous, or even mixed, which belonged to 10 systematic orders.

Based on the research undertaken, it was concluded that the species *Cydia pomonella*, has two generations per year in the area where the studies were conducted.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IMPACT OF SOIL MANAGEMENT SYSTEMS ON THE CHEMICAL COMPOSITION OF FRESH PLUM FRUIT OF 'KATINKA' CULTIVAR

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Abstract

The chemical composition of fresh plum fruits of 'Katinka' cultivar was studied for a 5-year period (2015-2020) in an experimental plum plantation of RIMSA Troyan. The soil surface in the plantation is maintained in three treatments - black fallow, natural grassland and artificial grassland. It was found that the content of dry matter and acids in the fruits of 'Katinka' were not affected by the way the soil surface was managed. These indicators varied relatively little over the years, in the range of less than 1.5% for dry matter and from 0.41% to 0.82% for acids. There was a higher percentage of total sugars in fruits in the artificial grassland for all years of the study period and varied from 9.05% to 11.60%. The most favourable values for the ratio between sugar and acids in fruits (glucoacidimetric coefficient) were reported in the artificial grassland, which is important for the formation of good taste qualities of the fruits with variation over the years from 13.89-27.07. In the case of fallow and natural grassland treatments, a high correlation dependence was established between the content of organic acids and inverted sugar.

Key words: *Plum, cv. 'Katinka', chemical parameters, soil management systems.*

INTRODUCTION

The plum crops occupy a major place in the foothills and mountain regions of Bulgaria. These are most often areas with sloping terrain, threatened by the development of water erosion. Overcoming the negative consequences of sloping terrain depends on the soil surface management systems. With a properly chosen management system, the risk of erosion can be reduced, soil fertility can be increased and good growth and high and quality yields of fruit plants can be ensured (Bozhanska et al., 2019, 2017; Hristova et al., 2017).

The impact of various soil management systems have been studying in the Research Institute of Mountain Stockbreeding and Agriculture for decades, monitoring their impact on tree reproductive capacity and fruit quality (Dinkova et al., 2004; Gergov et al., 2001).

Modern fruit production requires compliance with the quality requirements of fresh plums, according to European regulations (MAF, 2004).

The composition of the chemical elements in plums varies primarily depending on the cultivar, ecological conditions, the location of the garden and the latitude of a given area (Dzhuvinov et al., 2012).

Plums are rich in sugars, organic acids, tannins and dyes, pectin, vitamins and minerals.

An important indicator of fruit quality is the dry matter content.

Walkowiak-Tomczak et al. (2007) and Sosna (2010, 2012) found that plum genotypes had a significant effect on dry matter. Our results on the dry matter content of 'Katinka' cultivar are similar to those obtained by Blažek & PištӨkova (2009) and Milošević et al. (2012) for the same plum cultivar grown in the Czech Republic and Serbia respectively.

According to some scientists, the differences in sugar content in plums are due to different agro-climatic conditions (Nergiz & Yldz, 1997). According to others, the cultivar itself affects the sugar profile in fruits (Crisosto et al., 2004).

A summary indicator of the fruit chemical composition is the glucoacidimetric coefficient (the ratio between sugars and acids). It largely determines the fruit taste (Minev, 2002). According to Forni et al. (1992) the ratio between sugars and acids for good quality plums should be between 12-24. Our studies correspond to various studies, according to which the fruits of 'Katinka' cultivar have a good balance between sugars and acids (Milošević, et al., 2012) and are recommended mainly for fresh consumption, but are also suitable for processing and drying (Hartmann & Neumüller, 2006).

The objective of the present study is to establish the impact of different soil management systems on the chemical composition of fresh plums of 'Katinka' cultivar.

MATERIALS AND METHODS

The experiment was carried out in 2015-2020 at RIMSA-Troyan in a plum plantation of 'Katinka' cultivar, established in 2010 in an area of 8 da, on a pseudopodzolic soil, poorly stocked with nutrients. The exposure is northwestern with a slope of 4-5°. The planting pattern is 5 x 3 m.

The soil surface is managed in three variants:

1. Fallow - the inter-rows are maintained as a fallow by disking;
2. Natural grassland - the interrows are covered by turfgrass of natural perennial grasses;
3. Artificial grassland - interrows are covered by turfgrass of grass mixture from legume and grasses in ratio (1:1) with bird's-foot-trefoil and red fescue at a seeding rate of 5 kg/da.

The observation of the chemical composition of fresh fruits of 'Katinka' was conducted in the chemical laboratory of RIMSA-Troyan according to the following methods:

- dry matter according to Re (%);
- sugars (%) total, inverted and sucrose – according to Schoorl Regenbogen method;
- acids (%) - by titration with 0.1n NaOH;
- vitamin C (mg/%) - according to Fialkov method;
- anthocyanins (mg %) - according to Fuleki and Franciss method;
- tanning substances (%) - according to Levental method.

By mathematical data processing with ANOVA program in Excel, the dependences of correlation and regression among chemical elements in the different soil management variants were calculated.

RESULTS AND DISCUSSIONS

During the years of the study, no significant differences were found in terms of dry matter fruit content, depending on the soil surface management system. The clearest difference was in 2016 (4.9%), 1.5% in 2015 and 2017, for 2018 it was below 0.1% among the different variants. During the extremely dry year 2020, with the average amount of precipitation 386.2 mm, there was no difference - 15% in all variants (Table 1). The coefficient of variation was low 10.21%.

The dry matter content in the fruits of 'Katinka' variety was low during the whole period of research in all three variants. This corresponds to the data of Iliev (1988), Milosevic et al. (2012), according to which plum cultivars with early ripening period have a low dry matter content in the range of 12.75 to 17.53%; Zavisic end Rosic (2017) set the dry matter content in 'Katinka' fruits to 13%.

Carbohydrates are a major part of fruit dry matter, and the soluble sugars are the largest share of them. The plum fruits of 'Katinka' cultivar differ in the content of total sugars depending on the soil surface management systems. The highest percentage of total sugars was reported in the variant with artificial grassland (9.05-11.60%), followed by the fallow (7.85-11.30%), and the lowest content was in the variant with natural grassland (7.50-11.10%), with a low coefficient of variation 12.32%. During the years of the study, the content of sucrose and inverted sugar in fruits of 'Katinka' cultivar varied in very wide ranges of 3.70-6.85% (inverted sugar) and 1.43-5.94% (sucrose) with a coefficient of variation of 13.76% for inverted sugar and 33.27% for sucrose.

Table 1. Chemical analysis of fresh plums in different variants

	Soluble Solids (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Acids (%)	Glucoacidimetric index	vit. C (mg/%)	Tanning Substances (%)	Anthocyanins (mg/%)
2015									
1. Clean cultivation	13.50	9.05	3.70	5.08	0.35	25.86	3.52	0.104	17.42
2. Natural grassland	14.50	8.55	5.50	2.90	0.38	22.50	2.64	0.104	21.94
3. Artificial grassland	15.00	11.10	6.15	4.70	0.41	27.07	1.76	0.187	18.06
2016									
1. Clean cultivation	11.00	10.25	5.35	4.66	0.58	17.67	3.52	0.208	9.68
2. Natural grassland	15.70	9.90	5.20	4.47	0.45	22.00	6.16	0.187	15.00
3. Artificial grassland	15.90	10.75	4.50	5.94	0.45	23.89	4.40	0.187	8.71
2017									
1. Clean cultivation	14.00	11.30	6.35	4.70	0.62	18.23	8.80	0.056	8.87
2. Natural grassland	12.50	11.10	6.50	4.37	0.75	14.80	8.80	0.094	7.26
3. Artificial grassland	13.00	11.60	6.50	4.85	0.82	14.15	8.80	0.150	16.13
2018									
1. Clean cultivation	12.40	9.62	5.85	3.60	0.64	15.03	10.56	0.037	1.29
2. Natural grassland	12.50	9.55	5.85	3.52	0.57	16.75	10.56	0.056	0.48
3. Artificial grassland	12.50	9.90	5.85	3.85	0.64	15.47	8.80	0.075	3.23
2020									
1. Clean cultivation	15.00	7.85	6.15	1.62	0.60	13.08	14.08	0.145	14.35
2. Natural grassland	15.00	7.50	6.00	1.43	0.54	15.08	15.84	0.208	16.13
3. Artificial grassland	15.00	9.05	6.85	2.09	0.60	13.89	15.84	0.166	22.26
Average (2015-2020)	*13.83*	*9.80*	*5.75*	*3.85*	*0.56*	*18.36*	*8.27*	*0.130*	*12.05*
St. Dev. (2015-2020)	*1.41*	*1.21*	*0.79*	*1.28*	*0.13*	*4.51*	*4.46*	*0.06*	*6.79*
CV% (2015-2020)	*10.21*	*12.32*	*13.76*	*33.27*	*22.93*	*24.54*	*53.94*	*43.69*	*56.33*

Comparing the soil management methods, a slightly higher percentage (invert sugared and sucrose) was reported in the variant with artificial grassland, but these differences were insignificant for each year, with differences below one percent, except for 2015 with a difference of 2-3%.

Of the organic acids, malic predominates, the amount of which was from 0.35% in the fallow variant in 2015, to 0.82% in the artificial grassland in 2017 (CV = 22.93%).

The content of vitamin C in the fruits by years, according to the different variants of soil management, had similar values, with a difference of less than 2 mg/%. The lowest 1.76 mg/% and the highest 15.84 mg/% amount of vitamin C was reported in the artificial grassland variant, with a high coefficient of variation of 53.94%.

Tanning substance, which are most often tannins, have a specific effect on fruit taste. Their variation is the largest (43.69%) in the fruits in black fallow management system - from 0.037% (2018) to 0.208% (2016).

The presence of anthocyanins in plums has a positive effect on their healing properties. Significant differences were observed in terms of their values over the individual years. The highest value was reported in the artificial grassland with 22.26 mg% (2020), and the lowest in the fallow variant 0.48 mg% (2018). They had the highest coefficient of variation of 56.33%.

Fruit taste quality is one of the most important indicators characterizing the fruit cultivars. The fruits of plum cultivars containing sugars from 11% to 17% and not a high percentage of acidity from 0.16% to 0.74% With regard to acids, the fruits during all the years of the studied period in the different variants of soil management varied within these limits. Sugars only in 2017 are approaching the optimal values of 11%.

In a study of the chemical composition of plum fruits, Miloševi et al. (2012) found that 'Katinka' variety had the best characteristics between sugars and acids, compared to other German cultivars for the conditions of Serbia. Our experiments show that in the case of artificial grasslands the ratio of sugars and acids was close to the optimal values for the formation of good fruit taste, with a variation of the glucoacidimetric coefficient of 13.89-27.07 for the study period. The lowest value was reported for the fallow variant in the dry 2020 - 13.08, while for the grassed variants the values were higher 15.08% for the natural grassland and 13.89 for the artificial grassland.

For the period of the study, the indicators of the chemical composition of the fruits of 'Katinka' varied widely, as the average values in our study correspond to those of an eight-year period of research conducted by Iliev et al. (1977) for 'Kyustendilska sinia sliva' and 'Stanley' cultivars.

A high correlation was found between the acid content in fruits, in relation to the inverted sugar (r = 0.9521) in the fallow variant on the function y = 8.5556x + 0.0706, with regression coefficient R^2 = 0.9066 (Figure 1) and in the natural grassland variant (r = 0.9015), by the function y = 318516x + 4.0964 with regression coefficient R^2 = 0.8128 (Figure 2).

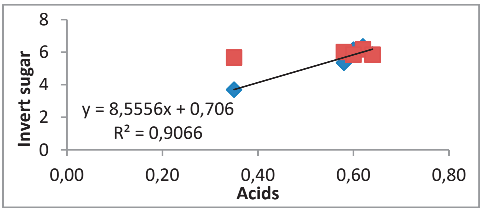

Figure 1. Graphic model of regression dependence between the content of acids and inverted sugar in fruit in clean cultivation

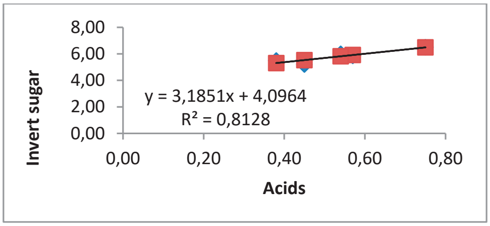

Figure 2. Graphic model of regression dependence between the content of acids and inverted sugar in the fruit in natural grassland

In the third variant (artificial grassland), there was a high correlation (r = 0.8595) and regression dependence (R^2 = 0.7388) between the content of tanning substances in fruit, by the function y = 27.012x + 10.147, relative to the dry matter, which is easily determined refractometrically (Figure 3).

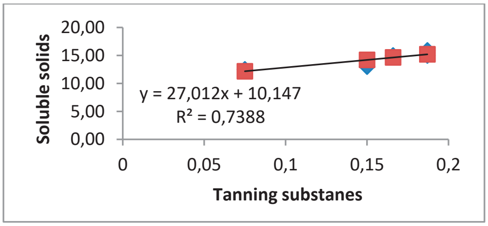

Figure 3. Graphic model of regression dependence between the content of tanning substances and soluble solids in the fruit in artificial grassland

CONCLUSIONS

The content of dry matter and acids in fruits of 'Katinka' cultivar was not affected by the soil surface management system (fallow, natural and artificial grassland) for the respective year.

When maintaining the soil surface in artificial grassland, the content of total sugars in fruit was higher 9.05-11.60%, compared to other variant. A similar trend is observed for inverted sugar (4.50-6.85%) and sucrose (2.09-5.94%).

In all studied variants, for the period 2015-2020 the ratio between sugar and acids (glucoacidimetric coefficient) was on average 18, which determines fruit taste qualities of 'Katinka' cultivar as good.

In the case of fallow and natural grassland, a high correlation dependence was established between the content of organic acids and inverted sugar.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCHES ON THE INFLUENCE OF STORAGE CONDITIONS ON BIOMETRIC AND PHYSIOLOGICAL INDICES OF PLUMS

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Abstract

The purpose of the study was to determine biometric and physiological indices during storage processes in four plum varieties, two of them originated from Romania ('Record' and 'Vinete Românești'), one from USA ('Stanley'), and one from Serbia ('Cacanska Lepotica'). The fruits were harvested at maturity and stored for 5 and 10 days at two different temperatures (4°C and 22°C, respectively). We analyzed 25 fruits for each variety and the following determinations were made: diameter, height, weight, sugar content, firmness, juice acidity and dry matter content. 'Record' and 'Cacanska Lepotica' plum varieties had the highest values in terms of fruit weight (60.37 g and 43.49 g, respectively), after 10 days of storage, at 22°C. Regarding the sugar content of the fruit, it was found that the 'Record' and 'Vinete Românești' varieties had a higher percentage, significantly positive $p < 0.01$ with 22.06 and 24.60%. After 10 days of preservation, the trend of increasing of the content of soluble carbohydrates continues possible due to the transformation of starch into glucose, following enzymatic hydrolysis processes. There was also found a pronounced decrease in fruits firmness, acidity of the fruit juice, but also an increase in fruits dry matter content.

***Key words**: plum, firmness, dry matter, acidity.*

INTRODUCTION

The plum is on the first place from the fruit tree species cultivated in our country, as regard as the cultivated area and concerning the fruit yield obtained (Coman et al., 2012). For the temperate zone and especially for the Balkan countries, plum is a fruit species of great economic importance, due to the rusticity of the species, its therapeutic value, the food value. So, due to its many uses, it was spread on a large area of culture (Iordănescu, 2008; Iordănescu & Olaru, 2014).
The pomological, physical and biochemical proprieties of the local genotypes or biotypes of the cultivated fruit tree species can be influenced by the pedo-climatic factors (Ionică et al., 2013; Iordănescu and Costea, 2014; Vâtcă et al., 2020a; Puia et al., 2017). This information about these particularities, are useful for breeding or growing processes, or to enlarge the assortment of local fresh, preserved or conditioning fruits (Vitanova et al., 2004; Okatan et al., 2017). Fresh plum fruits contain all the microelements necessary for the human body: calcium (Ca), potassium (K), iron (Fe), magnesium (Mg), phosphorous (P) etc. From the vitamins, the most representative is carotene, vitamin C, vitamins B1, B2, PP and others. However, plums are poor in lipids and protein, thus having a low caloric value (Scedei et al., 2019).
From known cultivars or local varieties there are different researches on fruits proprieties, such as the nutritional characteristics of local cultivars (Rop et al., 2009; Păcurar et al., 2018) and the changes that occur in their physical or chemical characteristic during the fruits ripening or their storage (Usenik et al., 2008; Miletic et al., 2012; Oltenacu and Oltenacu, 2014; Vâtcă et al., 2020b), the chemical compounds content and their contribution to the fruit aroma, color (Usenik et al., 2009; Pino and Quijano, 2012) or to the antioxidant activity (Donovan et al., 1998).
The excessive softening is the major factor limiting the shelf life of plums. (Crisosto et al., 2004).

When chilling injury symptoms or degradation are not limiting factors, the ripening-related process most sensitive to ethylene and a suitable predictor of potential shelf-life for plums is fruit softening (Lelievre et al., 1997). When are mature to a soft melting texture, plums are considered perfect to eat (Beinșan et al., 2019). Like on the other fruit species, during maturation, the cell wall extracted from plum fruit showed considerable increase in swelling and high pectin solubilization. (Redgwell et al., 1997). Plums are highly perishable at ambient temperatures and cannot endure long storage periods after harvest. Thus, pre-harvest treatment of the fruits can effectively be used to increase the storage life of their freshness with the plant growth hormones (like auxins, gibberellins), calcium chloride and growth retardants (like cycocel) which would retard the rate of deterioration in quality after harvest and thereby will increase the shelf life of the fruits (Kirmani et al., 2015, Shazia et al., 2013).

In this experiment we studied the influence of temperature, as well as the storage duration on biometric and physiological indices of four plum fruits varieties 'Cacanska Lepotica', 'Vinete Românești', 'Stanley' and 'Record'.

MATERIALS AND METHODS

The biological material

The biological material was represented by four plum varieties, with different origin: two Romanian ('Record' and 'Vinete Românești'), one from the USA ('Stanley') and one Serbian variety ('Cacanska Lepotica'). The plums varieties properties were summarized in Table 1.

The fruits were harvested from Vînju Mare locality, Mehedinți county, at full maturity, in 2019 and stored for 5 and 10 days at two different temperatures (4°C and 22°C).

The analyzed biometric and physiological properties

The fruits (25 fruits/variety) were harvested from different parts of the tree. The fruits diameter and their length have been measured with the help of a caliper. The weight was established by using an analytical balance.

Fruits sugar content, expressed as soluble carbohydrates (Brix degree - %) has been obtained with a digital refractometer (Kruss DR 201-95)

Table 1. Principal properties of tested plum varietes

Properties	Variety			
	'Record'	**'Vinete Românești'**	**'Stanley'**	**'Cacanska Lepotica'**
Origin	Romania	Romania	USA	Serbia
Size	very large	small	medium	large
Taste	appetizing	sweet and slightly astringent	sweet, slightly acidic and slightly aromatic fruit	sweet-sour
Fruit quality	very good	very good	very good, asymmetric	very good
Consumption	Fresh	Fresh/ Industrialization	Fresh/ Industrialization	Fresh/ Industrialization
Other	Non-stick pulp	ellipsoidal, dark-hunted fruit, with a green-yellow pulp, with a thick silvery plum, consistent	Weight 30-40 g, oval, dark-colored	Weight 50 g, spherical shape in shades of blue easily pulp removal
Source	https://fructifer.ro/prun-Record	https://www.pestre.ro/blog/pomi-fructiferi-tot-ce-trebuie-sa stii/#Soiuri_propuse_pentru_cultura_de_prun	http://www.horticultorul.ro/pomi-fructiferi/soiuri-de-prun-stanley/	https://www.gardenexpert.ro/arbori/pomi-fructiferi/prun-cacanska-lepotica.html

Fruit flesh firmness was determined with the penetrometer (FORCE GAUGE PCE-FM 200) and results have been expressed in lbr.

The fruit juice acidity was determined using a multiparameter analyzer - pH (CONSORT C933).

Dry matter content was determined with thermobalance Kern MLS in percent units.

All the results were expressed as average ± standard error (SE). Statistical analysis was made with RStudio software version 4.0.5 (31.03.2021), ANOVA and Fisher LDS for all the parameters.

RESULTS AND DISCUSSIONS

Significant differences $p < 0.001$ to all parameters were seen between different varieties. The parameters assessed soluble glucids, fruit acidity, dry matter and firmness show significant $p < 0.001$ differences between the storage methods (Table 2).

The interaction between varieties type and storage methods did not result in a significant difference within the parameters tested.

Table 2. Statistical result for all the tested parameters with ANOVA

Parameters	Variety		Storage		Variety × Storage	
	F	p.val	F	p.val	F	p.val
Small diameter	319.12	p < 0.001	0.44	0.78	0.00	1.00
Large diameter	456.40	p < 0.001	1.81	0.15	0.01	1.00
Height	405.56	p < 0.001	0.43	0.78	0.01	1.00
Weight	492.71	p < 0.001	1.11	0.36	0.01	1.00
Soluble glucids	756.57	p < 0.001	37.16	p < 0.001	0.71	0.73
Fruit acidity	252.92	p < 0.001	56.32	p < 0.001	0.78	0.66
Dry matter	552.27	p < 0.001	56.86	p < 0.001	0.30	0.98
Firmness	362.64	p < 0.001	43.82	p < 0.001	0.45	0.93

Looking at large and small fruits diameters we can observe that compared with the first determination - fd, all the values decreased due to water loss. Significant differences $p < 0.05$ between large and small diameters were observed for 'Vinete Românești' and 'Stanley' (except for small diameter parameter at fd) (Table 3).

Table 3. Large and small diameter of fruits in the studied plum varieties (average ± SE)

Parameter/treatment	Variety			
	Cacanska Lepotica	Vinete Românești	Stanley	Record
Large fruits diameter - fd	41.00±0.57 ^{b}	28.00±1.15 ^{d}	36.33±0.88 ^{c}	45.33±0.88 ^{a}
5 days at 22°C	40.18±0.47 ^{b}	27.14±0.83 ^{d}	35.56±0.86 ^{c}	44.60±0.75 ^{a}
5 days at 4°C	40.86±0.63 ^{b}	27.63±0.98 ^{d}	35.95±0.73 ^{c}	45.11±0.75 ^{a}
10 days at 22°C	39.64±0.62 ^{b}	26.65±0.73 ^{d}	34.93±0.63 ^{c}	43.98±0.86 ^{a}
10 days at 4°C	40.33±0.73 ^{b}	26.96±0.66 ^{d}	35.47±0.67 ^{c}	44.71±0.89 ^{a}
Small fruits Diameter - fd	37.33±0.88 ^{b}	25.66±0.88 ^{e}	34.00±0.57 ^{cd}	43.66±1.20 ^{a}
5 days at 22°C	36.87±0.86 ^{b}	25.21±1.01 ^{e}	33.53±0.57 ^{d}	43.17±1.22 ^{a}
5 days at 4°C	37.19±0.86 ^{b}	25.46±1.01 ^{e}	33.69±0.53 ^{d}	43.40±1.22 ^{a}
10 days at 22°C	36.46±0.85 ^{bc}	24.90±0.98 ^{e}	33.21±0.55 ^{d}	42.91±1.24 ^{a}
10 days at 4°C	36.61±0.88 ^{bc}	25.11±0.97 ^{e}	33.38±0.53 ^{d}	43.13±1.21 ^{a}

fd = first determination, different letters are significant at $p < 0.05$, LSD test

Significantly higher ($p < 0.05$) large (45.33±0.88 - fd) and small diameter (43.66±1.20 - fd) was seen at 'Record' variety.

The lowest large diameter (26.65 ± 0.73) and small diameter (24.90 ± 0.98) was observed to 'Vinete Românești' variety after 10 days of storage at 22°C.

'Record' and 'Stanley' varieties had the highest height and differ significantly ($p < 0.05$) from the other two varieties.

The weight was higher at Record (61.20 ± 0.37 g) and 'Cacanska Lepotica' (44.19 ± 0.83 g) with significant differences ($p < 0.05$) between the varieties not before storage days or temperature. 'Cacanska Lepotica' (37.63 ± 1.36 g) and 'Stanley' (41.07 ± 1.19 g) weight was assessed in a study and with not significant difference between them (Milošević at al., 2012). Another study place 'Cacanska Lepotica' (37.77 g) variety as with small fruit weight (Zamfirescu et al., 2019).

The lower weight was observed at 'Vinete Românești' variety after 10 days of storage at 4°C (Table 4).

Table 4. Experimental results regarding height and weight in the tested plum varieties (average ± SE)

Parameter/treatment	Variety			
	'Cacanska Lepotica'	'Vinete Românești'	'Stanley'	'Record'
Height - fd	45.66±0.88 ^{b}	35.00±1.15 ^{c}	51.00±0.57 ^{a}	52.66±0.88 ^{a}
5 days at 22°C	45.41±0.90 ^{b}	34.69±1.07 ^{c}	50.63±0.64 ^{a}	52.04±0.84 ^{a}
5 days at 4°C	45.51±0.91 ^{b}	34.86±1.09 ^{c}	50.84±0.62 ^{a}	52.32±0.83 ^{a}
10 days at 22°C	45.09±0.96 ^{b}	34.24±0.98 ^{c}	50.19±0.56 ^{a}	51.67±0.81 ^{a}
10 days at 4°C	45.34±0.92 ^{b}	34.69±1.07 ^{c}	50.66±0.63 ^{a}	52.12±0.82 ^{a}
Weight - fd	44.19±0.83 ^{b}	16.73±0.63 ^{d}	34.30±0.56 ^{c}	61.20±0.37 ^{a}
5 days at 22°C	43.88±0.80 ^{b}	16.27±0.55 ^{d}	33.86±0.62 ^{c}	60.89±0.37 ^{a}
5 days at 4°C	44.07±0.80 ^{b}	16.53±0.60 ^{d}	34.18±0.56 ^{c}	61.06±0.36 ^{a}
10 days at 22°C	43.48±0.82 ^{b}	15.67±0.60 ^{d}	33.45±0.63 ^{c}	60.37±0.49 ^{a}
10 days at 4°C	43.85±0.79 ^{b}	16.12±0.64 ^{d}	33.90±0.59 ^{c}	60.86±0.34 ^{a}

fd = first determination, different letters are significant at $p < 0.05$, LSD test

At the beginning of the experiment-fd, the highest content in soluble carbohydrates was recorded in the 'Vinete Românești' variety with 24.60 ± 0.36% Brix, and the lowest in Stanley (12.18 ± 0.12%). After 5 and 10 days of storage at different temperatures (4°C and 22°C) it can be seen a trend of sugar content increasing, due to the transformation of starch into glucose following enzymatic hydrolysis processes.

After 5 days of storage at 22°C, the soluble glucides increased significantly $p < 0.05$ compared with the fd. At 4°C, after 5 days of storage, the % BRIX was significantly higher from fd value in all varieties except to 'Vinete Românești' variety (Figure 1).

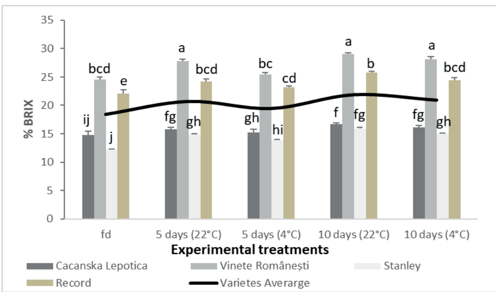

Figure 1. Results regarding the total content of soluble glucides in tested varieties (average ± SE); within overall different letters represent significant ($p < 0.05$, Fisher-LSD test) differences between the treatments

The highest sugar content after 10 days of storage at 22°C, was recorded in the varieties

'Vinete Românești' (29.08%) and 'Record' (25.78%), being significantly $p < 0.05$ higher than the other varieties 'Cacanska Lepotica' (16.72%) and 'Stanley' (15.92%). The sugar content values followed the same trend after 10 days of storage at 4°C.
The carbohydrate content increases higher to a temperature of 22°C than of 4°C.
The lowest amount of soluble solids overall varieties was observed at 'Stanley' variety with 15.92% with significant high difference compared to the initial record.
In the assessment of different plums hybrids, for 'Stanley' was obtained 17.95±1.34 % BRIX with 32% higher compared with our first determination for the same variety (Milošević at al., 2012). In case of 'Stanley' variety, the percent of soluble solids were 17.75±0.45 with only 16.79% higher that our first determination-fd (Milošević at al., 2012).
Here we can highlight and discuss also a better quality and higher sugar content to Romanian varieties like 'Vinete Romanești' and 'Record' with increased sugar content during storage, similar records were made to other Romanian varieties as 'Andreea' (19.30 % BRIX). However, to 'Andreea' variety, this high sugar contend was associated to the lowest yield production and number of fruits per tree, so not very productive (Zamfirescu et al., 2019).
Regarding the plum fruits pulp acidity, the lowest values were determined for 'Vinete Românești' and 'Record' varieties. The pulp acidity values compared with the fd determination decreased significantly $p < 0.05$, after 5 days of storage at 22°C for all the varieties and at 4°C only for 'Vinete Românești' with 5% (Figure 2).
The highest acidity of the plums pulp was highlighted in the 'Stanley' variety with an initial pH of 3.60, which decreased to 3.30 after 10 days of storage at 22°C and respectively 3.45 at 4°C. The initial obtained value for 'Stanley' variety was similar in other study respectively a pH of 3.66 ± 0.04 (Milošević at al., 2012). The lowest pH value was recorded at 'Vinete Românești' (3.12 initially), and decreased to 2.77 (at 22°C) and 2.86 (at 4°C), after 10 days of storage (Figure 2). Another reference similar with our finding place pH for 'Cacanska Lepotica' in a range between 3.42 ± 0.03 (fd) and 3.35 ± 0.04 (Milošević at al., 2012).

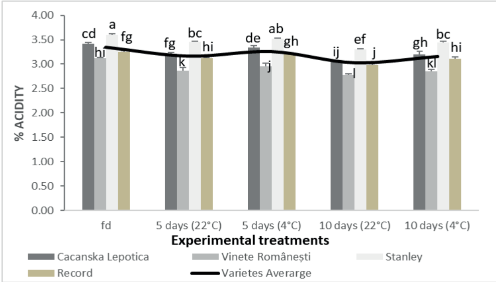

Figure 2. Results regarding the plum fruits acidity in tested varieties (average ± SE); within overall different letters represent significant ($p < 0.05$, Fisher-LSD test) differences between the treatments

The dry matter content first determination emphasis values between 13.82 ± 0.12% ('Stanley' variety) and 25.51 ± 0.34% ('Vinete Românești'). Between varieties type all the differences were significant $p < 0.05$ at the first determination. At a temperature of 22°C for 5 and 10 days of storage higher increases in dry matter content were seen compared with the storage at a temperature of 4°C.
Experiments have shown that dry matter content of the plum fruits tested show a significant increase during storage, both at 22°C and 4°C compared with the fd. At 22°C, after 10 days of storage, the lowest value was found in the 'Stanley' variety with 19.02 ± 0.53%, and the highest value was registered in the case of the 'Vinete Românești' variety with 31.36 ± 0.62%. After 10 days of storage, at 4°C, the 'Stanley' variety recorded the lowest value, of 16.28 ± 0.41%, and the highest also for 'Vinete Românești' (28.96 ± 0.55%) (Figure 3).

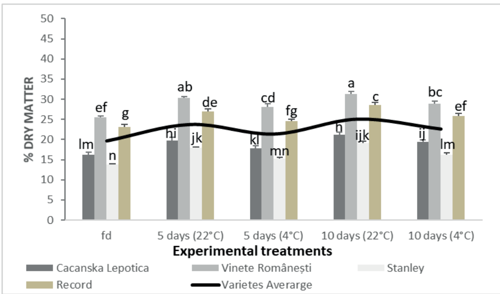

Figure 3. Results regarding the dry matter content in tested plum fruits varieties (average ± SE); within overall different letters represent significant ($p < 0.05$, Fisher-LSD test) differences between the treatments

Firmness is the best indicator of maturity. As the fruit matures, the pulp becomes softer.

The highest and significant ($p < 0.05$) firmness value was determined for ‘Record’ variety compared to the other varieties (Figure 4).
The lowest value in terms of firmness in plums, after 5 days of storage, both at a temperature of 4°C and at 22°C, is recorded at ‘Vinete Românești’ variety with 0.49 ± 0.01 lbr (22°C), while at the opposite pole is the ‘Record’ variety with 1.04 ± 0.02 lbr (22°C). After 10 days of storage, a reduction in fruit firmness can be observed, both at 4°C and at 22°C (Figure 4).

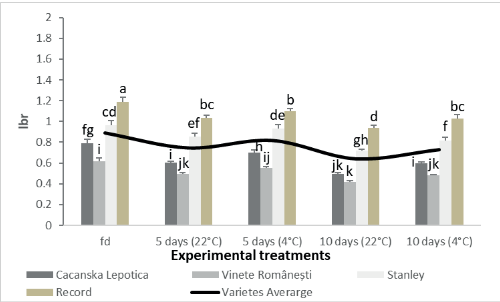

Figure 4. Results regarding the fruits firmness in tested plum varieties (average ± SE); within overall different letters represent significant ($p < 0.05$, Fisher-LSD test) differences between the treatments

CONCLUSIONS

All tested parameters were significantly different within the variety type.
Storage time produce significantly differences only for the following parameters: soluble glucids, fruit acidity, dry matter and firmness.
The parameters: small and large diameter, height and weight registered the highest values after 5 days of storage at 4°C with very low difference between the first determination. At 22°C, compared to 4°C, due to the post-ripening respiratory processes, a more drastic reduction of the fruit diameter was found.
After 5 and 10 days of storage at different temperatures (4°C and 22°C), the tendency to increase the sugar content continues, due to the transformation of starch into glucose following enzymatic hydrolysis processes.
The highest sugar content after 10 days of storage at 22°C, was recorded in the varieties ‘Vinete Românești’ and ‘Record’.
Regarding the plums acidity, the overall values highlight higher PH at ‘Stanley’ variety and decreased only after 5 days of storage at 22°C and after 10 days of storage at 4°C.
Although the ‘Vinete Românești’ variety registered the smallest dimensions in terms of diameter, length and weight of the fruit, it had the highest percentage in sugars.
At a temperature of 22°C for 5 and 10 days of storage higher increases in dry matter content were seen compared with the storage at a temperature of 4°C.
The highest and significant firmness value was determined for ‘Record’ variety.
There was also an accentuated decrease of the firmness, of the acidity of the juice from the fruit pulp, but in the same time the dry matter content increased.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

HYPERSPECTRAL IMAGING SYSTEM FOR MATURITY STAGE CLASSIFICATION OF DURIAN PULP USING BAYESIAN OPTIMIZED MACHINE LEARNING ALGORITHMS

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Abstract

Non-destructive classification of fruits based on the maturity stage is beneficial to the consumer and fruit industry. Improper ripening can lead to low eating quality and economic loss for the producers. In this research, a hyperspectral image (HSI) of durian pulp was obtained using a reflectance-based system. The mean raw spectra of the durian pulp were extracted and pre-treated using standard normal variate (SNV). An assessment of maturity stage classification (unripe, ripe, and overripe) on the full wavelength (900-1600 nm) was performed. The comparison among the machine learning (ML) algorithms (random forest (RF), support vector machine (SVM), and k Nearest Neighbours (kNN)) was carried out, where the hyperparameters were tuned using Bayesian optimization and the 3-fold cross-validation method. The samples were split into training (70%) and test (30%) set using stratified random sampling. In terms of overall classification accuracy and kappa coefficient, SVM (88.5%, 0.83) performed better than RF (84.6%, 0.77) and kNN (73.1%, 0.59). The results show that the classifiers (SVM and RF) can fairly differentiate the ripening stage of durian pulp using HSI.

Key words: *durian, hyperspectral imaging, maturity, machine learning, Bayesian optimization.*

INTRODUCTION

In Southeast Asian countries, durian is known as "the king of fruits". Durian is produced and consumed highly in Malaysia, Indonesia, Singapore, Thailand, the Philippines, and China (Siriphanich, 2011). Durian is popular for its appearance, taste, and distinct odor. Thailand is considered to be one of the largest durian producing country, as well as an exporter to the international market among the countries in the Association of Southeast Asian Nations (ASEAN) (Maninang et al., 2011; Somton et al., 2015). In some countries such as Malaysia, the fruit is likely to be consumed fully ripe with a soft texture and strong odor, whereas the majority of people from Thailand prefer just ripe fruit with a firm texture and mild odor (Siriphanich, 2011). The traditional methods for identifying the ripeness and maturity of durian before harvesting includes mostly visual inspection and destructive techniques (Ketsa et al., 2020). Uneven ripeness due to harvesting immature fruits develop low eating quality, and even after ripening, the fruit lacks the quality characteristic: aroma and flavour (Ketsa et al., 2020). Identifying the correct maturity is one of the biggest challenges that durian producers are facing currently.

Spectroscopy and imaging techniques have been applied on several fruits and vegetables for quality monitoring. Recently, hyperspectral imaging (HSI) is more in use for quality inspection as it integrates both, spectroscopy and imaging techniques (Park & Lu, 2015). Application of HSI has been done for decades in several fruits such as strawberry, peach, pear, banana, etc (Elmasry et al., 2007; Haiyan Cen, Renfu Lu, Fernando A. Mendoza, 2011; Khodabakhshian & Emadi, 2018; Rajkumar et al., 2012). HSI generates 3-dimensional hypercube where the first two dimensions contains spatial information and the last dimension stores the spectral information. Based on an approach in which the spatial information is acquired, the HSI sensor are classified into point scanning (whiskbroom), line scanning (push broom), and area scanning

(tunable filter). In pushbroom system configuration either the object or the imaging unit is moving which gives an advantage to be used in online systems for industrial application (Liu et al., 2007).
Similar to spectroscopic technique, HSI images are first preprocessed and then the classification/regression algorithms are implemented for classification and regression task along with feature extraction and dimensionality reduction. Machine learning algorithms such as support vector machine (SVM), random forest (RF), k nearest neighbor (kNN), discriminant analysis (linear discriminant analysis (LDA), partial least squares discriminant analysis(PLS-DA)) are popular for classification and regression analysis. In machine learning, some parameters control the learning process known as hyperparameters. Hyperparameter optimization can be done manually or with automatic search methods. One of the famous hyperparameter optimization methods is Bayesian optimization. It combines prior information about the unknown function with sample information, to obtain posterior information of the function distribution by using the Bayesian formula (Wu et al., 2019). Based on this posterior information, the optimal parameters combination can be known where the function outputs the optimal value (Wu et al., 2019). Bayesian optimization was proved to be a promising method to find the best hyperparameters for widely used machine learning models such as RF, SVM, and neural networks (NN) (Jones, 2001). Hyperparameter for SVM such as soft margin constant (C) should not be not too high or low. It has to be considered that large values for C lead to few training errors and narrow margin, whereas small values generate a larger margin, at the cost of more errors and more training points situated inside the margin (Eitrich & Lang, 2006). Similarly, the number of estimator trees (n-estimator) is one of the important parameters to be optimized in RF. If the value is too high for n-estimators, the strength can be improved but at the same time the error rate due to high inter tree correlation increases (Wu et al., 2019). If the value is low, the inter tree correlation and strength of individual tree goes down (Wu et al., 2019). Therefore, a proper optimization method plays a critical role in obtaining a good classification model with minimal error in machine learning.
The main objective of this research is to identify the potential of HSI system for the ripeness classification of the durian pulp by using machine learning algorithms: SVM, RF, and kNN. The hyperparameter tuning is performed by using Bayesian optimization for machine learning algorithms.

MATERIALS AND METHODS

Durian samples were collected from Trat Province, the eastern part of Thailand. The fruits were harvested at 99, 106, 113, 120, 127, and 134 days after anthesis (DAA). The total number of whole fruit harvested was 50 with each fruit consisting of approximately 5 to 6 pulps. In total, 260 pulps were used for the experiment. The experiment was conducted 2 days after the harvesting date. The fruits were divided in terms of ripeness level as unripe (99-106 DAA), ripe (113-120 DAA), and overripe (127-134 DAA).
The image was acquired using a pushbroom HSI system with a wavelength range from 900-1600 nm and the spectral resolution of 3.2 nm. The HSI system included an imaging spectrograph (Imspector N17E Specim, Spectral Imaging Ltd., Oulu, Finland) with a CCD camera (Xeva 992, Xenics Infrared Solutions, Belgium), and two 500 W tungsten–halogen light sources (Lowel Light Inc., New York, United States of America) at an angle of 45 degrees. The system was controlled by Specim's LUMO Software Suite (Spectral Imaging Ltd., Oulu, Finland). The integration time was set as 6. The pulp was placed on the translation stage moving at the speed of 10 mm s^{-1} and was guided by the bar on both sides to make it stable during the scan.
After hyperspectral image acquisition, the radiometric calibration was done. The white reference and dark reference acquired for each image in the HSI system were used for the radiometric calibration. To capture the white reference, the spectralon with relative reflectance of 99% was used. In the HSI system, the dark reference image was captured by the system automatically by closing the shutter of a camera.

$$R = \frac{Io - D}{W - D} \quad (1)$$

where R is the relative reflectance image, Io is the raw reflectance image, D is the dark reference image, and W is the white reference image.

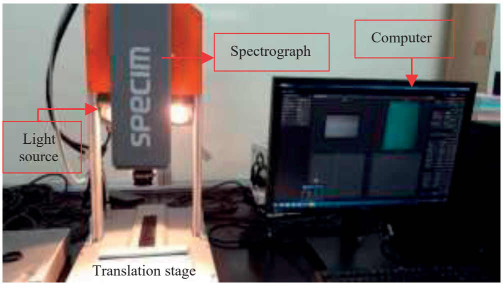
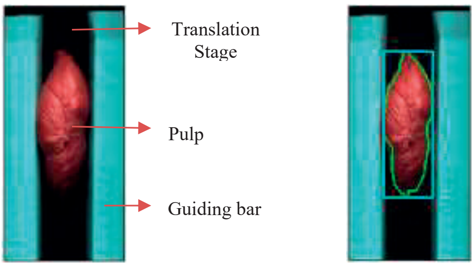


Figure 1. Image acquisition using hyperspectral imaging of durian intact fruit

The area of interest (AOI), pixels representing pulp region, was extracted from each radiometically corrected image. Since the durian pulp was supported by two guiding bars (Figure 2a) while scanning, a normalized difference index (NDR) (Equation 2) was computed to separate pulp from the bars and background (translation stage). Two wavelengths with high reflectance (1205 nm) and low reflectance (1450 nm) were selected for NDR computation.

$$NDR = \frac{(R_{1075.67} - R_{1450.18})}{(R_{1075.67} + R_{1450.18})} \quad (2)$$

The binary threshold operation was then applied to NDR image to separate the pulp from the background as shown in Figure 2b.



(a) (b)

Figure 2. Raw image acquired from hyperspectral imaging (a) Masked image by binary masking and morphological filter.

The threshold value was set to 0.5, with values greater than 0.5 representing the pulp. The morphological filters (dilation and erosion) were then applied to the binary image to extract the pulp pixels. From the extracted AOI, the mean spectra were calculated from every pixel within the boundary of the AOI.

The obtained mean spectra was then preprocessed using standard normal variate (SNV) technique as it minimizes the effects of scattering, particle size, and the difference in the global intensities of the signals (Barnes et al., 1993; Roger et al., 2020)

Supervised machine learning algorithms: SVM, RF, and kNN, were applied on the preprocessed mean spectra of durian pulp. The classification models were developed in Python 3.8 using Scikit-learn machine learning library. The hyperparameters: soft-margin constant (C), and gamma (γ) for SVM, the number of estimators trees in the forest (n-estimator), the number of features to consider for the best split, the maximum depth of the tree for RF, and number of neighbors (n) to calculate the nearest neighbor for kNN, were optimized by Bayesian optimization. In the case of SVM, the radial bias function (RBF) kernel function was used. The distance metrics in kNN was set as euculidean. The sample set was divided randomly into a training set and the test set in the ratio of 70:30. Overall accuracy (Equation 3), precision (Equation 4), recall (Equation 5), and Kappa coefficient (Equation 6) values were used to analyze the performance of each machine learning classifier. The Kappa coefficient measures the actual agreement (indicated by the diagonal elements of the confusion matrix) minus chance agreement (indicate by the product of the row and column marginal). It measures how the classification performs as compared to the reference data (Fung & Ledrew, 1988).

$$\text{Overall Accuracy (\%)} = \frac{TP + TN}{TP + TN + FP + FN} \times 100 \quad (3)$$

$$\text{Precision} = \frac{TP}{TP + FP} \quad (4)$$

$$\text{Recall} = \frac{TP}{TP + FN} \quad (5)$$

$$\text{Kappa coefficeint} = \frac{p_o - p_e}{1 - p_e} \quad (6)$$

		Predicted Class	
		Positive	Negative
Actual Class	Positive	True Positive (TP)	False Negative (FN)
	Negative	False Positive (FP)	True Negative (TN)

Figure 3. Confusion matrix

Where, TP is true positive, TN is true negative, FP is false positive and FN is false negative, p_o is the empirical probability of agreement on the label assigned to any sample, p_e is the expected agreement when both annotators assign labels randomly.

RESULTS AND DISCUSSIONS

The average raw spectra from the extracted AOI of the pulp are shown in Figure 4. With the change in path length due to the different sizes of the sample, the raw spectra showed a baseline shift. Therefore, the SNV preprocessing was applied in the raw spectra to improve the spectral characteristics. The SNV pretreated spectra show a dominant peak of water at the wavelength of 970 nm and 1205 nm (Figure 5). This wavelength represents O-H stretching due to the first overtone of water. Also, at 1450 nm there is a significantly lower reflectance band which represents the band of water and starch.

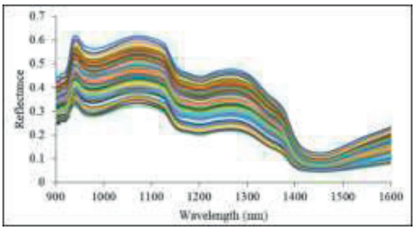


Figure 4. Average raw spectra of durian pulp

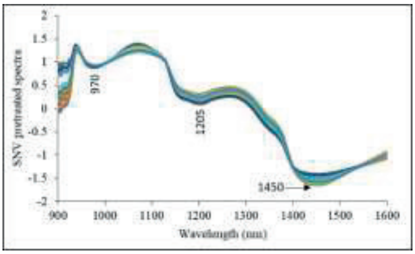


Figure 5. SNV pretreated spectra of durian pulp

Table 1 shows the selection of hyperparameters for SVM, RF, and kNN using Bayesian optimization.

Table 1. Selection of hyperparameters for SVM, RF, and kNN using Bayesian optimization

Classifiers		Hyperparmeter		
Support vector machine		C	gamma	Kernel function
	Input	0.01-1000	0.1-100	Radial base function, Linear, Polynomial
	Output	53	0.4	Radial base function
Random forest		n-estimator	Maximum feature	Maximum depth
	Input	25-500	2-20	2-20
	Output	93	8	14
k nearest neighbor		n- neighbor		Distance
	Input	2-20		Euclidean (default)
	Output	10		Euclidean

The best classification performance from the optimized hyperparameter classification models from SVM, RF, and kNN in terms of training accuracy, cross-validation accuracy, and test accuracy is shown in Table 2. SVM showed the highest test accuracy (91.8%) among three machine learning classifiers. The training accuracy of RF was significantly (100%) higher but the 3 fold cross-validation (86.8%) and test accuracy (84.6%) do not show the good performance of the RF model. Meanwhile, kNN obtained the lowest accuracy among all the machine learning classifiers.

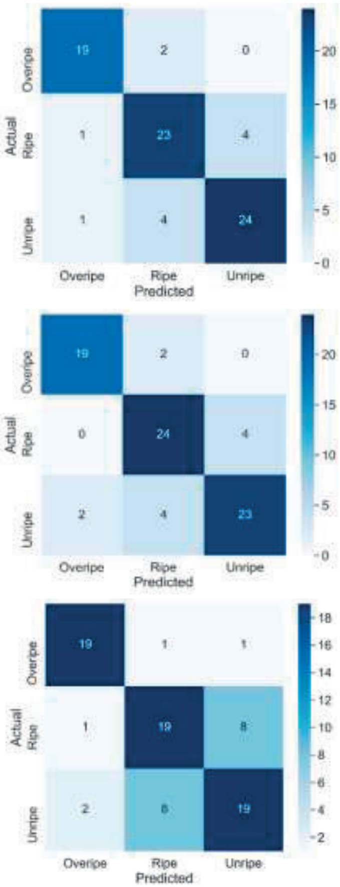
Table 2. Performance of machine learning classifiers using Bayesian optimization

Classifiers	Training accuracy	3-fold cross validation accuracy	Overall accuracy
Support vector machine	91.8%	87.9%	88.5%
Random forest	100%	86.8	84.6%
k nearest neighbour	84.1%	81.3%	73.1%

Table 3 shows the precision, recall and kappa coefficient of test set. Figure 6 shows the confusion matrix from each ML classifiers. The number of overripe, ripe and unripe in the test set were 21, 28 and 29, respectively, which is represented in confusion matrix. The precision, recall and kappa coefficient of overripe samples are higher for all classification models. With the advancement of the maturity stage, the moisture content of the pulp decrease. The significant difference in the moisture content between overripe samples and the other samples may have resulted in classification metrics value of overripe samples. The inter-class confusion between ripe and unripe can be seen for all the classification models with more classification error in the case of kNN. The similar spectral characteristics of ripe and unripe sample must have influenced the classification performance of kNN.

Table 3. Precision, recall and kappa coefficient obtained from the classification model by different machine learning classifiers

Classifiers		Precision	Recall	kappa coefficient
Support vector machine	Overripe	0.95	0.95	0.83
	Ripe	0.86	0.86	
	Unripe	0.86	0.86	
Random forest	Overripe	0.90	0.90	0.77
	Ripe	0.80	0.86	
	Unripe	0.85	0.79	
k nearest neighbor	Overripe	0.86	0.90	0.59
	Ripe	0.68	0.68	
	Unripe	0.68	0.66	



(a)

(b)

(c)

Figure 6. Confusion matrix from classification model of support vector machine (a), random forest (b), and k nearest neighbors (c)

Several supervised and unsupervised classification algorithms have been used for the maturity classification of fruits. The ripeness classification model of pear using short near-infrared (425-1000 nm) by three classification model soft independent modeling of class analogy (SIMCA), LDA, and PLS-DA were compared by Khodabakhshian & Emadi, 2018. The overall classification result showed that PLS-DA attained the best correct classification accuracy of 87.86% for pear ripeness classification (Khodabakhshian & Emadi, 2018). Hyperspectral image of navel oranges was obtained using the diffuse transmittance imaging-based system and a multispectral index was developed to identify the maturity with the hyperspectral technique by Wei et al., 2017. Linear discriminant analysis and kNN were used to classify the three maturity stages of navel orange, among which kNN attained the highest correct classification rate of 96.0%. Classification of maturity stages of cherry fruits by using the HSI system in the NIR region (874-1734 nm) was done by Li et al., 2018. The result showed the best correct classification ratio of 96.4% by the LDA classifier (Li et al., 2018). For different fruits, different machine learning algorithms showed the best results for maturity classification.

Durian maturity was evaluated by minimal destruction based on electrical impedance measurement (Kuson & Terdwongworakul, 2013). According to the findings of Kuson & Terdwongworakul (2013), selected impedance parameters using a stepwise regression could be used to classify durian samples into an immature class and mature class with less accuracy of 83.3%. Similarly, the research done by Timkhum & Terdwongworakul (2012) showed a good result to identify the characteristic changes in the durian spine with maturity using visible spectroscopy. Their result showed the best accuracy of 94.7% into only four maturity classes from 113-134 DAA. Tantisopharak et al. (2016) identified the potential of natural frequency due to electromagnetic scattering properties of durian to identify the fruit maturity. The findings shows that, the changes in natural frequencies were associated with the maturity of the fruit which resulted in a variation of resonant frequencies for classification of durian according to its maturity stages (Tantisopharak et al., 2016).

Several researches have been conducted on durian intact fruit and pulp for maturity identification and internal properties evaluation. However, the research using the HSI system had not been performed for the maturity classification of durian pulp. The results from this research show the potential to use the HSI system for ripeness classification. The result from SVM shows an overall accuracy of 88.5%, which is comparable to other researches that have been done.

CONCLUSIONS

The potential of the HSI pushbroom system with a spectral range from 900-1600nm for ripeness classification of durian pulp was evaluated in this research. The mean spectra extracted from the area of interest were preprocessed using SNV. Three machine learning classifiers: SVM, RF, and kNN were used for developing the ripeness classification of durian pulp where Bayesian optimization was used for tuning the the associated hyperparameters. SVM showed best classification with the training, cross-validation, and overall accuracy of 91.8 %, 87.9%, and 88.5%, respectively. The result shows the potential of the HSI system combined with machine learning algorithms for the ripeness classification of durian pulp. However, the overall accuracy should be improved further using more samples and applying other machine learning as well as deep learning approaches. Different wavelength selection algorithms: successive projection algorithm (SPA), genetics algorithm (GA), and competitive adaptive reweighted sampling (CARS), will be applied further for feature extraction and dimensionality reduction for improvement of classification performance.

ACKNOWLEDGEMENTS

The author would like to acknowledge NIR Spectroscopy Research Centre for Agricultural Product and Food (www.nirsresearch.com) and the Department of Agricultural Engineering, Faculty of Engineering, Kasetsart University Kamphaengsaen Campus for providing the necessary equipment for conducting the experiment. We would like to acknowledge King Mongkut's Institute of Technology Ladkrabang for the support.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MONITORING OF *POLISTIGMA RUBRUM, TRANZSCHELIA PRUNI SPINOSE, STIGMINA CARPOPHILA* IN PLUM ROOTSTOCK-CULTIVAR COMBINATIONS FOR THE TROYAN REGION

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Abstract

The study was conducted in the period 2017-2019 in an intensive plum plantation of the RIMSA Troyan. The susceptibility of the rootstock combinations to Polistigma rubrum (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis) *was studied. The infectious process of infection and development was monitored and the degree of attack of Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis)*, in plum rootstock combinations for the climatic conditions of the region of Troyan were reported. The climatic factors - temperature and precipitation during the infection and manifestation of the diseases have a significant influence on the degree of damage. Studies have found that in all treatments of the study, the highest values for the degree of attack of Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis) *were reported for 2019, due to the fact that the year is characterized by heavy and prolonged rainy periods, especially the month of June (234.6mm). During the 3 years of the study, the rootstock combinations with Wavit were characterized by a high attack index for the three diseases Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis)*. Stigmina carpophila occurs exclusively in the treatments with Brompton for the cultivars 'Hanita' and 'Stanley' and in the rootstock Wavit in 'Cacanska Lepotica' and 'Jojo'. The lowest degree of attack has the cultivar 'Hanita' (average 23%), and the highest is in 'Cacanska Lepotica' on Wavit (46.4%).*

Key words: *Plum, rootstock combinations, fungal diseases.*

INTRODUCTION

Fungal diseases, such as *Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis) occur annually in plum orchards in Bulgaria and everywhere in European countries (Alexandru et al., 2019; Molnár et al., 2018; Stoyanova et al., 2016; Miter et al., 2015b; Borovinova, 2002).

These are common diseases in the conditions of natural infection for the climatic conditions of a given area and cause significant losses.

Usually these diseases can be effectively managed by combining cultural technologies and growing resistant cultivars.

Proper disease management involves the selection and planting of genetically resistant cultivars. Iliev and Stoev (2011) show that *Polystigma rubrum* (Persoon) is one of the most common diseases on plums.

Vitanova et al. (2004) define 'Stanley' and 'Anna Spath' as tolerant to red leaf spots in the conditions of Bulgaria for the period 1996-2003.

There is an extremely strong manifestation of fungal diseases in years with high temperature, high humidity, heavy rainfall and intense clouds. Oroian et al. (2010) study, plantations from the Cluj region, Romania (2008 and 2009), on the emergence of specific pathogens in apples, pears and plums, taking as a main factor the average annual temperature, with the same phytosanitary measures in all variants.

As a result, it is reported that the attack and losses from fungal diseases increased in 2009, when it was warmer than in 2008 (the average temperature is 0.5°C higher), under the same conditions of maintenance and phytosanitary measures.

With additional studies to establish the correlation between climatic factors, represented mainly by temperature and the degree of attack of pathogens, appropriate strategies for integrated tree protection could be developed.

Polistigma rubrum (Persoon) De Candolle occurs annually and depends on the climatic conditions in the spring, when the infection occurs, causes greater or lesser damage to the plum leaves, which leads to a decrease in the quantity and quality of the plum crop and weakening of the plum trees.
According to Mitre (2015a), 'Anna Späth', 'Vinete romanesti', 'Vinete de Italia' and 'Tuleu timpuriu' had a high degree of attack by *Polystigma rubrum* (Persoon). These varieties have been shown to be very susceptible to *Polistigma rubrum* natural conditions of infection. The lowest attack rates of *Polystigma rubrum* were found in Top End, Jojo, Tophit, Toptaste, and Topfirst. The same cultivars are weakly attacked by *Stigmina carpophila* (Lev Ellis) (5.3-7.3% attack rate). These low levels of infection recommend the mentioned cultivars as possible donors in plum breeding programs, to create new genotypes with a good response to disease attack.
In another study by the same authors, Mitre et al. (2015b) under conditions of natural infection *Tranzschelia pruni-spinosae* (Persoon) Dietel studied the response of 13 plum cultivars to certain fungal diseases in Transylvania. With the best results, ie. low levels of rust infection were found in Jojo, Tophit, Topend, Anna Späth,
In UK orchards, O'brien and Berrie (2019) also studied plum rust caused by the fungus *Tranzschelia discolor*, which attacks plants of the genus *Prunus*. The first symptoms of rust are usually seen in July, although in years with hotter summers this can be delayed until August. Fungal spores are able to survive the winter in twigs or leaves, which means that this disease can continue for several growing seasons if sufficient control measures are not taken. Plum rust infections are most detrimental to yields when they appear at the beginning of the growing season from infected leaves. They greatly reduce their photosynthetic capacity and defoliate early, thus reducing the carbohydrate capacity of the tree, which causes lower fruit production the following year.
Soylu et al. (2003) observed rust in the eastern Mediterranean region of Turkey, where they encountered defoliation of plum trees (*Prunus cerasifera*) as a result of rust infections. The disease is observed in almost all orchards in the region on the leaves, but not on the fruits or twigs. The early symptoms of the disease are observed in late May, as distinct angular bright yellow lesions on the upper part of the leaves. By early September, the leaf lesions become dark brown.
Molnar et al. (2018), in a two-year study, determined the susceptibility of four plum cultivars to two fungal pathogens of plum (*Stigmina carpophila* (Lev Ellis) and *Polystigma rubrum* (Persoon) in two planting schemes (4 x 1.5 and 6 x 3). The results show that cvs 'Bluefre' and 'Stanley' were not affected in August 2016. In 'Čačanska Lepotica', the incidence was over 50% in both variants 2016. There is no significant difference between the two density systems.
Due to the significance of plum production for the Republic of Bulgaria and the damage caused by fungal diseases, it is important to determine the strength of their attack on plums during years with different climatic conditions. The obtained results can be used to determine the optimal period for application of pesticides in order to prevent the presence of diseases and to control them.
The objective of the present study was to trace the infection process and development and to register the degree of attack of *Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis), in plum cultivar rootstock combinations for the climatic conditions of the region of Troyan.

MATERIALS AND METHODS

The study was conducted in the period 2017-2019, in soil and climatic conditions of the region of Troyan. The altitude is 380 m, the exposure is west, with a slope of about 5-8°. The plantation was established in 2005 under the 4 x 1.7 m scheme, by planting in trenches trees of cultivars, such as 'Stanley', 'Čačanska Lepotica', 'Hanita' and 'Jojo', grafted on rootstocks, such as Brompton, GF 655-2, SJ A, Wavit, Wangenheims and *P. cerasifera* (control).
The crowns are free-growing and are maintained with annual winter pruning, the soil surface is covered with turf. The experimental plants are grown under non-irrigated

conditions, which are typical for the cultivation of plums in mountain areas.

Data on temperatures and precipitation, which are essential for the development of diseases, were gathered by the Meteorological Station of the Research Institute in Troyan.

The visual observation method was used to identify the diseases based on signs and symptoms shown by infected plants. The pathogens *Polistigma rubrum* (Persoon) De Candolle, *Tranzschelia pruni spinose* (Persoon) Dietel, *Stigmina carpophila* (Lev Ellis) were followed in this study. Each cultivar-rootstock combination is a treatment. The samples were taken 200 leaves from the middle floor by the 4 directions form 4 trees (replications).

The attack of the studied diseases was reported on the respective ball scales (Nedev et al., 1979), according to the spotting of the leaf blade. Mc Kenney's (1923) formula adopted in phytopathology was used to calculate the attack degree.

$$I=\Sigma \frac{n.k}{N.K} 100,$$

where:

I - infestation index of disease in %;

n - number of infested leaves of the respective degree;

k - the number of the degree;

N - number of degrees;

K - number of all reported leaves.

RESULTS AND DISCUSSIONS

For the course of the infectious process of the causative agent of red leaf spots *Polystigma rubrum* De Candolle, the climatic factors of the months of April and May cause a direct impact. A characteristic feature of *Polystigma rubrum* (Persoon) De Candolle is that it's monocyclic, i.e. there are no secondary infections. Ascospores mature and shoot almost together over a period of 2-4 weeks at temperatures of 10 to 26ºC and precipitation. Symptoms, such as relatively large-sized, rounded spots, which colour gradually changes during the growing season from pale green, pale yellow, red to blood red, concave on the upper and convex on the lower side, were observed only on young growing leaves up to 40 days of age. The tissue of the spots was 2-4 times thicker.

The spring months of 2017 are characterized by abundant rainfall, for the months of April (90 mm) and May (133 mm) and high average monthly T°C April 18°C, May 21°C (Figure 1). This favors the infection and development of *Polystigma rubrum* (Persoon) De Candolle.

The highest infestation index was registered in 3 of the variants of 'Čačanska Lepotica' (Brompton, Wangenheims) (28.7%) and SJ A (26%), with 'Jojo' ranging from 6% (Brompton) to 25.3% (Wavit), and with 'Hanita' from 2% to 20%.

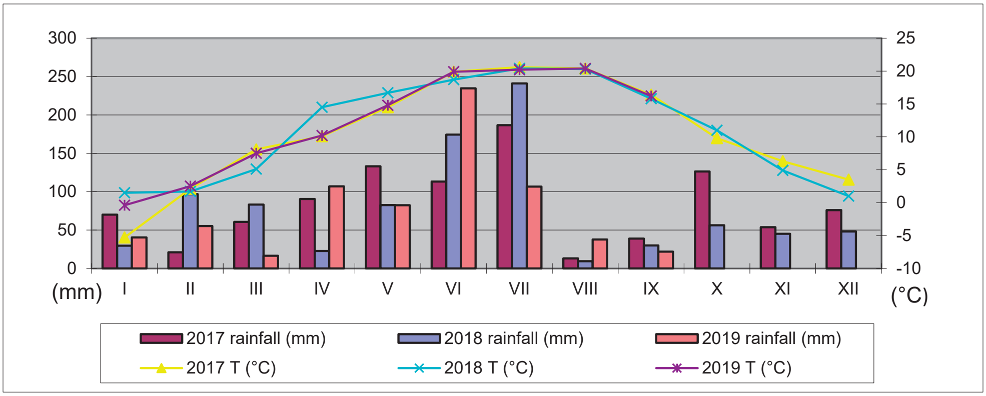

Figure 1. Climatic conditions (2017-2019)

In 2018, the reported infestation index was significantly lower than in 2017, due to climatic conditions, namely low rainfall (April - 22 mm, May - 82 mm) (Figure 1). An infestation of the disease was reported in one variant of 'Hanita' (SJ A 4.00%), in 'Čačanska lepotica' from 0.67% (Brompton) to 5.33% (Wangenheims) and in 'Jojo', on Wavit

(4.67%), and in the control *P. cerasifera* (1.33%).
For the conditions of 2019, no manifestation of the disease was registered in all studied variants. The low average monthly T(°C) (for April 10°C, for May 14°C) proved to be insufficient for mass infection and development of *Polystigma rubrum* (Persoon) De Candolle, despite the more precipitation, April 106 mm and May 82 mm, respectively.
'Stanley' was not affected by *Polystigma rubrum* (Persoon) in any of the years studied, in any of the variants (Figure 2).

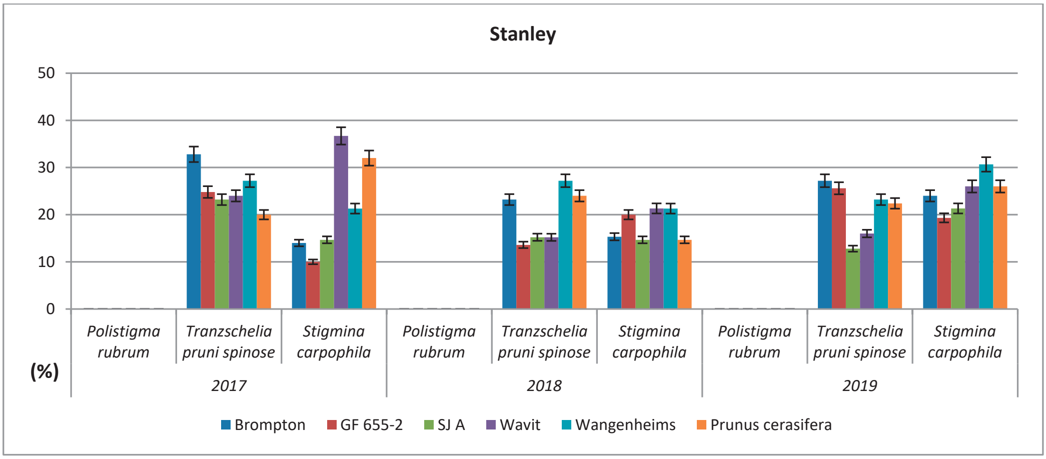

Figure 2. *Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis) degree of attack (%) on 'Stanley' (2017-2019)

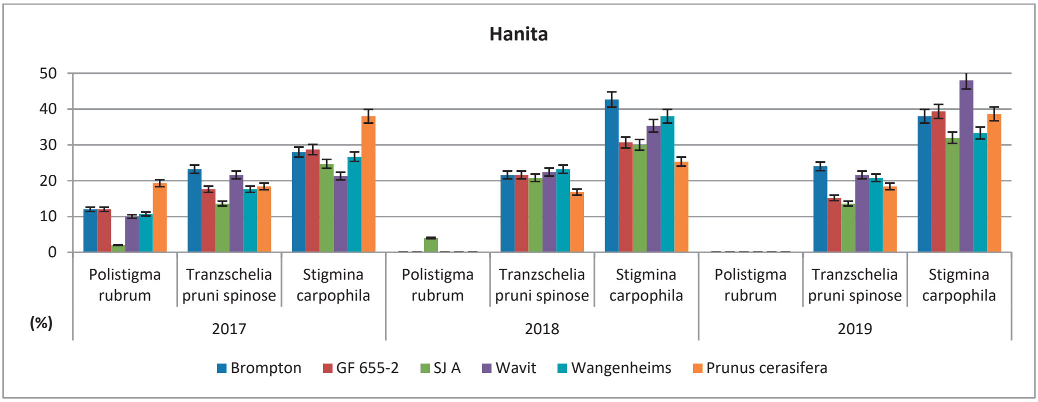

Figure 3. *Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis) degree of attack (%) on 'Hanita' (2017-2019)

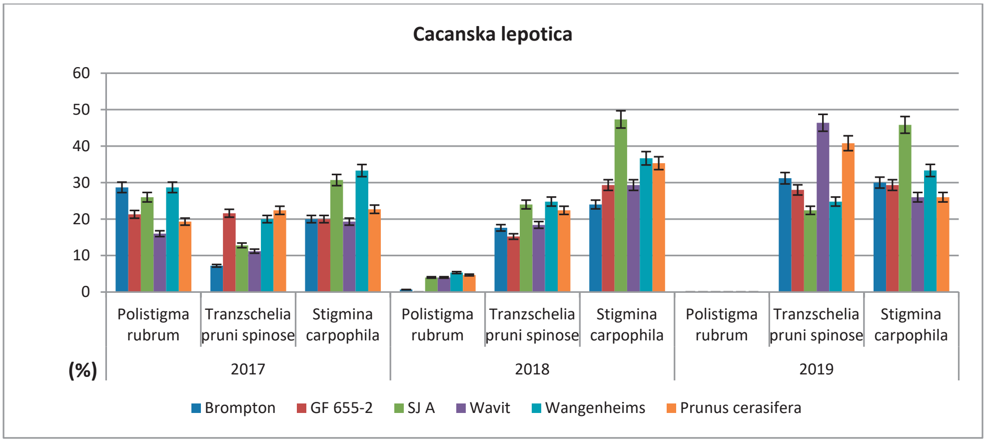

Figure 4. *Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel, *Stigmina carpophila* (Lev Ellis) degree of attack (%) on 'Cacanska Lepotica' (2017-2019)

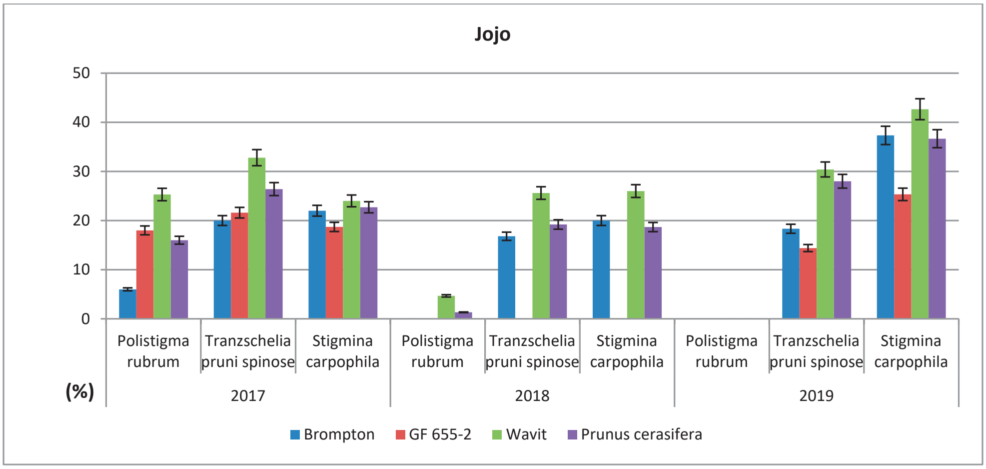

Figure 5. *Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel, *Stigmina carpophila (*Lev Ellis) degree of attack (%) on 'Jojo' (2017-2019)

The symptoms leaf rust of the pathogen ***Tranzschelia pruni spinose* (Persoon) Dietel** in plum cultivar rootstock combinations were observed on the leaves, on which small-sized, angular, yellowish or violet-red spots can be seen on the upper side. On the underside below them, in summer, uredosorus with light brown uredospores are formed, and in the autumn (September-October) black teliosorus with teliospores.

Infection in the spring passed at average monthly temperatures for April and May 20-23°C and humid weather (Figure 1).

Transzchselia pruni spinozae (Persoon) Dietel differs from other fungal diseases with its large uneven distribution during the current growing season, due to the strong influence of climatic conditions during the study period. The first symptoms of *Tranzschelia pruni spinose* (Persoon) Dietel were registered at the end of June, the disease developed more strongly in August, and the peak of the attack was reported in September and October. An exceptional manifestation of rust was reported in all rootstock combinations during the three years of the study.

For 'Stanley' on Brompton rootstock, the highest infestation index was in 2017 (32.8%) and 2019 (27.2%) (Figure 2).

The same trend was observed in 'Hanita' on Brompton in 2017 (23%) and 2019 (24%), as the lowest infestation index values of

susceptibility to rust for the three years were observed in ‘Hanita’ in all variants (*Tranzschselia pruni spinozae* (Persoon) Dietel) (average for the three years 20%) (Figure 3).
For ‘Čačanska Lepotica’ the data are different, the highest index was observed on Wavit rootstock - for 2019 - 46.4% (Figure 4).
In the case of ‘Jojo’ cultivar for the three years, Wavit rootstock also had the highest index (average 30%) (Figure 5).

***Stigmina carpophila* (Lev Ellis)**

The development of fungal shot hole disease (*Stigmina carpophila)* (Lev Ellis) on the leaves was observed almost throughout the growing season. The meteorological conditions in the region of Troyan in the months of April, May and June and during the three years of the study created favourable conditions for mass infection and strong development of the pathogen on the leaves in the observed rootstock combinations. The mass infestations took place at temperatures of 18-22°C and significant precipitations (Figure 1), as small-sized reddish-spotted spots were formed on the leaves, which reached 2-5 mm with a light central part. A characteristic feature is the appearance of a reddish-purple wreath around the spot. In the more susceptible cultivar rootstock combinations, due to the higher degree of infestation, the spots acquired an irregular shape, as the tissue in the center necrotized, fell off and we observed round perforations on the infested leaves.
For 2017 the highest indices for shot hole disease were reported for ‘Hanita’, on *P. cerasifera* (38.00%) (Figure 3), for 2018 ‘Čačanska Lepotica’ on SJ A (47.33%) (Figure 4), for 2019 ‘Hanita’/Wavit (48.00%) and ‘Jojo’/Wavit (42.66%).
In all variants of the study, the highest values for the *Stigmina carpophila* (Lev. Ellis) infestation index were reported in 2019, due to the fact that the pathogen is most common in years with heavy and prolonged rainy periods, such as June 2019. The amount of precipitation was 234.6 mm (Figure 1). This led to the creation of optimal conditions for the course of the infectious process and mass infections in almost all studied rootstock combinations.
The lowest values of the index were registered in the control cultivar ‘Stanley’ throughout the study period (Figure 2).
The highest index for the three years was reported for ‘Čačanska Lepotica’ for all rootstock variants, as in the combination with SJ A rootstock the maximum values were registered (30.70-47.30%) (Figure 4).

CONCLUSIONS

The climatic factors during the period of infection and development had a direct impact for the course of the infectious process of the causative agents of *Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis),
In all survey treatments, the highest values of the degree of attack of *Polistigma rubrum* (Persoon) De Candolle*, Tranzschelia pruni spinose* (Persoon) Dietel*, Stigmina carpophila* (Lev Ellis) were reported in 2019, due to the fact that the year is characterized by heavy and prolonged rainy periods, especially in June (234,6mm).
In 2018, the degree of attack of *Polistigma rubrum (*Persoon) De Candolle was lower than in 2017, due to climatic conditions, namely low rainfall.
For the period 2017-2019, *Tranzschelia pruni spinose* (Persoon) Dietel occured exclusively for ‘Hanita’ and ‘Stanley’ cultivars on Brompton rootstock and in ‘Čačanska Lepotica’ and ‘Jojo’ on Wavit rootstock. The lowest degree of infestation was registered in ‘Hanita’ on average 23%, and the highest is in 'Čačanska Lepotica’ on Wavit (46.4%).
‘Stanley’ and ‘Jojo’ can be defined as less attacked by the *Stigmina carpophila* (Lev Ellis), compared to ‘Hanita’ and ‘Čačanska. Lepotica’. The highest degree of attack was registered in ‘Čačanska lepotica’ for the whole period (2017-2019) especially on SJ A rootstock (30.70 - 47.30%). During the study period, rootstock combinations Wavit rootstock were characterized by a high degree of attack for *Stigmina carpophila* (Lev Ellis).

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VARIABILITY OF SOME *ROSA CANINA* L. GENOTYPES FROM SOUTHERN AREA OF OLTENIA

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Abstract

The aim of this paper was to identify valuable rosehip genotypes in the southern part of Oltenia region, Romania. Selected genotypes were studied in terms of fruit morphological characteristics such as fruit height, fruit diameter, fruit weight, fruit shape index and fruit volume. The height of the fruits varied between 11.35-32.14 mm, the diameter between 9.12-18.04 mm, the weight between 0.63-4.81 g, the fruit shape index between 0.92-2.25, the volume of 100 fruits between 130.00 cm^3 and 361.54 cm^3. The coefficient of variation in terms of fruit height varied between 6.41% and 18.99%, fruit diameter between 4.93% and 8.94%, fruit weight between 11.46% and 28.04%, fruit shape index between 5.51-14.37% and fruit volume was 31.49%. Knowledge of fruit morphological characteristics is important for the selection of biotypes that can be furthered used successfully in breeding programs but also in food and medicine industry.

***Key words**: dog rose, fruits, morphology, rosehips.*

INTRODUCTION

The genus *Rosa* includes over 100 species globally, widespread especially in Europe, Asia, Middle East and North America (Ercisli, 2007; Mármol et al., 2017). *Rosa canina* L., dog rose, is part of the *Rosaceae* family and is a deciduous shrub, decorative in flowers and fruits, climbing up to 2-3 m tall, with strong thorns, flowers (blooming in May - July) with 5 white or pink petals many stamens and pistils in a hypanthium and alternate serrate compound leaves. The pseudo-fruits, bright red hips resulting from thickening of the receptacle, are ellipsoidal, glabrous, edible, rich in vitamins important for human consumption. Inside the fruits are numerous hairy achenes, improperly called seeds. Dog rose can be found at the edge of forests, in fields, up to the bottom of the mountains, old pastures and at the edge of roads. Fruit species found in spontaneous flora have been used both for food and for medicinal purposes, due to their high content of bioactive compounds with antioxidant and antimicrobial action (Mármol et al., 2017; Cosmulescu et al., 2020). *Rosa canina* L. is used as a rootstock for roses, and for that, aromatic fruit genotypes with a high percentage of pulp, few thorns and numerous stems are preferred (Kazankaya et al., 2005). It is also a source of food during the cold season for many species of birds and mammals, this being the main vector of seed dispersal. In addition to being self-fertile, pollination is also made by insects. It is an unpretentious species to climatic conditions and adapts very well to almost any type of soil. Prefers sunny exposures, does not tolerate shading and the soil needs to have a good drainage. The large phenotypic adaptability of the species while many are remarkably polymorphic with different geographical biotypes have attracted the interest of many researchers (Singh et al., 2020; Stoenescu & Cosmulescu, 2020; Popa et al., 2020). The morphological characteristics have been frequently studied by many authors, and this paper aims to add information on existing biotypes found in the southern part of Oltenia region, Romania, important for both food and medicine industry and also for breeding programs of valuable genotypes.

MATERIALS AND METHODS

21 genotypes were selected for fruit harvesting taking into account the quality and quantity of fruits obtained per individual. Determinations were made regarding the maximum height of the shrub with a measuring tape, the existing stems within each genotype were counted, while the geographical coordinates and altitude were determined with GoogleEarth application. 100 carefully selected fruits from each genotype were harvested during their ripening period for morphological determinations. The height and diameter of the fruits were determined with a digital caliper (accuracy ± 0.1 mm), the weight of the fruit with the analytical balance Radwag AS220.R2, and the volume of 100 fruits was determined using a graduated cylinder. The fruit shape index (FSI) was calculated by the ratio between the fruit height (length) and the fruit diameter, and according to Brewer et al. (2006), a value higher than 1.00 indicates elongated fruits, equal to 1.00 indicates round fruits and a value lower than 1.00 indicates flattened fruits. The obtained data was processed in Excel descriptive statistics and represents the mean (X), standard deviation (SD), variation limits and coefficient of variation calculated with the formula CV% = SD/X*100.

RESULTS AND DISCUSSIONS

The phenotypic characteristics of the genotypes can be found in Table 1. All studied genotypes are classified by habit as shrubs, with many thorns, having an average height of 212.52 cm with a minimum value of 109.00 cm at genotype 19 and a maximum value of 420.00 cm at genotype 6. The number of stems ranged from 5 at genotype 17 to 27 at genotype 2 with a mean value for this characteristic of 13.52. The coefficient of variation for these two characteristics was 48.00% for the number of stems and 39.61% for the plant height. The high coefficient of variability indicates differences from one genotype to another, an aspect that can be influenced by the age of the plant, climatic conditions, land exposure, proximity to the groundwater or in some cases even the nearby watercourses. The altitude varied between 54 m and 61 m, studied genotypes being located at the edge of a forest ecosystem. Other representative fruit species located near dog rose was *Prunus spinosa* L. and *Crataegus monogyna* (L.) Jacq. Fruits are the useful part of the plant, with food and medicinal importance, therefore, their quality, expressed by morphological characteristics (size, weight) is important for the selection procedures. The morphological determinations of the fruits can be found in table 2. The height of the fruits varied between 11.35 mm corresponding to genotype 11 and 32.14 mm at genotype 20, with an average for this characteristic of 21.43 mm.

Table 1. Phenotypic characteristics of the identified *Rosa canina* L. genotypes

Genotype	Number of stems	Plant height (cm)	GPS coordinates	Altitude (m)
1	7	254	44°06'N 23°53'E	56
2	27	286	44°07'N 23°54'E	54
3	7	410	44°06'N 23°53'E	55
4	11	196		56
5	14	245		57
6	18	420		61
7	21	203	44°06'N 23°54'E	54
8	9	149		54
9	23	262		54
10	11	214		57
11	9	194		55
12	8	157		54
13	20	267		54
14	7	138		54
15	17	170		54
16	18	159	44°07'N 23°53'E	56
17	5	110		56
18	23	205		56
19	11	109		55
20	9	177	44°06'N 23°53'E	55
21	9	138	44°07'N 23°53'E	56
X±SD	13.52 ± 6.49	212.52 ± 84.20		
Variation limits	5 - 27	109.00 - 420.00		
CV%	48.00	39.61		

The fruits diameter had values between 9.12 mm at genotype 12 and 18.04 mm at genotype 20 with an average value for this parameter of 13.00 mm. The results are similar to those obtained by Demir & Özcan (2001) who had an average fruit height of 17.29 mm and 19.68 mm and an average fruit diameter of 11.16 mm and 13.20 mm for fruits from spontaneous flora genotypes in two Turkey regions. At the same time, Kazankaya et al. (2005) reported at dog rose selections in eastern Anatolia, Turkey, values between 12.30-44.30 mm for fruit height and 10.30-26.60 mm for fruit diameter which are larger than those obtained in this study.

Table 2. Morphological characteristics of studied *rosehips* genotypes

Genotype	Descriptive statistics	Fruit height (mm)	Fruit diameter (mm)	Fruit weight (g)	Fruit shape index (FSI)	Volume/100 fruits (cm^3)
1	X±SD	23.10 ± 1.82	13.41 ± 1.01	2.26 ± 0.39	1.73 ± 0.14	180.85
	Variation limits	19.50 – 26.52	11.66 – 16.71	1.61 – 3.50	1.46 – 1.95	
	CV%	7.87	7.53	17.25	8.09	
2	X±SD	22.11 ± 2.78	12.71 ± 0.89	1.93 ± 0.39	1.74 ± 0.20	200.00
	Variation limits	17.19 – 27.28	10.87 – 15.48	1.19 – 3.14	1.38 – 2.20	
	CV%	12.57	7.00	20.20	11.49	
3	X±SD	19.20 ± 2.32	12.55 ± 0.80	1.64 ± 0.30	1.53 ± 0.16	164.00
	Variation limits	14.47 – 25.71	10.90 – 14.40	1.11 – 2.67	1.12 – 1.82	
	CV%	12.08	6.37	18.29	10.45	
4	X±SD	18.02 ± 1.86	12.65 ± 0.88	1.67 ± 0.31	1.43 ± 0.13	156.00
	Variation limits	11.78 – 21.69	10.49 – 14.68	1.12 – 2.34	0.92 – 1.80	
	CV%	10.32	6.95	18.56	9.09	
5	X±SD	19.91 ± 1.50	13.48 ± 0.94	2.06 ± 0.30	1.48 ± 0.12	137.50
	Variation limits	17.92 - 23.67	11.48 – 15.07	1.51 – 2.59	1.30 – 1.77	
	CV%	7.53	6.97	14.56	8.10	
6	X±SD	26.65 ± 1.71	14.15 ± 1.13	3.03 ± 0.58	1.89 ± 0.13	300.00
	Variation limits	23.78 – 30.72	11.92 – 16.80	2.10 - 4.32	1.62 – 2.25	
	CV%	6.41	7.98	19.14	6.87	
7	X±SD	17.89 ± 1.98	11.44 ± 0.81	1.35 ± 0.30	1.56 ± 0.12	130.00
	Variation limits	14.75 – 22.26	9.75 – 13.49	0.90 – 2.09	1.24 – 1.89	
	CV%	11.06	7.08	22.22	7.69	
8	X±SD	19.82 ± 2.41	13.31 ± 1.19	1.94 ± 0.50	1.49 ± 0.14	166.67
	Variation limits	15.74 – 25.33	11.23 – 15.94	1.23 -3.11	1.32 – 1.88	
	CV%	12.15	8.94	25.77	9.39	
9	X±SD	21.23 ± 1.51	11.75 ± 0.58	1.57 ± 0.18	1.81 ± 0.14	156.00
	Variation limits	16.59 – 24.20	10.57 – 13.35	1.16 – 2.02	1.46 – 2.08	
	CV%	7.11	4.93	11.46	7.73	
10	X±SD	25.87 ± 2.27	15.13 ± 0.85	2.87 ± 0.43	1.71 ± 0.15	300.00
	Variation limits	19.24 – 29.75	13.42 – 17.42	2.20 – 3.77	1.30 – 1.98	
	CV%	8.77	5.61	14.98	8.77	
11	X±SD	20.27 ± 3.85	12.36 ± 0.95	1.64 ± 0.46	1.67 ± 0.24	176.79
	Variation limits	11.35 - 28.04	10.43 – 15.20	1.00 – 2.89	1.02 -2.08	
	CV%	18.99	7.68	28.04	14.37	
12	X±SD	18.97 ± 2.22	11.40 ± 0.97	1.35 ± 0.33	1.65± 0.19	138.00
	Variation limits	13.56 – 24.41	9.12 – 13.52	0.63 – 2.14	0.95 – 1.93	
	CV%	11.70	8.50	24.44	11.51	
13	X±SD	20.50 ± 2.76	11.50 ± 0.80	1.48 ± 0.31	1.79 ± 0.23	137.50
	Variation limits	13.66 – 26.21	9.57 – 12.94	0.93 – 2.33	1.11 – 2.11	
	CV%	13.46	6.95	20.94	12.84	
14	X±SD	22.25 ± 1.75	13.05 ± 0.69	1.97 ± 0.25	1.71 ± 0.13	170.00
	Variation limits	18.50 – 26.08	11.20 – 14.26	1.37 – 2.47	1.37 – 1.98	
	CV%	7.86	5.28	12.69	7.60	
15	X±SD	20.17 ± 2.35	12.65 ± 1.12	1.73 ± 0.41	1.60 ± 0.18	180.00
	Variation limits	16.27 – 25.24	11.00 – 16.04	1.08 – 2.88	1.30 – 1.93	
	CV%	11.65	8.85	23.69	11.25	
16	X±SD	21.77 ± 2.54	13.11 ± 0.74	2.07 ± 0.38	1.66 ± 0.15	205.36
	Variation limits	17.00 - 27.92	11.48 – 14.79	1.46 – 2.91	1.31 – 1.97	
	CV%	11.66	5.64	18.35	9.03	

17	X±SD	21.90 ± 1.54	13.37 ± 0.94	2.19 ± 0.35	1.64 ± 0.12	225.00
	Variation limits	18.80 – 25.20	11.59 – 15.56	1.67 – 2.89	1.44 – 1.90	
	CV%	7.03	7.03	15.98	7.31	
18	X±SD	19.68 ± 1.98	13.60 ± 1.07	2.02 ± 0.45	1.45 ± 0.08	202.38
	Variation limits	16.54 – 24.35	11.42 – 15.70	1.28 – 3.07	1.28 -1.66	
	CV%	10.06	7.86	22.27	5.51	
19	X±SD	19.40 ± 2.31	12.12 ± 0.86	1.66 ± 0.36	1.60 ± 0.16	154.39
	Variation limits	14.92 – 24.78	10.18 – 13.95	0.87 – 2.81	1.30 – 1.99	
	CV%	11.90	7.09	21.68	10.00	
20	X±SD	27.02 ± 2.94	16.10 ± 0.92	3.48 ± 0.52	1.68 ± 0.19	361.54
	Variation limits	21.04 – 32.14	13.88 – 18.04	2.66 – 4.81	1.28 – 2.17	
	CV%	10.88	5.71	14.94	11.30	
21	X±SD	22.14 ± 1.73	13.78 ± 0.89	2.36 ± 0.39	1.61 ± 0.12	180.00
	Variation limits	17.90 – 25.28	12.22 – 16.59	1.77 – 3.29	1.31 – 1.85	
	CV%	7.81	6.45	16.52	7.45	
All genotypes	X±SD	21.43 ± 3.53	13.00 ± 1.53	2.01 ± 0.70	1.65 ± 0.20	191.52 ± 60.32
	Variation limits	11.35 – 32.14	9.12 – 18.04	0.63 – 4.81	0.92 – 2.25	130.00 – 361.54
	CV%	16.47	11.76	34.82	12.12	31.49

Another study with similar results to those obtained was performed by Celik et al. (2009) which reported values between 23.53-33.83 mm for fruit height and 13.11-18.40 mm for fruit diameter while Dogan & Kazankaya (2006) reported in the basin of Lake Van, Turkey an average fruit height of 20.70 mm and an average fruit diameter of 13.20 mm. Other similar results were obtained by Rosu et al. (2011) with an average fruit height of 25.00 mm in genotypes from the northeastern part of Romania, in Erzurum, Turkey, Ercişli & Eşitken (2004) had fruit height results between 23.40-34.36 mm and a fruit diameter between 15.30-21.14 mm while Šindrak et al. (2012) in a study from Zagreb, Croatia, had values in terms of fruit height between 20.40-25.30 mm and fruit diameter between 13.10-16.00 mm. Soare et al. (2015) recorded values for rosehips in Oltenia region, between 14.20 mm and 24.90 mm in terms of fruit height, and for fruit diameter between 9.10 and 14.40 mm. In another study by Soare et al. (2014) also in Oltenia region, the diameter of the fruits was between 9.50 mm to 15.10 mm and the fruit height between 14.30 mm to 30.20 mm. Roman et al. (2013) had values for rosehip fruits in Transylvania area between 12.00 mm and 46.00 mm for fruit height while Ghiorghiţă et al. (2012) recorded values for fruits in the area of Moldova between 18.50-24.75 mm for fruit height and 11.86-16.80 mm for fruit diameter. Bilgin et al. (2020) had results in terms of morphological characteristics of rosehips an average of 19.29 mm for fruit height and 11.17 mm for fruit diameter while Ancu et al. (2012) had maximum values for genotypes from Pitesti, Romania of 26.80 mm for fruit height and 19.36 mm for fruit diameter. The weight of the fruits varied between 0.63 g corresponding to genotype 12 and 4.81 g in genotype 20 with an average of studied genotypes of 2.01 g. Kazankaya et al. (2005) reported, for rosehip selections higher values between 1.02-6.10 g for fruit weight and also mentions values close to those studied in this paper in other Turkey regions, of 1.65-5.49 g in Gumushane, 1.28-2.20 g in Izmir, 0.82-2.22 g in Bursa, 1.74-3.99 g in Gevas and Ahlat and 0.60-4.95 g in other areas. Similar values for fruit weight are mentioned by Celik et al. (2009) with 2.60-4.95 g and Chrubasik et al. (2008) between 1.25 g and 3.25 g. Another study by Dogan & Kazankaya (2006) reports rosehips with an average weight of 2.35 g, a value similar to the average obtained in this study. Ercişli & Eşitken (2004) had values between 3.64-4.62 g, Šindrak et al. (2012) between 1.88 g and 2.96 g, Soare et al. (2015) between 1.06 g and 2.74 g, Roman et al. (2013) up to 3.25 g, and 1.24 g for fruit weight studied by Bilgin et al. (2020). Ancu et al. (2012) had maximum values at studied genotypes of over 3.7 g in terms of fruit weight. The morphological results of the studied fruits are similar to the representative values of the *CAN* variety which has fruits of 2.4-3.4 g, with a height of 23.00-32.00 mm and a diameter of 12.00-17.00 mm. The fruit shape index had limits of variation between 0.92 at genotype 4 meaning slightly flattened round fruits and 2.25 at genotype 6 with an average value of all genotypes of 1.65

which indicates elongated fruits according to Brewer et al. (2006) classification. Similar values were obtained by Dogan & Kazankaya (2006) with 1.63 and Šindrak et al. (2012) with values between 1.48 and 1.86. The volume of 100 fruits varied between 130.00 cm^3 at genotype 7 and 361.54 cm^3 at genotype 20, with an average value for all studied genotypes of 191.52 cm^3. The coefficient of variation in terms of fruit height varied between 6.41% at genotype 6 and 18.99% at genotype 11, with 16.47% value for all genotypes. Similar values were obtained by Soare et al. (2015) with a coefficient of variation between 5.37% and 18.54% for this characteristic. The diameter of the fruits had a minimum CV% of 4.93% at genotype 9 and a maximum of 8.94% at genotype 8, and the value of all genotypes was 11.76% while Soare et al. (2015) obtained higher values with a coefficient of variation between 7.13% and 24.25% for this characteristic. In terms of fruit weight, the coefficient of variation was between 11.46% in genotype 9 and 28.04% in genotype 11 with a CV % corresponding to the studied genotypes of 34.82% which indicates a high variability within individuals. Similar values were obtained by Soare et al. (2015) with a CV% for this characteristic between 9.98% and 41.87%. The fruit shape index had a coefficient of variation between 5.51% in genotype 18 and 14.37% at genotype 11, with a value of all genotypes of 12.12%. The volume of fruits registered a coefficient of variation of 31.49% among the studied genotypes. The determination of the coefficient of variation for all studied morphological characteristics indicates a degree of variability within the genotypes. In order to establish the relationships between these characteristics, coefficient correlations were calculated (Table 3).

Table 3. Determination coefficient (R^2) and correlation (r) between the analyzed morphological parameters

Fruit morphological parameters	R^2	r
Diameter – Weight	0.859	0.927
Height - Weight	0.723	0.850
Height - Diameter	0.473	0.688

A positive correlation is between the fruit diameter and fruit weight with r = 0.927 and R^2 = 0.859 which means that the diameter of the fruits influences their weight in a fairly large percentage (85.9%). Regarding the correlation between fruit height and fruit weight, the correlation coefficient had a value of r = 0.850 and R^2 = 0.723 while between the height of the fruits and their diameter was r = 0.688 and R^2 = 0.473. It turns out that the size of the fruit influences their weight in high percentage (72.3-85.9%).

CONCLUSIONS

In conclusion, the degree of variability of the rosehip genotypes identified in the southern area of Oltenia presents fruits with intermediate values compared to those identified in the literature, which highlights the genetic diversity of this species. Using this diversity, valuable genotypes can be selected for their fruits morphological characteristics important in food and medicine, but also biotypes with representative features for breeding programs in order to obtain new varieties or improve the existing ones.

ACKNOWLEDGEMENTS

This work was supported by the grant POCU380/6/13/123990, co-financed by the European Social Fund within the Sectorial Operational Program Human Capital 2014-2020.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

AUCHENORRHYNCHA FAUNA ASSOCIATED WITH ABANDONED APPLE AND PLUM ORCHARDS IN NORTHERN BUCHAREST IN 2020

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Abstract

The species of the Auchenorrhyncha group represent one of the most important risks for apple and plum orchards, some of them being the vectors of major pathogens such as phytoplasmas. This paper presents results on the presence and structure of species of the Auchenorrhyncha suborder recorded in two unmanaged orchards of apple and plum from the northern part of Bucharest (Băneasa area) in 2020. The insects were collected on yellow double-sticky traps (two traps / orchard) placed in the canopy of apple and plum trees and replaced every week from early April until late November. In total, 25 species have been identified in both orchards, accounting 4834 individuals (4036 in the apple orchard and 798 in the plum orchard). In apple, the most abundant species were Orientus ishidae with 3096 specimens (76.7%), followed by Erasmoneura vulnerata with 380 specimens (9.41%), Zygina flammigera with 177 specimens (4.38%) and Anoplotettix fuscovenosus with 156 specimens (3.86%). The most numerically relevant species in the plum orchard were Zygina flammigera with 371 specimens (46.49%), Erasmoneura vulnerata with 115 specimens (14.41%), Orientus ishidae with 106 specimens (13.28%) and Fieberiella florii with 91 specimens (11.4%). Dynamics of adult populations was performed for the most abundant species in both fruit orchards.

Key words: *apple and plum orchards, invasive insects, leafhoppers, planthoppers.*

INTRODUCTION

Worldwide, apple and plum crops represent very important food sources. According to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), in 2019, the total area harvested in the world for apples was 4,717,384 ha, and for plums and blackthorns 2,727,745 ha. In Romania, in 2019, the area harvested for apples was 52,740 ha and for plums and blackthorns 65,580 ha. For both crops, the Auchenorrhyncha fauna represent an economically important category of pests. Auchenorrhyncha with the common name 'true hoppers' is one of the largest groups of Hemiptera order and includes leafhoppers, planthoppers, treehoppers, froghoppers and cicadas. Currently, there are approximately 42,000 species of Auchenorrhyncha described worldwide (Deitz, 2008). In the European continent, more than 2000 species, mostly pests, have been reported. Among them, a total of 32 invasive species have been detected in Europe (Šćiban & Kosovac, 2020). They are phytophagous insects (Waloff, 1980) and equipped with piercing-sucking mouthparts. Their economically importance status is given by the fact that they cause direct damage by sucking the sap and indirect damage because some of them are vectors of pathogens, including phytoplasmas, bacteria and viruses. Over 200 species of 'true hoppers' are vectors of phytoplasmas and 70 species transmit viruses (Weintraub & Beanland, 2006). They can feed either with phloem, xylem or parenchyma (Bartlett et al., 2018) and both mature adults and nymphs can transmit plant pathogens (Weintraub & Beanland, 2006). The presence and role of abandoned orchards and crops have been studied in different papers, as they represent important sources of insect vectors and diseases (Altieri & Schmidt, 1986; Brown et al., 1988; Ricci et al., 2009; Grimová et al., 2016).

Investigations on the fauna of Auchenorrhyncha in fruit orchards in Romania are almost non-existent. The only study on this subject in autochthonous literature was in 2012 in a modern young pear orchard in Bucharest area (Cean & Cean, 2013). The authors described 6 species *Metcalfa pruinosa* (Say 1830), *Reptalus panzeri* (Low, 1883), *Fieberiella florii* (Stal, 1864), *Psammotettix notatus* (Melichar, 1896), *Zyginidia pullula*

(Boheman, 1845) and *Neoaliturus fenestratus* (Herrich-Schäfer, 1834) and 3 families, Flatidae, Cixiidae and Cicadellidae. Some of them were described as potential vectors of different diseases where they have been identified.

The aim of this paper was to comparatively evaluate the Auchenorrhyncha community in two abandoned orchards (apple and plum) in the northern Bucharest (44°30'21" N 26°04'01" E, 90 m), southern Romania in 2020. The species structure, abundance and adult population's dynamics of most abundant species and other ecological parameters have been calculated.

MATERIALS AND METHODS

Two abandoned old orchards, apple and plum, in the former experimental field of the Research-Development Institute for Plant Protection in the northern Bucharest were sampled in 2020. The area which includes the two orchards contains a mixture of different other herbaceous and woody plants grown spontaneously: European dwarf elder (*Sambucus ebulus* L.), American pokeweed (*Phytolacca americana* L.), common privet (*Ligustrum vulgare* L.), old man's beard (*Clematis vitalba* L.), wild and common grapevine (*Vitis* spp.), species of *Parthenocissus*, common barberry (*Berberis vulgaris* L.), dog rose (*Rosa canina* L.), common hawthorn (*Crataegus monogyna* Jacq.), blackthorn (*Prunus spinosa* L.), mulberry (*Morus* sp. L.), common oak (*Quercus robur* L.), Turkey oak (*Quercus cerris* L.), common walnut (*Juglans regia* L.). In addition, different species of *Prunus* belonging to the former experimental fields were present, including apricot (*P. armeniaca* L.), sweet cherry (*P. avium* L.) and sour cherry (*Prunus cerasus* L.).

Insects were collected on yellow double-sticky traps (2 traps/orchard), fixed inside the trees canopy at approximately 2 m above ground level and replaced every week from May to late November. A total of 56 traps per plantation were used. The insects were identified under a stereomicroscope SZ 61 in laboratory, according to morphological features in literature. In some cases, the nymphs were not identified to the genus level and therefore were not included in the total captures of this study.

The ecological parameters of abundance (A), dominance (D%), constancy (C%) and ecological significance (W%) have been calculated using specific formulas (Simionescu, 1983; Stan, 1994; Baban, 2006).

$$D\% = \frac{A \times 100}{n},$$

where: A - number of individuals in a species (abundance);

n - total number of individuals of all species in a sample;

For the dominance values, species were classified as subreceding species (D1 < 1%), receding species (D2 = 1-2%), subdominant species (D3 = 2-5%), dominant species (D4 = 5-10%) and eudominant species (D5 > 10%).

$$C\% = \frac{ns \times 100}{n},$$

where: ns - number of samples with one species;

n - total number of samples.

Regarding constancy values, species were classified as accidental species (C1 = 1-25%), accessory species (C2 = 25-50%), constant species (C3 = 50-75%), and euconstant species (C4 = 75-100%).

$$W\% = \frac{D \times C}{100}$$

For the ecological significance, species were classified as accidental species (W1 < 1%), accessory species (W2 = 1-5%) and characteristic species (W3 > 5%).

To measure the specific diversity of the species collected in the two orchards, two equations of Shannon function were used, modified by Mac Arthur and corrected by Lloyd and Ghelardi (Baban, 2006).

$$H(S) = \frac{K}{N}\left(N \log_{10} N - \sum_{r=1}^{S} Nr \log_{10} Nr\right),$$

where: H(S) - real diversity (observed);

K - conversion factor for changing the base of the logarithm from 10 to 2, having the value: 3.321928;

N - total number of specimens;

S - total number of species;

Nr - number of individuals of the species r (abundance);

$$H(S)_{max} = K \log_{10} S$$

where: $H(S)_{max}$ - maximum diversity (hypothetical);

$$H_r = \frac{H(S)}{H(S)max}$$

H_r - relative diversity (equitability).

The similarity of Auchenorrhyncha species in the two orchards was appreciated calculating the Spearman index (r_s) and Jaccard coefficient (JC). In addition, the Venn diagram was carried out (Heberle et al., 2015).

$$r_s = 1 - \frac{6\sum d^2}{n^3 - n}$$

where: r_s - calculated Spearman coefficient; d = differences between the ranks of a registration, n = number of observations. Its value is between -1 and +1, indicating a perfect negative/positive relationship between the two communities. The statistical significance of the values of the Spearman index is represented by the correlation coefficient (r_s). This coefficient is an effect size which describe the strength of the correlation according to the following values: 0-0.19 (very weak), 0.20-0.39 (weak), 0.40-0.59 (moderate), 0.60-0.79 (strong), 0.80-1.0 (very strong).

$$JC\% = \frac{C}{(A+B)-C}$$

where: A - number of species found only in the apple orchard;

B - number of species found only in the plum orchard;

C - number of species found in both orchards;

When JC = 0, the samples are entirely different from each other; when JC = 1, the samples are entirely similar.

Seasonal dynamics was realized for first five most abundant species at least in one of the investigated orchards.

RESULTS AND DISCUSSIONS

In this study, the Auchenorrhyncha species collected from two old unmanaged orchards (apple and plum) was compared.

The two orchards have not been managed since 2009 in terms of phytosanitary treatments for pest and diseases control and technological maintenance, so they became abandoned.

A total of 4834 leaf and plant - hopper specimens (adults and larvae together) were captured on yellow double-sticky traps from May to October 2020, of which 4036 specimens (83.5%) in the apple orchard and 798 specimens (16.5%) in the plum orchard (Table 1).

These belonged to 25 species and six families Membracidae, Cixiidae, Aphrophoridae, Cicadellidae, Acanaloniidae and Flatidae.

Table 1. Auchenorrhyncha captures on yellow double-sticky traps on apple and plum trees in 2020

Taxa	Apple							Plum						
	A	D		C		W		A	D		C		W	
	(no)	Class	%	Class	%	Class	%	(no)	Class	%	Class	%	Class	%
Cixiidae/Cixinae														
Cixius wagneri China, 1942	1	0.02	D_1	3.44	C_1	0.0006	W_1		-	-	-	-	-	-
Reptalus quinquecostatus Dufour, 1833	-	-	-	-	-	-	-	5	0.62	D_1	11.53	C_1	0.047	W_1
Membracidae/Smiliinae	-	-	-	-	-	-	-							
Stictocephala bisonia Kopp & Yonke, 1977	-	-	-	-	-	-	-	2	0.25	D_1	7.69	C_1	0.019	W_1
Aphrophoridae/Aphrophorinae														
Philaenus spumarius L., 1758	13	0.32	D_1	27.58	C_2	0.088	W_1	9	1.12	D_2	15.38	C_1	0.172	W_1
Cicadellidae/Agalliinae														
Anaceratagallia ribauti Ossiannilsson, 1938	2	0.04	D_1	6.89	C_1	0.002	W_1	13	1.62	D_2	19.23	C_1	0.311	W_1
Cicadellidae/ Deltocephalinae														
Scaphoideus titanus Ball, 1932	17	0.42	D_1	17.24	C_1	0.072	W_1	12	1.50	D_2	11.53	C_1	0.172	W_1
Fieberiella florii Stal, 1864	33	0.81	D_1	37.93	C_2	0.307	W_1	91	11.40	D_5	76.92	C_4	8.768	W_3
Anoplotettix fuscovenosus Ferrari, 1882	156	3.86	D_3	24.13	C_1	0.931	W_1	14	1.75	D_2	7.69	C_1	0.134	W_1
Neoaliturus fenestratus Herrich-Schäffer, 1834	-	-	-	-	-	-	-	1	0.12	D_1	3.84	C_1	0.004	W_1
Orientus ishidae Matsumura, 1902	3096	76.70	D_5	44.82	C_2	34.37	W_3	106	13.28	D_5	38.46	C_2	5.107	W_3
Euscelidius variegatus Kirschbaum, 1858	-	-	-	-	-	-	-	2	0.25	D_1	7.69	C_1	0.019	W_1
Platymetopius major Kirschbaum, 1868	3	0.07	D_1	10.34	C_1	0.007	W_1	-	-	-	-	-	-	-
Phlogotettix cyclops Mulsant & Rey, 1855	12	0.29	D_1	20.68	C_1	0.059	W_1	-	-	-	-	-	-	-
Allygus atomarius F., 1794	24	0.59	D_1	31.03	C_2	0.183	W_1	12	1.50	D_2	30.76	C_2	0.461	W_1
Allygus modestus Scott, 1876	2	0.04	D_1	6.89	C_1	0.002	W_1	4	0.50	D_1	15.38	C_1	0.076	W_1
Psammotettix sp.	1	0.02	D_1	3.44	C_1	0.0006	W_1	1	0.12	D_1	3.84	C_1	0.004	W_1
Cicadellidae/Typhlocybinae														
Zygina flammigera Fourcroy, 1785	177	4.38	D_3	65.51	C_3	2.869	W_2	371	46.49	D_5	65.38	C_3	30.39	W_3

Taxa	Apple							Plum						
	A (no)	D		C		W		A (no)	D		C		W	
		Class	%	Class	%	Class	%		Class	%	Class	%	Class	%
Zyginella pulchra Löw, 1885	13	0.32	D_1	20.68	C_1	0.066	W_1	-	-	-	-	-	-	-
Erasmoneura vulnerata Fitch, 1851	380	9.41	D_4	55.17	C_3	5.191	W_3	115	14.41	D_5	34.61	C_2	4.987	W_2
Arboridia sp.	93	2.30	D_3	44.82	C_2	1.030	W_2	25	3.13	D_3	23.07	C_1	0.722	W_1
Cicadellidae/Cicadellinae	-	-	-	-	-	-	-							
Cicadella viridis Linnaeus, 1758	-	-	-	-	-	-	-	2	0.25	D_1	7.69	C_1	0.019	W_1
Cicadellidae/Eurymelinae														
Macropsis fuscula Zetterstedt, 1828	2	0.04	D_1	6.89	C_1	0.002	W_1	-	-	-	-	-	-	-
Acericerus ribauti Nickel & Remane, 2002	-	-	-	-	-	-	-	5	0.62	D_1	7.69	C_1	0.047	W_1
Acanaloniidae														
Acanalonia conica Say, 1830	5	0.12	D_1	17.24	C_1	0.020	W_1	1	0.12	D_1	3.84	C_1	0.004	W_1
Flatidae/Flatinae														
Metcalfa pruinosa Say, 1830	6	0.14	D_1	13.79	C_1	0.019	W_1	7	0.87	D_1	23.07	C_1	0.200	W_1

The range of species was about the same for both orchards, 19 in the apple and 20 in the plum orchard, and were members of 5 and 6 families respectively.

The insect catches in the apple orchard were five times higher comparative to plum, although the two orchards are very close (approximately 70 m distance between them).

Seasonal trend of the number of species and specimens captured during the growing season from May until November are illustrated in Figure 1.

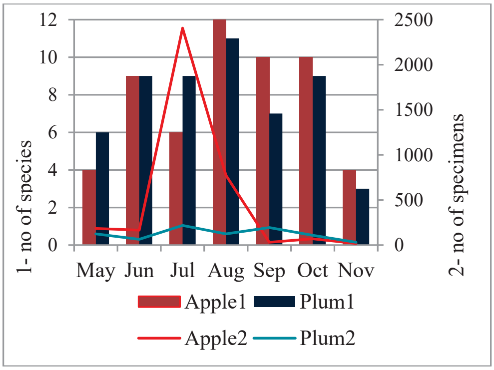

Figure 1. Number of species and specimens on yellow sticky traps recorded in the abandoned apple and plum orchards monitored in 2020

By comparing the collecting data from the two orchards we can observe that both number of species and captures were variable throughout the collecting period. The maximum number of species was in August for both orchards but high values were also reached in the first two months of autumn. Few species were found in May and November for both orchards.

Seasonal dynamics of the captures on yellow traps showed a maximum in July for both orchards, with a higher density of Auchenorrhyncha activity in this period. In terms of percentage, this maximum accounted 59.61% and 27.32% of all Auchenorrhyncha insects captured in apple and in plum, respectively.

The Cicadellidae family was the most abundant in both orchards, constituting 99.38% in apple and 96.99% in plum and included 15 species in apple but also in plum. Under this family, the leafhoppers of the Deltocephalinae and Typhlocybinae subfamilies were the most abundant in both orchards: Deltocephalinae accounted 82.86% in apple and 30.45% in plum and Typhlocybinae recorded 64.04% in plum and 16.43% in apple orchard.

The first four species per plantation, considered as main species, in descending order of relative abundance, were *Orientus ishidae* (76.7%), *Erasmoneura vulnerata* (9.41%), *Zygina flammigera* (4.38%) and *Anoplotettix fuscovenosus* (3.86%) in the apple orchard, and *Z. flammigera* (46.49%), *E. vulnerata* (14.41%), *O. ishidae* (13.28%) and *Fieberiella florii* (11.4%) in the plum orchard.

The values of dominance, constancy and ecological significance of the Auchenorrhyncha species collected from apple and plum orchards are presented in Table 1.

In the apple orchard, *O. ishidae* was found to be eudominant species and *E. vulnerata* dominant. Also, 3 subdominant species and 14 subreceding species were evaluated. Values of constancy highlighted the constant presence in samples of 2 species (*Z. flammigera* and *E. vulnerata*), 5 accessory and 12 accidental species. The ecological significance index showed 2 characteristic species (*O. ishidae*,

E. vulnerata), 2 accessory species (*Arboridia* spp., *Z. flammigera*) and 15 accidental species.
In the plum orchard, four species were evaluated to be eudominant (*F. florii*, *O. ishidae*, *Z. flammigera*, *E. vulnerata*). The *Arboridia* genus was subdominant and the other species were subreceding and receding. The highest two values of the frequency in the samples had *F. florii* (euconstant species) and *Z. flammigera* (constant species). The other 18 species were accessory and accidental. The ecological significance index indicated 3 characteristic species (*F. florii*, *O. ishidae*, *Z. flammigera*), one accessory (*E. vulnerata*) and 16 accidental species.
The relative diversity and equitability of the Auchenorrhyncha species collected in the apple and plum orchards are presented in Table 2.
The comparative analysis of the Shannon diversity index [H(S)] and equitability (Hr) values showed that these varied during the vegetation period for both orchards.
Overall, the community from plum plantation proved to be more stable, registering a total value of the Shannon index (2.57) and equitability (0.59) higher than the community from apple plantation (1.37 and 0.32, respectively).
The high relative abundance of only one species (*O. ishidae* 76.7%) in apple trees has led to a reduction in the Shannon index.
Generally, the ecological imbalance of the two Auchenorrhyncha communities was mainly influenced by large populations of 1-2 species.

Table 2. Trend of number of species, relative diversity and equitability of the Auchenorrhyncha community in abandoned apple and plum in 2020

Month	No of species/S		H(S)		H(S)max		Hr	
	Apple	Plum	Apple	Plum	Apple	Plum	Apple	Plum
May	4	6	0.71	0.98	1.99	2.55	0.35	0.38
June	9	9	1.53	2.61	3.15	3.15	0.48	0.82
July	6	9	0.20	1.80	2.55	3.15	0.07	0.57
Aug	12	11	0.49	2.20	3.55	3.45	0.13	0.63
Sept	10	7	2.80	0.77	3.32	2.79	0.84	0.27
Oct	10	9	2.34	1.84	3.32	3.15	0.70	0.58
Nov	4	3	1.81	1.42	1.99	1.56	0.90	0.91
May-Nov	19	20	1.37	2.57	4.21	4.31	0.32	0.59

The intensity of association degree of the two insect communities given by the values of Spearman coefficient (r_s) and Jaccard coefficient is presented in Table 3. Compared by the total value of the r_s index (0.87), the two communities can be appreciated to be in a good direct proportional interdependence. The significance of the correlation coefficient was examined by comparing the obtained values of t_{calc} and F_{calc} with the tabulated values available in the books of Snedecor (1956) and Ceapoiu (1968) in limits of 0.05% at n-1 degrees of freedom.
The result of similarity Jaccard coefficient showed a good resemblance of the two communities, more than half of the total of 25 species identified being common.

Table 3. Nominal and calculated values of the Spearman similarity index and their statistical significance (F and t tests)

Comparative communities	Spearman index		Significance test				Signification	Jaccard
	Obs.	Correct.	t test		F test			
	r_s	r_c 0.05	calc.	0.05%	calc.	0.05%		
May A/P	0.50	-	0.57	4.30	0.32	19.0	ns	0.42
June A/P	0.30	0.90	0.54	2.77	0.29	6.39	ns	0.38
July A/P	0.40	-	0.61	3.18	0.37	9.12	ns	0.36
Aug. A/P	0.85	0.82	3.20	2.57	10.24	5.05	*	0.45
Sept. A/P	0.83	0.90	2.53	2.77	6.40	6.38	*	0.31
Oct. A/P	0.67	0.71	1.99	2.44	3.96	4.28	ns	0.58
Nov. A/P	0.50	-	0.57	4.30	0.32	19.0	ns	0.75
A/P	0.87	0.46	6.02	2.16	36.24	2.46	ns	0.56

r_c - corrected Spearman coefficient.

The seasonal relationship established between the two Auchenorrhyncha communities can be visualized in the Venn diagram in Figure 1.

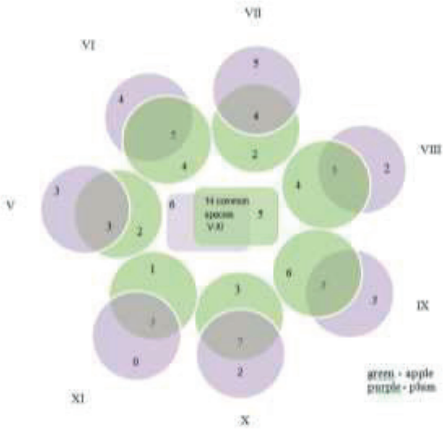

Figure 1. The Venn diagram

Seasonal dynamics of the captures were calculated only for principal species with high relative abundance in at least one of the investigated orchards. These species were *Orientus ishidae, Erasmoneura vulnerata, Zygina flammigera, Anoplotettix fuscovenosus* and *Fieberiella florii*.

O. ishidae (Cicadellidae, Deltocephalinae) was the most abundant species (3202 specimens, 76.04%) in apple orchard while in plum this was the third most abundant species (106 specimens, 13.28%). The presence of this invasive species of Asian origin was confirmed in Romania since 2016 (Chireceanu et al., 2017) even in the area where this study was carried out. This is considered an economically important pest especially after it was associated with the Flavescence dorée phytoplasma in Italy and Slovenia (Mehle et al., 2010; Gaffuri et al., 2011). Moreover, this species was already reported as potential vector of the peach yellow leafroll phytoplasma in U.S.A. (Rosenberger and Jones, 1978) and recently have been found to be infected with '*Ca. Phytoplasma mali*' (Oppedisano et al., 2017). It is a highly polyphagous species on many dicotyledonous plants in Europe including the genera *Malus* and *Prunus* (Seljac, 2004; Guglielmino, 2005; Nickel, 2010; Lessio et al., 2016).

The dynamics of *O.ishidae* (Figure 2) showed that the flight activity of adults started at the end of June-beginning of May and ended in mid-September for both orchards. For the apple orchard, the number of specimens was much higher with a massive increase in population during July. Fot both orchards, the population peak was reached at the end of July, with 894 specimens in apple and only 37 specimens in plum.

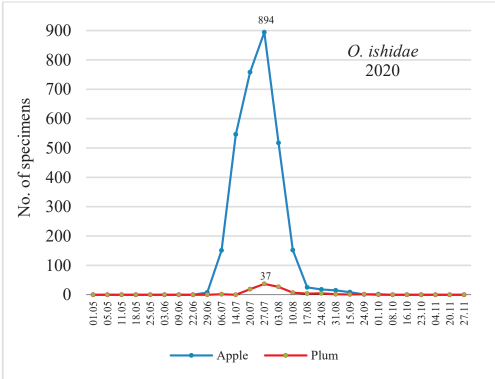

Figure 2. Population dynamics of *O. ishidae* in 2020

Zygina flammigera (Cicadellidae, Typhlocybinae) (Figure 3) was first the most abundant species in plum (371 specimens, 46.49%) and the third in apple orchard (177 specimens, 4.38%). This leafhopper is a polyphagous species native to Europe, considered a common species of fruit trees. It was reported as minor pest of peach in Italy (Viggiani et al., 1992) and Spain (Torres et al., 2000) and serious pest on peach and almond in Tunisia (Chaieb et al., 2011).

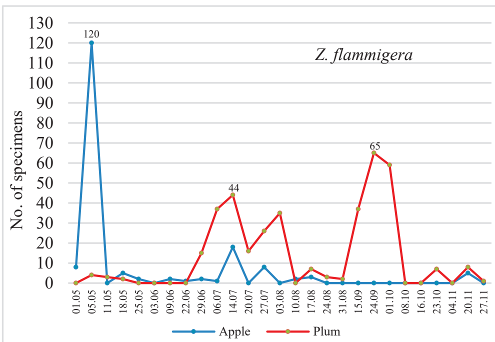

Figure 3. Population dynamics of *Z. flammigera* in 2020

The population dynamics of this species showed different peaks throughout the year. Its adults on yellow traps were present in the beginning of May, when there is the principal peak in the apple orchard. In the plum orchard there were three peaks of the captures, the first one coinciding with the second one of the apple orchard (middle of July). The second peak was in the beginning of august and the third peak was in the end of September. Last adults were captured in both orchards until the end of November.

Erasmoneura vulnerata (Cicadellidae, Typhlocybinae) reached the second large captures on apple (9.41%) and on plum (14.41%) being represented by 380 specimens and 115 specimens respectively. The population dynamics of this leafhopper (Figure 4) indicates an activity of adults more intense in May. In the plum orchard, it was only one peak that coincided with the second peak in the apple orchard (beginning of May), with a maximum of 59 adults. Adults were also sparsely captured from the beginning of June until the middle of November with numbers varying between 1 and 4. This is an invasive species from North America detected on Italian grapevine in 2004 (Duso et al., 2005). *E. vulnerata* is mentioned as a new pest of grapevine in Europe with an increasing damage potential (Duso et al., 2020). Presence of this species in Romania was observed in the area of this study from 2016 and also on grapevine in various sites in the country (Chireceanu et al., 2020). The host plants for this species are mainly plants of the *Vitis* and *Parthenocissus* genera (Girolami et al., 2006; Duso et al., 2019).

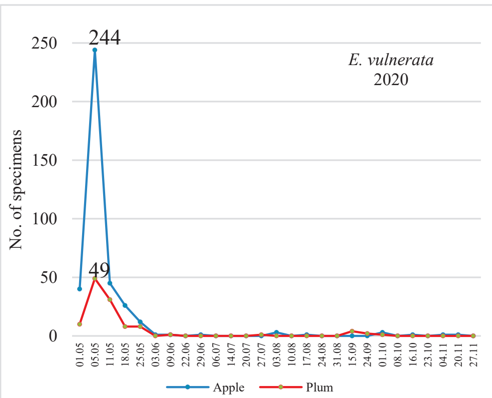

Figure 4. Population dynamics of *E. vulnerata* in 2020

Although this species was not yet associated with apple and plum, the number of specimens captured in this study was relevant. The presence of adults on traps in these orchards is probably the result of the yellow color of the traps attractive to them. The adults on their specific host plants voluntary grown in or close to monitored orchards were possibly lured in the orchards.

Anoplotettix fuscovenosus (Cicadellidae, Deltocephalinae) amounted 156 specimens in apple orchard. Native to Europe, this species is associated with grapevine and considered a potential vector of yellows phytoplasmas (Alma, 1995). This leafhopper was found to be associated to shrubs, trees but also to herbaceous hosts from urban ecosystems (Guglielmino et al., 2015). The flight activity of adults (Figure 5) on apple was between early June and the middle of August. The first peak was on late June with a maximum of 63 adults and a smaller peak on early August with a maximum of 5 adults. In plum, adults were captured very sparsely with only one maximum of 11 adults in the middle of June.

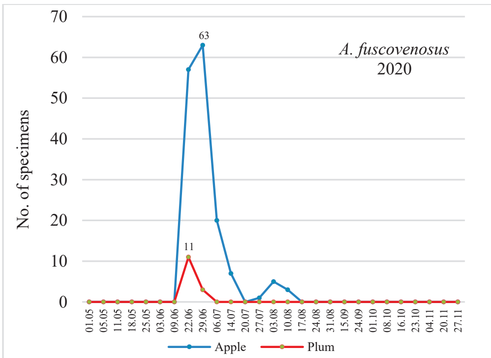

Figure 5. Population dynamics of *A. fuscovenosus* in 2020

The privet leafhopper *Fieberiella florii* (Cicadellidae, Deltocephalinae) was relatively abundant with 91 specimens in plum and only 33 specimens trapped in apple. This species is a major vector of the apple proliferation phytoplasma, one of the most economically important pathogens in apple orchards in Europe (Tedeschi & Alma, 2006). *F. florii* was found on many woody plants near hedges, including *Prunus* species but also on fruit trees

(*Prunus mahaleb* L., *P. avium* L., *Malus domestica* Borkh, *Cydonia oblonga* Mill.) and grapevine (*Vitis vinifera* L.). The dynamics of captures (Figure 6) showed visible fluctuations in the flight activity of adults in both orchards. In apple, there was only one maxim of only 8 adults in the middle of October. In plum, two maximums have been reached, one throughout the first half of July with 11 adults and another one in mid-October with 9 adults. For both orchards, the maximum values were very low compared to the population dynamics of the other 4 dominant species of leafhoppers.

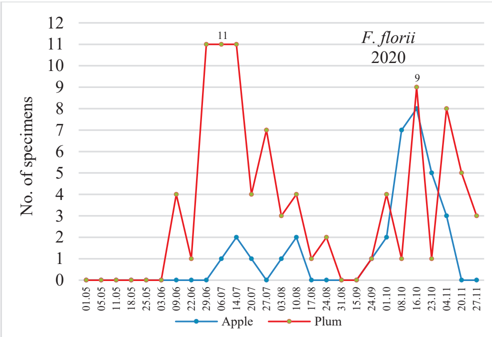

Figure 6. Population dynamics of *F. florii* in 2020

Other species sporadically captured were the invasive species *Scaphoideus titanus*, *Phlogotettix cyclops*, *Acanalonia conica* and *Metcalfa pruinosa*.

Regarding *S. titanus*, while living exclusively on grapevine (*Vitis vinifera* L.), it was also observed occasionally on other plants that grow in close proximity to vines. Chuche & Thiéry (2014) observed it on basket willow (*Salix viminalis* L.) and peach (*Prunus persica* Batsch). In North America, it was also recorded in various environments including forests, meadows, orchards and bogs (Chuche & Thiéry, 2014).

CONCLUSIONS

Comparative analyses of the Auchenorrhyncha communities monitored in 2020 in two old unmanaged orchards (apple and plum) revealed follows:

A total of 4834 specimens (adults and larvae together) belonging to 25 species and six families Membracidae, Cixiidae, Aphrophoridae, Cicadellidae, Acanaloniidae and Flatidae were captured on yellow double-sticky traps from May to October 2020, of which 4036 specimens (83.5%) in the apple orchard and 798 specimens (16.5%) in the plum orchard.

The most abundant species belonged to the Cicadellidae family: *Orientus ishidae*, *Zygina flammigera*, *Erasmoneura vulnerata*, *Anoplotettix fuscovenosus* and *Fieberiella florii*.

According to the Shannon diversity index and equitability values, the Auchenorrhyncha community from plum plantation proved to be more stable than those in apple plantation.

The two insect communities can be appreciated to be in a good direct proportional interdependence.

The high relative abundance of only one species (*O. ishidae* 76.7%) in apple trees has led to a reduction in the Shannon index. Generally, the ecological imbalance of the two Auchenorrhyncha communities was mainly influenced by large populations of 1-2 species.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE BEHAVIOR IN THE POLLINATION PROCESS OF SOME NATIVE APRICOT VARIETIES

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Abstract

Sharka virus disease is extremely difficult to control and cause severe economic loss. A long-term solution is to propose new hybrid combinations where, as parents, local genotypes better adapted to the climatic conditions of the culture area are involved. This paper aims to study the behavior in the process of pollination of native apricot varieties, 'Amiral', 'Dacia', 'Siret' in different hybrids combinations. The highest number of pollinations flowers was performed on the combination 'Dacia ♀ x Rareş ♂' with 243 flowers, then in descending order follows the combination 'Excelsior♀ x SEO ♂' with 210 flowers, 'Siret ♀ x Amiral ♂' 198 flowers, 'Bucovina ♀ x Harcot ♂' 178 flowers. At the end, a steady fall is recorded again in all combinations, but a fall rate of 100% was observed in the combination of 'Bucovina ♀ x Harcot ♂'. The lowest rate of fall of the remaining studied fruits was noticed in the combination 'Dacia ♀ x Amiral ♂' of 67.98%.

Key words: *apricot, genotypes, hybrids, combination.*

INTRODUCTION

Plum pox virus that causes the disease called Sharka brings devastating damage in the culture of stone fruits worldwide. It is thought to be a pathogen agent of phytosanitary quarantine, listed in OEPP documents (Damsteegt et al., 2001).

Quality in fruits is a critical component of the research programs as related to the fruit-bearing trees in the stone fruit group in the *Prunus* genus (peach, apricot, plum, cherry) where the criteria of 'resistant to disease and pest' carries a peculiar significance within the program of plant/pathogens interaction. Along this line, Sharka (pox virus) is a severe issue for Europe, since the fruit production and quality are fully ravaged (Krska et al., 2002). Sharka has recently traced in Asia, South America and North America (Kobber, 2000). As a consequence, creating varieties with a genetically resistance to this disease is a major objective to improve the *Prunus* genus (Dosba et al., 1994).

Apart from its economic interest, *Prunus*/Sharka pathosystem is likely to bring a meaningful contribution to the understanding of the plant/pathogen interaction mechanism. On the other hand, the 'perennial' and 'stone' features of the host plant (fruit-bearing trees) may have a vast impact upon the plant/pathogen interaction and evolution, which is why this particular genus of plants is less studied than the herbaceous plants (such as *Nicotiana ebontamiana*, *Arabidopsis thabana*, *Pisum sativum*). (Desvignes et al., 1999)

Thorough research studies on this virus worldwide have been assisted with substantial financial support so as not to create other infection areas and with identification of measures most efficient to eradicate it.

While important progress has been made for decoding the molecular and epidemiological aspects of the virus, which enabled a control upon the virus spreading, further investigation is conducted to scale down the effect of this virus upon the stone species, since there is still uncertainty in terms of epidemiology and the relation with the vector-borne virus (Kegler et al., 2000).

The paper herein examines different methods to eradicate the PPV impact upon the *Prunus* genus cutivated species by using parents from certain apricot varieties resistant to PPV, rootstocks with strong resistance, regulation of the population of vectors, use of the biocontrol chemicals, virus visualization via high-resolution electron microscopy, as well as the improvement of the detection molecular techniques (Kölber, 2001).

MATERIALS AND METHODS

For the purpose of the study, Romanian and foreign apricot varieties have been used, of different traits to be monitored during the research on the collection in the experimental field of the Faculty, along with apricot varieties at the Variety Testing Centre in the Ulmi locality, Dambovita county.

1. 'Dacia' - registered in 1989, an early ripening variety. It is a high-vigor tree, of an increased yielding potential.
The fruit is round in shape, slightly flattened on top and a large peduncle, heavier than 50 g. The pulp is light orange in color, non-adherent to the stone, average firmness, light orange skin covered in crimson red on the sunlit side, the stone is large and round, with a sweet core.
It is a self-fertile variety.
2. 'Amiral' - a high-vigor tree, self-fertile.
It has a high resistance to PPV. The fruit is in an oval shape, with the orange skin in a carmin red hue, a fine and aromatic pulp of a medium succulence, while the core has a bitter taste.
The ripening time interval ranges from June 26 to July 5.
3. 'Rareș' - a Romanian variety, obtained at SCDP Băneasa, registered in 2002, resistant to diseases and freezing temperature, a large fruit of 60-65 g, in the shape of an elongated sphere with the seam line very visible, a yellow-orange skin in color, splashed with crimson red, a light yellow-orange succulent pulp, a sweet core.
It is a high-yielding variety, low vigor, early ripening, good resistance to PPV.
4. 'Bucovina' was created by USAMV Bucharest.
The fruit is large, in an egg-shaped form. Pubescence is absent. The background skin color is medium orange, while the coating color is red. The pulp is light orange in color, with an average firmness and non-adherence to the stone. Fruits reach consumption maturity in the second half of month of July.
5. 'Harcot' - an American origin variety, registered in 1991.
It is an early ripening variety, maturing between 10 and 15 of July, high vigor. The pulp is yellow-orange in color, succulent, non-adherent to the stone, dry substance 11.5-14.5%, average to large fruit, 50-55 g, yellow-orange skin with a red spot on the sunlit side, a large stone of a bitter taste. It is a self-fertile variety.
6. 'Excelsior' - a Romanian variety, obtained at SCDP Băneasa in 1994.
It is a tree of a medium vigor, resistant to the variations in temperature and frost, large and very large fruit, spherical - ovoid shaped, slightly flattened on the side. The skin is finely pubescent, yellow-orange in color. Yellow pulp, fleshy, with a balanced taste. Ripening takes place towards the end of month of July.
7. 'SEO' - a tree of a weak vigor, early ripening, large fruit size, yellow-orange in color with reddish spots, resistant to disease and pests.
8. 'Siret' - Romanian variety, an average vigor tree, large size fruit that are yellow and orange in color, with reddish spots on the sunlit side.
Ripening occurs in the first half of the month of July. The work method lies in the controlled pollination among different varieties of Romanian origin in two distinct locations, adopting the following crossing combination schemes:

In the USAMV orchard, pollinations were conducted as in the below crossing combination scheme:
1. Crossing combination 'Dacia ♀x Rareş♂'
2. Crossing combination 'Dacia ♀ x Amiral ♂'
3. Crossing combination 'Siret ♀ x Amiral ♂'

At the Variety Testing Center in the Ulmi locality, Dambovita county, the following combinations of varieties were pollinated:
1. Crossing combination 'Bucovina ♀ x Harcot ♂'
2. Crossing combination 'Excelsior♀ x SEO ♂'.

RESULTS AND DISCUSSIONS

As seen in Chart 1, the largest number of pollinations flowers was performed for the combination 'Dacia ♀ x Rareş ♂', with 243 flowers, and in a descending order, the combination 'Excelsior ♀ x SEO ♂' with 210 flowers, 'Siret ♀ x Amiral ♂' with 198 flowers and 'Bucovina ♀ x Harcot ♂' with 178 flowers in the year 2019. The lowest number of pollinations was done on the combination 'Dacia ♀ x Amiral ♂', with 153 flowers.

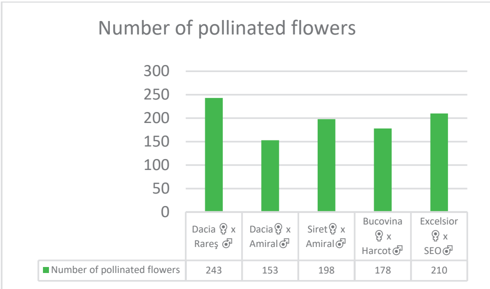

Figure 1. Number of pollinated flowers

According to the data in Figure 1 regarding the behavior in the pollination process for Romanian and foreign apricot varieties, following the already established crossing, a number of 243 flowers were pollinated in the hybrid combination 'Dacia ♀ x Rares ♂', with 183 pollinated flowers, with a percentage of 75.31 for fruits set. The lowest number of pollinated flowers was recorded for the combination 'Dacia♀x Amiral ♂', with 153 flowers and a fruits set percentage of 77.13%. As for the number of fruits obtained at the end of observation, the 'Bucovina♀ x Harcot ♂' combination was left with no fruit, 'Excelsior ♀ x SEO ♂' has 10 fruits left and, in an ascending order, are the combinations 'Dacia♀ x Rareş ♂' with 38 fruits and 'Dacia ♀ x Amiral ♂' with 49 fruits. The combination preserving the most fruits in the end (58) is 'Siret ♀ x Amiral ♂'.

Table 1. Behavior of the studied combinations during the pollination process

No.	Hybrid combination	Number of pollinated flowers	Number of fruits set	Fruit set percentage (%)	Number of fruits resulted at the end of observations
1.	Dacia♀ x Rareş ♂	243	183	75.31	38
2.	Dacia♀x Amiral♂	153	118	77.13	49
3.	Siret ♀x Amiral♂	198	161	81.32	58
4.	Bucovina♀ x Harcot♂	178	111	62.36	0
5.	Excelsior ♀x SEO♂	210	95	45.24	10

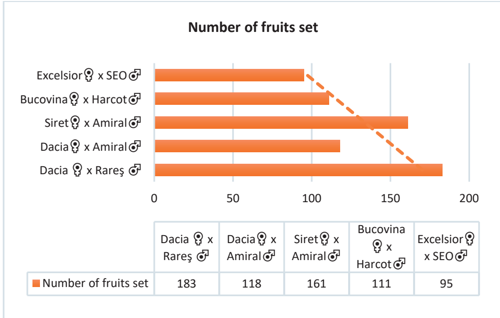

Figure 2. Number of fruits set per crossed combinations

As seen in Figure 2, the highest number of fruits set is in the hybrid combination 'Dacia♀ x Rares ♂', with 183 hybrid fruits. The fewest fruits set were derived from the combination 'Excelsior ♀ x SEO ♂', with 93 in number.
The highest fruits set percentage has been obtained in the combination 'Siret ♀ x Amiral ♂', with 81.32%, whereas the lowest fruits set percentage was recorded for the combination 'Excelsior ♀ x SEO ♂', 45.24%, despite having had a number of 210 flowers pollinated (Figure 3).

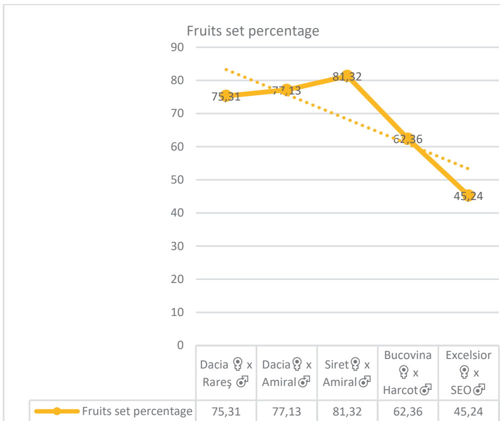

Figure 3. Hybrid fruits set percentage (%)

To have a better picture of the number of fruits set and of the decreasing rate of them in all five combinations, observations were made every 7, 14, 21 days and at the end of the process.
The highest number of fruits set at 7 days was calculated for the combination 'Dacia ♀ x Rareş ♂', while the lowest number of fruits set was in the combination 'Excelsior ♀ x SEO ♂'.

Table 2. Number of hybrid fruits set monitored at the 7-day time interval

No.	Hybrid combination	Number of fruits set 11.04.	Number of fruits set 18.04	Number of fruits set 24.04.	Number of fruits set 01.05.
1.	Dacia♀ x Rareş ♂	163	92	78	38
2.	Dacia ♀x Amiral♂	108	74	65	49
3.	Siret♀ x Amiral♂	151	82	72	58
4.	Bucovina♀x Harcot ♂	98	75	44	0
5.	Excelsior ♀x SEO♂	87	68	25	10

The combination 'Siret ♀ x Amiral ♂' had a number of 151 hybrid fruits set, whereas the combination 'Dacia ♀ x Amiral ♂' the number was 108. During the following week (14 days), the most fruits are noticed to be still in the combination 'Dacia♀ x Rareş ♂'and the lowest number (68) was again detected in the combination 'Excelsior ♀ x SEO ♂'. In the descending order in the number of fruits set and left afterwards, we have 'Siret ♀ x Amiral ♂' with 82 fruits, 'Bucovina ♀ x Harcot ♂' 75 and 'Dacia ♀ x Amiral ♂' with 74 fruits. For the third observation week (21 days), the number of fruits set to be left is 78 in the hybrid combination 'Dacia♀ x Rareş ♂', while 'Siret ♀ x Amiral ♂' has 72. The lowest number of fruits left was recorded in the combination 'Excelsior ♀ x SEO ♂', i.e. 25. For 'Dacia ♀ x Amiral ♂', there are only 65 and 44 for the combination 'Bucovina ♀ x Harcot ♂'.

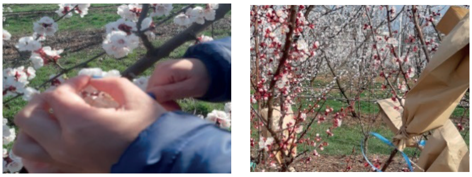

Figure 4. Pollination in the experimental field

CONCLUSIONS

At the final of the observations concerning the number of fruits set and left, it is noticed that there is no remaining fruit for the combination 'Bucovina ♀ x Harcot ♂'. In regards to this aspect, what can be considered is the incompatibility between the two parents, plus the torrential rains, strong winds and low temperatures that likely occurred in Ulmi locality at that time. The most fruits left in the study were in the combination 'Siret ♀ x Amiral ♂', 58, while the others are in a descending order - 'Dacia ♀ x Amiral ♂', with 49, 'Dacia ♀ x Rareş ♂', 38 and 'Excelsior ♀ x SEO ♂' with 10 fruits.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON BIOMETRIC STUDIES ON APRICOT HYBRID FRUITS

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Abstract

Plum pox virus (PPV) gen. Potyvirus, agent of Sharka disease, is the most devastating viral pathogen of stone fruits. The identification and exploitation of PPV-resistant sources represents the main eligible strategy for the long-term protection of the apricot cultivation. In this work, biometric studies were performed on hybrid fruits compared to the parents. Four hybrid combinations were studied, 'Dacia ♀ x Rareş ♂', 'Excelsior ♀ x SEO ♂', 'Siret ♀ x Amiral ♂' and 'Bucovina ♀ x Harcot ♂'.

***Key words**: hybrids, combination, apricots, resistance.*

INTRODUCTION

Plum pox virus has been acknowledged as the most destructive viral pathogen for the stone tree species. This is a consequence of the decrease in quality of the fruits and the financial loss from their premature fall (Polák, 1994). Up to current date, seven PPV strains have been identified and serologically and molecularly characterized - strain D (Dideron) isolated for first time on an apricot in South-East France; strain M (Marcus) identified on an apricot in North Greece (Pelet, 1968; Myrta et al., 1998); strain Ea (El amar) described in Egypt for apricot (Wetzel et al., 1991a); strain SoC (sour cherry) detected in the Republic of Moldova (Mircetich et al., 1982); strain SwC (sweet cherry) identified in Italy (Quiot et al., 1995); strain PPV-Rec coming from the recombination of the two major strains (M and D), discovered in Albania, Bulgaria, Czech Republic, Germany and Slovak Republic (Ranković et al., 1995). Strain PPV-W (Winona) was identified in Canada (Roy, 1994).

The virus was artificially transmitted to the sour and sweet cherries, yet the infections remained local, as there was no proof of them having spread further (Pop, 1975). Natural infections in *P. cerasus* species were reported by Revers et al. (1999), but PPV infection is known as extremely unusual since it is hardly present in most Europe. Infection with this virus can trigger a considerable loss.

Around 100 million trees in this stone species are infected in Europe, to the point that certain sensitive trees can drop their fruit in a percentage between 80 and 100 (Vilanova et al., 2003).

In the East and Central Europe, the sensitive plum species can exhibit premature fruit fall and fruit cracking. The exploitation of the natural resistance to PPV can play an important role in the fight against the Plum Pox Virus, especially when the strategy of the pathogen-derived resistance, transgenesis - induced, will not be accepted as an alternative to the conventional improvement (by the member states in the European Union) (Ravelonandro et al., 2000).

MATERIALS AND METHODS

For the study purpose, Romanian and foreign varieties, with different characteristics that have been monitored during the research work conducted on the collection in the experimental field of the Faculty, as well as apricot varieties within the Variety Testing Centre in Ulmi locality, Dambovita county. The following hybrid combinations have been obtained:

1. Crossing combination 'Dacia ♀ x Rareş ♂' (LIV)

2. Crossing combination ‘Dacia ♀ x Amiral ♂’ (PAN)
3. Crossing combination ‘Siret ♀ x Amiral ♂’ (BTP)
4. Crossing combination ‘Bucovina ♀ x Harcot ♂’ -
5. Crossing combination ‘Excelsior♀ x SEO ♂’ (MON)

As work methods, determinations have been made in regards to:
1. Size of the fruit (height and base) (cm)
2. Weight of the fruit (g)
3. Weight of the stone (g)
4. Pulp/stone ratio
5. Firmness (kgf/cm^2)
6. Soluble dry substance (g/100 g)
7. Description of the hybrid fruits (aspects related to phenotype)

RESULTS AND DISCUSSIONS

1. Description of the hybrid fruits derived from the crossing combination ‘Dacia ♀ x Rareş ♂’.
From this combination, 9 hybrid fruits, called LIV 1 - LIV 9 have been obtained. Fruits are large, ovoid, symmetrical, yellow - orange in color and topped by burgundy on the sunlit side. They have the mucron on the outgrowth area, slightly symmetrical.
2. Description of the hybrid fruits from the crossing combination ‘Dacia ♀ x Amiral ♂’.
A number of 11 hybrid fruits, called PAN 1 - PAN 11, have been derived from this combination. Fruits are medium in size, yellow-orange in color, with reddish spots on one side.
3. Description of the hybrid fruits from the crossing combination ‘Siret ♀ x Amiral ♂’
From this combination, 10 hybrid fruits were obtained, called BTP 1 - BTP 10. Fruits are medium in size, spheroid in shape, slightly symmetrical, tricolor background (yellow, orange, red), covered in reddish on a third of its surface. An excrescent mucron shows on the apex of the fruit.
4. Description of the hybrid fruits from the crossing combination ‘Bucovina ♀ x Harcot ♂’.
Fruits from this combination have not yet reached maturity.

5. Description of the hybrid fruits derived from the crossing combination ‘Excelsior♀ x SEO ♂’.
A number of 5 hybrid fruits, called MON 1 - MON 5, were obtained from this combination.
The fruit is large, ovoid, symmetrical, yellow-orange color as background covered in raspberry red and burgundy in the shape of spots, on the sunlit side.

Table 1. Results concerning the average values of the fruit size in the hybrid combinations of interest

No.	Hybrid combination	Fruit size		Weight (g)
		Height (cm)	Base (cm)	
1.	Dacia♀ x Rareş ♂ **LIV**	5.3	3.7	43.8
2.	Dacia ♀ x Amiral ♂ **PAN**	4.3	3.4	34.8
3.	Siret ♀ x Amiral ♂ **BTP**	4.7	3.5	33.2
4.	Excelsior ♀ x SEO ♂ **MON**	5.5	4	66.4

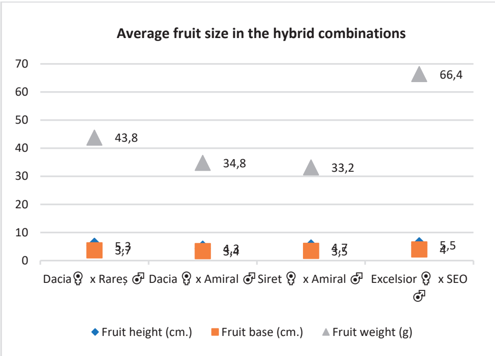

Figure 1. Average fruit size in the hybrid combinations

In terms of the average size of the hybrid fruits from the four combinations of interest, the chart above shows that the height ranges from 4.3 to 5.5 cm, with the highest in the ‘Excelsior ♀x SEO ♂’ combination, measuring between 3.4 and 4 cm in the fruit base. The fruit weight from the combination varies a great deal.
The maximum value still held by ‘Excelsior ♀ x SEO ♂’, 66.6 g, whereas the minimum is in the Siret ♀ x Amiral ♂ combination, i.e. 33.2 g.
The conclusion to be drawn is that these hybrid fruits fall within the medium to large sizes category.

Table 2. Results concerning the average values for the stone weight and the pulp/stone ratio

No.	Hybrid combination	Stone weight (g.)	Pulp/stone ratio %
1.	Dacia♀ x Rareş ♂	3.3	8.6
2.	Dacia ♀ x Amiral ♂	3.2	9.4
3.	Siret ♀ x Amiral ♂	3.1	9.5
4	Excelsior ♀ x SEO ♂	4.2	8.9

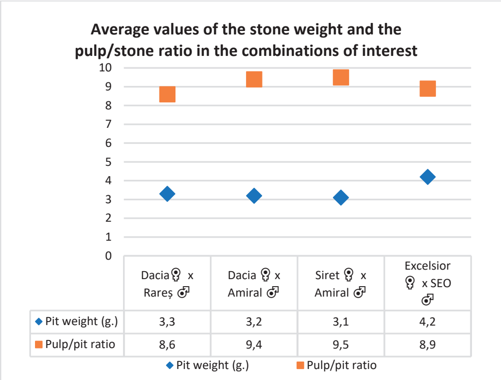

Figure 2. Results on the average values for the stone weight and the pulp/stone ratio

As seen in the chart, the hybrid combinations 'Dacia♀ x Rareş ♂', 'Dacia ♀ x Amiral ♂' and 'Siret ♀ x Amiral ♂' are noticed to have close values when it comes to the stone weight (3.1-3.3 g), while the combination 'Excelsior ♀ x SEO ♂' has a stone of 4.2 g.

In regard to the pulp/stone ratio, it is evident that the ratio is clearly favorable for the hybrid combinations 'Dacia ♀ x Amiral ♂', with 9.4 and 9.5 for 'Siret ♀ x Amiral ♂', followed by 'Excelsior ♀ x SEO ♂', 8.9 and 8.6 for the combination 'Dacia ♀ x Rareş ♂'.

Tabel 3. Results concerning the average values of the pulp firmness (kgf/cm^2) and the soluble dry substance (%)

No.	Hybrid combination	Pulp firmness (kgf/cm^2)	Soluble dry substance (g./100 g.)
1.	Dacia♀ x Rareş ♂	2.1	12.1
2.	Dacia ♀ x Amiral ♂	2.7	11.4
3.	Siret ♀ x Amiral ♂	1.9	12.3
4.	Excelsior ♀ x SEO ♂	1.4	12.4

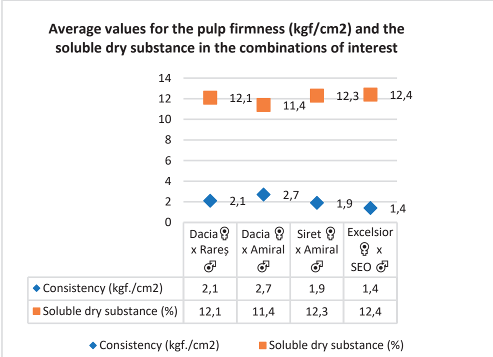

Figure 3. Average values for the pulp firmness (kgf/cm^2) and the soluble dry substance (%)

As for the pulp firmness, the chart 3 shows that the highest value is held by 'Dacia ♀ x Amiral ♂' with 2.7 kgf/cm^2, whereas the lowest is for the combination 'Excelsior ♀ x SEO ♂', with 1.4 kgf/cm^2; 'Dacia♀ x Rareş ♂' has the value of 2.1 kgf/cm^2 and 'Siret ♀ x Amiral ♂' has 1.9 kgf/cm^2.

Similar values are noticed for the soluble dry substance in three hybrid combinations, namely 12.4 kgf/cm^2 for 'Excelsior ♀ x SEO ♂', 12.3 kgf/cm^2 in 'Siret ♀ x Amiral ♂' and 12.1 kgf/cm^2 for the combination 'Dacia ♀ x Rareş ♂'. The hybrid combination 'Dacia ♀ x Amiral ♂' has a percentage of soluble dry substance of 11.4 kgf/cm^2. It is worth mentioning that 'Dacia' and 'Amiral' cultivars are early varieties, while 'Excelsior' falls into the late category.

CONCLUSIONS

The conclusion to this article is that the hybrid fruits place themselves into the class of medium to large size. These fruits are larger compared to their parents, but this description can also come from the fact that there are fewer on the branch, selected during the pollination process and many of them have fallen amid the physiological drop process. These fruits are larger, sweeter, with a better pulp/stone ratio.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SECONDARY METABOLITES AND HEALTH IMPORTANCE OF *PUNICA GRANATUM*. AN OVERVIEW

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Abstract

Originally from Central Asia, pomegranate (Punica granatum L.) is one of the oldest fruit crops cultivated, with a high geographical distribution. It is one of the most important crops in the Mediterranean area, some areas of Asia, former soviet countries or countries such as Argentina, Chile or the United States of America. In Romania, the species in cultivated in southern and south western part of the country, mostly as an ornamental plant, but it has potential for crop production. Despite the fact that the genetic diversity of Punica granatum totals more than 500 varieties, only 50 cultivated for their commercial value, reducing the cultivated germplasm. Punica granatum is grown in temperate and subtropical regions and it is highly appreciated in the food industry as a fresh fruit or as preservative, due to its high content in citric acid. Numerous studies mention the antiviral, anticancer, antibacterial, anti-diabetic, anthelminthic and immunomodulatory effects. Latest research identifies Punica granatum as a technical plant, of avail in nanoparticles synthesis due to its high content in antioxidants.

Key words: *Punica granatum, micropropagation, disinfection, culture medium, oxidative reaction.*

INTRODUCTION

Pomegranate is one of the most exotic and culinary fruits consumed all over the world and belongs in the *Lythraceae* family, which only has one genus - *Punica*, and two species - *Punica granatum* and *Punica protopunica* (syn. *Socotria protopunica*, *Punica spinosa, Punica florida*)- which is endemic and can only be found in the island of Socotra, located in the Arabian Peninsula. *Punica protopunica* is considered to be either an ancestor of the species (The Plant List - *Lythraceae*), either as an independent genetic line (Kosenko, 1985). *Punica granatum* is a diploid species, with a haploid number of chromosomes = 8, 2n = 16 for ‘Dholka’, ‘Ganesh’, ‘Kandhari’, ‘Muskat White’ varieties and 2n = 18 for the double flowered varieties ‘Vellodu’ and ‘Kashmiri’ (Mars, 2000). A tetraploid clone was identified in the spontaneous flora of India, a clone whose flowers exceed the standards and whose pollen sterility reaches 85.4%, compared to 7.4 % in the diploid varieties (Chandra et al., 2010). Chandra (2010), places the species as being one of the first to be domesticated by humans, cultivated in 4000-3000 B.C. and one of the oldest edible fruits, mentioned in the Bible and Coran.
According to Levin (2006), the wild species has three main origin basins and five macro-centers (Middle Eastern, Mediterranean, Eastern Asian, American and South African). Studies show that in the endemic micro zone of the species, Kandahar (Afghanistan), grows a variety with the biggest seedless fruit, and in the area of Dashnabad (Uzbekistan), the most resistant to cold variety was found.
Initially cultivated between 41° N and 42° S, commercial plantations are now found in the Mediterranean basin and Asia (Bar-Ya’akov et al., 2008), as well as in countries from the southern hemisphere, Australia, South America and South Africa (Holland et al., 2009). This shows the high level of adaptability this species has to climatic variations. In the area of Romania, the species is found cultivated for ornamental and pomological purposes in the areas of the southern part of the country or in depression areas with warmer weather, as well as in the campus of the Faculty of Horticulture from USAMV Bucharest, in a collection of 14

genotypes from Turkey ('Hicaz', 'Ähmar'), Siria ('Malissi', 'Kandahar', 'Shahvar'), Crimeea ('Nikitski Ranni'), Spain ('Mollar'), Italy ('Dolce', 'Nana' and 'Dolce di Sicilia') and Bucharest (local selection), collection established in 2012.

According to historiography, various preparations from parts of the plant were used in the days of Dioscorides, who indicated in "*de Materia medica*" that flower decoct was helpful to prevent dental loss, the juice of kernels mixed with honey was used for ulcers and the root decoct utilised to eliminate tape worms. Dioscorides points out the difference between *Punica granatum* and *Punica protopunica*, the wild variety being used for its astringent properties (de Materia Medica, 2000).

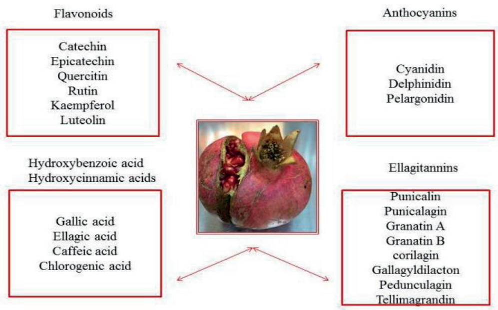

Figure 1. Chemical structure of *Punica granatum* compounds

Pomegranate is recognised now as a superfruit, because of its nutritional values and active principles. The fruit and peel have antioxidant properties and the juice, peel and oil extracted have a mild estrogenic action, which makes the plant useful for the treatment of menopause. Also, it interferes in the proliferation of cancer cells and their multiplication, being associated with other plants with anti-inflammatory role.

The main purpose of this study is to synthetise the main published works and research that indicates the pharmacology importance of this species and the main biocomponents from pomegranate, reviewing only the phenolic compound, tannins, flavonoids, anthocyanins, fatty acids and alkaloids.

I. Chemical composition of pomegranate

The different parts of pomegranate - leaves, fruit, seeds, root or bark have different compounds with remarkable chemical properties (Lanskisi et al., 2007). The main groups of compounds that are found in the plant are synthetized in table 1, grouped depending on their primary localization.

The edible part of the fruit represents about 50% of the whole fruit and it is formed by 40% of the juice generated by the aril and 10% of seeds. While the aril contains about 80 % water and 10% sugars (fructose and glucose) (Mphahlele et al., 2016), organic acids and bio compounds, cafenols, flavonoids, the seeds are a rich source of lipids, fiber and ash, with an average content of 6% pectin and 4.7 % total sugars (El-Nemr et al., 1990). While iron (Fe), copper (Cu), sodium (Na), magnesium (Mg) and zinc (Zn) are found mostly in seeds, the juice has a high potassium (K) content - 49.2% (İncedayi, 2010).

Table 1. Chemical compounds of *Punica granatum*

Source of compounds	Compounds
Juice	anthocyanin (Du et al., 1975), glucose, ascorbic acid (nutrition data); ellagic acid, gallic acid, caffeic acid (Amakura et al., 2000); catechin, EGCG (de Pascual et al., 2000); quercetin, rutin (Gómez-Caravaca et al., 2013); minerals, particularly iron (Lansky et al., 2007); amino acids (Waheed et al., 2004);
Roots and bark	punicalin and punicalagin (Tanaka et al., 1986); piperidine alkaloids (Wu et al., 2017);
Flower	gallic acid, ursolic acid (Li et al., 2008) triterpenoids, including maslinic and asiatic acid (Johanningsmeier et al., 2011);
Leaf	tannins (punicalin and punicafolin) (Yan et al., 2017); flavone glycosides, including luteolin and apigenin (Nawwar, 1994);
Pericarp (peel, rind)	phenolic punicalagins; gallic acid and other fatty acids (Amakura et al., 2000); catechin, EGCG (de Pascual-Teresa et al., 2000); quercetin, rutin, and other flavonols (Gómez-Caravaca, 2013); flavones, flavonones (Nawwar et al., 1994); anthocyanidins (Nodaet al., 2002);
Seed oil	95-percent punicic acid (Schubert et al., 1999); ellagic acid (Rahimi et al., 2020); other fatty acids (Wu & Tian, 2017); sterols (Choi et al., 2006);

II. Phenolic compounds

Phenolic compounds were identified as a result of research on peel, fruit, root system (Akkiraju et al., 2016; Sharma & Akansha Chauhan, 2018), seeds (Derekhshan et al., 2018), rid (Moorthy et al., 2013), mesocarp, exocarp, aril (Jaiswal et al., 2010), juice (Derakhshan et al., 2018), flowers (Yisimayili et al., 2019) and leaves (Yan et al., 2017). Depending on the cultivar or variety, the total content of polyphenols varies (Hmid et al., 2017), being higher in peel than in seeds, leaves and flowers (Elfalleh et al., 2012) and much higher in juice (Akhavan et al., 2015).

According to Li et al. (2006) pomegranate leaves extract induces apoptosis and inhibits migration and invasion of cancers cells. The peel extract is about 10 times richer in polyphenols than the one obtained from pulp, and the extraction in H_20 + EtOH (1:1 v/v) and temperature control of the probes has improved the efficiency (Venkataramanamma et al., 2016).

Generally, the total content of polyphenols from a plant is reported in the content of gallic acid, the highest level being reported in kale (*Brassica olerace*a var. *sabellica* - 16.3-18.8 mg GAE/g) and other vegetables such as tomatoes (*Solanum lycopersicum*), rhubarb (*Rheum rhabarbum*), spinach (*Spinacia oleracea*) and broccoli (*Brassica oleracea* var. *italica*) (Zhou & Yu, 2006). Also, the studies on the antioxidant capacity of the molasses obtained from pomegranate indicated a concentration of 52.6 mg GAE/g dry mass (Yılmaz et al., 2007), compared to the values of other economically important species such as Fuji apple variety *(Malus domestica*), kiwi (*Actinidia* sp.), pear (*Pyrus communis)*, orange (*Citrus sinensis*), where values of phenolic compounds varied between 1.2 and 5.1 mg GAE/g dry mass. Malic acid, glusides derivated such as hydroxybutanedioic acids were reported in the structure of pomegranate, alongside with quinic acid, quinic acid methyl ester and acetyl glucoside derivates. (Al-Rawahi et al., 2014).

The presence of gallic acid, togheter with cafienic acid was reported in chinese cultivars with values of 2.53 and 0.03 mg/100 mg (Song et al., 2016), while studies on peel indicated values up to 8.91 mg/g (Ma et al., 2015) of gallic acid or 30.4 mg/g in metanolic extract for the turkey variety. Poyrazoglu et al., in 2002, determined the main acids present in 13 pomegranate cultivats from the mediterranean area of Turkey. Citric acid was determined as the most present organic acid, found in an average concentration of 4.85±2.83 g/L, followed by mallic acid, in an average concentration of 1.76 ± 1.59 g/L, and oxalic and tartric acid, found in a concentration of 1.16 ± 2.07 g/L and 0.87 ± 0.75 mg/L, respecttively. Analysing the main phenolic compounds, the following compounds and concentrations resulted: gallic acid 4.55 ± 8.55 mg/L, protocatechuic acid 0.84 ± 0.64 mg/L, catechin 3.72 ± 2.29 mg/L, chlorogenic acid 1.24 ± 1.42 mg/L, caffeic acid 0.78 ± 0.79 mg/L, p-coumaric acid 0.06 ± 0.07 mg/L, ferulic acid 0.01 ± 0.02 mg/L, o-coumaric acid 0.17 ± 0.08 mg/L, phloridzin 0.99 ± 1.47 mg/L, quercetin 2.50 ± 1.96 mg/L (Poyrazoglu et al., 2002).

The presence of gallic acid, quercitin, catechin, chlorogenic acid and o-coumaric acid was previously signaled in peel and aril (Poyrazoğlu et al., 2020). Caffeic acid (3.88-75.19 μg/g), p-coumaric acid (0.12-14.87 μg/g), ferulic acid (0.15-8.84 μg/g), sinapic acid (2.13-3.58 μg/g), syringic acid (15.17-88.24 μg/g) and vanillic acid (65.87-108.36 μg/g) were identified as the main phenolic acids from pomegranate peel in a study on some cultivars from Pakistan (Mushtaq et al., 2015).

III. Tannins

Tannins and flavonoids are found in high quantities in the pericarp and mostly in wild cultivars, compared to the commercial ones (Tzulker et al., 20078). In pomegranate fruits, the concentration of tannins can reach 19.3%, having the one of the highest concentrations, after *Rhus semialata* (47%) and *Acacia catechu* (41.2%) (Cai et al., 2004). The study identified that in *Punica granatum*, tannins are both hydrolysable and condensed, and the condensed ones have a more complex structure and are more widespread in plant compared to the condensed ones. Hydrolysable tannins are found in the whole plant, in juice, in fruit or seeds, leaves or bark (Tanaka et al., 1985), in peel, in the form of ellagitannins or gallotannins (Çam & Hışıl, 2010), with a value of 262.7 mg tanninic acid equivalents (TAE)/g extracted with pressurized water extraction method. If analysed comparatively, the content of tannins from peel obtained from aqueous, methanol and ethanol extracts from four Turkish cultivars shows that the highest concentrations were obtained from the methanol extraction (124.10-183.18 µg TAE/ mg) (Orak et al., 20212) For four cultivars of pomegranate from Tunisia, the reported values were between 470.7-504.8 mg TAE/g (Elfalleh et al., 2012).

In 1985, a new ellagitannin, punicafolin, was isolated from the leaves of *Punica granatum* and punicalin and puninalagin were also identified in the roots and bark (Tanaka et al., 1985). In a cultivar from Peru, ellagitannins were identified in peel, in concentration of 44 g/kg (the most representative being punicalagin - 10.5 g/kg, pedunculagin I - 3.5 g/kg, granatin B - 5.9 g/kg, punigluconin - 3.8 g/kg, lagerstannin C - 3.9 g/kg) and gallatonnin (digalloylhexoside) in concentration of 4.3 mg/kg (Fischer et al., 2011). Punicalagin is the most studied compound from pomegranate, having multiple pharmacological properties and it is identified as the main phenol in Chinese pomegranate peel (Song et al., 2016). Hydrolysable tannins that contain isomers punicalagin are responsible of about half of the oxidative response of the pomegranate juice, followed by punicalin, ellagic acid and gallic acid (Tzulker et al., 2007).

A concentration of 39.6 mg/g and 32 mg/g concentration of punicalagin and ellagic acid from the extract of pomegranate leaves inhibited the cellular proliferation in the case of non-small epithelial pulmonary cancer and flow crytometry technology revealed the fact that the extract interfered with the progress of H1299 cells in G2/M and generated apoptosis (Li et al., 2016).The punicalagin content of the peel extract from three cultivars from Pakistan - 'Badana', 'Desi' and 'Kandhari' on dry weight was 88.70, 110 and 118.60 mg/g (Khalil et al., 2017). Punicalagin-β was isolated from the extract of three cultivars from Maroc - 'Beni Mellal', 'Berkane' and 'Settat', sin concentrations higher than 200 mg/g (Sabraoui et al., 2020). The present variations of punicalagin depend on the cultivar/variety and culture conditions. A comparative study from peel, flower, seed and leaf on 'Gabsi' variety from Tunisia showed that there are remarkable differences between the total content of hydrosoluble tannins, which explains the interest of traditional medicine of (using) pomegranate as a medicinal plant, especially for its anti-ischemic activity: peel (139.63 ± 4.25), seeds (29.57 ± 4.54), leaves (128.02 ± 4.49) and flowers (148.24 ± 10.29) – results expressed in mg TAE/g DW (Elfalleh, 2012).

Using the methanol extraction technique, ellagic acid was isolated from the peel of six Spanish cultivars in concentrations between 9.8-16.5 mg/g and the antimicrobian and antifungal activity was tested for *Aspergillus flavus* CECT 2686, *Aspergillus parasiticus* CECT 2947, *Gibberella fujikuroi var. fujikuroi* CECT 2987 (syn. *Fusarium verticillioides*), *Alternaria alternata* CECT 20560, *Botryotinia fuckeliana* CECT 20754 (Syn. *Botrytis cinerea*) (Rosas-Burgos et al., 2017). The comparative results regarding the isolation with the three classical extraction methods (aqueous, methanol and ethyl acetate) shows that pressurized water extraction method may also be successfully used, limiting the residue and the toxic remains from the methanol and ethanol extraction (Derakhshan et al., 2018). In 2006, Wang isolates a new compound from the pomegranate leaves, named pomegranatate 1, togheter with ellagic acid, derivates of ellafic acid 3,3′,4′-tri-O-methylellagic acid and phyllanthusiin E (Wang et al., 2006). Traditional Iranian medicine frequently uses pomegranate flowers for its therapeutic effects and gallic acid was first synthetized from two local genotypes, 'Ghojagh' and 'Golnar', and its content was 25.94% for 'Ghojagh' and 15.19 mg gallic acid equivalents per gram of dry powder (Hajimahmoodi et al., 2013). Studies have shown that the antioxidative capacity of pomegranate juice is stronger than the one in red wine or green tea, possible as a result of the presence of hydrolysable tannins from rid, anthocians and ellagic acids and its derivates (Gil et al., 2000).

IV. Flavonoids

Widespread in the biochemical structure of plants, flavonoids are classified in flavones, flavonols, flavanones, flavanonols, chalcones, isoflavonoids (mainly isoflavones), anthocyanins (anthocyanidins) and bioflavonoids (dimer of flavones, flavonols and flavanones) (Cai et al., 2004).

The extract from the whole fruit of pomegranate (aqueous extract, ethyl acetate extract and ethanol extract) has 30 % more favonoides than the peel extracts. Research showed that there is a correlation between the total antioxidant activity and the high flavonoids content and the type of extraction (Masci et al., 2016). The same research evidentiates that, in general, the extracts from the whole fruit have a higher bioactive potential than the ones obtained from the peel. Although there are differences between varieties and cultivars (Maroc) regarding the content in polyphenols, the value of correlation (R2=0.9) between flavonoids and the oxidative capacity of pomegranate juice showed that flavanoides are one of the most important compounds that contribute to the oxidative capacity of the species (Hmid et al., 2018). Compared to the apple juice, the content of flavonoids is almost double, from 92 mg/L in apple to 174 mg/L in pomegranate, being highly recommended for its antioxidant activity, especially for older people (Guo et al., 2008).

The antioxidant and anti-bacterian effect of flavonoids was reported as a result of the identification of chelating capacity of the iron and/or copper ions that launches the hypothesis that flavonoids can prevent the cellular damage caused by the free radicals. Therefore, a comparison of antioxidant activities of juice, peel and seed of pomegranate and inter-relationships with the total phenolic, tannin, anthocyanic and flavonoid contents was carried out (Oral et al., 2012). A good correlation evidentiated that in pomegranate juice, only flavonoids ($r = 0.410$) are the ones that contribute to the chelating capacity of metals, and, similarly, in the seed extract, the total content of flavonoids and the correlation value ($r = 0.623$) is also responsible for the chelating capacity of metals.

Catechin, gallocatechin and procyanidin B were identified using chromatography-mass spectrometry (LC-MS) analysis in the peel of a Tunisian cultivar of *Punica granatum* var. *Nana* (Wafa et al., 2016). Catechin was found in peel in concentrations that varied from 76.5 mg/100 g and 12.66 mg/100 mg in varieties from India and China (Singh et al., 2016; Song et al., 2016). Research identified the presence of flavonols, quercitin and rutin (Shams Ardekani et al., 2009), flavonones and flavones (Newwar et al., 1994) in pericarp. Anthocians, flavonones, flavones, flavonols and isoflavonols were identified in the flower extracts of two Tunisian cultivars (Fellah et al., 2018), while Al-Rwahi isolated Kaemferol 3-orutinoside, kaempferol derivatives, isorhamnetin and hexahydroxydiphenoyl -glucosideacetyl glucoside derivatives. (Al-rawahi et al., 2014)

A study on nine Spanish cultivars (Fernandes et al., 2017) identified a total content of flavonoids between 20.8 and 189 mg QE/100 ml juice, where two cultivars 'Katirbasi' and 'CG8' have shown the highest level, and 'Parfanka', 'Wonderful 2' and 'Cis 127' with the lowest level of total flavonoids. All the values in the study were slightly higher than the ones obtained in the Turkish varieties - 38.78 and 45.50 mg QE/100 ml aqueous extract (Orak et al., 2012).

V. Anthocyanins

Anthocyanins, class of flavonoids, are responsible for the colours that can vary from orange to blue in flowers, leaves, fruits, seeds or other tissues. Most of the times, carotenoids and anthocyanins are present together in the same tissue or organ, which gives increased a intensity and a variety of colours, next to the pH and the metal ions that are present (Tanaka et al., 2008). In pomegranate, anthocyanins are the main class of pigments and are responsible of the aril and peel colour, depending on the complex of glicosides that are present. Only 3-glicosides and 3,5-diglucosides of cyanidin, delphinidin and pelargonidin, are identify in peel, the species having a high concentration of anthocyanins in comparation to other fruits (Masci et al., 2016). Research on the anthocyanins content in the fruit skin identified only cyaniding and pelargonidin derivates (Tanaka et al., 2008). The increased interest for the anthocyanins in pomegranate comes from their role in the antioxidant activity: preventing the formation of free radicals by chelating iron and scavenging of free radicals (Fischer et al., 2011). The total content of anthocyanins varies depending on the cultivar, the maturation stage and the type if exposure, an increase of anthocyanins concentration being reported in the last stage of fruit ripening (Fernandes et al., 2017) (Figure 2).

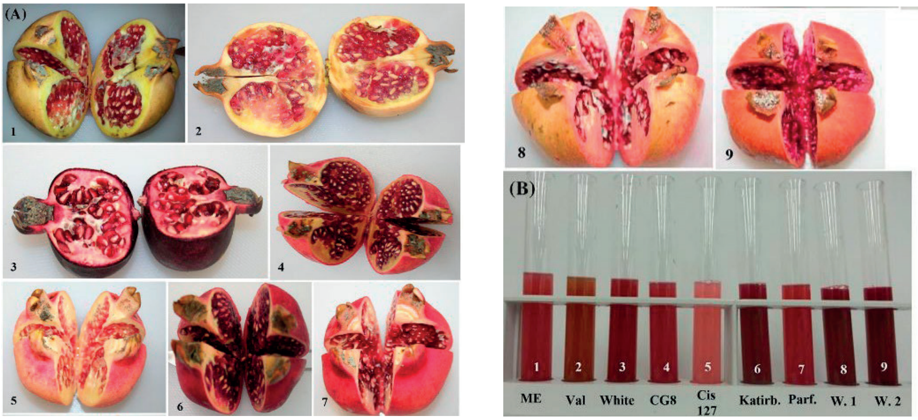

Figure 2. Pomegranate fruits (a) and juices (b) of nine cultivars grown in Spain: 1 - Mollar de Elche, 2 -Valenciana, 3 - White, 4 - CG8, 5 - Cis 127, 6 - Katirbasi, 7 - Parfianka, 8 - Wonderful 1, 9 - Wonderful 2 (Fernandes et al., 2017)

3,5-diglucosides predominate in the early ripening stage and delphinium based derivates are mostly found, while in the later ripening stages, the concentration of monoglicosides raises, and cyanidin based derivates become predominant (Gil et al., 1995).

Research by Fischer et al. (2011) has revealed a series of anthocyanins in the peel of some unknown Peruvian cultivars: the most representative - delphinidin 3,5 diglucoside (10.8 mg/kg), followed by cyanidin 3,5-diglucoside (157.8 mg/kg), pelargonidin 3,5-diglucoside (145.8 mg/kg), delphinidin 3-glucoside (13.3 mg/kg), cyanidin hexoside (1.7 mg/kg) and cyanidinpentoside (1.4 mg/kg), cyanidin 3-glucoside (41.2 mg/kg) pelargonidin 3-glucoside (56.7 mg/kg), cyanidin 3-rutinoside (18.4 mg/kg).

They reported that there still are molecular structures that are not identified in this species, as cyanidin 3-rutinoside and cyanidin-pentoside were not mentioned in the literature at the time of the study. Antocians were not found in the aril and peel of green or immature fruits, and seed analysis evidentiated the presence of delphinidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3,5-diglycoside, cyanidin 3,5-diglucoside, pelargonidine 3,5-diglucoside and pelargonidine 3-glucoside (Sreekumar et al., 2014)

The activity of delphinidin, cyanidin, and pelargonidin on H_2O_2 induced lipid peroxidation in rats and the values of 0.7, 3.5, and 85 íM, respectively, showed that this three antocianidini contribute to the antioxidant activity of the pomegranate fruit, delphinidin being considered the main constituent that gives the juice the inhibitory effect on H_2O_2 – induced lipid peroxidation. (Noda et al., 2002).

VI. Fatty acids

Fatty acids are present in different concentrations in pericarp (Jurenka, 2008; Moorthy et al., 2013), in leaf (1.7 ± 0.96%) (Yan et al., 2017), fruit peel (1.2%), seeds (4.8%), whole fruit (1.4%) (Sharma et al., 2018) and juice (Liu et al., 2009). The quantity of oil/kg of seeds varies not only depending on the genotype but also on the extraction method (Abbasi, 2008). The saponification point of pomegranate oil is 188.9 and the high breakdown rate of pomegranate oil can be attributed to the high trans-fatty acid content (El-Nemr et al., 1990). Although pomegranate seeds have a low content of polyphenols (Singh et al., 2016), the oil extracted from them has multiple nutraceutic uses, having a high content of phytosterol and punicic acid. In the majority of industrial extraction processes used for processing pomegranate, the seeds are discarded, despite the fact that these are an important source of polyunsaturated fatty acids, sugars, proteins and other bioactive compounds (Yoshime et al., 20169). Recent studies on pomegranates from Iran, Turkey, Spain and China evidentiated the antioxidant activity of pomegranate oil, and the composition of fatty acids gained interest for further research. In the oil obtained from the seeds, 83.6% of the fatty acids are saturated and 16.3% unsaturated

(Momeni et al., 2021). The lipid content of seeds varies between 140-270 g/kg dry weight, hence they are rich in lipid (Lansky & Newman, 2007). Johanningsmeier & Harris (2011) mentioned that the oil extracted from the seeds of 15 Turkish cultivars contains α-eleostearic, linoleic, oleic, catalpic, palmitic, stearic, β-eleostearic, gadoleic, arachidic, and behenic acids and punicinic acid represents between 70-76% or the oil composition (Johanningsmeier and Harris, 2011).
The average content of seeds is between 37-143 g/kg fruit, depending on the ripening stage, culture conditions, geographic location and cultivar (Fernandes et al., 2017). A consistent variability between genotypes was identified in a study on pomegranates from Puglia, Italy, the values being between 10.7 % (‘ModTri’) and 26.8% (‘Ako’) in case of sweet genotypes and between 4.9% (‘SouTri’) and 17.4% (‘Wond’) for the sour genotypes (Ferrara et al., 2014). Pomegranate oil contains phyto-estrogens, which are very similar with the ones produced by the human body (Abbasi et al., 2008). Van Elswijk et al. (2004) isolated from the pomegranate oil steroidal estrogens (g-tocopherol, 17-a- oestradiol, stigmasterol, β-oestriol sitosterol and testosterone) and non-steroidal compounds (compestrol, coumestrol) (van Elswijk et al., 2004).

VII. Alkaloids

The presence of alkaloids was reported in the fruit peel, seeds, bark and in the whole fruit (Sharma et al., 2018), but their identification begins in 1994, when an unusual alkaloid was isolated from pomegranate leaves: N-(20,50 - dihydroxyphenyl)-pyridinium chloride (Schmidt et al., 2005). In 2016, a new alkaloid was discovered, pyrrolidine, isolated from the rid of *Punica granatum*. Pyrrolidine was tested on MDR *Klebsiella pneumonia* and it had efflux inhibition activity at a concentration of 6 mg (Rafiq et al., 2016). The four most studied alkaloids isolated from *Punica granatum* are pelletierine, pseudopelletierine, isopelletierine and methylisopelletierine, the one with the best anthelmintic activity being pelletierine (Wibaut et al., 1954). Piperidine alkaloids were isolated from the roots of different pomegranate cultivars (Jurenka, 20018). Pelletierine, pseudopelletierine, isopelletierine and methylisopelletierine are considered the most importants alkaloids found in *Punica granatum*.

CONCLUSIONS

An analysis of over 100 scientific papers or research from literature highlights the importance of pomegranate for human health and we selected over 70 for this paper. Having a wonderful taste, either sweeter or sour, the pomegranate fruit and its derivates (molasses, juice, concentrate) have a remarkable antioxidant activity compared to other fruits and have a capacity to reduce free radicals. Worldwide, pomegranate is an edible species that still needs to be studied regarding its chemical compounds that are still not totally identified.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VIRUS INFECTIONS IN NEW PLUM ORCHARDS FROM MOLDOVA, ROMANIA

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Abstract

A survey to assess the phytoviral status of eleven new plum orchards established in Moldova region from Romania was carried out in 2020. Sampling trees were tested by DAS-ELISA for the presence of six viruses: Plum pox (PPV), Prune dwarf (PDV), Prunus necrotic ring spot (PNRSV), Apple chlorotic leaf spot (ACLSV), Apple mosaic (ApMV) and Myrobalan latent ringspot (MLRSV). Ten out of eleven surveyed plum orchards proved to be infected by at least one virus. PPV infections have been present in ten orchards, with a rate between 0.5-79%, which reflects a rather serious situation if we take into account that the orchards are young. Infections with PDV were present in two orchards with a rate between 5-15%, while PNRSV was confirmed in other two orchards with a rate between 15-30%. Overall, the average of infection rate of PPV in the surveyed orchards from Moldova was 19.4%, of PDV was 1.8% and of PNRSV was 4.1%. No infection with ACLSV, ApMV and MLRSV was found in the surveyed orchards.

***Key words**: plum, propagation material, survey, DAS-ELISA, viruses.*

INTRODUCTION

The plum has an important economically impact in Romania due to its domination between fruit species (FAOSTAT, 2018).

In most European countries, including Romania, plum is highly affected by Sharka disease, produced by *Plum pox* virus (PPV), known as the most detrimental viral pathogen that affect stone fruits (Barba et al., 2011; Cambra et al., 2006). That because it reduces the quality of the fruits and causes premature dropping, being the most significant factor that limits the plum production (Dunez, and Sutic, 1988; Nemeth, 1994, Stoev et al., 2004). Sharka disease was described for the first time in Bulgaria at the beginning of the 20th century (Atanasoff, 1932), and since then, has progressively spread to a large part of the European continent, being found in America (Chile, Argentina, USA and Canada) and Asia (India, China, Pakistan, Kazakhstan, Iran and Japan) (Capote et al., 2006; García, and Cambra, 2007). In Romania, PPV was found in all plum-growing areas causing serious yield losses (Zagrai et al., 2010).

Other viruses, such as *Prune dwarf* (PDV), *Prunus necrotic ring spot* (PNRSV), *Apple chlorotic leaf spot* (ACLSV), *Apple mosaic* (ApMV) and *Myrobalan latent ringspot* (MLRSV) also might cause direct or indirect damages, as growth reduction, loss of plant vigour, a decrease of quality values, and overall with a negative effect on productive parameters of the crops (Hadidi and Barba, 2011).

A proper management of virus diseases represents a priority in any strategy to limit their damages on the fruit yield. In case of infection with viruses, trees can no longer be treated in the orchard. Therefore, the prevention measures are very important to control virus diseases, such as using resistant cultivars and rootstocks, planting material with virus free status, establishing the new orchards far away from sources of infection, applying treatments against virus vectors. Also, preventing viruses introduction into new area is essential because no eradication by any methods is possible once these pathogens infect an area where trees are growing (Reed, and Foster, 2011). However, there are often situation when the virus infection overcome these prevention measures and escape in the new orchards.

In spite of European regulatory and EPPO standards requested for plant certification, there are cases with deficiencies in its implementation. In addition, free movement of propagating material within the European Union increases the risk of introduction of new viruses or viral strains in new areas. Thus, new virus outbreaks may occur and can create serious issues in new orchards and, sometimes, even can compromise the investment. Once a virus infection accidentally occurs in the young orchards, removing of the infected trees remain the main measure for limiting the virus spreading. Therefore, early identification of infections in new orchards can sometimes be plum tree life-saving. Thus, the monitoring of viruses in the new orchards, followed by suitable measures for limiting their spreading depending on the specific phytoviral situation observed, may reduce the damage caused by viruses.

New plum orchards were established in the last years by using planting material produced both in Romania and in different European countries. This allowed us to get information about initial virus status of planting material and potential outbreaks by assessing the incidence of the viruses in the young orchards.

MATERIALS AND METHODS

Eleven young plum orchards from Moldova region were the subject of the survey in the summer of 2020. Six of these were established by using propagated material in Romania, and the other five with material from Austria, Czech Republic, Netherlands, Italy and Hungary.

Two blocks with a total of 200 trees (each block of 100 trees) from each orchard were first monitored by visual observation of viral symptoms development. The surveys were mainly focused on typical PPV symptoms on leaves that allowed getting a preliminary evaluation on the incidence of PPV based on the visual observations. Then, ten trees from each block were sampled for virus diagnosis by serological assays, as follow: when PPV incidence based on visual observations was lower than 10%, one symptomatic and nine asymptomatic trees were randomly sampled. When the visual incidence was between 10 and 20%, two symptomatic and eight asymptomatic trees were sampled, and so on, so that when PPV visual incidence was between 90-100%, ten symptomatic trees were sampled. In the case of no symptomatic trees was founded, ten trees were randomly sampled from each block. Because PPV-M, known as the most epidemic strain of PPV was not reported so far in Romania (Zagrai et al., 2010), additional samples with typical PPV symptoms were collected from young orchards established with planting material from abroad in order to check its potential overcoming of the borders (data not show). For virus diagnosis by serological assays a minimum of ten leaves per tree were randomly collected throughout the canopy. In PPV symptomatic trees, only symptomatic leaves were collected. If symptoms were limited to particular branches, leaves were only sampled from symptomatic branches.

A total of 220 trees were sampled for virus diagnosis. Serological tests were performed by Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Clark and Adams, 1977) using a commercial polyclonal antiserum to PPV, PDV, PNRSV, ACLSV, ApMV (Bioreba, Switzerland), and MLRSV (Sediag, France) according to the manufacturer's instructions. Absorbance values were measured at 405 nm after 1 h substrate hydrolysis. Samples were considered positive if their absorbance values were more than twice those of the negative control. Then, a rate of infection was established for each virus.

If was the case, the nearby plum orchards (1-200 m) were visually checked and has been established the incidence of PPV based on the observed typical symptoms in order to check the potential presence of nearby outbreaks/sources of infection (data not show).

RESULTS AND DISCUSSIONS

The results of plum young orchards surveyed for the presence of viruses by visual monitoring and serological assays are show in Table 1.

The data collected revealed that ten out of eleven orchards have viral infections with at least one virus, *Plum pox virus* infections being the most troublesome.

Although the level of infection rate was different from one orchard to another, in some cases it was quite similar. Thus, six orchards showed a PPV infection rate between 0.5-10%, two orchards between 11-20%, and the other two between 71-80%. The serological analyses against ACLSV, ApMV and MLRSV revealed that these viruses were not present in none of the samples tested.

Plum orchards established with planting material produced in Romania (no. 1, 3, 4, 7, 8 and 11). PPV was visually observed by symptoms development, and then infection was confirmed by serological tests in all six plum orchards, with an incidence between 6% and 79%. PDV was found in two out of six surveyed orchards, with an incidence between 5% and 15%, and PNRSV in one orchard, with an incidence of 30%.

A high rate of PPV infection (73% and 79%, respectively) founded in two orchards (no. 3. Scobinti and no. 4. M. Bucium) correlated with the young age of the orchards, and also a virus spreading throughout the canopy suggests that most part of the planting material was provided as infected from the nurseries. This hypothesis is also supported by the fact that an external source of the inoculum was observed at a distance of over 200 m, and it was represented only by isolated trees in family gardens that could not contribute to such critical situation. These orchards represent an outbreak and a source of PPV for new potential orchards set up around it. Applying a PPV eradication strategy in such cases would mean the destroying of such orchards. This radical measure is not in agreement with the orchard owners' management expectation because of the missing of governmental compensation. Thus, this kind of recommendation is not acceptable and remained just theoretically.

Table 1. Incidence rate of viruses based on visual observation and DAS-ELISA assay on eleven plum orchards from Moldova region

Orchard no./ location (county code)	Provenance of plant material	Visual rate of PPV (%)	Virus incidence (%) based on serological tests (DAS-ELISA)					
			PPV	PDV	PNRSV	ACLSV	ApMV	MLRSV
1. Itcani (BC)	Romania	23.5	23.5	0	0	0	0	n/a
2. Plopana (BC)	Italy	0.5	0.5	0	15	0	0	n/a
3. Scobinti (IS)	Romania	79	79	0	0	0	0	0
4. M. Bucium (IS)	Romania	78	73	5	0	0	0	0
5. Podul Iloaiei (IS)	Austria /Czech Rep.	2.5	2.5	0	0	0	0	0
6. Padureni (IS)	Netherlands	0.0	0	0	0	0	0	0
7. Icusesti (NT)	Romania	12.5	15.5	15	0	0	0	n/a
8. Husi (VS)	Romania	6	6	0	0	0	0	n/a
9. Husi (VS)	Hungary	0.5	0.5	0	0	0	0	n/a
10.Grumezoaia (VS)	Hungary	7	7	0	0	0	0	n/a
11. Crasna (VS)	Romania	6	6	0	30	0	0	n/a
Average		19.6	19.4	1.8	4.1	0	0	0

Two other orchards (no. 7. Icusesti and no. 1 Itcani) revealed a rate of PPV of 15.5% and 23.5% respectively. In both situations, the overwhelming proportion of trees with widespread infections throughout the canopy suggests a high probability that part of the planting material has been infected since the plum nurseries. Also, the presence of trees with partly infections revealed the potential occurring PPV both from infected trees within the orchard, and from its vicinity. In these cases, the removing of infected trees in order to limit the impact of PPV is not an economical solution for the owners. Given that the orchards have varieties that tolerate the PPV damages on fruits, the plantation can be economically profitable, but its profitability will be greatly diminished.

The lower rate of PPV infection was recorded only on two orchards established with planting material propagated in Romania, with an infection rate of 6% (no. 8 Husi and no. 11. Crasna). The occurred PPV infections throughout the canopy of infected trees, correlated with the absence of nearby sources of infection and the young age of the plantations (2-3 years), suggests that the planting material has been infected since the plum nurseries. In these cases, it was recommended to extend the monitoring of orchards, to eliminate the trees that show typical symptoms of the *Plum pox virus* and to replace them with healthy trees. Monitoring these orchards in the following years to eliminate any subsequently infected trees and applying additional treatments to control aphid vectors could significantly contribute to the containment of PPV spreading and its impact in these young plum orchards.

Plum orchards established with planting material produced in other European countries (no. 2, 5, 6, 9 and 10). Four out of five orchards were found to be infected by PPV with an incidence between 0.5% and 7%, and one orchard was founded with a rate of 15% infection by PNRSV.

Two of these orchards (no.2 Plopana and no. 5. Podu Iloaiei) are located in the proximities of trees infected by PPV. Thus, the infections in the new two orchards were most likely caused by the presence of scattered trees in the vicinity that have facilitated the transmission of the virus especially because they did not receive any treatments to control aphid vectors. This is also supported by the fact that the infected trees in the two orchards developed PPV sporadical symptoms, just on a few shoots, the most part of the canopies remaining symptomless.

The other two orchards (no. 9. Husi and no. 10. Grumezoaia) are very well isolated for external PPV sources being located far away from any potential host for this virus. The fact that one or two years old trees showed PPV symptoms throughout canopy, since no infection sources in the vicinity of orchards were present, suggests that the infected trees acquired the virus before planting.

Overall results revealed that ten out of eleven young plum orchards surveyed in Moldova region were found infected by at least one virus. One orchard (no. 6. Padureni) did not express any symptoms and did not confirm any virus by serological tests, thus proved to be virus free (Figure 1).

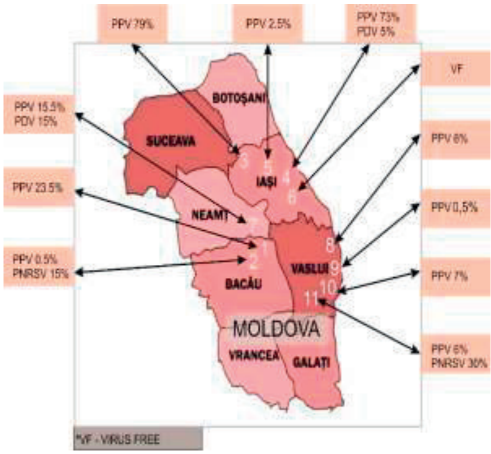

Figure 1. The map of virus incidence on young plum orchards from Moldova region

The average virus incidence at the Moldova region level in the surveyed plum orchards were as follow: 19.4% of PPV, 1.8% of PDV, and 4.1% of PNRSV. ACLSV, ApMV and MLRSV was not confirmed in any young plum orchards surveyed in Moldova region (Figure 2).

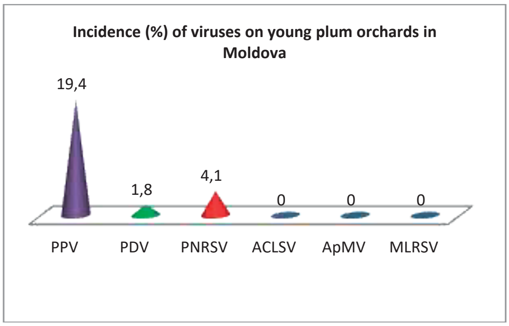

Figure 2. The incidence of viruses in young plum orchards from Moldova

Interestingly, the comparative analysis of the results revealed insignificant differences between the results obtained based on visual observation and serological assays related to *Plum pox virus* in young plum orchards (19.6%, respectively 19.4%). This finding has a practical importance because the owners can proceed themselves periodically surveys by visual monitoring of PPV with the condition to know how the virus express typical symptoms especially on leaves.

Given that PPV is a virus that causes a disease with a major economic impact, farmers' awareness of personal involvement in the early identification of this virus in orchards can significantly contribute to limiting of PPV, of course alongside of other measures depending of the situation.

CONCLUSIONS

Ten out of eleven plum new orchards from Moldova region proved to be infected by at least one virus by using serological assay. PPV was the prevalent virus, followed by PNRSV and PDV. No infections with ACLSV, ApMV and MLRSV were found.

ACKNOWLEDGEMENTS

This work was supported by Romanian Ministry of Agriculture, contract ADER 7.3.13 / 2019.

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VITICULTURE AND OENOLOGY

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRELIMINARY STUDY ON THE INHIBITION OF ALCOHOLIC FERMENTATION USING OCTANOIC AND DECANOIC ACIDS TO OBTAIN AROMATIC WINES WITH RESIDUAL SUGAR

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Abstract

Aromatic wines produced from 'Tămâioasă românească' variety can be fermented to dryness, but they are usually preferred with residual sugar. To preserve some of the sugar unfermented the natural alcoholic fermentation can be stopped by several methods. One of the newest methods researched is the use of medium chain fatty acids, which are naturally produced by yeasts and are also inhibitors of fermentation. In this study doses of 10-30 mg/l of octanoic and decanoic acids, as well as combinations of both acids in doses of 15 mg/l were used to inhibit the fermentation of wines inoculated with 2 different yeast strains. The final sugar content of the resulted wines along with some other physico-chemical and sensory parameters were determined and compared. Our preliminary observations showed that the inhibition is dose dependent and also that the decanoic acid tends to be more inhibitory than octanoic acid.

Key words: *'Tămâioasă românească' wine, alcoholic fermentation inhibition, medium chain fatty acids, octanoic acid, decanoic acid.*

INTRODUCTION

Sweet wines are, in most cases, intended for dessert. The high content of sugar makes this type of wine a suitable drink for the end of every meal as it can stimulate digestion and quench the appetite. In addition, some sweet wines can be served as appetizers.
Sweet wines require a greater addition of SO_2 not only for conservation, but also for stopping the fermentation to preserve some of the natural sugars in the wine. Over time, many researchers have focused on reducing the amount of SO_2 in wine by partially or totally replacing it with various oenological materials such as: lysozyme, potassium sorbate, dimethyl dicarbonate and, more recently, medium chain fatty acids (Antoce et al., 2005; Babikova et al., 2012, Santos et al., 2012; Baron et al. 2017).
Medium chain fatty acid derivatives (MCFAs, containing 6-14 carbon atoms) are natural compounds found in high concentrations in some foods and are a source of energy for the body (Michelle et al., 2019). Due to their ubiquity, they are used in various applications such as for the production of cosmetics, lubricants, biodiesel products or cleaning products.
Yeasts can also produce some MCFA and some of them have proven anti-fungal properties (Guilloux-Benatier et al., 1998; Antoce et al., 1997, 1998). However, few studies are available in the literature (Froissard et al., 2015).
Octanoic acid (C8) and decanoic acid (C10) belong to the group of Medium Chain Fatty Acids (MCFA). They are naturally present in wine, even though in a low concentration, being produced by yeasts during alcoholic fermentation as by-products of lipid synthesis (Legras et al., 2010). In oenology, they are studied as inhibitors of alcoholic and malolactic fermentation (Baron et al., 2011; 2014). During the fermentation of the must, the toxicity of these fatty acids for the microorganisms is increased by ethanol and low pH, which favours their penetration into the cells by passive diffusion through membranes in a non-

ionized form, dissociating afterwards at higher internal pH, leading to a decrease of the intracellular pH and disturbing homeostasis (Antoce et al., 1998; Legras et al., 2010).

Beside their antimicrobial activity, MCFA, together with their esters formed during alcoholic fermentation, can have a significant flavour effect on wine due to their higher solubility and volatility (Waterhouse et al., 2016).

For this study, 'Tămăioasă românească' variety was selected as a model for the production of sweet white wines. This aromatic muscat-type grape variety is very suitable for the production of natural sweet wines, due to its high sugar accumulation.

In order to obtain sweet wines by preserving some of the natural sugar from grapes, often the fermentation should be stopped. Usually, the fermentation is stopped by adding high doses of SO_2 and lowering the temperature in the fermentation tank. In an attempt to reduce the effects of high doses of SO_2 which may be less tolerated by some people, but still obtain a similar effect, in this study we used various doses of MCFA and lower doses of SO_2 to halt the alcoholic fermentation.

MATERIALS AND METHODS

'Tămăioasă românească' musts were produced from grapes harvested at 230 g/l sugar, crushed, and macerated for 8 hours at 14-15ºC.

Two winemaking protocols were used, the main difference among them being the yeast inoculated for the fermentation: ERSA (*Saccharomyces cerevisiae* provided by Enologica Vason) and TR313 (*Saccharomyces cerevisiae bayanus* from Rennaisance Yeast), respectively.

The addition of the MCFA for the inhibition of alcoholic fermentation was performed in the must, when the fermentation was med-way and the sugars reached about 10% Brix (more precisely, 10.0% Brix for the musts with TR313 yeast and 10.8% Brix for musts with ERSA).

The reagents used were as follows: octanoic acid (n-caprylic acid) from Kishida Chemicals, Osaka, Japan, purity >98.0%, decanoic acid (n-capric acid) from Wako Pure Chemical Industries, Osaka, Japan of purity >98% and sulphur dioxide solution of 6% from Miflachim Group, Romania.

The doses used for inhibition of alcoholic fermentation are summarised in Table 1, for each yeast experimental variant resulting 9 samples and 3 repetitions each.

The sugar consumption during fermentation was closely followed in each batch and at around 10% Brix the fermenting musts were treated with the specified doses (Table 1) of SO_2, octanoic acid and decanoic acid.

After the wines were clarified with 0.6 g/l bentonite and racked, all the samples were analysed in laboratory. The main wine parameters were determined in accordance with the OIV methods: alcoholic concentration was determined by distillation method OIV-MA-AS312-01A, sugar concentration in must by refractometric method OIV-MA-AS2-02 and in wine by the chemical method OIV-MA-AS311-01A, total acidity and pH by potentiometric method OIV-MA-AS313-01 (OIV, 2019).

Table 1. Wine samples coding and the doses of the inhibitors used for halting the alcoholic fermentation

No.	Repetitions	Samples coding*		Octanoic acid mg/l	Decanoic acid mg/l	SO_2 mg/l
		Yeast TR313	Yeast ERSA			
1-3	3	TR_313_0	TR_ERS_0	-	-	120
4-6	3	TR_313_OC10	TR_ERS_OC10	10	-	60
7-9	3	TR_313_OC20	TR_ERS_OC20	20	-	60
10-12	3	TR_313_OC30	TR_ERS_OC30	30	-	60
13-15	3	TR_313_DE10	TR_ERS_DE10	-	10	60
16-18	3	TR_313_DE20_r1	TR_ERS_DE20	-	20	60
19-21	3	TR_313_DE30_r1	TR_ERS_DE30	-	30	60
22-24	3	TR_313_OC10DE10	TR_ERS_OC10DE10	10	10	60
25-27	3	TR_313_OC15DE15	TR_ERS_OC15DE15	15	15	60

*TR= 'Tămăioasă românească' variety; 313 is abbreviation for the yeast TR313 used for fermentation (not to be confused with the variety name only 313 from the yeast name was used); ERS is abbreviation for the yeast ERSA. Also, octanoic and decanoic acids were abbreviated as OC and DE.

RESULTS AND DISCUSSIONS

For the wine samples prepared with several combinations of MCFA the influence of some major wine parameters was determined and discussed.

Influence of MCFA on pH

The management of pH in winemaking is of importance because this parameter impacts the colour stability, the precipitation of tartaric salts, the preservation of aroma compounds of the wine, being involved in the chemical reactions that are related to the formation, degradation and loss of several compounds in the wine. At the same time, the pH can influence directly or indirectly the protection against microorganisms that can alter the wine.

pH is the main parameter which determines the antimicrobial activity of the sulphur dioxide. The lower the pH, the higher the activity of sulphur dioxide used, because the same dose of free SO_2, at lower pH, has a higher ratio of molecular SO_2, which is the active form against microorganisms (Viegas et al., 1989).

Because MCFA are organic acids, they may also have influences on the pH of the final wines. Also, their inhibitory action is correlated to their molecular form (Antoce et al., 1997), which interferes at the membrane level of the microorganisms, disturbing the absorption of nutrients (Stevens and Hofmeyr, 1993).

The influence of the used doses of MCFA are presented, for both yeast fermentations, in Figure 1.

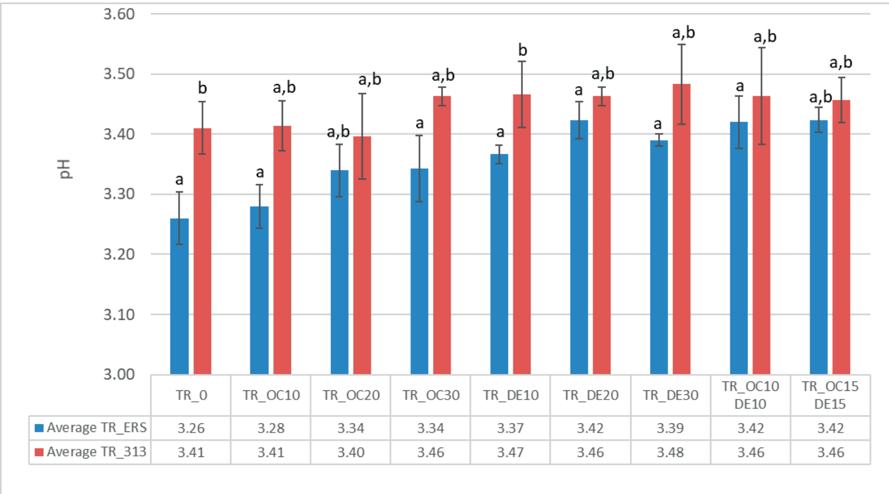


Figure 1. Mean ± SE of the pH of 'Tămâioasă românească' wine samples in which octanoic (OC) and decanoic (DE) acids in doses of 10, 15, 20 and 30 mg/l were used to stop alcoholic fermentation. Blue bars represent wines fermented with ERSA yeast and the red bars represent wines fermented with TR_313 yeast. Different letters represent significant differences of the means at the level of $p \leq 0.05$ in accordance with the post-hoc Tukey's Test run for 2-way-ANOVA analysis

At the MCFA doses employed, only small pH differences are determined, with no statistical differences among samples fermented with the same yeasts. However, the yeast used for fermentation induced statistically significant differences in the final wine pH, from 3.3 to 3.4 for ERSA and 3.4 to 3.5 for TR_313. It is clear that these differences were induced due to different rates of fermentation and metabolites produced by the specific yeasts and not by the doses of MCFA used, as the control wine pHs are, for the same yeast, in the same range as the samples treated with fermentation inhibitors.

Irrespective of the yeast, a small tendency to increase the pH is observed in the samples treated with MCFAs, but the fermentation medium had sufficiently high buffering power to prevent the pH increase. Thus, irrespective of the octanoic or decanoic acid dose used to inhibit fermentation, the pH of the final wine did not rise sufficiently to be statistically significant.

However, this tendency of the acids to impact the medium pH, even in a very discrete, statistically not significant way, may have a slight contribution to the inhibition power of these acids, as well as of the SO_2, for all these inhibitors lower doses being necessary to have the same antimicrobial effect at a lower pH than at higher ones.

Influence of MCFA on total titratable acidity

Closely related to the pH, which is the real acidity perceived, the total titratable acidity is a parameter with more complex influence on the product stability and sensory characteristics. The amount of the typical acids in must and wine can vary within fairly wide limits, depending on the climatic conditions of the year, the degree of ripeness of the grapes, soil type, phytosanitary status of grapes, the processing of grapes, the storage of wine and more. Beside the influence on the pH, stability of salts, phenols and colour, chemically, these acids can interact with alcohols and form esters, some contributing to wine aroma as well. As also stated before, many acids directly influence the growth of microorganisms, being beneficial for the final product stability.

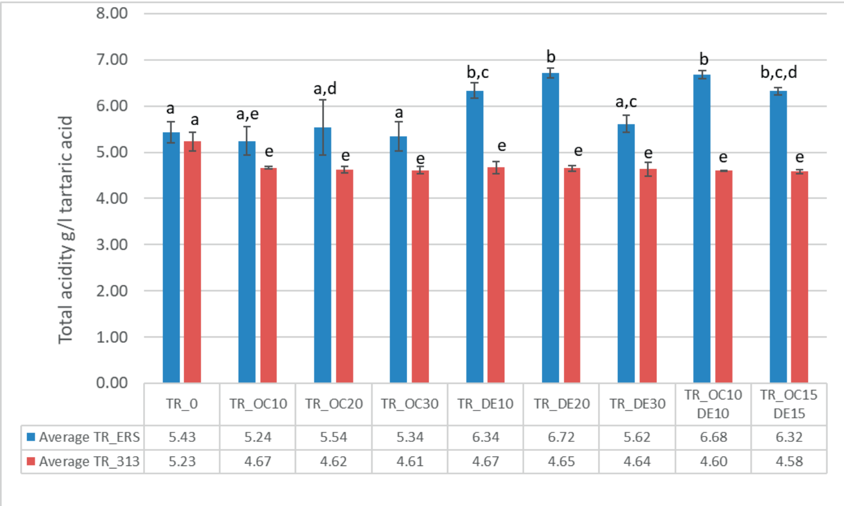


Figure 2. Mean ± SE of the total titratable acidity (expressed in g/l tartaric acid) of 'Tămâioasă românească' wine samples in which octanoic (OC) and decanoic (DE) acids in doses of 10, 15, 20 and 30 mg/l were used to stop alcoholic fermentation. Blue bars represent wines fermented with ERSA yeast and the red bars represent wines fermented with TR_313 yeast. Different letters represent significant differences of the means at the level of $p \leq 0.05$ in accordance with the post-hoc Tukey's Test run for 2-way-ANOVA analysis

Thus, the acidity of the wine can directly or indirectly influence the inhibition of alcoholic fermentation and act synergistically with the added MCFA and SO_2.

In correlation with the observation on the overall wine pH, the total acidity (Figure 2) was higher in the samples with lower pH. This inverse correlation is especially very clear in most of the samples fermented with ERSA yeasts. The wines fermented with TR_313 yeasts and added with any of the octanoic or decanoic acid dose found their stability at lower levels as compared with the control fermented in the absence of MCFA (5.2 g/l total acidity). This effect was correlated with the tartaric acid salts precipitation in these wines and led to a quite similar final acidity, at around 4.6 g/l.

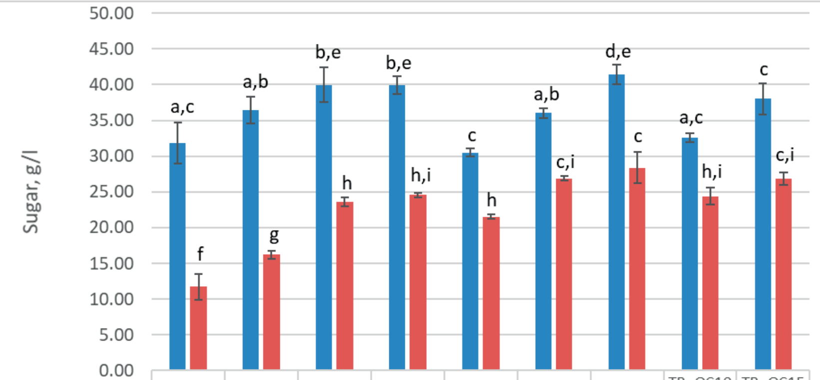
In the wines fermented with ERSA yeasts we observed that the addition of the MCFA led to increases in total acidity (5.5-6.7 g/l total acidity in tartaric acid units) as compared to the control wine fermented with the same yeast (5.4 g/l total acidity), especially the wines added with decanoic acid alone or in combination with octanoic acid having statistically higher acidities as compared with control. This observation may indicate that the inhibitory action of decanoic acid may be more important than the one exerted by octanoic acid. Thus, it is to be expected that decanoic acid is better at inhibiting the alcoholic fermentation than its inferior homologues in the saturated fatty acid series.

Influence of MCFA on residual sugar content

The residual sugar content, that is the sugar concentration naturally remaining in wine after the cessation of fermentation, is a parameter mostly determined for the classification of wine from a legislative point of view in dry (< 4 g/l sugars as glucose and fructose), half-dry (4.01-12 g/l sugar), half-sweet (12.01-45 g/l sugar) and sweet (>45 g/l sugar). At the same time sugar plays an overwhelming role in the sugar-acidity balance of wines and in the general sensory perception.

As a consequence, sweet wines are preferred for certain food association and consumption patterns, being increasingly popular in the world as niche products, suitable for fast consumption, but also for long aging. For this reason, this study focuses on finding an alternative method to stop the alcoholic fermentation and leave a certain amount of unfermented sugar in the final wine.

The sugar content of the must in fermentation is often correlated with the existing population of active yeasts and, obviously, inversely correlated to the alcohol formed from it by the yeasts. Choosing the optimum moment for the inhibition of alcoholic fermentation is not an easy task, as it depends very much, beside the composition of the must, on the fermentation rate of the population of active yeasts. The higher or stronger the population of active yeasts, the higher the doses of inhibitors required to stop the fermentation.



	TR_0	TR_OC10	TR_OC20	TR_OC30	TR_DE10	TR_DE20	TR_DE30	TR_OC10 DE10	TR_OC15 DE15
Average TR_ERS	31.84	36.45	39.99	39.99	30.50	36.04	41.38	32.60	38.01
Average TR_313	11.74	16.24	23.62	24.57	21.52	26.87	28.40	24.38	26.87

Figure 3. Mean ± SE of the sugar content of 'Tămâioasă românească' wine samples in which octanoic (OC) and decanoic (DE) acids in doses of 10, 15, 20 and 30 mg/l were used to stop alcoholic fermentation. Blue bars represent wines fermented with ERSA yeast and the red bars represent wines fermented with TR_313 yeast. Different letters represent significant differences of the means at the level of $p \leq 0.05$ in accordance with the post-hoc Tukey's Test run for 2-way-ANOVA analysis

The inhibition should be started when the sugar concentration is 10-20 g/l higher than the final desired content, to allow for the inhibition process inertia. Higher doses of MCFA, coupled with sulphur dioxide addition, are proven to be more efficient in rapid inhibition, which correlates with higher residual sugar content in the final wine (Figure 3).

Even though the moment of inhibition was similar for all the wine samples, the highest sugar concentrations remained in the wines fermented with ERSA yeast (between 32-41 g/l sugar), as opposed to wines fermented with TR-313 yeast (between 12-28 g/l sugar). For either yeast, the control wine, inhibited only with SO_2 (120 mg/l), showed the lowest residual sugar content of the series, in the same range of concentrations specific for the wines fermented with that yeast, but also inhibited by the addition of 10-30 mg/l doses of MCFA plus a half dose of SO_2 (60 mg/l). Accordingly, these results can be correlated with specific yeast behaviour, showing that TR-313 yeast, a *Saccharomyces cerevisiae bayanus* yeast, is more resistant to inhibitors and a faster fermenter than ERSA yeast, a classical *Saccharomyces cerevisiae* yeast. Thus, the cessation of fermentation needs to be started earlier in case of stronger yeasts such as TR-313, to accommodate for the longer inhibition inertia and to obtain wines with higher concentration of sugar, as it happens for more sensitive yeasts, such as ERSA. The inhibition inertia observed for the two yeasts can be explained mostly on their different resistance to sulphur dioxide, rather than to MCFAs. This fact is clear for the behaviour of the control wines, which were only added with sulphur dioxide as an inhibitor, at the same stage of their fermentation, but led to a quicker cessation of alcoholic fermentation in the case of ERSA (31.8 g/l sugar remaining) and a slower cessation in the case of TR-313 (11.7 g/l sugar).

It is worth noticing that the inhibition of fermentation tends to be dose-dependent. The effect was more evident in the case of ERSA, but also sufficiently evident in the case of TR-313 yeast.

As seen in Figure 3, in the range of 10-20 mg/l, the higher the concentration of the acid, the lower the sugar content was in the final wine. The doses of 30 mg/l have also a tendency to exert a higher effect, but in most cases the result obtained with 30 mg/l octanoic or decanoic acid was not significantly different than in the case of 20 mg/l of the same acid and same fermentation yeast. Only for ERSA 30 mg/l decanoic acid produced higher residual sugar in the final wine (41 g/l) as compared to 20 mg/l decanoic acid (36 g/l).

Although it was expected to observe a clearer higher effect with the decanoic acid, which has a higher molecular weight, our experiment did not lead to a conclusive result. Doses of 30 mg/l decanoic acid were more inhibitory than 30 mg/l of octanoic acid in both type of wines, irrespective of the fermentation yeast the results being a higher concentration of residual sugar when inhibition occurred in the presence of decanoic acids. For doses lower than 30 mg/l the results are mixed and more research is needed to confirm that decanoic is indeed more inhibitory for alcoholic fermentation in wine. The results produced by mixtures of octanoic and decanoic acids fall closely to the results obtained with single acids of similar doses, for the same fermentation yeast.

At the same time, the effect of these acids is amplified in samples that have a much lower pH and a much higher alcohol concentration, such as TR-313.

Influence of MCFA on alcohol content

Following the inhibition of alcoholic fermentation at a certain point to obtain wine with a desired residual sugar, alcohol concentration produced is lowered, in direct correlation with the sugar remained unconsumed. The alcohol concentration however is the main wine parameter, with legal and sensory importance. Moreover, even the inhibition of fermentation based on the addition of MCFAs is dependent on the alcohol concentration, their effect against yeasts being potentiated by each other. The higher the concentration of alcohol in the medium, the better is the inhibitory effect on microorganisms exerted by the addition of MCFA and/or SO_2. Thus, for a faster effect, the addition of octanoic and decanoic acid should be done when alcohol is already produced in sufficient quantities.

In the final wines, clearly correlated with the sugar concentrations remained unfermented, the alcohol concentration is lower in the sweeter wines. Accordingly, as explained in the previous section, the wines produced with the yeast more resistant to sulphur dioxide inhibition, TR-313, were able to ferment longer, even in adverse conditions, therefore contained in the end lower sugar concentrations (Figure 3), but higher concentrations of ethanol (Figure 4-red bars).

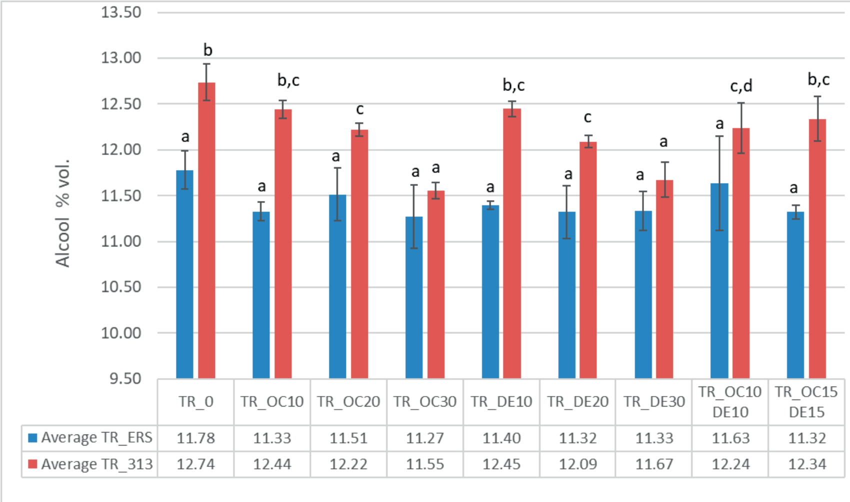


Figure 4. Mean ± SE of the alcohol content of 'Tămâioasă românească' wine samples in which octanoic (OC) and decanoic (DE) acids in doses of 10, 15, 20 and 30 mg/l were used to stop alcoholic fermentation. Blue bars represent wines fermented with ERSA yeast and the red bars represent wines fermented with TR_313 yeast. Different letters represent significant differences of the means at the level of $p \leq 0.05$ in accordance with the post-hoc Tukey's Test run for 2-way-ANOVA analysis

The less resistant yeast, ERSA (Figure 4-blue bars) is easily inhibited by both 120 mg/l SO_2 (control wine) and 10-30 mg/l doses of MCFA plus 60 mg/l SO_2, fermentation being stopped relatively at the same alcohol concentration (11.3-11.8% vol./vol. ethanol). No statistical difference was observed for the mean alcoholic fermentation of ERSA yeast samples, irrespective of the inhibitors or their doses.

In the case of the resistant yeast, TR-313, the values recorded for alcohol content in the final wines were significantly different (11.6-12.7% vol./vol. ethanol). The lower doses of MCFA (10 mg/l octanoic, 10 mg/l decanoic acid and some mixtures) did not lead to alcoholic concentrations significantly different than the control (range 12.4-12.7% vol./vol. ethanol). However, starting with 20 mg/l MCFA doses, the mean concentrations became significantly different than the control, the higher the dose, the less alcohol determined in the final wine. Also, the doses of 30 mg/l produced stronger significant effects than 20 mg/l doses, irrespective of the fatty acid used. The effect of the combinations of octanoic and decanoic acids however, did not display an additive effect, but rather behaved as the single 10 mg/l doses of MCFA. More research is needed to confirm or not this effect and to understand the potential mechanism.

As control samples for both yeasts contained the highest alcohol concentrations of their wine series, it is clear that sulphur dioxide alone, even in dose of 120 mg SO_2, was not as effective as the MCFA addition combined with a lower SO_2 dose of 60 mg/l.

CONCLUSIONS

The results performed on the 'Tămâioasă românească' variety highlight the efficiency of MCFA as antifungal agents able to inhibit alcoholic fermentation and leave some of the natural sugar unfermented. MCFA addition is thus a good alternative method to produce sweet of half-sweet wines.

The study showed that to halt the alcoholic fermentation the dose of medium chain fatty acid used is mainly correlated to the inhibitory effect, but also the type of acid (octanoic or decanoic in our case) can have influences, especially due to their potentially different effect on total acidity and pH. With a higher size and more clear influence on acidity, decanoic acid was expected to be more inhibitory than octanoic, but in the case of final sugar content in wines this assumption proved to be correct only for the higher doses (30 mg/l). Doses of singular acids of 10 mg/l are effective, but 20 mg/l are better, while 30 mg/l seem to lead in many cases to similar effects as 20 mg/l. Also, the combinations of both acids do not prove to be better in any way than single acid, but more research may be needed in this respect.

Most importantly, the study showed that the yeasts used for wine fermentation react differently to the endeavours to stop the fermentation, their resistance to sulphur dioxide inhibition having a decisive role, as sulphur dioxide in lower doses is used to complement the action of MCFA. Irrespective of the yeast, however, by using MCFA the dose of sulphur dioxide required to stop alcoholic fermentation can be lowered.

To obtain best results, the process of fermentation cessation should be started earlier during fermentation for the yeasts more resistant to the action of sulphur dioxide, to compensate for the time required to overcome the inertia to the inhibition, during which the sugar is still consumed and transformed in ethanol.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

USE OF ANALYTIC HIERARCHY PROCESS ON THE CHOICE OF GRAPEVINE VARIETIES SUITABLE FOR A SUSTAINABLE VITICULTURE AND MARKET DEMANDS IN THE VITICULTURAL CENTER STEFANESTI

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Abstract

The aim of this paper was to apply the AHP method for ranking grapevine varieties, evaluating the market demands and for developing a program for setting up new vineyards in a near future. Eight grapevine varieties ('Perlette', 'Muscat d'Adda', 'Fetească albă', 'Muscat Ottonel', 'Pinot gris', 'Cabernet Sauvignon', 'Burgund', 'Merlot') and nineteen quantitative and qualitative criteria have been used in the AHP exercise. The analyses were carried out using the Expert Choice Desktop software package. Based on the results, consistent with the recognition of the grapevine growing area and in the frame of climatic change conditions, Ștefănești viticultural centre requires further assessments regarding the cultivated grapevine genotypes, having a great potential for table grapes and white wine varieties.

Key words: *AHP, pairwise comparisons, ranking, Region III, Vitis.*

INTRODUCTION

From the beginning of this Century the global agricultural market has evolved to satisfy increased demand.
The Analytic Hierarchy Process (AHP) is one of the most used multicriteria decision making method that was firstly developed by Saaty (1977, 2008). This is a method that derives ratio scales from paired comparisons. AHP allows some small inconsistency in judgment because humans are not always consistent.
Being a simple and powerful tool, AHP was used both by decision makers and researchers in large areas of activity (social, personal, education, manufacturing, engineering, industry, commerce, government, sports, banking), and for different purposes (selection, evaluation, cost-benefit analysis, allocation, planning and development, priority a ranking, decision making, forecasting, medicine and other related fields) (Vaydia and Kumar, 2006; Enescu, 2017).
Also, the technique can be used for a sustainable development of the viticulture and oenology fields. AHP was applied to determine the pesticide using preferences of grapevine growers from three districts of Manisa, Turkey between environment friendly and conventional pesticide groups (Karabat and Atis, 2015). The identification of the best adaptation measure of the Tuscan viticulture under the conditions of climatic changes was realized using the same method by which three options of adaptation are specified: selection (establishing a genetic selection program so that the cultivated varieties are more suitable for the climatic conditions), relocation (relocation of vineyards to higher elevation, to reduce the impact of temperature changes and to maintain cultivation under similar conditions to the present), and switch (switch to other southern varieties, more suitable for warmer and drier projected conditions for the region) (Trombi et al., 2011). Dlbokić et al. (2017) used a SWOT – AHP hybrid model to discuss the possibilities of defining a strategy for further developing viticulture in the Jablanica district, as a sector which can potentially drive the overall development of agriculture in this region of Serbia.

In order to choose a vineyard for the production of superior quality wine, an AHP exercise included the following processes: the choice of grapevine plantation, the criteria picking, selection and significance of the examination, rating of the opinions and final aggregated priorities (Beltrán et al., 2010). This multicriteria decision method was perfectly applicable to rank wines, with fully satisfactory consistency degrees. Even though the wines are very close in quality, it is possible to obtain a more precise ranking, despite subjectivity and complexity (Pinto et al., 2016). In China, the wine industry was evaluated at Yalu River valley, to find out the main indicators affecting its competitiveness. The study explored implications and suggestions to strengthen small wineries and to empower wine companies to form a wine cluster under the Yalu River valley strategy (Yi and Lee, 2019).

In this study, the objective is the application of a hierarchical methodology for evaluating and ranking grapevine varieties, giving a scientific contribution to the viticultural market, including both table and wine grapevine varieties, by taking into consideration the Stefanesti viticultural centre tradition and new climate conditions.

MATERIALS AND METHODS

Stefanesti-Arges National Research and Development Institute for Biotecnology in Horticulture is located in the Stefanesti vineyard, in the southern central part of the Subcarpathian Mountains (Figure 1).

Figure 1. Location of Stefanesti town, Arges County, Romania (source:https://ro.wikipedia.org/wiki/%C8%98tef%C4%83ne%C8%99ti,_Arge%C8%99)

According to the Order no. 1205/2018 for the approval of the Nomination of the viticultural areas and the classification of the localities by viticultural regions, vineyards and viticultural centers, Stefanesti vineyard is part of Hills of Vallachia and Oltenia viticultural region (Region III).

Currently, the institute has a surface with vineyards that cover 156.12 ha, predominantly in the following cities: Stefanesti 34.39 ha; Calinesti 56.98 ha; Topoloveni 14.67 ha; and Pietroasa 50.08 ha. At the same time, it has an area of 400.97 ha under conservation, which can be set up as new vineyards, occupying the platform area, the slopes, the glacis and part of the Arges meadow. From a geographical point of view, the location is situated between 44°42" and 44°55" north latitude, covering the area of the Getic Piedmont, formed on the Candesti gravels and covers the left slope of the Arges River for a distance of almost 30 km. To the south, the boundary is formed by the Arges river corridor with a 2-3 km wide meadow between Pitesti and Gaesti. To the north, it climbs to the wide, smooth hills between the valleys that fragment the piedmont platform with altitudes of over 350 m. Specific to the western part of the territory is the fragmentation of the platform of deep valleys, both in the piedmont and in glacis and higher altitudes (400 m), while the eastern part of the territory is characterized by the greater extension of the unfragmented platform, crossed by valleys and lower altitudes (325 m).

A multitude of soils can be found on the territory owned by NRDIBH Stefanesti, both in a natural state as well as with deranged profiles created through management and slope levelling works. Due to the battered relief, they are mainly distributed in soil complexes. Based on the Romanian Soil Taxonomy System (SRTS), established in 2003 by the Institute for Research in Pedology and Agro-chemistry, Bucharest, the classification is in conformity with the requests present in "World Reference Base for Soil Resources" (Tarziu et al., 2000; Sparchez et al., 2013). This classification groups soils based on their characteristic genetic process and the diagnosed horizons. As such, NRDIBH Stefanesti has the following soil classes and types: Protisol class (unevolved soils) with Regosol and Aluviosol types;

Umbrisol class with Eutricambisol type; Luvisol class with Typical Preluvisol, Typical Luvosol, White Luvosol, and Planosol types; Antrisol class (blunted and rutted) with Antrosol type. Generally speaking, with the exception of Regosol and Aluviosol, all profiles are very well developed in depth, with very well differentiated horizons per soil profile (Dakers et al., 1998; Toti et al., 2017).

The viticultural recognition and the area's popularity were given over time by high quality, dry white wines ('Fetească regală', 'Fetească albă'), aromatic wines ('Tămâioasă românească') and less by the red ones that have expanded in the eastern part of the territory in the last decades of the last century.

The studied grapevine varieties were grouped in three categories: table grapes ('Perlette', 'Muscat d'Adda'), white wine ('Fetească albă', 'Muscat Ottonel', 'Pinot gris'), and red wine ('Cabernet Sauvignon', 'Burgund', 'Merlot').

The most important grapevine varieties were established with the help of 19 criteria, with a scale of 8 levels each were used in the AHP exercise, as follows: criterion 1 - time for the crop gathering (from 1: the lowest time to 8: the longest time); criterion 2 - portfolio of secondary products (from 1: few secondary products to 8: a lot of secondary products); criterion 3 - the amount of grapes harvested by a workmen in 8 hours (from 1: the lowest to 8: the highest); criterion 4 - the price of crop collecting (from 1: the lowest price to 8: the highest price); criterion 5 – expertise for identification (from 1: most identifiable variety to 8: hardest identifiable variety); criterion 6 - expertise for collecting crop (from 1: the less expertise to 8: most expertise); criterion 7 - ustensil for collecting the crop (from 1: the least to 8: the more); criterion 8 - difficulty of collecting crop (from 1: lowest to 8: highest); criterion 9 - apportionment interval (from 1: lowest to 8: highest); criterion 10 - market demand (from 1: low to 8: high); criterion 11 - the cost of raw product (from 1: lowest to 8: highest); criterion 12 - the cost of the derived product (from 1: smallest to 8: biggest); criterion 13 - transport from the field to the deposit (from 1: simple to 8: difficult); criterion 14 - perishability (from 1: lowest to 8: highest); criterion 15 - "notoriety" of the product on the market (from 1: the smallest extent to 8: the most notorious); criterion 16 - market request (from 1: lowest to 8: highest); criterion 17 - biotic menace (from 1: the rarest menace to 8: the most menace); criterion 18 - abiotic menace (from 1: the rarest menace to 8: the most menace); criterion 19 - improvement of the harvesting procedure (from 1: unimprovement to 8: to the highest degree of improvement).

Having a high degree of generality, these criteria have been also used in other fields (Braga and Dinca, 2019). Taking into account the climatic changes influences reported in the viticulture area (Buciumeanu et al., 2018; Dinca et al., 2018b), and the need to formulate solutions (Vizitiu, 2019) and recommendations (Dinca et al., 2018a), three criteria referred closely to these new climatic conditions (1 - harvesting period, 17 - biotic threats, 18 - abiotic threats).

The results were obtained with the aid of the Expert Choice Desktop software (v. 11.5.1683).

RESULTS AND DISCUSSIONS

The AHP alternative ranking, derive from experts' judgment, is presented in Table 1.

According to the AHP results, the grapevine varieties with the highest potential for Stefanesti viticultural centre were: 'Perlette', 'Muscat d'Adda' and 'Fetească albă', while the less important ones were red wines varieties (Figure 2).

Table 1. AHP alternative ranking

Criterion	Grapevine varieties							
	Perlette	Muscat d' Adda	Feteasca alba	Muscat Ottonel	Pinot gris	Cabernet Sauvignon	Burgund	Merlot
1	1	2	4	3	5	8	7	6
2	7	5	8	6	3	1	2	4
3	1	6	7	4	8	3	5	2
4	1	7	8	6	2	4	5	3
5	2	3	8	4	7	6	1	5
6	8	6	4	5	7	3	1	2
7	3	1	5	4	2	8	7	6
8	8	5	1	7	6	4	3	2
9	1	2	8	5	3	6	7	4
10	3	4	8	2	1	5	7	6
11	6	7	4	8	5	3	2	1
12	8	5	1	6	7	3	4	2
13	8	5	7	6	4	1	2	3
14	8	5	3	6	7	2	4	1
15	1	2	7	8	6	5	4	3
16	3	2	6	7	8	1	5	4
17	6	4	5	3	8	1	7	2
18	8	6	1	2	4	5	3	7
19	5	7	6	8	1	4	3	2

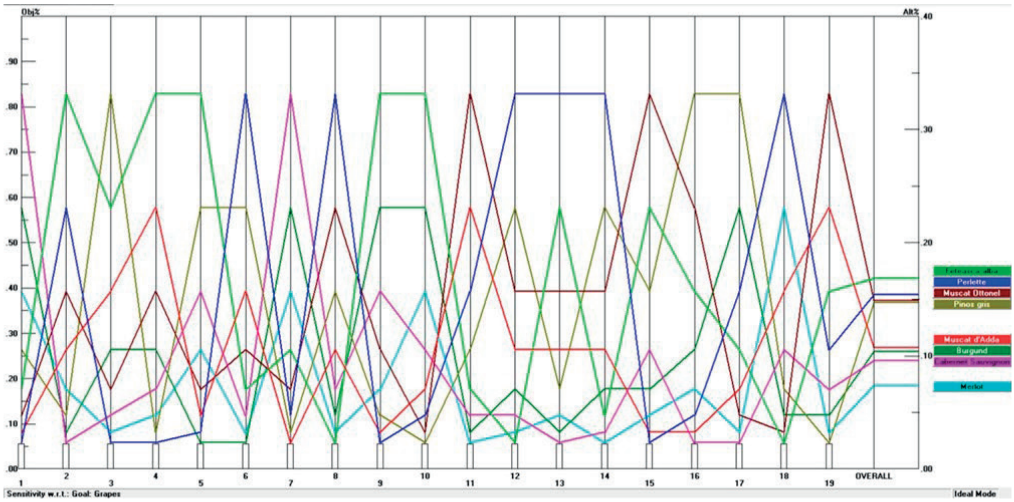

Figure 2. The ranking of the eight grapevine varieties

'Perlette' variety (seedless) is grown in the all viticultural centres with very favourable conditions for the cultivation of table varieties from the varietal conveyor. It has low frost tolerance (-18°C) and is very sensitive to downy mildew, gray mould, anthracnose, and, also, to wasp attacks. The grains crack easily, even if the overall climatic conditions are favourable during the ripening of grapes (high temperature, low rainfall) (Stroe, 2012).

Considering the consumers' demands, a special attention was paid in Stefanesti, as well as in Dragasani and Pietroasa in obtaining seedless varieties. Compared to other varieties for raisins, Perlette variety has a high production.

Perlette 10 St (clonal selection of 'Perlette' variety, obtained at NRDIBH Stefanesti-Arges/Certificate no. 1701/2008) has a good tolerance to drought and a lower frost tolerance; it has medium tolerance to the downy mildew and powdery mildew and the gray mould, due to the compactness of the grains on the bunches.

'Muscat d 'Adda', a variety for table grapes, is grown in most viticultural centres in the south and on restricted areas in the west of the country (51 centres). It has a relatively low tolerance to the action of low temperatures, but has a better drought resistance. It is a very sensitive variety to downy mildew that attacks the inflorescences just before flowering, the attack intensifying proportionally with canopy density (Stroe, 2012).

'Muscat d'Adda 22 St.' (clonal selection of 'Muscat d´Adda' variety, obtained at NRDIBH Stefanesti-Arges/Certificate no 4419/2009) has good resistance to drought and disease (downy mildew, powdery mildew, gray mould).

'Fetească albă', a white wine variety, is widespread being cultivated in many viticultural centres, 77 - after some authors, 127 - after others, spread in the Transylvania plateau, Moldavia and Muntenia. It is a good variety of sugar accumulator (200-240 g/l), with a good acidity in the northern areas, around 5.6‰, and decreasing in the southern areas (due to acid combustion) (Stroe, 2012).

Under normal conditions, 'Fetească albă' 97 St. (clonal selection of 'Fetească albă', obtained at NRDIBH Stefanesti-Arges, Certificate no 1699/2008), has a medium tolerance to frost and some cryptogamic diseases (downy mildew and gray mould).

Oenotherapy (therapy with wine) and uvotherapy (therapy with grapes) have been placed together with the balneological conditions in different specialized sanatorium, based on the treatment of gastric, cardiac, pulmonary, renal diseases and many others illnesses (Gaina, 2000; Nicolaescu et al., 2008).

Wine, grapes and other grapevine derivatives (grape seeds, leaves and dried marc) are used not only in naturist medicine, but also in cosmetics.

Despite information that moderate red wine consumption has beneficial effects on health due to its content in bioactive substances (resveratrol, hydroxytyrosol, melatonin) (Fernández-Mar et al., 2012), red wine varieties were ranked on the latest positions.

By specific methods, the breeders improved the plant resistance to diseases, pests and unfavourable environmental factors and, most often, the results have led to increasing the plant's production potential.

CONCLUSIONS

By analysing eight grapevine varieties grown in Stefanesti viticultural centre from the point of view of AHP method, which took into consideration 19 different criteria (including the request on the market, knowledge for recognition, and biotic and abiotic threats that can influence the crop) the most appreciated in this region were table grapes varieties ('Perlette', 'Musca d'Adda') and 'Fetească albă' variety for white wine.

The result is consistent with the recognition of the area for dry white wines production. Despite information that moderate red wine consumption has beneficial effects on health due to its content in bioactive substances, red wine grapevine varieties were ranked on the latest positions. Arising a great potential for table grapes, Stefanesti viticultural centre requires further assessments regarding the cultivated grapevine varieties, considering the conditions of climatic changes.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research, Innovation and digitalization, UEFISCDI, project code PN-III-P2-2.1-PED2019-5098.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON SOME METHODS OF CANOPY MANAGEMENT TO MITIGATE THE EFFECTS OF CLIMATE WARMING AT GRAPEVINE

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Abstract

The study was conducted in 2017-2019 in the experimental plantation of the USAMV Bucharest, on the most widespread Romanian grape variety, 'Fetească regală clone 21 Bl', grafted on the rootstock 'Kober 5 BB', to monitor how green operations - severe shoot topping and leaf removal above the bunch area - causes an effective delay in the accumulation of sugars in the grapevine berries. The green operations have been performed when berry sugar content reached about 14-15.5°Brix. The severe shoot topping at beginning of veraison increased competition of the lateral shoots, contributed to the delay of grapes maturity. Defoliation consisted in removing 30-40% of the vine leaves located above the grape floor (the most active photosynthetically). The results obtained on the qualitative parameters were compared with the control on which no intervention was applied. During the three years, there were lower accumulations of sugars in the berries at the time of harvesting the grapes, both in the case of severe shoot topping (21.8°Brix) and in leaf removal (21.5°Brix), compared to the control (22.8°Brix).

Key words*: canopy management, climate change, grapevine.*

INTRODUCTION

The multitude of effects of global warming has led, in the last decade, to the development of strategies for adapting the grapevine culture, which provide for a series of measures in the long, medium and short term.

Short-term measures refer to the application of pruning (Novello & de Palma, 2013; Frioni et al., 2016; Zheng et al., 2017; Moran et al., 2018), to the application of green operations (Stoll et al., 2009; Filippetti et al., 2011; Martinez de Toda & Balda, 2013; Poni et al., 2013; Palliotti et al., 2014; Quénol et al., 2014; Parker et al., 2016; Caccavello et al., 2019; Silvestroni et al., 2019; Holzapfel et al., 2020; Lopez-Granados et al., 2020), as well as in soil maintenance works (Brîndușe et al., 2013; Șerdinescu et al., 2013; Enache & Donici, 2014; Șerdinescu & Brîndușe, 2014, and others).

Good results were also obtained by using plant growth regulators (Davies et al., 2015) and antitranspirants (Palliotti et al., 2013b; Gatti et al., 2016).

The main measures are to delay the ripening of grapes leading to a reduction in excessive accumulations of sugars, maintaining titratable acidity at normal values, which leads to obtaining wines with a medium, balanced alcohol content.

Lower accumulations of sugars in berries can also be obtained by reducing the "leaf area: fruit weight ratio" to values less than 0.5-0.8 m^2/kg by some works and green operations (defoliation, shoot topping etc.).

The research carried out in Valea Călugărească, for the 'Cabernet Sauvignon' variety, confirmed that at low values of the "leaf area:fruit weight ratio" (6-8 cm^2/g grape, respectively 0.6-0.8 m^2/kg) obtained sugar concentrations of 180-185 g/L, compared to 197-205 g/L, at optimal values of 1.0-1.7 m^2/kg (Belea, 2008).

Research by Palliotti et al. (2013a) for the Sangiovese variety in central Italy, with the removal of 30-35% of the leaf surface of the vine (from the middle - upper part) when the sugar concentration of the must was 16-17°Brix determined for the variety 'Sangiovese' reduced it by 1.2°Brix when the grapes were harvested, without affecting the titratable acidity, the content of anthocyanins and total polyphenols.

Shoot topping and increased competition from grapevine lateral shoot can contribute to delaying grape ripening. Through the late shoot topping one week after the grape veraison, Filippetti et al. (2011) obtained a significant reduction in the concentration of sugars in the must, without changing the pH, organic acids, the content of anthocyanins in the skins of the berries and the tannins in the seeds.

The aim of this study was to investigate the influence of some green operations (shoot topping and leaf removal) on delay of grape maturation, as a measure to mitigate the effects of global warming.

MATERIALS AND METHODS

Plant material and experimental conditions

The experiment was carried out during the vegetation period of 2017, 2018 and 2019 in the experimental vineyard located in the southern part of Romania, at the University of Agronomic Sciences and Veterinary Medicine Bucharest (N Lat.: 44° 47' 07"; E Long.: 26° 07' 28"; alt. 87 m).

The plantation where the experiment took place was established in 1994, with 'Fetească regală' variety, clone 21 Bl, grafted on 'Kober 5 BB' rootstock, spaced by 2.2 m (inter-row) and 1.2 m (intra-row), with a density of 3787 plant ha-1. The vines are trained as bilateral cordon with a spur pruning system and loading of 12 buds/m2 and the support system is of the vertical monoplane type. The plantation is located on a plane surface with reddish preluvosol soil and rows direction N-S. Phytosanitary treatments against to control diseases and pests have been applied in accordance with local standard practice.

Phenological data

The four main phenophases (budburst, flowering, veraison, harvesting maturity) were followed, according to BBCH (**B**ioligische **B**undesansalt und **Ch**emische Industrie), modified under the COST Action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding" (Rustioni et al., 2014).

Data were recorded at which 50% of buds, flowers, grapes reached the respective phenological stages (BBCH 008 - budburst; BBCH 605 - flowering; BBCH 801 - veraison and BBCH 809 - berries ripe for harvest).

Canopy management techniques used in research

On three consecutive rows, three intervals were selected (3 repetitions of 10 vine), on which three different foliage management techniques were applied, including control, severe topping and leaf removal above the floor with grapes.

→ Control (C) - no green operations (topping shoots or leaf removal) were applied to these vines.

→ Severe shoot topping (T) and leaf removal (LR) have been applied on 28.07.2017, 24.07.2018 and 31.07.2019 (Figures 1 and 2).

The green operations were performed manually in the veraison phenophase, corresponding to the phenological stage BBCH 85 - the berries become translucent or colored and softening (Lorenz et al., 1994) and up to an average concentration of soluble sugars in berries between 14.0-15.5°Brix.

The severe topping of the shoots applied in July and the increased competition of the lateral shoot contributed to the delay of the ripening of the grapes.

Also, the defoliation applied after veraison, by removing the 50-60 day old leaves (the most photosynthetic active), from the middle part of the vegetal wall, determined a slowing down of the sugar accumulation. Defoliation consisted in the removal of 30-40% of the leaves of the vine, and created a 35-40 cm vertical window without leaves above the bunch area, keeping a few leaves at the canopy apex.

Quantitative and qualitative parameters examine in research

The grapes were harvested during the three years of observations between September 4-12.

At harvesting, for control and each experimental variant, determinations were made on quantitative (grape weight - grams, berry weight - grams, yield - kg/vine) and qualitative parameters (sugar content - ºBrix, titratable acidity - g/L tartaric acid). Sugar concentration in grapes was measured by using an Atago digital refractometer. The results were expresed in ºBrix. Titratable acidity was determined by titrating with 0.1 N NaOH using an Pellet digital biurette, and expresed as g/L tartaric acid.

Statistical analysis
Each data set was analyzed using analysis of variance, the statistical One Way ANOVA, and the mean values of the experimental and control variants were compared post-hoc Tukey HSD $p<0.05$. Results of the seasonal evolution of sugar content and titratable acidity are shown as means ± standard error.

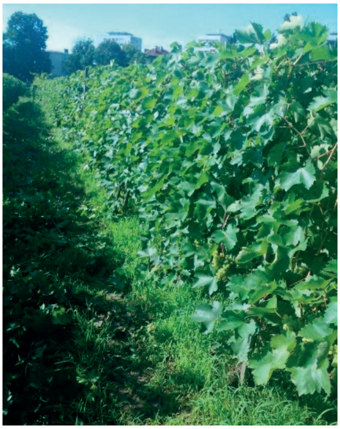

Figure 1. The topping of shoots of 'Fetească regală' variety

Figure 2. The leaf removal above the bunch area at 'Fetească regală' variety

RESULTS AND DISCUSSIONS

Climatic conditions
The climatic indicators were determined for experimental period (2017-2019) comparatively with reference period (1981-2010) after the recommendations of National Meteorological Administration (Dima et al., 2019).
Table 1 shows an obvious global warming during the experimentation period, especially during the vegetation period of the vine. Thus, the largest differences are found in the case of maximum annual temperatures (1.4°C), those during the growing season (1.38°C) and during the summer (June-August) (1.1°C). At the same time, there was a decrease in rainfall during the vegetation period (on average by 14%), especially in 2018 (by 27%).

Table 1. The main climatic parameters and bioclimatic indices during the experimentation period (2017-2019) compared to the reference period (1981-2010)

Climatic parameters and bioclimatic indices	Average 1981-2010	Years 2017	2018	2019	Average 2017-2019
Average annual temperature, ºC	11.55	11.74	12.21	12.92	12.29
Average temperature in the growing season (IV-X), ºC	18.07	17.99	19.33	18.53	18.62
Average temperature in summer (VI-VIII), ºC	22.50	22.78	22.83	23.05	22.90
Average temperature in the warmest month, ºC	23.32	23.80	23.87	23.60	23.74
Average annual minimum temperature, ºC	5.03	5.44	6.38	6.57	6.13
Average minimum temperature, in the growing season (IV-X), ºC	10.42	10.80	12.01	11.40	11.40
Average minimum temperature in summer (VI-VIII), ºC	14.47	15.60	15.84	15.60	15.66
Average of absolute minimum temperature, ºC	-17	-20	-12.2	-15.4	-16.1
Average annual maximum temperature, ºC	17.05	18.04	18.03	19.28	18.45
Average maximum temperature in the growing season (IV-X), ºC	24.46	25.22	26.66	25.63	25.84
Average maximum temperature in summer (VI-VIII), ºC	29.01	30.01	29.81	30.50	30.11
Average maximum temperature in the warmest month, ºC	29.87	30.16	28.87	29.64	29.56
Annual total precipitation, mm	608	661	623	529	604
Total precipitation in the growing season (IV-X), mm	428	415	312	385	371
Total precipitation in summer (VI-VIII), mm	198	155	228	142	175
Huglin index (HI)	2346	2408	2646	2458	2504
Winkler index (WI)	1726	1710	1997	1825	1844
Cool night index (CNI)	10.45	12.16	11.30	10.60	11.35

Values below 2400 units of the Huglin index, determine a titratable acidity generally higher than 5.5-6 g/L, while Brix is generally lower than 22%, which lead to harmonious light-bodied wines, with less than 13% vol. alcohol, typical for 'Fetească regală' variety. Values higher than 2400 units lead to more unbalanced wines with higher alcohol concentration, sometimes over 13.5% vol. alcohol and the mandatory need for acidity corrections (Bucur et al., 2019).

The development of the main phenophases

The greatest variation from one year to another was found in flowering and harvest maturity, these phenophases being more influenced by climate variability (Table 2). It can also be seen, that, grapes veraison (50% of berries softening) was recorded on average on DOY 209 (July 28) with variations between DOY 205 (July 24) and DOY 212 (July 31).

Table 2. Mean day of year (DOY) of the phenological stages (budburst; flowering; veraison and harvest) and the corresponding standard deviations (SD in days), for 'Fetească regală' variety (2017-2019)

Year	Budburst (50%)	Flowering (50%)	Veraison (50%)	Harvest maturity
2017	101	148	210	247
2018	103	136	205	255
2019	104	151	212	251
Mean	103 ± 1.53 (April 13)	145 ± 7.97 (May 25)	209 ± 3.61 (July 28)	251 ± 4 (September 8)

Larger differences were found in harvest maturity, on average on DOY 251 (September 08), with variations between DOY 247 (September 04) and DOY 255 (September 12).

The effect of green operations applied

Table 3 shows insignificant differences between variants each year, in terms of the average weight of a grape and yield.

Defoliation resulted in a significant reduction in the average weight of the berry each year (on average 2.17 g compared to 2.29 g in the control).

Regarding the accumulation of sugars in the berries, in each year of experimentation a significant reduction of its content was found, on average from 22.8°Brix at control, to 21.5°Brix at leaf removal. A similar reduction was found in the case of the application of severe shoot topping (21.8°Brix).

Compared to the control, in which every year there was an accentuated reduction in titratable acidity (4.8-4.9 g/L tartaric acid). In the case of severe shoot topping this parameter was maintained at normal values (6.6-6.8 g/L tartaric acid).

Following the application of defoliation, the acidity took on low values (5.1-6.3 g/L tartaric acid), but higher than the control.

Acidity values less than 6 g/L tartaric acid, lead to flat white wines, fade which requires corrections of this parameter.

Table 3. Grapes quantitative and qualitative parameters at harvesting time for Fetească regală variety* (2017-2019)

Year	Variant	Grape weight, grams	Berry weight, grams	Yield, kg/vine	Brix, %	TTA, g/L tartaric acid
2017	Control	100.7 ± 2.4^{a}	2.25 ± 0.1^{a}	3.9 ± 0.1^{a}	22.6 ± 0.1^{b}	4.9 ± 0.1^{b}
	Severe shoot topping	112.5 ± 4.3^{a}	2.28 ± 0.1^{a}	4.2 ± 0.2^{a}	21.7 ± 0.2^{a}	6.8 ± 0.3^{a}
	Leaf removal	105.6 ± 4.3^{a}	2.16 ± 0.1^{a}	4.2 ± 0.2^{a}	21.3 ± 0.2^{a}	5.3 ± 0.4^{b}
2018	Control	101.9 ± 2.4^{a}	2.40 ± 0.1^{b}	3.9 ± 0.2^{a}	22.7 ± 0.1^{b}	4.8 ± 0.1^{b}
	Severe shoot topping	107.3 ± 5.2^{a}	2.42 ± 0.1^{b}	4.3 ± 0.3^{a}	21.8 ± 0.3^{a}	6.6 ± 0.2^{a}
	Leaf removal	101.2 ± 5.0^{a}	2.20 ± 0.1^{a}	4.0 ± 0.2^{a}	21.5 ± 0.2^{a}	6.5 ± 0.1^{a}
2019	Control	104.4 ± 2.2^{a}	2.22 ± 0.1^{a}	3.9 ± 0.1^{a}	23.0 ± 0.2^{b}	4.9 ± 0.1^{b}
	Severe shoot topping	103.1 ± 7.3^{a}	2.25 ± 0.1^{a}	3.9 ± 0.2^{a}	22.0 ± 0.3^{a}	6.7 ± 0.2^{a}
	Leaf removal	101.1 ± 4.6^{a}	2.14 ± 0.1^{a}	3.8 ± 0.3^{a}	21.7 ± 0.1^{a}	5.1 ± 0.3^{b}
Overall	Control	102.3 ± 1.3^{a}	2.29 ± 0.1^{b}	3.9 ± 0.1^{a}	22.8 ± 0.1^{b}	4.9 ± 0.1^{c}
	Severe shoot topping	107.6 ± 3.2^{a}	2.32 ± 0.1^{b}	4.1 ± 0.2^{a}	21.8 ± 0.2^{a}	6.7 ± 0.1^{a}
	Leaf removal	102.6 ± 2.4^{a}	2.17 ± 0.1^{a}	4.0 ± 0.2^{a}	21.5 ± 0.1^{a}	5.6 ± 0.3^{b}

*One Way ANOVA, post-hoc Tukey HSD $p<0.05$.

CONCLUSIONS

Global warming which has been more pronounced in recent years has determined an exaggerated accumulation of sugars in the grapevine berries, under the conditions of accentuated degradation of the titratable acidity (under the 6 g/L tartaric acid) which required corrections.

As a means of avoiding this situation, winegrowers have the application of green operations at their disposal which have in these circumstances, through their application and intensity, favourable effects to avoid excessive accumulations of sugars, with the possibility of obtaining balanced wines.

In the conditions of degradation of the titratable acidity, the defoliation (on a portion of 35-40 cm above the grapes) and the severe topping when the grapes reached maturity proved to have a favourable influence (14-15.5°Brix).

Both green operations have proved their efficacy in the normal ripening of grapes under experimental conditions.

The reduction of the average weight of the grape berries, induced by the defoliation, can have a positive influence on the accumulation of aromatic compounds in the peel, especially in the varieties subjected to a pre-fermentative maceration.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE ECONOMIC IMPACT OF SUBSTRATE MIXTURE ON THE PRODUCTION OF GRAFTED AND POTTED 'FETEASCĂ REGALĂ' VINES

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Abstract

Grapevine planting producing material is focused to produce healthy and long-lived vines. This study analyzes the strategic choices of a nursery to obtain the best profit rate on grafted and potted vines 'Fetească regală' grape variety grafted on Oppenheim Selection 4 root stock was paraffined 8-chinolinol wax (before callusing), and silver color blue color (after grafting callusing and before planting in pots). The unit cost price was calculated based on total expenses and the yield of grafts obtained in the vine greenhouse nursery. The grafts were potted in three rooting mixtures (BP: forest ground 50% + red peat 30% + river sand 15% + conifer sawdust 5%; RP: forest ground 60% + black peat 25% + river sand 10%+ conifer sawdust 5%; RS: forest ground 70% + river sand 15% + conifer sawdust 15%. Variant RP generated the highest yield of the potted grafted vine with a rate of profit of 174.94%, while variant RS, generated the lowest yield directly related with cost production, and rate of profit (133.34%). The results are useful for the small-to-medium grapevine nurseries to choose the best strategies and to enhance their competitiveness and survive in their activity.

***Key words**: 'Fetească regală', grafted vines, rooting mixture, black peat, red peat, river sand.*

INTRODUCTION

Given the perennial nature of some horticultural crops (grapes especially), their high establishment costs, variability production in quality and prices, it is important to pay attention to risk management (Pozzan et al., 2012). Grapevines are mainly propagated by vegetative methods from cuttings, from which, more than 80 % are grafted vines (Ollat et al., 2012). In the last years, the global planting producing material and wine industry was transformed from small, traditionally oriented, family-owned enterprises to an industry dominated by multinational corporations (Waite et al., 2018). The individual component of risk management may be affected by price, yield, and product quality (Waite et al., 2015).

Rooting media (chemical and physical characteristics) is an important factor for graft production in the greenhouse and directly affect graft quality and efficiency (Sengel et al., 2012). The rooting substrate must be easy to handle, free of weeds and pathogens, had good water capacity and drainage (Rajkumar et al., 2017). Due to its hydrological, physiochemical and agronomic characteristics, peat is commonly used in the callusing stage after grafting (Aroca et al., 2010; Assunção et al., 2019), but also for rooting in pots or other containers (Corbean et al., 2011). For alternatives to peat utilization and to improve agricultural sustainability of rooting media, vineyard winter pruning, and grape stalks are valorized in nursery greenhouses for grapevine planting material production (Ronga et al., 2019; Popescu et al., 2015). Rooting grafts in pots method had a high degree of intensity and require the complete assurance of the material base, complete and thorough knowledge of the technology, and its correct application. The production of grafted vines in nutritional pots includes a large number of agro technical links and requires a high volume of qualified works, justifying itself economically only in cases where a very valuable biological material is multiplied.

Increasing the market potential for wine and grapes increased the demands for planting materials by the improvement and effective employment of techniques which allow the rapid expansion of propagating stock.

A good planting material, regardless the method use to obtained, begins in the previous year. Carbohydrates are reserve substance stored in the amiloplastele of liberian parenchyma, medullar and wooden, under the form of grains. The content of dosed carbohydrates (soluble sugar and starch) in cuttings is a accurate indicator of laboratory for assessing the degree of maturation of canes (rootstock and scion) (Iliescu et al., 2012). The processes of rooting and callusing are energy-intensive processes that cannot be taken over than through hydrolysis of poliglucid located in reserve rootstock cuttings. Therefore, the material with total carbohydrate content below 12% is rejected from propagating (Iliescu et al., 2012; Corbean, 2011).

The yield obtained is directly influenced by the quality of the biological material used (scions and rootstocks), by the ecological conditions (temperature, humidity, ventilation) of the solarium, by the nutrient substrate for rooting, by the phytosanitary substances, and the rooting stimulants. All these factors have the role of reducing the stress of vines when planting in pots (Cookson et al., 2013; Corbean, 2011).

The soil mixture used for potting must be relaxed to allow carbon dioxide release and oxygen access. The ratio between the gap space and the solid parts of the mixture should be 4:1, as opposed to a soil of the chernozem type (which contain a high percentage of humus), where this ratio is usually 1:1 (Corbean, 2011).

Becker, 1975, shows that pure peat, the yield was 56%, and when pure sand was used, the yield was only 12%. That in the case of cultivating grafted vines in nutrient pots, when using peat and sand mixtures, the yield in good grafted vines to be planted is higher as the percentage of peat is higher.

The vigor of the shoot is very important for obtaining a quality vineyard planting material. This is conditioned by the content in reserve substances of the two partners (Iliescu et al., 2012; Warschefsky et al., 2016), the degree of nutrition of the rooting substrate (Ronga et al., 2019; Popescu et al., 2015) and, the fertilization in the vine school (Bozzolo et al., 2017). In the experience, the foliar and root fertilization were used equal doses and concentrations for the three variants, the influence being only of the soil mixture.

Zamanidis et al., 2013, used for the rooting of grafted vines (Rkatsiteli grafted on rootstocks 5 BB and 101-14) different nutrient substrates: soil, pearlite, sawdust; rice bran + fallow + sand (1: 1: 1); peat + fallow + sand (1: 1: 1); loose soil + fallow + sand (1: 1: 1), the percentages of vines good for planting are satisfactory in all variants.

Potted vines should be planted early enough to benefit from a long growing season rather than suffer from a short one. The season should be sufficiently long for the vine to develop self-supporting root and shoots and develop frost-tolerant wood before the winter season (Mudge et al., 2019). At planting, the root system should fill the pot and have an active shoot tip growth with a good basal caliper.

The main objective of the research was to determine the influence of the three types of soil mixture(black peat, read peat and river sand) on the production of grafted vines for ‘Fetească regală’. Based on economic data, the profitability rate was analyzed for each of the three variants. The results may be useful for the producers to obtain grapevine material with the highest profit rate.

MATERIALS AND METHODS

The experience was established at the Jidvei Company, in Alba County, Romania (46°13′20″N 24°06′41″E, elevation 400 m) during 2018. Jidvei Company it is knownas the main grapevine planting producing material in the country, with 1.5-3 million grafts produce per year (Călugăr et al., 2020). The experiment involved the analysis of the rooting substrate in pots on 'Fetească regală' graft rates and its economic efficiency. 'Fetească regală' is a representative grape variety for Târnave vineyard. In Romania is planted on 17.47% of total surface grapevine planted (Antoce and Călugăru, 2017). Scions cuttings of 'Fetească regală' variety were harvested from mother plantation of graft canes of Jidvei Company during the previous fall. Prior to grafting, the degree of maturation of the graft cords and rootstocks was determined by the carbohydrate content and soluble sugars using the method of the anthrona reagent. By this method, the extraction of soluble sugars is made with 80% volume alcohol solution, the starch with 52% volume perchloric acid solution and then treatment with anthrona ($C_{14}H_{10}O$) 0.2% solution. 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).

The 'Fetească regală' scions cutting canes of 8 buds were kept till grafting at 2-4°C and 90% humidity in cold rooms. Rootstock canes of Oppenheim Selection 4, clone 762 were imported from France. Rootstock canes were cut at 35 cm and scions at 5 cm (1 bud - 1 cm above bud and 3 cm below bud). Pre-grafting hydration and soaking propagation material (scions and rootstock) were made in tanks containing drench water and captan to reduced pathogens infection. Grafting was made using an omega-cut grafting machine in mid-April. The paraffin used after grafting was the paraffin with 8-chinolinol. Stratification was made with pine sawdust, wetted and disinfected with a concentration of 0.01% $CuSO_4$. Grafts were vertically positioned in wooden boxes walls lined with porous polyethylene film. The exchange of humidity and temperature between inside and outside the box was provided by the polyethylene sheet. The capacity of a box is about 2000 grafts (Corbean, 2011). The boxes, after filling with grafts, were covered with a thin canvas over which was placed a 5 cm layer of wet sawdust. The temperature rose from 10 to 32°C, in the first three days of forcing, then went down to 28-30°C, keeping at this level until the end of the callusing (14th day). Air humidity was rose from 65% to on a high level of 85%, in the first three days. After the beginning of the formation of callus mass at the grafting point, after the 3 - 4 days, air humidity has decreased to 75-80% and remained constant until the end of forcing. The grafts forcing was made without light. During callusing, phytosanitary treatment to avoid the attack of *Botrytis cinerea* and saprophytic fungal species of the genus *Fusarium*. At the end of callusing, grafts were classified in two categories: Category I and inadequate (Council Directive 68/193/EEC). Category I grafts have new well defined and formed formations (callus around grafting point, shoots, roots), measured and appreciated according to Celik et al. (1998) and previously shown by Călugăr et al. (2019). The grafts in Category I were paraffined with a second layer with paraffin containing aluminum particles (silver color) before planting in the nutritive pots. After the second layer of paraffin, 30000 grafts were manually transferred into Fertil pot pressed cardboard (7/9/11 cm). Fertil Pot pots made of pressed cardboard have the property of optimum water retention in the soil mixture and allow the roots to easily penetrate the cardboard casing. The pots were filled with three different soil mixtures from local sources. Each soil mixture variant was 10,000 pots, arranged in a randomized design with 10 replicates/variant. The highest weight of mixing pots was in variant RS, where the percentages of forest ground (70%) and sand (15%) were higher than the variants RP and BP (Table 1).

Table 1. Variants of mixture nutritive substrate

Variant	Forest ground	Red peat	Black peat	River sand	Conifer sawdust	Nutritive pot weight
RP	50%	30%	-	15%	5%	247
BP	60%	-	25	10%	5%	274
RS	70%	-	-	15%	15%	323

Rooting grafts in Fertil Pot were made in a tunnel greenhouse covered with plastic foil of width - 6 m, length 50 m and height 4 m. The pots were put on perforated polyethylene foil on the ground over a drainage layer (5 cm depth) of gravel (0.5-1 cm diameter) and clean river sand. Inside the greenhouse tunnel, was appointed 4 alleys, of 1.2 m width and a passing aisle between alleys of 60 cm width.

The delimitation of the alleys where pots were placed was made of concrete slabs with the square section with the side of 8 cm, which ensures good lateral stability of the alleys (Corbean, 2011). During experience, verification of the irrigation system was made, the verification of the covering sheet, the tracing of the layers on which the pots are placed. To prevent excessive heating inside, they were covered with a protective mesh.

The planting of vines was made in the greenhouse, with the base in the nutritional mixture on a depth of 5-6 cm. After planting, the pots were watered with 15-20 L/m² water to ensure the contact of the grafts with the rooting mixture.

During the rooting period of grafted vines in nutrient pots, the temperature, in the first week after planting was maintained at 25-30°C, then, lowered to 20-22°C. The hygroscopicity of the air remained around 85% during the first two weeks, until the appearance of the first leaves, then lowered to 60%. On hot days, when the humidity of the airdropped below 50%, 2-3 artificial watering systems, of short duration, were made (Corbean, 2011).

The moisture content of the nutrient mixture in the pots was maintained within optimal limits of 28-30% of the weight of the rooting mixture, by repeated watering. Root and foliar fertilization were made with water-soluble fertilizers based on nitrogen, phosphorus, potassium, and with micro-elements.

The control of diseases and pests was carried out by weekly treatments with contact and systemic products. The grafts were classified according to root appearance and root output from the pot, growth vigor of grafted vines - shoot length (cm) and diameter (mm).

Grafted Yield of Potted Vines % =
(Number of Grafted vines in First Category/
Number of Grafted Vines planted in Pots)
x 100

After 4 weeks in greenhouse, all the shoots started from the rootstocks were removed. The grafts in the pots where the growths of the shoots were at least 20 cm long and with a minimum of 4 mm diameter, were selected to be planted in the vineyard.

Economic efficiency calculations were made on the basis of the technology fiches and material costs provided by the producer. The most important economic indicators were calculated as follows:

Profit per unit = Sell price per unit - Cost price per unit (1)

Total profit = Income from sales - Total production expeses (2)

Profit ratio = Total profit / Total incomes (3)

ANOVA (Analysis of Variance) and LSD (Least Significance Difference) test were used to calculate the significant difference between the variants and the mean of experience of yield grafted grapevine and potted vines. The interaction between variants and mean of experience was evaluated at 95%, 99 % and 99.9 % in order to determine the significance. The statistical interpretation of the results was made using Excell 2010, USA.

RESULTS AND DISCUSSIONS

The degree of wood maturation in rootstocks (carbohydrates content: soluble sugars and starch) is an important indicator, which allows both the assessment of physiological processes during the whole vegetation period and the quality of the initial material used to produce grafts (Iliescu et al., 2011). The quality of the initial material (graft and rootstock) is determined by the agricultural techniques applied.

In the Târnave vineyard, the period of active vegetation of the grapevine is lowering (compared to vineyards in southern Romania) due to low heliothermal resources (Bora et al., 2015; Călugăr et al., 2018).

Tabel 2. The content of total carbohydrates of rootstock and scion cuttings before grafting

Variety	Soluble sugars g %	Starch g%	Total carbohydrates g%
Fetească regală	7.95	6.77	14.72
SO4, clone 762	11.11	3.69	14.80

The determination of carbohydrates is a reliable laboratory indicator for assessing the degree of maturation of rootstock and scion cuttings for grafting. Carbohydrate content studied variants recorded values of 14.72 g% for 'Fetească regală' variety and of 14.80 g % for SO4, clone

762 rootstock (Table 2). According to Hunter et al. (2004) and Iliescu et al. (2012), these content of carbohydrates determined in grafting material ranks in category of sufficiently matured (between 14 to 16 g%). Values over 16 g% carbohydrates is considered good matured grafting material. During hydration, grafted material could loss some carbohydrates, between 0.5 to 1.5 g%, depending on the number of days of hydration (Iliescu et al., 2012).The aspects studied are related to the influence of the type of nutritive mixture, potted graft yield, and production costs with a direct impact on the cost per unit of product, but also on economic efficiency. The management of the process of forcing is aimed at obtaining the highest percentage of grafted vines, in which the two partners joined by grafting are congruent by circular callus, the main bud being started in the vegetation and at the basal pole of the rootstock to present moderate callus, formed around with emerged roots. Obtaining quality grafted vines, as well as a growth vigor, is also influenced by the type of paraffin used. Classification of grafted vines was made in two categories: Category I and inappropriate. Grafted vines form Category I have the new formations well defined and shaped (calus, shoots, roots). As previously shown in our research, the yield of 'Fetească regală' grafts in the Category I was 82.8% (Călugăr et al., 2019). Other studies recorded a higher yield of 90%-92.5% of grafted vines after callusing for Storgozia (local variety) grafted on SO_4 rootstock, testing Aktigref, Rebvaks VF, and Proaktigref (Iliev et al., 2014). Teker et al., 2014 using paraffin (name or other characteristics not mention) obtained the highest graft ratio, after callusing, for Cardinal on 5 BB (80.00%) and Michele Palieri on 1103 P (78.33%).Based on the accounting data, the cost of production for 'Fetească regală' grafts after callusing was reported for one hectare of field nursery (250,000 grafts/hectare).The expenses with the necessary materials in the production technology of grafted vines in nutritional pots and the obtained yields was used to calculate the cost price per unit of the product. Due to the data regarding material expenditure, costs of the biological material, paraffin and labor, but also the yield of quality vines obtained after callusing, the cost per unit was calculated (for grafted vine) as 0.27 euro/graft. The labor cost included the harvest of scions buds from mother scion vine, deposit till mechanized grafting, paraffining, maintenance during callusing, sorting and second paraffining (paraffin with aluminium particle - silver color). The low callusing rate after stratification may cause higher costs of biological material (scion buds and rootstock) (Călugăr et al., 2019).

The 8-quinolinol/Silver color paraffin variant was choosen for rooting in different substrates mixtures due to the highest yield 82.8 % after callusing. Potted vines are grafted and dispatched in the same year. The production of vines in nutritious pots is necessary for the newly established plantations during summer. In Romania, this practice is not frequent, mainly this type of planting material is used to replant dead vines in the young vineyards or sell in garden-center for planting in people yards. The vigor and efficiency of the planting are related to the support, respectively the nutritional pot which consists of stimulants existing in the rooting substrate. The fertilization must start from planting in the pot, until planting in the vineyard (Corbean, 2011). Potted vines could be a good solution to establish a vineyard one season quicker than dormant vines, in warmer areas (Santos et al., 2020). Potted vines need more attention than a dormant vine when planting in the field, and during their management for the first season. Watering is essential for potted vines as they are planted during summer when new, tender plants need the most nutrition (Corbean, 2011).In the experience, the foliar and root fertilization were used equal doses and concentrations for the three variants, the influence being only of the soil mixture. The 35-40 day period, after the second layer of paraffin has been applied and potted, is considered sufficient to obtain potted vines ready to be planted in the vineyard. Fertil Pot type of cardboard ensures good resistance, is not destroyed due to the humidity or during the transport period, it decomposes in the ground after one month after planting (Nechita et al., 2010). The texture of the rooting mixture is influenced by the quality of the peat. The rooting mixture for variant BP (red peat), with a lighter texture, drainage and, permeability of the water of upper irrigation, facilitates heat exchange of medium - pots and creates aeration to prevent infestation of roots with saprophytic fungi (Grohs et al., 2017). At the end of period of rooting, after 40 days, were analyzed the influence of the three variants of the nutritional mix on potted vines yields. The results showed that the best yield of potted vines was obtained in the black peat variant (BP) (91.5%), followed by the red peat variant (RP) (86.2%), and the lowest yield was obtained in the (RS) variant (77.4%), which consisted of only forest land, river sand, and fir sawdust. The results are close to the ones obtained by Călugăr et al., 2020, for the 'Muscat Ottonel' variety for the same variants mixtures (85.4% - subtrate with red peat; 89.1 % -subtrate with black peat and 75.8% - substrate with river sand). The composition in different proportions in the nutritious mixture of peat (red, black), sand, fallow, and sawdust gave different costs to the three variants. The costs of human labor (preparing the rooting mixture, laying the drain bed, filling the pots with the rooting substrate, wetting the pots, planting vines in the pots, irrigating and performing phytosanitary treatments, but also some works on the plant) were equal for the variants with peat (red and black). Due to the greater weight of the pots with sand in variant RS, they were handled more difficult and needed more labor days (Table 3). The yield of quality potted grafts was different due to different cost prices between the three variants. The lowest cost production (0.61 euro/potted vine) was for the rooting substrate with 60% woodland, black peat 25%, sand 10%, sawdust 5% (variant BP). The cost price of variant RP was 0.65 euro/potted vine. The highest cost price was for variant with sand RS (forest land 70%, sand 15%, sawdust 15%), with 0.72 euro/potted vine. The low yield of quality vines (77.4%) recorded by the variant RS caused the higher cost price for this rooting mixture (Table 3).

Generally, the price of the potted vine is higher than grafted vine from field nursery (Borsellino et al., 2010). The selling price of the grafted vine may vary depending on variety, type and economic aspects. Waite et al, 2018 states that at the level of 2017, the selling price for a grafted vine was very different in countries with viticultural tradition. There by, in Hungary the price for one grafted vine was 0.90 euro, in Austria - 1.30-1.50 euro, in France 1.40-1.65 euro, in Germany 1.55 euro, in Italy 1.50-1.60 euro, in South Africa 1.00-1.50 euro, in Spain 1.40-1.65 euro to 2.72-3.00 euro in Australia, 3.00-3.10 euro in New Zealand, 3.16-4.36 euro in Switzerland and 3.20-5.00 euro in the USA.

The selling price was considered equal for each variant, of 1.68 euro/potted vines. After selling the potted vines, variant BP (black peat) could generate a rate of profit of 174.94%. Variants RP and RS could also generate a profit rate of over 100% (158.74% for variant RP and 133.34 % for variant RS). The values of profit ratio were directly influenced by yield of quality of potted vines (root system, grafting point, length and diameter of the main shoot).

During graft planting material production, some losses (2-50%) occur in different stages (grafting, callusing, rooting, and uprooting from the nursery field) (Iliescu et al., 2012).

To increase the quality of grafts material, the hydroponic systems are used in greenhouses to minimize nursery losses (Sengel, 2005).

Table 3. The yield and economic data of potted vines

Variant / EE	RP	BP	RS
	euro/10,000 potted vines		
Vine grafts	2,700.00	2,700.00	2,700.00
FP	1,286.54	1,286.54	1,286.54
FS	22.45	29.83	41.09
BP	-	116.20	-
RP	124.63	-	-
RS	19.47	13.68	25.26
CS	4.47	5.21	17.88
[a]Water		2.43	
[b]Water		29.71	
SB		9.09	
SF		59.30	
Labour 60NDL	1,263.16		1,312.50
FFA		90.00	
OE	5,597.04	5,590.94	5,572.75
QPV (n=10)	86.20±0.26***	91.50±0.13***	77.40±0.41^{ooo}
CP(euro /pcs)	0.65	0.61	0.72
SP		1.68	
IS	14,481.68	15732.00	13003.20
Profit	8,884.56	9,781.06	7,430.45
Profit rate %	158.74	174.94	133.34
MQV %		85.03	
LSD 5%		0.38	
LSD 1%		0.59	
LSD 0.1%		1.00	

EE - Expenses elements; FP- Fertil Pots; FS -fertil soil; CS- conifer sawdust;[a] - water for wetting the pots; [b] - water for irrigation and phytosanitary treatments; SB -Sand for draining bed; SF - Soluble fertilizer; FFA - Fixed fonds amortized; OE - Overall expenses; QPV - Quality of potted vine yield; CP - cost price; SP - Selling price; IS - Income from selling; MQV - Mean quality vine yield; NDL= Normated Day Labour (8 hours/day); Values of quality potted vines % are expressed as mean ± standard deviation $^{*,\,o}$: $p < 0.05$; $^{**,\,oo}$: $p < 0.01$; $^{***,\,ooo}$: $p < 0.001$.

From an economic point of view, the production of vines in pots should be less expensive, due to the shortening of the production cycle, respectively the rooting in the field nursery, phytosanitary treatments, irrigation, mechanical works, additional costs with labor, the surface of the land, different materials additional, and not least, of the last stage, uprooting from field nursery. Some of these stages continue to be carried out, at the definitive planting site, with the specific works and treatments specific to a vineyard.

CONCLUSIONS

The cost of good planting material is amortized during the life of the vineyard. The improved propagation practices that rise grafts quality, compensate any additional cost of production, and will not threaten the viability of planting material businesses. Some propagators may say they receive a low price for grafted vines, so for nurseries are important to adopt practices designed to obtain high yielded grafts and a low production cost. As a general rule, dormant vines are preferable to green-potted vines.

The yield of quality potted grafts was different and generated different cost prices between the three variants. The rooting substrate with 60% woodland, black peat 25%, sand 10%, sawdust 5% (variant BP) had the lowest cost production (0.61 euro/potted vine). The highest cost price was for variant with sand RS (forest land 70%, sand 15%, sawdust 15%), with 0.72 euro/potted vine. The low yield of quality vines (77.4%) recorded by the variant RS caused the higher cost price for this rooting mixture. Profit rates were over 100% for all variants, with the highest for variant with black peat (BP) of 174.94%.

The study focused on a nursery with a specific business model and was conducted to analyze the profitability of the potted vines. This study has territorial limitations, although Jidvei company plays an important role in Romanian grafted grapevine producers. This research may be considered as an exploratory study. It deserves further investigation into different strategic choices within the company system by performing cross-case comparisons. Results obtained in this study may be useful in choosing strategies of small-to-medium grapevine planting materials producers.

The grape plant materials have a high value mainly due to the competitive nature of the grape nursery business, but also of the rapid globalization of the grape and wine community. This results in some fundamental changes in the availability of some grape selections, varieties, and clones that are appropriate for the different climates in the world.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MODULATION BY YEASTS OF AROMA PROFILE OF 'FETEASCĂ REGALĂ' WINES PROTECTED WITH LOW CONCENTRATION OF SULPHUR DIOXIDE

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Abstract

Fermentation of grape juice with various yeasts inherently leads to wines with different aromatic profiles, which get superimposed onto the varietal aroma and may or may not be well suited to the grape variety used. Determining which commercial wine yeast to use for a certain grape variety is a challenge and a risk for the winemaker, especially when working with low doses of sulphur dioxide or when the yeast was not used before in the winery. A good option to select a suitable yeast is to perform micro-vinification to compare the sensory profile of the resulted wines. The sensory analysis can then be completed by a flash GC analysis to determine the differences in the odour intensity and in the volatile profile of the wines, compared with the wine variant fermented with a classic well-known yeast. In this study 5 yeasts were compared in order to select the most suitable ones to modulate the aroma of Romanian variety 'Fetească regală' so that the temperate climate fruits aroma, specific to this variety, is intensified. At the same time, it was important to also identify the yeasts able to minimize the oxidative aroma when the sulphur dioxide concentration used in winemaking was low. Among the yeasts used 2 were recommended for their ester producing aroma (Anchor Alchemy I and Renaissance Allegro AL-48), while the other 3 are mainly used for their ability to release thiols (Lalvin QA23, Anchor VIN7 and Renaissance TR-313). The sulphur dioxide added in all variants was only 70 mg/l in total. Sensory analysis performed 3 years after bottling showed that some, but not all, yeasts were able to produce intense fruity aroma which was well preserved. Our study shows that for 'Fetească regală', ester releasing yeasts are more suitable to produce stable aroma, less affected by oxidation during storage under preservation with low sulphur dioxide concentrations.

Key words: *Heracles GC electronic nose; 'Fetească regală'; commercial wine yeast, aromatic profile, low* SO_2.

INTRODUCTION

Aroma is an essential attribute involved in the recognition of quality of food products and beverages and its complexity is often directly linked with quality and consumer preference (Wang and Spence, 2018). Aroma is generated by the mixture of the volatile compounds present in the food matrix, which can be perceived by the nose and is evaluated by the brain. A volatile profile which induces a more pleasurable reaction will distinguish a specific product from other similar ones. Thus, in wine, for example, the process of winemaking is usually conducted to optimize the volatile compounds extracted from the grapes, as well as produced during fermentation and aging. Avoiding the oxidation of the volatile compounds is also important (Zironi et al., 2010), as the oxidation of aroma is perceived as a loss of quality. Beside the grape variety, which brings to wine specific aromatic compounds (primary aroma), yeast strains used for fermentation play a significant role in the final product aromatic profile (secondary aroma). As primary aroma is relatively constant for a grape variety grown in a certain region, intervention with an appropriate yeast for fermentation can induce more changes in the final volatile profile (Swiegers and Pretorius, 2005; Graham, 2008) and lead to a more or less favourable result, also depending on the winemaking conditions (Morgan *et al*., 2019). To evaluate the influence of interventions, yeasts which generate either more ester compounds or more thiol compounds were compared. Two yeast strains for ester production and three yeast strains for thiol production were selected to be used to modulate the aromatic profile of 'Fetească regală' white wine produced in the region of Bucharest, Romania. Considered among the semi-aromatic varieties, 'Fetească regală' benefits sometimes from maceration of the grape skins, as more aroma precursors are extracted, but at the same time, this skin contact also brings into the must more phenols, increasing bitterness and astringency as well. In order to limit phenolic extraction, separation of the skins immediately after grape crushing is the most used winemaking procedure, which, in turn, reduces the varietal aroma of wines. Therefore, to obtain a more intense aromatic profile, selecting suitable wine yeasts for fermentation is of utmost importance. Moreover, because the consumers also prefer to have wines with reduced SO_2 concentrations (Amato et al., 2017), to compensate for the less antioxidant protection it is desirable to select yeasts which generate compounds less oxidisable or confer aroma protection for longer times. The present study evaluates the aroma profile generated by 4 yeasts as compared to another one usually employed and considered the control, also evaluating the sensorial profile of these wines after 3 years from bottling with low SO_2 concentrations.

MATERIALS AND METHODS

The wine used for the experiment was obtained from grapes cultivated in the plantation of the University of Agronomic Sciences and Veterinary Medicine of Bucharest and harvested in 2018 on September 11th. The wine variants were prepared in accordance to white wine technology. The harvested grapes were destemmed, crushed and the free run juice separated in a hydraulic press and treated with a dose of 50 mg/l SO_2 and then with a dose of 1 g/hl commercial pectolytic enzyme Zimafruit from Enologica Vason (web source 1). The collected must was left one day for settling in a stainless-steel tank. The limpid must, with a turbidity of 113 NTU, sugar of 23.2% Brix and titratable acidity of 3.94 g/l tartaric acid, was split in 5 smaller tanks, 40 l each and inoculated with selected commercial yeasts, as follows: Lalvin QA23 from Lallemand (web source 2), VIN7 (web source 3) and Alchemy I (web source 4) from Anchor Oenology, and TR-313 (web source 5) and Allegro (AL-48) (web source 6) from Renaissance. For these yeasts, the main characteristics are summarized in Table 1. The alcoholic fermentation was conducted in each tank at a temperature of 16 ± 1.5°C and lasted about 4 weeks. Afterwards, the newly obtained wines were racked on October 17th and left for maturation on the fine lees for another 4 months, in the first two months also being homogenised with the lees twice a month. Adjustments of acidity were made twice (one week after racking and 2 months after racking, respectively), with 1 g/l tartaric acid each time. A small dose of 50 mg/l sulphur dioxide was added in each tank 2 months after racking and this was supplemented two months later with another 20 mg/l on the occasion of bottling. The wines thus prepared were left for aging in bottle for 2 years.

Table 1. Main oenological characteristics of selected yeast strains

Comercial name	**Lalvin QA23**	**Anchor VIN7**	**Renaissance TR-313**	**Anchor Alchemy I**	**Renaissance Allegro (AL-48)**
Genus, species and variety	*S. cerevisiae var. bayanus*	*S. cerevisiae x S. kudriavzevii*	*S. cerevisiae*	*S. cerevisiae (mixture)*	*S. cerevisiae var. bayanus*
Aroma profile	Thiolic	Thiolic	Thiolic	Esteric	Esteric
Latency	Medium	Short	-	-	Short
Kinetics	Fast	Very fast	Fast	Fast	Moderate
Optimal temperature	14-18°C, without peaks over 28°C	13-16°C	14-18°C, without peaks over 25°C	13-16°C	15-18°C, without peaks over 28°C
Cold tolerance	<10°C	12°C	13°C	12°C	13°C
Alcohol tolerance	16.0% vol.	14.5% vol.	16.0% vol.	15.5% vol.	16.0% vol.
Conversion factor	16.5	16.2	16.3	16.2	16.3
Glycerol, g/100 ml ethanol	Medium (3.5-5)	Medium (3.5-5)	High (>5)	-	Medium (3.5-5)
Volatile acidity, acetic acid, g/100 ml ethanol	Low (<0.25)	Medium (0.25-0.4)	Low (<0.25)	Medium (0.25-0.4)	Low (<0.25)
Nitrogen requirements, mg N/g sugars	Low (0.75)	High (1.25)	Low – Medium (0.85)	Medium (0.90)	Medium (0.90)
Killer factor	Active (K2)	Sensitive	Active	Active / Neutral	Active
Flocculation	Low	Low	High	-	High
Foam production	Low	Moderate	Low	Low	Low
SO_2 production	Low (<20 ppm)	Low (<20 ppm)	Low (<20 ppm)	Low (<20 ppm)	None (0 ppm)
H_2S production	Low	-	None	-	None

The aroma profile of the wines was evaluated 2 years later by sensory analysis by a panel of winetasters, using a specific tasting sheet (Antoce and Namolosanu, 2007; Antoce and Cojocaru, 2017a) containing intensity scales for the main parameters (acidity, sweetness, astringency, bitterness, extract, colour intensity and aroma intensity) as well as discontinuous scales for various identified aromas.

The volatile compounds with main influence on the wine profile were also evaluated using a 2-column gas-chromatograph from Alpha MOS, France (Heracles e-nose), working on the principle of an electronic nose. A more detailed description of the apparatus and the method used is available elsewhere (Antoce and Namolosanu, 2011; Antoce and Cojocaru, 2017b, 2017c; Cojocaru and Antoce, 2019). The e-nose has its own software used for data acquisition and data analysis (AlphaSoft 12.42).

The main statistical analysis performed were the PCA (Principal Component Analysis), which allowed for non-hierarchic grouping of wines fermented with different yeasts strains and SQC (Statistical Quality Control analysis), which compares the total quantity of volatile compounds of a control wine with the ones of the other wines, based on the major volatile compounds determined to have discriminant powers above 0.5.

For the identification of the compounds separated by the chromatograph an integrated database, AroChemBase and Flavornet database (web source 7) were used. For each wine the chromatographic analysis was run 3 times.

RESULTS AND DISCUSSIONS

The Heracles e-nose, endowed with two short chromatographic columns of different polarities, allows for the separation of different volatile compounds on each column, but sometimes the same compound is separated and identified in both columns. The column DB5 is mainly suitable for separation of alkanes and other less-polar compounds, while the column DB1701 is better for the separation of alcohols, diols, esters, carboxylic acids, ethers, ketones, thiols or amines.

In Table 2 are included the volatile compounds separated and clearly identified with our flash chromatograph on the non-polar column DB5 and on the medium-polar column DB1701, respectively, to be important for the discrimination of the 'Fetească regală' wines fermented with the 5 selected yeasts. Kovats indices, necessary for the identification of the chromatographic peaks are also included, as reported in the literature (web source 7) and as determined previously by us on this Heracles apparatus (Cojocaru and Antoce, 2019). For each peak, the discrimination power calculated by the AlphaSoft show the impact of these compounds for the differentiation of wine samples fermented with the 5 different yeasts. Sensory descriptors of these volatile compounds were taken from ArochemBase and Flavornet (web source 7).

Table 2. Compounds identified on non-polar column DB5 and low/mid-polarity column DB1701 in 'Fetească regală' wine variants

Average RT	DB5 Kovats	DB1701 Kovats	Actual Heracles Kovats*	Compounds	Discrimination power (R^2)**	Sensory descriptors
8.96	769	-	769.08-*1*	*cis*-2-Penten-1-ol	0.718	green, plastic, rubber
9.84	795	-	795.32-*1*	Ethyl butyrate	0.891	banana, ethereal, pineapple, apple
11.54	-	859	858.98-*2*	Ethyl butyrate	0.921	banana, ethereal, pineapple, apple
12.93	874	-	873.77-*1*	Isoamyl acetate	0.947	banana, pear
14.80	-	940	940.60-*2*	Isoamyl acetate	0.952	banana, pear
16.63	967	-	964.51-*1*	5-Methylfurfural	0.890	sweet, almond, caramel, spicy
7.08	-	729	729.25-*2*	Isoamyl aldehyde	0.960	fruity
30.86	-	1354	1,354.30-*2*	2-Decenal	0.632	tallow, orange
33.38	-	1424	1,423.45-*2*	4-Oxodecanal	0.677	fatty

*DB5 = column 1 (suffix -1); DB1701 = column 2 (suffix -2); **Coefficient of determination R^2 (COD);

By taking into account these determined discriminant peaks it can be observed that the wine variants can clearly be separated by the PCA analysis (Fig. 1), with a high positive Discrimination Index calculated by AlphaSoft.

Wines produced with esteric-aroma releasing yeasts (Alchemy I, Allegro AL-48) are placed in distinct parts of the biplot as compared to the wines produced with thiolic-aroma releasing yeasts (QA23, TR-313). VIN7 yeast however is placed between the thiolic and esteric-aroma producing yeasts, leading to wines with the lowest ethyl-butyrate concentration, but with moderate production of isoamyl-acetate (the highest observed among the 3 tested thiolic yeasts), confirming that this strain is also a good ester-producer, not only a thiol-releaser (Hart et al., 2017).

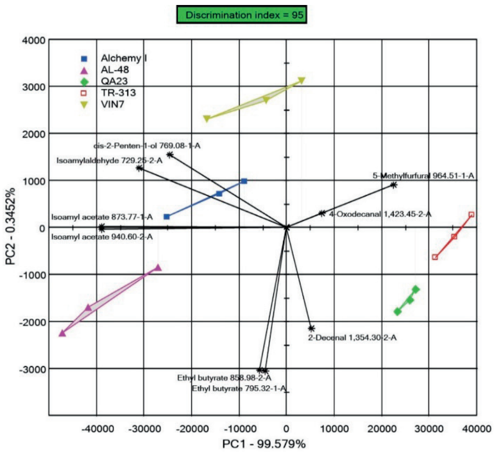

Figure 1. PCA biplot of wines fermented with 5 selected yeast strains and the main volatile compounds identified chromatographically

The other thiolic yeasts (QA23 and TR-313) lead to wines which were more easily affected by oxidation, the PCA biplot showing that especially TR-313 is clearly differentiated by 5-methylfurfural, a compound which is developed in aged (Dumitriu et al., 2019) or oxidized/prematurely aged wines (Escudero, et al., 2002; Tarko et al., 2020).

As seen from Table 1, in spite of the fact that thiol-releasing yeasts were used in some experimental variant, no thiols were chromatographically identified as being clearly present or discriminant for the variants. One explanation is that 'Fetească regală' variety has lower accumulation of thiols or precursors than the notorious Sauvignon Blanc variety or even other varieties such as Pinot Gris, Riesling, Chenin blanc, Colombard, Gewurztraminer, Semillon, Koshu and so on, which are known for the presence of thiols in the grapes (Roland et al., 2011). However, another explanation is also the lower protection from oxidation conferred by our attempt to lower the doses of sulphur dioxide in the final wine. Thus, the thiols, which are more sensitive to oxygen (Coetzee and Du Toit, 2015) or indirect oxidetion by means of catechins (Blanchard et al., 2004), had surely a higher rate of destructtion than the esters, which are more resistant to oxidation. Many thiols disappear in the first months of wine storage in bottles (Herbst-Johnstone et al., 2011).

This does not necessarily mean that the thiolic yeasts may not positively modulate the final aroma of 'Fetească regală' wines, given the right conditions.

Albeit the concentration of grape aroma precursors is highly important (Lee et al., 2008), the concentration of some thiols in wines are not strictly correlated with the must precursors (Pinu et al., 2012).

The magnitude of the volatile compounds' concentration was evaluated based on the peak areas, which are presented in Table 3.

Table 3. Peak area of the compounds identified in wines produced with different selected yeasts

Identified compounds	***Peak Area (± Standard error of mean)**					**Effect size (ω^2)**
	Wines fermented with thiolic yeasts			**Wines fermented with esteric yeasts**		
	QA23	**VIN7**	**TR-313**	**Alchemy I**	**AL-48**	
cis-2-Penten-1-ol (DB5)	596 ± 174^{b}	1291 ± 131^{a}	774 ± 128^{ab}	1345 ± 106^{a}	1017 ± 87^{ab}	0.589
Ethyl butyrate (DB5)	7508 ± 148^{a}	4827 ± 306^{c}	6547 ± 284^{ab}	6002 ± 180^{bc}	7857 ± 364^{a}	0.839
Ethyl butyrate (DB1701)	7067 ± 99^{a}	4584 ± 183^{c}	5988 ± 225^{b}	5650 ± 201^{b}	7384 ± 290^{a}	0.883
Isoamyl acetate (DB5)	50272 ± 962^{c}	75231 ± 4777^{b}	43297 ± 1791^{c}	82898 ± 3699^{b}	100179 ± 4653^{a}	0.920
Isoamyl acetate (DB1701)	41019 ± 663^{c}	60057 ± 3344^{b}	34236 ± 1328^{c}	66842 ± 3059^{b}	81266 ± 3822^{a}	0.928
5-Methylfurfural (DB5)	103 ± 5^{b}	182 ± 37^{b}	425 ± 44^{a}	163 ± 5^{b}	104 ± 0^{b}	0.829
Isoamyl aldehyde (DB1701)	3252 ± 85^{c}	5733 ± 184^{a}	2593 ± 167^{c}	4136 ± 196^{b}	5189 ± 188^{a}	0.940
2-Decenal (DB1701)	140 ± 19^{a}	n.d.**	122 ± 30^{a}	63 ± 32^{a}	132 ± 44^{a}	0
4-Oxodecanal (DB1701)	334 ± 17^{a}	297 ± 37^{a}	n.d.**	266 ± 58^{a}	n.d.**	0

*One Way ANOVA, post-hoc Tukey HSD $p<0.05$; **Groups excluded from statistical analysis.

To determine the statistical differences among the wines for each compound/ chromatographic peak, One-Way ANOVA ($p<0.05$) with Tukey HSD post-hoc was applied. For some of the discriminant compounds, ANOVA analysis too showed significant differences ($p<0.05$) among the wines (Table 3). For 2-decenal and 4-oxodecanal, which could not be identified in all samples, it seems that the influence of the yeast in their production is limited. Although the peak area values are small, it may be of importance to notice that 4-oxodecanal was not detected in the wines produced with any yeast from Renaissance, irrespective of their classification as a thiol or ester releasers. The other compounds, with a calculated effect size ($\omega^2>0.14$) can be safely be considered as being influenced by the fermentation yeasts, especially on the cases of the compounds with high ω^2 values, which explain a high percentage of variation among samples (94% for Isoamyl aldehyde, 92% for Isoamyl acetate, 88% for Ethyl butyrate). The fruitiness and banana-like aroma expected to be induced by the Isoamyl aldehyde and Isoamyl acetate is more present in both esteric-yeasts rather than in the other 3, the thiolic ones. Also, the banana-pineapple-like aroma of ethyl-butyrate is expected to be present in all wines, but with a lowest intensity in the wines fermented with VIN7.

The sensory analysis revealed that the major parameters of the final wines were not significantly affected by the yeast employed for the alcoholic fermentation (Table 4). The only noticeable exception was the total aroma intensity, which, after two years in bottles, was perceived as being lower for the wines fermented with the yeast conferring an esteric profile, especially in the case of Alchemy I.

Table 4. Main sensory characteristics of wines produced with different selected yeasts analysed after 2 years of aging in bottle

Sensory parameter*	Wines fermented with thiolic yeasts			Wines fermented with estric yeasts	
	QA23	VIN7	TR-313	Alchemy I	AL-48
Acidity	6.7 ± 0.6^{a}	6.6 ±0.9^{a}	5.2 ± 0.4^{a}	6.1 ± 0.6^{a}	6.7 ± 1.1^{a}
Sweetness	0.4 ± 0.6^{a}	1.8 ± 0.9^{a}	0.5 ± 0.6^{a}	1.3 ± 1.1^{a}	1.2 ± 1.0^{a}
Astringency	3.8 ± 1.5^{a}	5.3 ± 0.8^{a}	4.3 ± 1.2^{a}	5.5 ± 0.6^{a}	6.2 ± 1.1^{a}
Bitterness	1.2 ± 1.1^{a}	0.9 ± 0.6^{a}	2.5 ± 1.0^{a}	1.4 ± 1.1^{a}	2.4 ± 0.5^{a}
Extract	5.1 ± 0.4^{a}	4.7 ± 0 .7^{a}	4.0 ± 1.1^{a}	5.5 ± 0.8^{a}	6.3 ± 1.2^{a}
Colour intensity	5.0 ± 1.6^{a}	6.2 ± 0.8^{a}	5.2 ± 1.2^{a}	5.0 ± 1.4^{a}	5.3 ± 0.8 ^{a}
Aroma intensity	4.5 ± 0.8 ^{a,b}	5.9 ± 1.1^{a}	5.9 ± 0.9^{a}	3.7 ± 0.5^{b}	4.3 ± 1.3^{a,b}

* The values represent the sensory evaluation on intensity scales of maximum 10, expressed as means ± standard error of means.

In spite of a lower aroma intensity, the quality of aroma and the aromatic profile of wines was however more appreciated in the wines fermented with Alchemy I, being correlated with specific vegetal, lime and some fresh floral aroma, while the aromatic profile of wines fermented with the thiolic yeasts QA23 and TR-313 were more correlated with oxidized aromatic compounds described as sweet apple, caramel and toasted nuts (Figure 2). In sensory analysis too, the yeast VIN7 behaved differently than the typical thiolic or esteric yeasts, the wine aromatic profile generated by this being the most complex, with attributes related to flower, citric fruits, spices and temperate climate fruits (quince, apricots and pears), but with an overwhelming overripen apple aroma, showing that the low protection with sulphur dioxide was not beneficial for this particular wine.

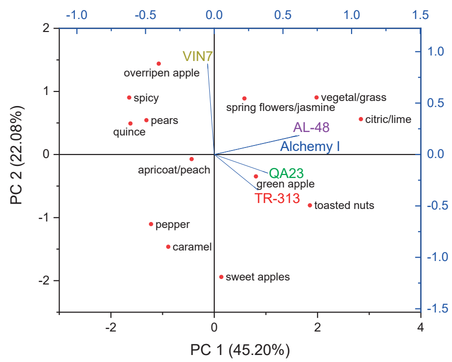

Figure 2. PCA biplot of wines fermented with 5 selected yeast strains and the main aroma descriptors identified by sensory analysis

Overall, the odor intensity conferred by the volatile compounds identified as being discriminatory for the wine fermented with the 5 selected yeasts proved that the esteric yeasts Alchemy I and Allegro AL-48 were clearly differentiated by a higher distance from the control QA23. Figure 3 shows diagram of odor distances of all wines fermented with selected yeasts as compared to the wines fermented with the QA23 control yeast obtained by statistical quality control analysis (SQC). The SQC analysis has taken into account only the peaks (representing sensors for the electronic nose) identified as having a high discriminative power (those presented in Table 2, with the exception of 2-Decenal and 4-Oxodecanal, which were not directly associated with yeast strains used). Figure 3 also shows that the thiolic TR-313 yeast was placed in a lower range than the control yeasts, proving again that the compounds it generates and releases are more sensitive to oxidative degradation than in the case of the estetic yeasts.

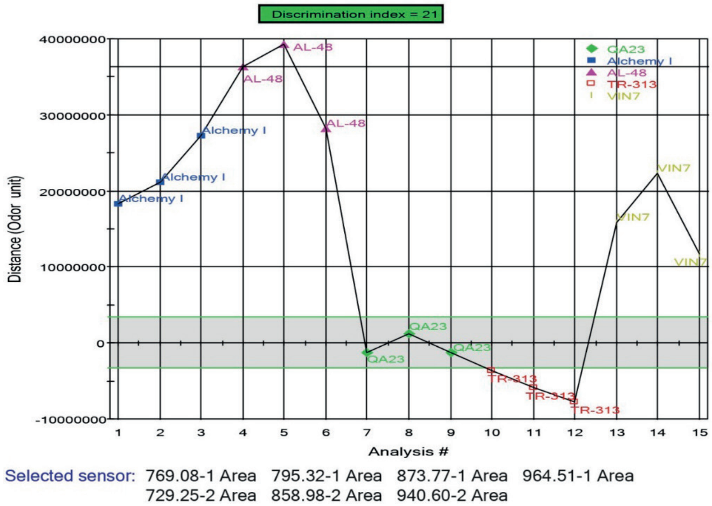

Figure 3. Diagram of odor distances of the wines fermented with several selected yeasts as compared to the wines fermented with the QA23 control yeast

Moreover, it should also be noted that a higher odor distance from the control is not necessarily associated with a higher odor quality. As it was the case of the wines fermented with VIN7, the odor distance determined by the electronic nose was mostly due to compounds showing premature aging/oxidation.

CONCLUSIONS

This experiment showed that the aromatic profile of the semi-aromatic variety ‘Fetească regală’ can indeed be influenced by winemaking, and the selection of the fermentation yeast leaves a specific mark on the final wine, also in direct dependence of the oxidative status of the wine. Selected yeasts classified as ester-releaser and thiols releasers, respectively, were compared with the classical QA23 thiolic yeast usually employed for this variety fermentation. While in other experiments, in which the wines were protected with the normal levels of sulphur dioxide permitted by legislation, the thiolic yeasts tended to confer a more complex and pleasant aromatic profile for ‘Fetească regală’, in the present experiment, lowering of the sulphur dioxide dose used at the bottling pointed to a more stable aromatic profile conferred by the esteric yeasts. Thus, to comply with the trend in lowering the sulphur dioxide concentrations in the bottled wines, the winemakers may need to resort to esteric yeasts, such as Alchemy I or Allegro AL-48.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF THE BIOCHEMICAL COMPOSITION OF VINE CANES ON COLD RESISTANCE OF BUDS IN DIFFERENT 'SYRAH' CLONES

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Abstract

The studied 'Syrah' clones are in full fruiting and are planted in area with a transitional-continental climate. They were grafted on the Berlandieri x Riparia SO4 rootstock. A spur pruning system was used. The clones were chosen because of their widespread distribution in Bulgaria, as each of them is characterized with identity, biological and production potential, valuable economic and technological qualities. The aim of the study is to determine the supply of vine canes with nutrients and their impact on the cold resistance of winter buds. The 'Syrah' variety has medium cold resistance, and the SO4 rootstock provides better wood maturation of the grafted vines. There is a difference of winter buds frost resistance in the vine. It depends on the variety, the tissue maturation degree and the buds dormant stage. The establishment of differences in cold resistance for some 'Syrah' clones participating in the study will be used as a zoning criteria in places with low temperatures during the autumn-winter period and will be indicative to adapt to changing climate conditions.

Key words: *clones, cold resistance, 'Syrah' variety, vine canes, winter buds.*

INTRODUCTION

The dormant period of the vine depends not only on the weather conditions during the autumn and winter months, but also on the conditions of development during the growing season.

Resistance to low temperatures increases from late autumn to mid-January. The 'Syrah' wine variety has medium cold tolerance (Mills L. et al., 2006). In Bulgaria it is widely practiced manual pruning and pulling-out the shoots from the trellis. After pruning, the shoots are extracted with adapted cultivators (Zapryanov Z., et al., 2009).

Clonal selection is an important tool for genetic improvement in viticulture and the production of high quality vines (Atak, A. et al., 2014). In all regions of the world where Phylloxera develops, new vineyards are planted with grafted vines of Phylloxera-resistant rootstocks (Angelov L. et al., 2016).

When the graft is perfect and both components involved in grafting complement each other, there is an improvement in overall vegetative growth, leading to higher nutrient accumulation and more plant biomass (Csikasz-Krizsics & Diofasi, 2008).

The rootstock affects the changes in the biochemical components in the grafted vine that helps the plant to store enough nutrients. Grafted vines show variations of primary nutrients content, emphasizing the possibility of choosing a better rootstock for sustainable nutrition management (Türkmen C. et al., 2011).

When studying the chemical composition of vine trunks, was found that compared to non-woody plants, they contain larger amounts of extractible substances, such as lignin and similar in content of cellulose. Elemental analysis of the ash shows that the main ingredients are mineral elements K and Ca (Mansouri S., 2012). The main buds accumulate twice as much raffinose as the replacement ones, which makes them more resistant to cold.

The accumulation and concentration of raffinose in the buds can be an early step in the hardening process (Grant & Dami, 2015). Starch content increase and thickening of the meristem cell walls, which occurs in sleeping buds, may be associated with structural and metabolic changes that favor the subsequent acquisition of cold resistance (Rubio S. et al., 2016).

This study characterized the influence of the biochemical composition of the vine shoots during the period of deep dormancy to the buds and their tolerance to cold, in the clones of the 'Syrah' variety included in the study.

MATERIALS AND METHODS

The experimental work included four clones of 'Syrah' coded 100, 174, 470 and 524, which were grafted on Berlandieri x Riparia SO4 rootstock. They were planted in April 2011 in the experimental vineyard of the Agricultural University - Plovdiv, located near the village of Brestnik.

The vineyard is in full fruiting period. The planting distance is 3.0 m between rows and 1.00 m between vines inside the row. The vines are grown tall.

The training system is a modified Lenz-Moser. The loading of the vines in all variants is carried out through a spur pruning, with 12 winter buds per vine (Figure 1).

Figure 1. 'Syrah' clones in the vineyard

When setting the attempt to establish the chemical composition and soil pH in the vineyard, soil samples were taken for analysis in a humus horizon from 30 to 60 cm.

To determine the nutrient supply of the vine shoots three weeks after leaf fall, at the end of November 2020, average samples were taken from the four clones of the 'Syrah' variety. The one-year mature growth weight after pruning was measured, in 4 repetitions x 15 vines.

The average yield per hectare is calculated on the basis of 3330 plants per hectare. In order to establish the degree of buds cold resistance in field conditions during February 2021, average samples of annual shoots were taken from the studied clones.

Each sample consists 50 shoots, cut from the base, with a length of more than 15 winter buds. The main and replacement buds were accounted through thin layers made with sharp knife, longitudinal from the top to the base of the bud. The dynamics of shoot maturation of pre-marked vines was monitored.

The studies of the main elements in the soil and the chemical composition of the shoots were made in an Accredited Laboratory Complex at the Agricultural University - Plovdiv. Intra-laboratory methods were used, observing all the requirements according to the adopted methodology like follow (Tables 1 and 2):

Table 1. Soil analysis

Soil reaction, pH	BDS ISO 10390:2011	**Soil quality - Determination of pH.** Specifies an instrumental method for the routine determination of pH using a glass electrode in a 1:5 (volume fraction) suspension of soil in water (pH in H_2O), in 1 mol/l potassium chloride solution (pH in KCl) or in 0,01 mol/l calcium chloride solution (pH in $CaCl_2$). The method is applicable to all types of air-dried soil samples, for example pretreated in accordance with ISO 11464.
Electrical	BDS ISO 11265:2002	**Soil quality - Determination of the specific electrical conductivity.** Specifies an instrumental method for the routine determination of the specific electrical conductivity in an aqueous extract of soil. The determination is carried out to obtain an indication of the content of water-soluble electrolytes in a soil.
Movable nitrogen (sum of all forms)	ISO/TS 14256-1:2003	**Soil quality - Determination of nitrate, nitrite and ammonium in field-moist soils by extraction with potassium chloride solution Part 1: Manual method.** Method for the determination of nitrate, nitrite and ammonium in a 1 mol/l potassium chloride extract of field-moist soil samples. The method is applicable to all types of soils homogenized by suitable methods.
Mobile phosphorus (P_2O_5)	GOST 26209:1991	**Soils. Determination of mobile compounds of phosphorus and potassium by Egner-Riem method (DL-method).** This standard establishes a method for the determination of mobile compounds of phosphorus and potassium in soils.
Mobile potassium (K_2O)		
Carbonates (active/general)	BDS EN ISO 10693:2014	**Soil quality - Determination of carbonate content Volumetric method.** Applicable to all types of air-dried soil samples.

Table 2. Biochemical analysis

Dry substance, % Water contents, %	BDS ISO 6496:2000	Determination of moisture and other volatile matter content
Total sugars, %	BDS 7169:1989	Determination of total sugar content
Starch, %	BDS EN ISO 15914:2005	Enzymatic determination of total starch content
Total nitrogen, % Protein, %	BDS EN ISO 5983-1:2011	Determination of nitrogen content and calculation of crude protein content - Kjeldahl method (ISO 5983:1997)
Phosphorus, %	BDS EN 15959:2011	Determination of extracted phosphorus
Potassium, %	BDS EN 1134:2000	Determination of sodium, potassium, calcium and magnesium content by atomic absorption spectrometry (AAS)

RESULTS AND DISCUSSIONS

The purpose of the preliminary soil analysis (Table 3) is necessary to determine the content of nitrogen, phosphorus and potassium. The soil reaction (pH) from the performed research shows that the alkalinity is determined by the total carbonates. They help for normal development of the roots and increase the amount of sugars and aromatic substances in the grapes.

Table 3. Main elements content and soil reaction, pH

Parameter measured	Unit	Contents
Soil reaction, pH	pH units	7,60
Electrical conductivity	$\mu S/sm^{-1}$	119,70
Movable nitrogen (sum of all forms)	mg/кg	8,55
Mobile phosphorus (P_2O_5)	mg/100g	5,46
Mobile potassium (K_2O)	mg/100g	26,27
Carbonates (general)	g/kg	62,42
Carbonates (active)	g/kg	15,00

The shoot ripening is of great practical importance. It is associated with cold resistance of buds and shoots. Maturation dynamics was monitored in all four clones included in the study by weekly measurement of marked shoots from leveled vines.

The ripening process began in late July (Figure 2) shows the most intensive process takes place in the vines of clone 524, followed by clones 100 and 470.

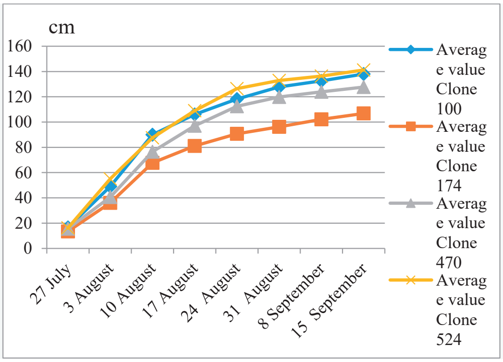

Figure 2. Shoot maturation dynamic

The slowest shoot maturation was found in clone 174. The process takes 45-50 days - from the end of July to the first ten days of September. The bark color becomes typical for the variety.

The last ten days of August and the first of September show a slower ripening rate, which is associated with both reaching technological maturity and the impact of climate change in late summer. The change in the dry substance content and the correlatively related change in the water content are one of the most characteristic features of the maturation process. The ripening degree of the shoots depends on the ecological conditions, on the biological characteristics of the variety, as well as on the load of the vines with grapes.

The concept "strength of the vine" means the totality of root system volume, annual shoots growth, as well as the amount of stock nutrients contained in the plant. The one-year growth weight is one of the important criteria for the potential possibilities of the individual varieties to bear fruit. It gives an idea of the growth strength at the end of the growing season after harvest, during the pruning.

From Figure 3, it can be seen that the highest average mass of mature one-year growth wood is in clone 524, followed by clone 100.

Obtaining high and quality yields of grapes is possible only with optimal loading of vines with winter buds during pruning. This determines their growth strength and their potential during the growing season. The load is influenced by the growing area, the cultivation method, the agro-technical measures, the ecological conditions and the biological features of the variety.

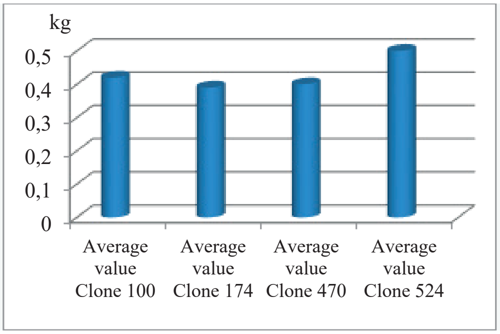

Figure 3. Average mass of one-year mature growth of vines, kg - 2020

Grape yield is an important indicator that determines the economic efficiency of the vineyards (Figure 4). For a plantation that is in the process of full fruiting, it is relatively high in the four studied clones. Clone 100 (12370 kg/ha) stands out with the highest yield, followed by clones 174 and 470, and clone 524 (10130 kg/ha) with the lowest yield.

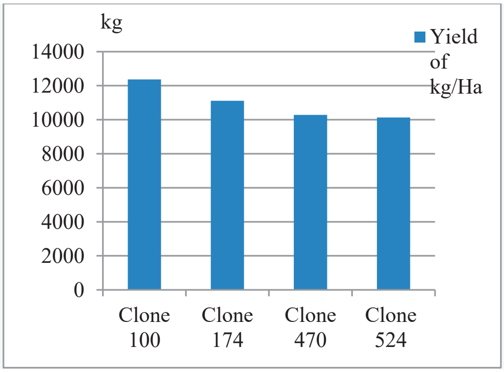

Figure 4. Average grape yield per hectare, 2020

The high activity of these processes affects the formation of stable and quality yields of grapes, as well as the timely preparation and adaptation of plants to adverse winter conditions.
The amount of directly reducing sugars and pure protein is a prerequisite for increasing the cold resistance of plant tissues.

The results obtained from the biochemical analysis of the shoots (Table 4) show that clones 100 and 524 have the highest sugar content. This is directly related to the winter resistance of the buds in the same clones, which is supported by the data in Figure 4.

Table 4. Biochemical analysis - 2020

Indicator \ Clone	Clone 100	Clone 174	Clone 470	Clone 524
Dry substance, %	61.00	56.23	55.61	56.16
Water contents, %	39.00	43.77	44.39	43.84
Total sugars, %	7.20	6.00	3.60	8.40
Starch, %	10.18	11.51	10.85	10.19
Total nitrogen, %	0.64	0.62	0.71	0.92
Protein, %	4.00	3.87	4.44	5.75
Phosphorus, %	0.23	0.16	0.15	0.16
Potassium, %	0.79	0.95	1.31	1.01

The transition from vegetation state of relative dormancy is a biological feature of the vines associated with their preparation for winter. Then the total water content in the tissues decreases and the sugars increase at the expense of the hydrolyzed starch. In the shoots and perennial trunk parts of the vine, the main components are water, organic compounds and minerals. The different degree of load leads to significant changes in the intensity of the growth processes. This has an impact on the activity of some biochemical processes such as accumulation and hydrolysis of starch, nitrogen metabolism and others.
The dry substance content is almost the same. It is highest in the clone 100. The protein content of clone 524 is 5.75%, which is the highest. This gives us reason to assume that the vines are well prepared for a possible more drastic deterioration of climate conditions in the autumn - winter period.
For the successful overwintering and the preservation of the individual organs and parts of frost, their cold resistance plays a decisive role.

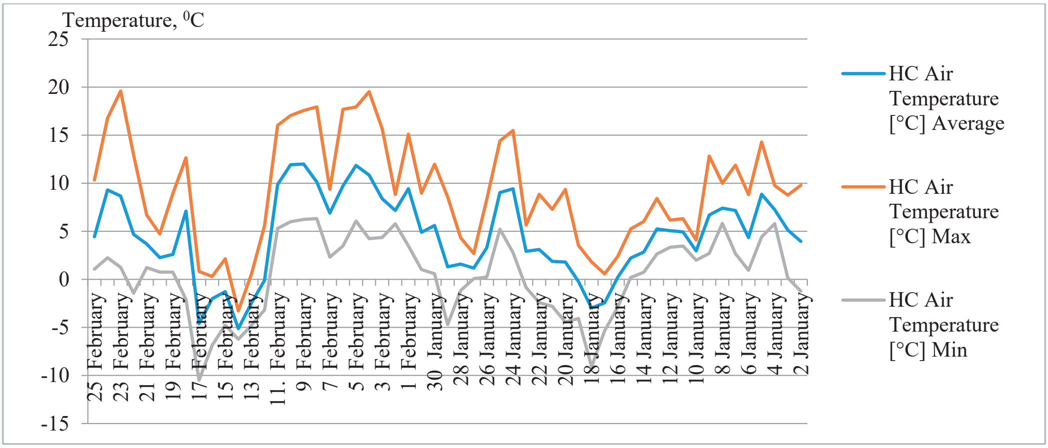

Figure 5. Air temperature in January and February, 2021

The presence of less sugars in clones 174 and 524 creates a precondition for the lower cold resistance of main and replacement buds in the winter eyes (Figure 6). This is established by the impact of low negative temperatures in field conditions (Figure 5).

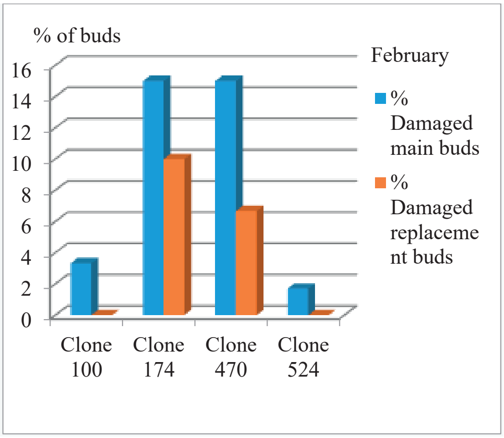

Figure 6. Damage to the winter buds, 2021

In areas with moderate-continental climate, the vines have the highest cold resistance in late December and early January. In February, as a result of reducing the resistance, the vines become more sensitive to frost at lower negative temperatures.

CONCLUSIONS

The shoot maturation takes 45-50 days, starting at the end of July and ending in the first ten days of September. The most intensive process takes place in the vines of clone 524, followed by clones 100 and 470. The slowest one is 174.

The field experiment conducted under conditions of scarce content of the macronutrients Phosphorus and Nitrogen, as well as the lack of irrigation at yields over 10000 kg/ha are a prerequisite from a practical point of view for the risk of normal winter resistance. The protein content from the biochemical analysis of vine shoots in clone 524 was 5.75%, which is more than other clones in that experiment.

The amount of mature growth and the presence of more sugars in clones 100 and 524 is a prerequisite for supplementing the resistance of tissues to the degree of frost. This is the reason for the vine plant resistance before its entry into deep dormancy and the impact of adverse external conditions.

The reported lower sugar and protein content in clones 174 and 470, in combination with the higher water content, is the reason for higher percentage of dead buds in 1st, 2nd and 3rd node, taking into account the physiological state of winter buds after dormant season.

Based on this research we can recommend that all four clones can be grown almost everywhere in Bulgaria, with similar soil and climate conditions. Clones 174 and 470 are characterized with better cold resistance. Clone 100 is economically profitable with high yield. All four have a high tolerance to soil drought, because they are grown under non-irrigated conditions.

ACKNOWLEDGEMENTS

This research was carried out with the support of the Center for Research, Technology Transfer and Intellectual Property Protection at the Agricultural University-Plovdiv, and the publication funded by Project 03-20, 2021.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON CLIMATE TRENDS IN THE AREA OF ODOBEŞTI VINEYARD

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Abstract

The reality of climate change is accepted by the vast majority of the scientific community (IPCC, 2014). Among human activities, agriculture - especially viticulture - is highly dependent on climatic conditions during the growing season. This paper aims to address a complex study on the evolution / trend of the main annual climate indicators and during the vegetation period (air temperature, precipitation, insolation, air hygroscopicity) and climate indicators with synthetic character (Ihr, CH, Ibcv , IAOe) in the viticultural area of Odobeşti vineyard. The climatic data analyzed in this study were recorded at the weather station of R.S.V.O. Odobeşti, in the period 1946-2020, during 75 years. The analysis of the recorded data highlighted clear trends in the evolution of climatic factors in the Odobeşti wine ecosystem, which certifies the reality of phenomena specific to climate change, with a direct impact on the vegetative and productive potential of vineyards. There is an increase in the annual and average values of the analyzed climatic indicators and an increasing frequency of extreme climatic phenomena.

Key words*: climate change, climate indicators, grapevine, Odobeşti.*

INTRODUCTION

Climate plays a vital role in the terroir of a particular wine region, as it strongly controls the canopy microclimate, vine growth, vine physiology, grain yield and composition, which together determine the attributes and typicality of wine (Santos et al., 2020).

Each main wine region of the world can be characterized by average climatic conditions that are the drivers of the typicality of the region's wine. However, new challenges are expected to arise due to climate change, as the cultivation of vines is deeply dependent on weather and climate conditions.

Global warming is a phenomenon that has strongly characterized the last decades with the effect of disrupting the evolution of natural factors of ecosystems. In the case of wine ecosystems, changes in temperature and expected rainfall can lead to changes in vegetation periods, variety zoning and many other changes that cannot be predicted at present, but which can increase over time and have a negative impact. Predictions based on global climate patterns show that we can expect more frequent occurrence of extreme weather events, and the associated risks and harms may become significant (Van Leeuwen et al., 2016). The main effects of climate change are: increased temperature during the growing season; increase in growing degree-days; increase in mean temperature during fruit ripening; increase in mean temperature of the warmest month of the growing season; increase in mean temperature of the coldest month of the growing season; increass in length of the growing season (frost-free days); occurrence of extreme winter minimum temperatures; increases in precipitation for July through October; increase in precipitation seasonality (coefficient of variation); change in the aridity index (Gladstones, 2011; Roehrdanz and Hannah, 2016).

These changes may reshape the geographical distribution of wine-growing regions, while the typicality of wine may also be threatened in most cases. Climate change will thus require the implementation of timely, appropriate and cost-effective adaptation strategies, which must be sufficiently planned and adapted to local conditions for effective risk reduction (Fraga et. al., 2012).

In this context, the paper present an analysis of the evolution of the main climate indicators and climate indicators with synthetic character recorded in 1946-2020 period in the viticultural area of Odobeşti vineyard.

MATERIALS AND METHODS

Odobeşti vineyard is located in the area of the curvature subcarpathians whose climate strongly influences the climate of the vineyard, having three wine centers: Odobeşti, Jariştea and Boloteşti. It occupies the hilly area parallel to the Vrancea Mountains, which includes the city of Odobeşti, under the shelter of the highest hill, Măgura Odobeştilor (996 m) with the famous plains Șarba, Vărsătura, Pădureni and Scânteia (Figure 1). The Odobeşti wine center is delimited by the parallel 45° 46" north latitude and the meridian 27° 40" east longitude at an altitude of 150 m.

In order to evaluate and characterize the climatic tendencies in the viticultural area of Odobeşti vineyard, the evolution of the main annual climatic indicators was analyzed (average air temperature, sum of precipitations, sum of sunlight hours, hygroscopicity of air, etc.), thermal and hydric regime of vegetation period (average temperature, amount of precipitation, average temperature of the hottest month - July, average maximum temperatures in August, number of days with temperatures above 30ºC) and synthetic climate indicators: heliothermal index - Ihr (Branas, 1946), the hydrothermal coefficient - CH (Seleaninov, 1936), the bioclimatic index of the vine - Ibcv (Constantinescu, 1964), the oenoclimatic aptitude index - IAOe (Teodorescu, 1978).

The climatic data recorded at the weather station of R.S.V.O. Odobești in the years 1946-2020 were analyzed. The data collected over 75 years were centralized in a database used to calculate the main climate balance and synthesis indicators. The values for two periods were calculated and compared: the period 1946-2000 (55 years) and 2001-2020 (20 years).

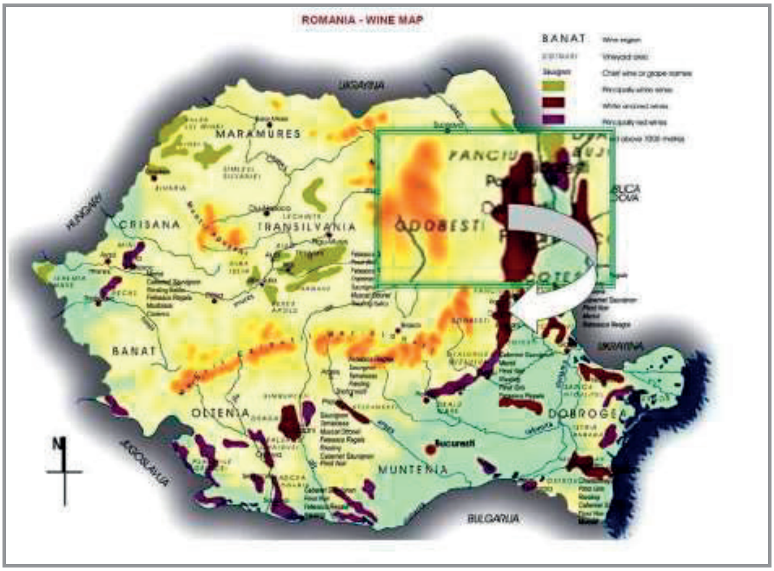

Figure 1. The viticultural area of Odobeşti vineyard

RESULTS AND DISCUSSIONS

The evolution of the thermal regime. Global climate warming, a phenomenon that has strongly characterized the last decades, has considerably influenced both the evolution of the annual thermal regime and the thermal regime during the vegetation period in the viticultural ecosystem of Odobeşti vineyard (Figure 2). The analysis of the data recorded during the study period (1946-2020) found that the average annual temperature had a general upward evolution with a clear upward trend in the last 20 years, when a surplus of 0.7ºC was recorded, compared to the average for the period 1946-2000 (Table 1). If in the period 1946-2000 (55 years), 11 years were recorded with values of the average annual air temperature above 11ºC, representing a frequency of 20.00%, in the last two decades (2001-2020), were recorded recorded 14 years with values of average annual air temperature above 11ºC, representing 70.00%. The increase of the average annual air temperature has been demonstrated more and more strongly in the last 7 years (2014-2020), a period in which values of over 12ºC, and even 13ºC (2015, 2020) were constantly registered.

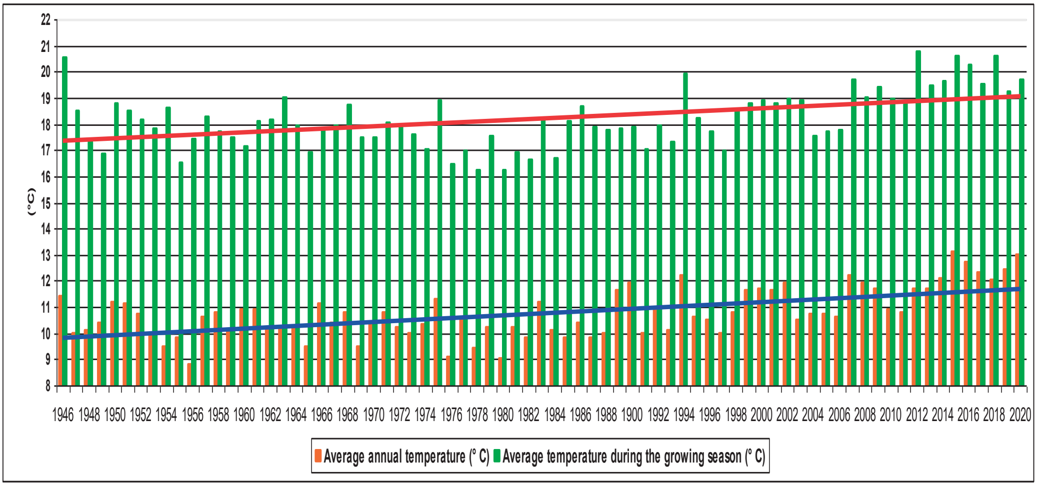

Figure 2. Evolution of the average annual temperature and of the average temperature during the vegetation period (Odobeşti, 1964-2020)

Table 1. Characteristics of the evolution of the thermal regime in the Odobeşti vineyard (1946-2020)

Climate indicator	Characteristics	Period 1946-2000 (55 years)	2001-2020 (20 years)
Average annual air temp. (°C)	Mean ºC	10.4	11.7
	No. years with temp.. > 11ºC	11	14
	Frequency (%)	20.00	70.00
Average air temp. during the growing season (°C)	Mean	17.8	18.3
	No. years with temp. > 19ºC	2	11
	Frequency (%)	3.64	55.00
Average air tem. in the warmest month (July)	Mean	21.7	22.1
	No. years with temp. > 23ºC	7	11
	Frequency (%)	12.73	55.00
Average air temp. of the maxime in August	Mean	27.3	28.1
	No. of years with temp.> 29ºC	10	13
	Frequency (%)	18.18	65.00
Nr. days with temperatures > 30ºC (average value)		23..3	42.9
∑ºt active during the veg. period		3143	3295
∑ºt useful during the veg. period		1488	1660

The values of the average air temperature during the vegetation period (April-September) also registered an ascending evolution, more accentuated in the last two decades (18.3ºC), with a surplus of 0.5ºC compared to the period 1946-2000 (17.8ºC). The incidence of years with average air temperature during the vegetation period > 19ºC was 55.00% (11 years) in the last twenty years, compared to 3.64% (2 years) in the period 1946-2000. From the analysis of the data presented in figure 2 it is found that if in the period 1946-2000 the years with values of average air temperature above 19 ºC were exceptions, in the last twenty years they have become a constant.

The average temperature of the warmest month (July) increased on average by 0.4 ºC during the years 2001-2020 (22.1ºC), compared to the average value recorded in the period 1946-2000 (21.7ºC). Also, the average maximum temperatures in August increased by 0.8ºC in the period 2001-2020 (28.1ºC) compared to the average value in the period 1946-2000 (27.3ºC), and the frequency of years with average temperatures of August highs > 29ºC increased from 18.18% (10 years) in the period 1946-2000 to 65.00% (13 years) in the period 2001-2020. Another important indicator that confirms the intensity of the global warming phenomenon is represented by the number of days with temperatures higher than 30ºC, whose average value increased from 23.93 days in the period 1946-2000 to 42.90 days in the period 2001-2020, representing an average increase of 18, 97 days/year (Figure 3).

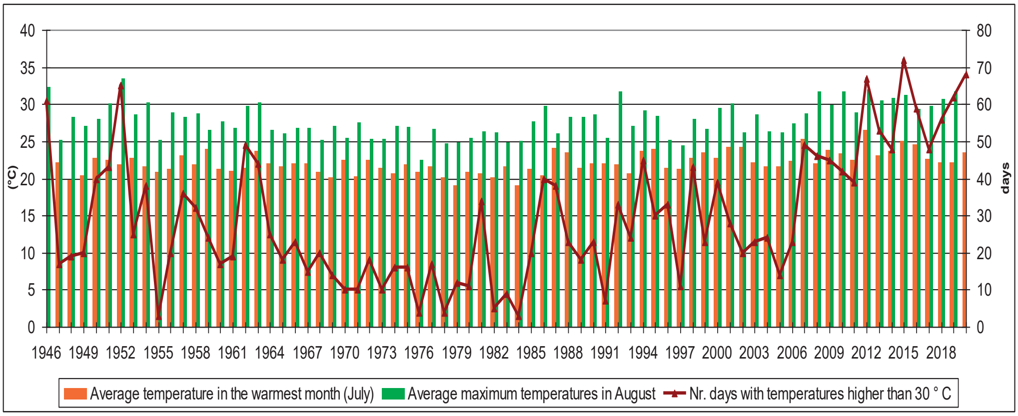

Figure 3. Evolution of the indicators of the thermal regime during the summer (Odobeşti, 1964-2020)

Thermal balance indicators during the vegetation period, respectively the sum of the degrees of active temperature (∑ºt active) and the sum of the degrees of useful temperature (∑ºt useful) also had a more ascending evolution in the last two decades (Figure 4).

The analysis of the data presented in table 1 shows an increase in the value recorded for the active heat balance during the vegetation period on average by 152 ºC in the last two decades (3295ºC), compared to the period 1946-2000 (3143ºC).

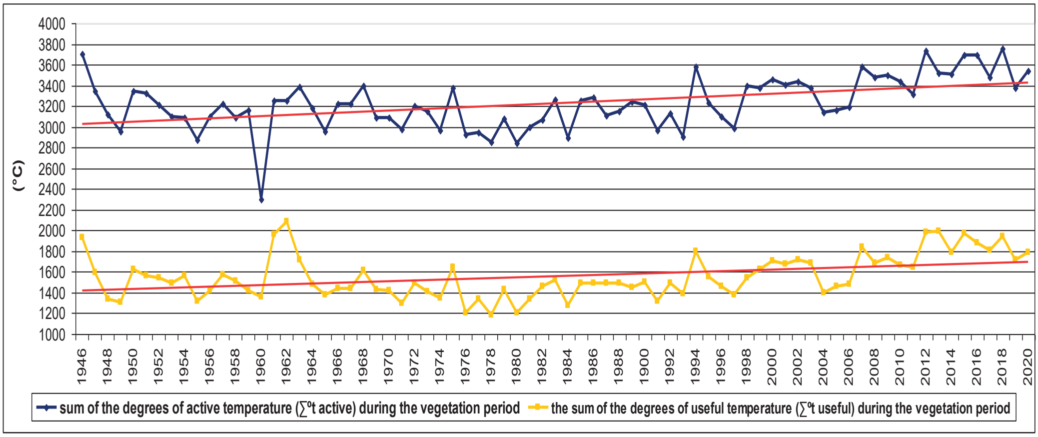

Figure 4. Evolution of thermal balance indicators during the vegetation period (Odobeşti, 1964-2020)

The evolution of the precipitation regime. The sum of the annual precipitations and the amount of precipitations fallen during the vegetation period registered an ascending trend (Figure 5). It is observed that in the first part of the period studied (1946-1968), with two exceptions (years 1954 and 1966), the values for the sum of annual precipitation did not register large differences from one year to another. Starting with 1969 year, the values recorded for this indicator showed significant differences, alternating a period of 2-4 rainy years with 1-2 years drier. The driest years recorded in the study period (1946-2020) were recorded in years 1946, 1952, 1986, 1994 and 2020.

The average amount of annual precipitation recorded in the last 20 years (644.6 mm) increased compared to the average value for the period 1946-2000 (608.0 mm) by 36.6 mm (Table 2). The incidence of years with total annual rainfall greater than 600 mm increased by 6.36% in the last two decades (50.00%) compared to 1946-2000 period (43.64%).

Also, the frequency of years with annual rainfall less than 450 mm increased by 5.91% in the last 20 years (15.00%) compared to 1946-2000 period (9.09%).

Thus and the value for the average amount of precipitation recorded during the vegetation period has increased in the last two decades (411.0 mm) by 30.3 mm compared to the average value for the period 1946-2000 (380.7 mm).

The frequency of years with precipitation values recorded during the vegetation period less than 250 mm decreased by 2.73% in the last 20 years compared to 1946-2000 (12.73%).

Table 2. Characteristics of the evolution of the rainfall regime in the Odobeşti vineyard (1946-2020)

Climate indicator	Characteristics	Period	
		1946-2000 (55 years)	2001-2020 (20 years)
The annual rainfall	Average amount (mm)	608.0	644.6
	No. years with average amount > 600 mm	24	10
	Frequency (%)	43.64	50.00
	No. years with average amount < 450 mm	5	3
	Frequency (%)	9.09	15.00
The rainfall during the growing season	Average amount (mm)	380.7	411.0
	No. years with average amount < 250 mm	7	2
	Frequency (%)	12.73	10.00
	Percentage of annual rainfall (%)	62.64	64.18

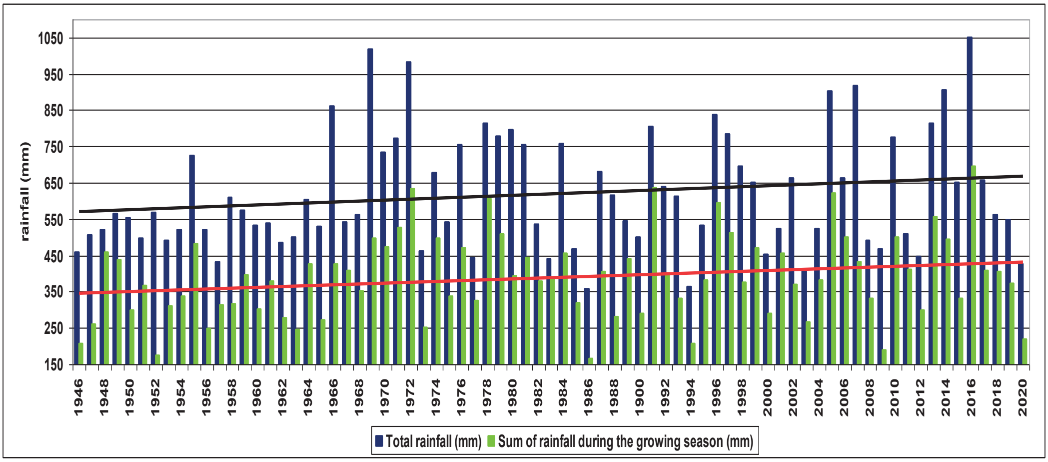

Figure 5. Evolution of the precipitation regime in the area of Odobeşti vineyard (1964-2020)

A very important indicator regarding the precipitation regime is the distribution of precipitations during the year. In the period of years 1946-2000, the amount of precipitation registered during the vegetation period of the vine represented on average 62.64% of the total annual precipitation, increasing on average by 1.54% in the last 20 years (64.18%). The unbalanced distribution of annual precipitation reported to the vegetation period was recorded in 1946, 1952, 1956, 1969, 1986, 1988, 2007 and 2009.

The evolution of the insolation and air hygroscopicity. The sum of the hours of sunshine and the hygroscopicity of the air during the vegetation period are other important indicators for the grapevines (Figure 6).

The average value calculated for the duration of sunshine during the vegetation period in the last two decades is 1586 hours, on average 126 hours higher compared to the average of years 1946-2000 (1586 hours).

Thus, and for the humidity of the air during the vegetation period there was an average increase of 3.4%, from 63.1% average value in the period years 1946-2000 to 66.5% in the last two decades (2001-2020).

Table 3. Characteristics of the evolution of the sum of sunlight hours and the hygroscopicity of air in the vegetation period in the Odobeşti vineyard (1946-2020)

Climate indicator	Characteristics	Period	
		1946-2000 (55 years)	2001-2020 (20 years)
The sum of sunlight hours in the vegetation period	Average value (hours)	1460	1586
The hygroscopicity of air in the vegetation period	Average value (%)	63.1	66.5

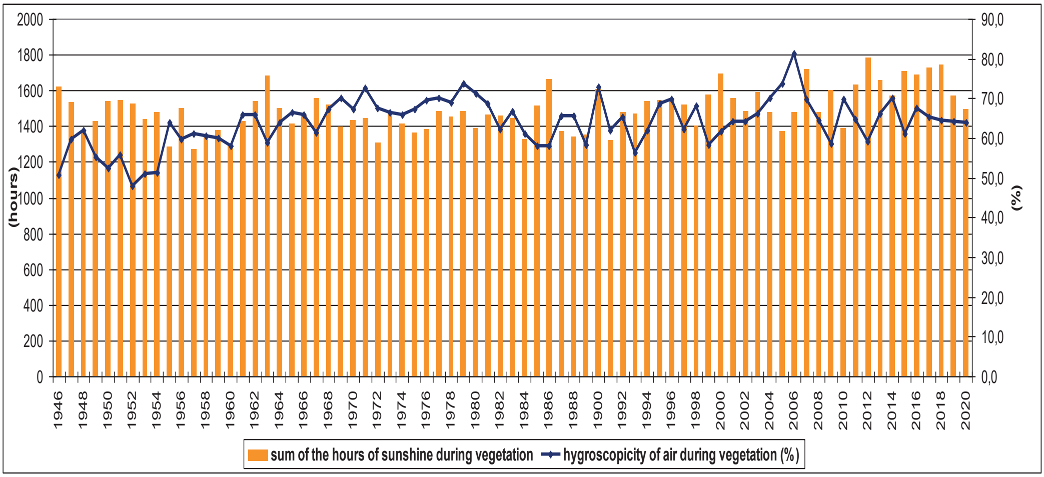

Figure 6. Evolution of the insolation and air hygroscopicity in the area of Odobeşti vineyard (1964-2020)

The evolution of synthetic climate indicators. For the assessment of the heliothermal and hydric resources of a vineyard, a series of synthetic indicators are used that integrate the combined action of two or three ecoclimatic factors (Ţârdea and Dejeu, 1995). The main climatic indicators with synthetic character important for the vine culture are: the heliothermal index - Ihr (Branas, 1946), the hydrothermal coefficient - CH (Seleaninov, 1936), the bioclimatic index of the vine - Ibcv (Constantinescu, 1964), the aptitude index oenoclimatics - IAOe (Teodorescu, 1978). The evolution of the synthetic climatic indicators in the viticultural area of the Odobeşti vineyard in the period 1946-2020 is presented in Figure 7.

The real heliothermal index (IHr) is calculated according to the formula:

$$IH_r = \sum t^o{}_u \times \sum i_r \times 10^{-6}$$

where: $\sum t^ou$ - the sum of the useful temperature degrees during the vegetation period; $\sum ir$ - the sum of the hours of insolation during the vegetation period.

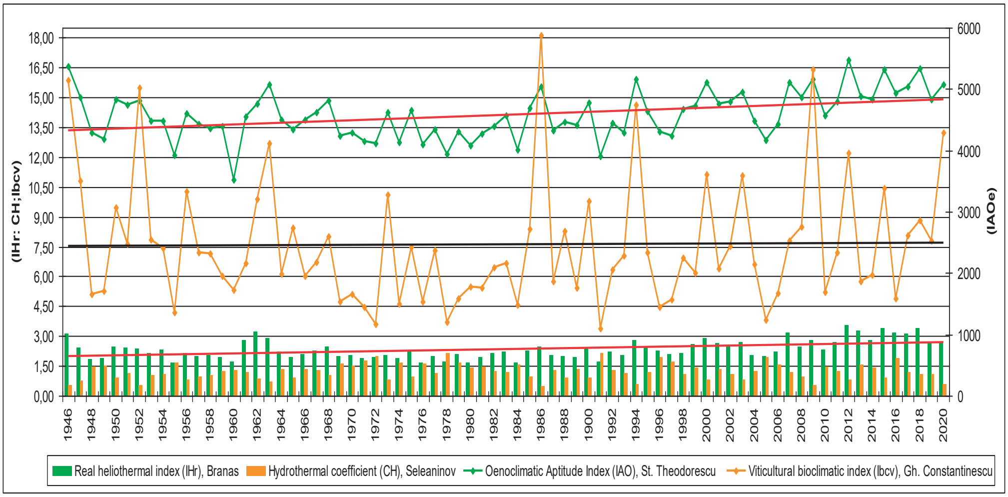

Figure 7. Evolution of the synthetic climate indicators in the area of Odobeşti vineyard (1964-2020)

In the environmental conditions of our country, the real heliothermal index varies between the limits of 1.35-2.70, the highest values being found in the southern vineyards with increased heliothermal availability (Oşlobeanu et al., 1991).

In the ecoclimatic conditions of the Odobeşti vineyard, the real heliothermal index registered an ascending evolution, the average value calculated for the last 20 years (2.78) increased by 0.6 compared to the average value calculated for the period years 1946-2000 (2.18) (Table 4).

The hydrothermal coefficient (CH) is calculated according to the formula:

$$CH = (\sum p / \sum t^o{}_a) \times 10$$

where: ∑p - the sum of the precipitations during the vegetation period; ∑ta - the sum of the degrees of active temperature during the vegetation period.

CH values in Romania vary between 0.6 and 1.8, and at minimum values (0.6-0.7) the vine culture becomes economical in irrigated regime (Ţârdea and Dejeu, 1995).

Under the conditions of the Odobeşti vineyard, the hydrothermal coefficient registered a slightly downward evolution, the average value calculated for the last 20 years (1.19) decreased by 0.4 compared to the average value calculated for the period years 1946-2000 (1.23).

The bioclimatic index of the vine (Ibcv) integrates the combined action of temperature, insolation and humidity and is calculated according to the formula:

$$Ibcv = (\sum t^o{}_u * \sum i_r) \times (\sum p * N_{zv})/10$$

where: ∑ta - the sum of the degrees of useful temperature during the vegetation period; $\sum i_r$ - the real insolation during the vegetation period; ∑p - the sum of the precipitations during the vegetation period; Nzv - the number of days in the vegetation period.

In the conditions in our country the Icbv values vary between 4 and 15 (Ţârdea and Dejeu, 1995). In the Odobeşti vineyard the values of the viticultural bioclimatic index had a slightly ascending trend, the average value for the last two decades (8.15) increasing by 0.75 compared to the average value calculated for the period years 1946-2000 (7.40).

The oenoclimatic aptitude index (IAOe) determines the degree of climate favorable that a region has to synthesize anthocyanins in grapes and, in general, to produce red wines.

The oenoclimatic aptitude index is calculated according to the formula:

$$IAOe = T + I - (P - 250)$$

where: T - sum of degrees of active temperature in the range 01.IV - 30. IX; I - the sum of the hours of insolation in the same interval; P - the sum of precipitation from the same period.

Table 4. Characteristics of the evolution of synthetic climate indicators in the Odobeşti vineyard (1946-2020)

Synthetic climate indicator	Period /average value	
	1946-2000 (55 years)	2001-2020 (20 years)
The heliothermal index - IHr (Branas, 1946)	2.18	2.78
The hydrothermal coefficient - CH (Seleaninov, 1936)	1.23	1.19
The bioclimatic index of the vine - Ibcv (Constantinescu, 1964)	7.40	8.15
The aptitude index oenoclimatics - IAOe (Teodorescu, 1978)	4472	4890

On the Romanian territory, the values of this index are between 3700 and 5200, the viticultural areas with values of over 4600 present favorable conditions for the production of red wines (Teodorescu et al., 1987).

Due to the climatic conditions registered in the Odobeşti vineyard in the last 20 years, the value of the oenoclimatic aptitude index also had an ascending evolution.

It increased on average from 4472 in the period years 1946-2000, to 4890 in the last two decades, changing the classification of the viticultural area of the Odobeşti vineyard in the region with medium favorability for the production of red wines in a favorable area for this production direction.

CONCLUSIONS

The annual thermal regime and during the vegetation period registered a surplus of 0.7ºC, respectively 0.5ºC in the last two decades compared to the period 1946-2000.

Other indicators that confirm the intensity of the global warming phenomenon are the average temperature of the warmest month (July), the average maximum temperatures in August and the number of days with temperatures higher than 30ºC, whose value increased on average by 18.97 days/year in the last 20 years.

The regime of annual precipitation and during the vegetation period registered an increase in the last two decades, with the accentuation of the unbalanced distribution, alternating 2-4 rainy years with 1-2 drier years.

Climate indicators with synthetic character (Ihr, Icbv, IAOe) have had an ascending evolution in the last two decades, the value of IAOe classifying the viticultural area of Odobeşti vineyard in the area with favorable conditions for obtaining red wines.

The analysis of the data registered in the last 20 years in the Odobeşti viticultural ecosystem, highlighted clear trends in climate evolution, which certify the reality of the phenomena specific to climate change.

ACKNOWLEDGEMENTS

To accomplish this work we want to thank all who contributed to the recording of climate data since 1946 from the Laboratory of Meteorology of R.S.V.O. Odobeşti.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GENETIC DIVERSITY THE VITICULTURAL GERMPLASM FUND OF ROMANIA – NEWS ACCESSIONS

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Abstract

In contemporary society, the viticultural germplasm fund, vine (varieties) and wine - are elements of universal and national heritage, and it must be known, evaluated, capitalized and last but not least, preserved - according to unitary, equidistant and unanimously recognized concepts by specialists. The accentuated variability of the varieties belonging to the species Vitis vinifera spp sativa, their number and the vast geographical space on which they are fund, provide genetic improvement ameliorators with a background of germplasm and an equally vast genetic resource. In the current practice of genetic improvement vines, it has always been taken into account the manifestation of exceptional heredity in offspring, for one or more characters, either from the spectrum of biological characters or from the spectrum of characters related to behaviour and production quality, as well as resistance to diseases and pests, drought resistance, frost resistance, extreme phenomena, etc. In general, the new grape varieties for table and wine, obtained in Romania are obtained by intraspecific hybridization, by self-pollination, by free pollination, by natural selection, as well as by fixing valuable mutations. In most of them, however, there are new characters, clearly superior to those found in the parent varieties. As the study shows, the particularities of new creations register a higher predictable genetic gain, incorporating a high genetic value, often even having a different production direction compared to the parents.

Key words*: diversity, germplasm fund, grape, varieties, yield.*

INTRODUCTION

The improvement of grape vine assortment (table grapes, white, red, rose wine grapes, varieties with biological resistance, stock varieties) was realized gradually over the years, starting in 1946 (Constantinescu et al., 1959; 1960; 1962; 1965; 1966; Constantinescu and Negreanu, 1960; Dvornic, 1960; 1974), continuing with a prolific period between years 1970 and 2000 (Constantinescu et al., 1975, Gorodea et al., 1976; Lepadatu, 1979; Gorodea, 1983; Ioniță et al., 1981; Toma and Ispas, 1991), followed by a period more constant in results after 2000 (Toma and Ispas, 2008; Antoce et al., 2004; Glăman et al., 2018). The programme and the objectives of the activity of genetic amelioration of grape vine in our country were coordinated by the Research-Development Institute for Viticulture and Wine Making Valea Călugărească, by the combined activity of the 12 viticulture and wine making research stations and concerned mostly the creation of high quality varieties, with high productivity, with different maturation periods (table grapes), with resistance to diseases and pests and to weather conditions and extreme phenomena, as well seedless grape varieties (Brânduşe and Ionescu, 1992; Indreaș and Vișan, 2000; Ţârdea and Rotaru, 2003; Stroe, 2013; Glăman et al., 2018). This institutions possess ampelographic collections and contest plantations and carry out research activity, identification, acquisition, inventorying, registration and maintenance, activity that requires a complex and interdisciplinary approach. This collections contain old varieties, local varieties, varieties removed from the cultivated assortment, traditional varieties (agroecotypes) that are cultivated in a restrained area, wild varieties and species related to the cultivated ones, selected elite genotypes, natural mutations selected for their valuable features or obtained from genetic transformation (loss of genetic information), local ancestral varieties that are a national wealth that must be protected and used in breeding, as well as the grape varieties cultivated nowadays and the new varieties obtained using different breeding techniques.

Generally, these are considered true “gene banks”, being a source of germplasm that helps the study and the knowledge of the agrobiological and technological aspects of the varieties used in the breeding and selection of the grape wine, at the same time maintaining the genetical diversity of the species. The biological material may be available at any time, for scientific and economical purposes. Practically, in contemporary society, the viticultural germplasm resource, the varieties and the wine, are elements of universal and national heritage, and this genetic heritage must be recognised, known, evaluated, utilized, and lastly, conserved. In this context, using different breeding techniques (interspecific hybridization, fixation of valuable mutations), 70 varieties of *Vitis vinifera* were obtained in Romania, creations for different production directions, mostly table grapes - 31 and white wine grape varieties - 21, followed by red wine grape varieties -15, seedless grape varieties - 4 and rootstock varieties - 3.To this numbers, 14 varieties with increased resistance to pests and diseases and to natural stress factors can be added.

A special position, contribution in Romanian viticulture is attributed to the clonal selection – a tool utilized to avoid the genetic erosion of the varieties and a way to ensure the conservation of the clonal and local varieties and the ones of different origins (104 clonal selections). Our institution, as a part of this national breeding program of *Vitis vinifera*, possess an ampelographic collection composed of over 130 varieties, local varieties, ancestral, as well as varieties from the international assortment, new varieties obtained from Romania, with different directions of production. Practically, from the 84 newly obtained varieties, the U.A.S.V.M. Bucharest collection possess 55.95 % of the national new varieties, and if adding the biological resistant ones, 67.14% can be found. Among the main achievements that were obtained here, is the creation and (homologation) of two early ripening table grape varieties: ‘Muscat Timpuriu de București’ and ‘Augusta’, two table grape varieties with middle ripening - ‘Chasselas de Baneasa’ and ‘Triumf’ and two late ripening varieties - ‘Select’ and ‘Coarnă neagră selecționată’ (Stroe, 2016). Contextually, referring only to this point of view, they represent 19.35 % of the total number of table grapes created in Romania. The present study aims to highlight the elements that define the quality of these new varieties - visual quality (hedonic approach) and that are found in these collection, in the southern part of Romania, to popularize them, because, expect for a few varieties - ‘Victoria’, ‘Crâmpoșie selecționată’, ‘Șarba’, ‘Novac’, ‘Negru de Drăgașani’, ‘Columna’ (Antoce et al., 2004; Stroe and Cojanu, 2018; Antoce et al., 2017; Cichi et al., 2019), few are known and cultivated at national and international level, which indicates the lack and the inefficient utilization of the genetic resources of Romania. As a result, the marketing activity and the more coherent and aggressive promovation of this varieties would be a win for both grapevine growers and consumers. But, unfortunately, these newly obtained varieties, although very valuable, both productively and qualitatively, will be destined to anonymity, failing to transcend the boundaries of the area where they were created, even in the context of the over-publicized globalization. Furthermore, recent data shows that in the last 30 years, the total area of table grapes in Romania has decreased dramatically, representing only 6.9% of the total cultivated area (wine varieties representing 93.1%).

MATERIALS AND METHODS

The present study was approached starting from the need to know the performances of these varieties from a visual and organoleptic perspective, in order to promote them, at least at national level. In order to achieve the objective presented above, research took place in the ampelographic collection in the experimental field of U.A.S.V.M. Bucharest, with institute code ROM06 in www.vivc.de. The data about this varieties and the paternal varieties can be found in Vitis International Variety Catalogue (www.vivc.de), as a result of the scientific papers confirming the origin and genetic paternity of those (Maul et al., 2012; Lacombe, 2013; Rustioni et al., 2013; Rustioni et al., 2014a, 2014b; Popescu, 2017; Popescu and Crespan, 2018). Although established in 1984, and renewed in proportion of 65%

between 2006 and 2018, from many points of view, the ampelographic collection of this institution corresponds to the standards of VITI-GENET 14-539. "OIV guidelines for recognising grapevine collections", of which we can mention: it holds a large number of varieties (the minimum number of varieties in an ampelographic collection must not be less than 20); each variety must be maintained in a minimum number of (4) plants, grafted on the same rootstock; must not contain quarantine microorganisms; must contain the varieties of reference ('Bicane', 'Cabernet Sauvignon', 'Chardonnay', 'Chasselas blanc', 'Merlot', 'Pinoit Noir', 'Riesling Weiss', 'Rkatiteli', 'Sauvignon'); adequate pruning system: Guyot simple/double, a planting distance chosen to promote the vegetative development of each plant (1m, 1.2 m/2 m, 2.2 m); the varieties must be grouped, according to their use. The varieties in the collection are pruned using the Guyot technique, with a load of 42 buds/vine, planted at a distance of 1.2/2.2 m, and the control plants for each category were chosen according to the VITI-GENET 14-539- "OIV guidelines for recognising grapevine collections" standard, Rustioni et al., 2013, which takes in account the maturation epoque and the direction of production.

For this analysis, we used 8 cultivars of 'Cardinal', 'Chasselas dore', 'Muscat Hamburg' 'Kişmiş alb', 'Crâmpoşie', 'Fetească regală', 'Băbească neagră' and 'Cabernet Sauvignon', on which traits were measured repeatedly, using the same standard protocol mentioned across sites and years. The list of traits considered for this analysis were: berry colour (OIV descriptor); sugar content (Brix); berry weight (mg); colour of skin, flavour, conform OIV (2008a; 2008b; 2013).

The graphic representation of the frequency of utilisation of this very known varieties in the creation of new ones was realized with Word Cloud type graphics, generated using the add - on for Microsoft Word Pro Word Cloud. The graphics disclose the frequency of utilisation of some varieties for each category (if a variety was more utilised more frequently, it will be represented bigger in the generated image).

The phenological maturation of the berries - Brix was chosen according to the cultivar (= the optimum moment of harvesting or N stage on Baggiolini scale, stage 89 on BBCH scale, stage 38 on Eichiorn K. W. and Lorenz H. scale) (Baggiolini, 1952; Meier, 2001; Pierrot and Rochard, 2013). To assess the descriptive parameters of production quality, 10 primary climate parameters and bioclimatic indices for 2019 were calculated, in order to correctly appreciate the context of sugar content (Brix), which are visibly quite high, as it can be observed in Tables 1 - 7): annual average temperature - 12.92^{0}C; average temperature in the growing season (IV-X) – 18.5^{0}C; average temperature in summer (VI-VIII) - 23.05^{0}C; average maximum temperature in July - 29.64^{0}C; average minimum temperature in January - (-4.55) ^{0}C; annual total precipitations – 528.6 mm; summer total precipitations -142.0 mm; Huglin Index; $\Sigma[(Tavg-10°C) + (Tmax-10°C)] / 2 \times k$ - 2458; Winkler Index; $\Sigma[(Tmax+Tmin)/2 - 10°C]$ - 1825; Cool Night Index; $Tmin_{sept}$-10.60 - (Climate records from the National Agency of Meteorology and Hydrology Bucharest).

RESULTS AND DISCUSSIONS

The ampelographic collection in the experimental field of U.A.S.V.M. Bucharest holds in cultivation 48 mature varieties, out of the total number of 70 - (61.14 %): 22 table grapes out of a total of 31 - (70.96 %), 3 seedless varieties out of 4 - (75 %), 13 for white wine, out of 21- (61.90 %), 10 for red wine, out of 15 - (66.66 %). For a correct presentation, in order to popularize this varieties, we considered that it was necessary to determine the rate of use of the most know cultivars used as genitors for this new Romanian creations, this remarkable record of new varieties, practically foreshadowing their qualitative panel, because the descendants exceed the parents of in most cases.

According to Figure 1, in Romania, the following genitors stand out in the creation of new table grapes: 'Afuz Ali', 'Alphonse Lavallée', 'Coarnă albă', 'Coarnă neagră', 'Regina viilor', 'Cardinal', 'Muscat de Hamburg', 'Italia', 'Bicane' etc. Regarding the breeding of seedless grapes, the most valuable genitors are: 'Braghină', 'Perlette', 'Sultanină', 'Maria Pirovano', 'Tămâioasă românească'. Concerning the breeding of varieties for white wine, as shown in Figure 2, the most utilised varieties come from the international and national assortment: ‘Crâmpoșie’, ‘Fetească regală’, ‘Grasă de Cotnari’, ‘Tămâioasă românească’, ‘Iordană’, ‘Aligoté’, ‘Chardonnay’, ‘Riesling italian’, ‘Pinot gris’, ‘Traminer roz’, ‘Furmint’, etc., and table grape cultivars, such as ‘Hamburg Muscat’, ‘Muscat Perla de Csaba’, ‘Chasselas doré’, ‘Coarnă albă’, ‘Coarnă neagră’, ‘Bicane’, ‘Silvania’ and varieties for red wine: ‘Băbească neagră’.

Figure 1. The patterns of the varieties for table grapes

Figure 2. The patterns of the varieties for white wines

Figure 3. The patterns of the varieties for red wines

Analysing the data from Figure 3, we can observe that in regard to the breeding of new varieties for red wines, regardless of their destination, the most used genitors are the local varieties ‘Băbească neagră’ and ‘Negru vârtos’, but also international varieties: ‘Alicante Bouschet’, ‘Roșioară’, ‘Pinot noir’, ‘Cabernet Sauvignon’, ‘Merlot’, ‘Saperavi’. Table grapes varieties have also been evidenced - ‘Hamburg Muscat’, international varieties for white wines - ‘Chardonnay’ and international varieties for aromatic white wines - ‘Muscat Ottonel’. The cultivars studied in the conditions of the experimental filed of U.A.S.V.M. Bucharest - situated in the south eatern part of Romania, presents promising perspectives, due to the rather high degree of adaptation. The success of their integration and extension in culture at national level, depends on finding adaptation to zonal climate change and lasting solutions of culture technologies, oenological practices, etc. From the analysis of sugar accumulation, it was found that the 25 varieties for table grapes, out of which 3 are seedless and 2 have mixed characteristics, recorded a fairly high sugar concentration for the year 2019, with values above those specified in the literature for some of them (Ţârdea and Rotaru, 2003; Stroe, 2012; Glăman et al., 2018; Stroe, 2020). Concerning the early ripening table grape varieties, the limits of this values are between 16.7 and 19°Brix: ‘Augusta, ‘Victoria’, ‘Timpuriu de Cluj’ (mixed characteristics), and ‘Muscat Timpuriu de București’ recorded a maxim absolute 20.2°Brix. The same trend was observed for the accumulation of sugars in the case of the varieties with middle and late ripening (16.5-19.4°Brix) and ‘Milcov’, ‘Silvania’, ‘Istrița’, followed by ‘Splendid’ and ‘Transilvania’ from the same group (Table 2) and ‘Xenia’, ‘Tamina’ and ‘Coarnă neagră selecționată’ from the late ripening group. It can be appreciated that from this point of view, the cultivars from the three groups differentiated according to the maturation period, exceed the reference values for each group (‘Cardinal’, ‘Afuz Ali’, except the values accumulated in the control ‘Muscat de Hamburg’). Practically, it does not equal the control regarding the soluble sugars, but it compensates by size, shape, uniformity of the berries and discrete aroma – qualities that overcome overcome the organoleptic barrier at the first contact of the consumer.

Table 1.Varieties grape table early ripening - qualitative parameters

Varieties Code VIVC	Parents	^{0}Brix (as Total Soluble solids, g/L)	Use	Carpometrical analysis berry			
				Weight of berry (g)	Shape	Color of skin	Flavour
Muscat Timpuriu de Bucureşti, 8256	Coarnă albă x (Regina viilor x Muscat Perlă de Csaba)	20.2	Table grape early ripening 6	4.8	ovoid	B	muscat
*Timpuriu de Cluj, 12451	Crâmpoşie x Frumoasă de Ghioroc	19		4.3	globose	B	muscat
Augusta, 14781	Italia x Regina viilor	16.7		6.28	ovoid	B	none
Victoria, 13031	Cardinal x Afuz Ali	16.8		7.8	ovoid	B	none
Timpuriu de Pietroasa, 21603	Alphonse Lavallée x Regina viilor	16.3		4.3	ellipsoid	N	foxy
*Bujoru, 26664	Băbească gris x Muscat Perlă de Csaba	19.9		2.18	ovoid	Rs	other flavour - frank
Cardinal Control, 2091	Ahmeur bou Ahmeur x Alphonse Lavallée	15.3		5.3	globose	Rg	other flavour - frank

Color of skin, N black, Rg red, Rs rose, B white, Use: table/wine/seedless; Early ripening - about 2 weeks before Chasselas doré; Middle - ripening at the same time as Chasselas doré or 1-2 weeks after; tardive ripening (Late) - 3-4 weeks after Chasselas doré; Very tardive ripening (Very late) -5-6 weeks after Chasselas doré.
*varieties with mixed propertie

According to OIV, 2008 standards, table grape varieties can be harvested at concentrations of sugar smaller compared to the varieties for wine, but not less than 16°Brix, except for few derogations depending on the climate of each country. The data that refers to the carpometrical parameters, also indicates values that prove that most of the varieties have qualities that achieve and sometimes exceed the parental phenotype (specified in Figure 1).

Table 2. Table grape varieties with middle ripening - qualitative parameters

Varieties Code VIVC	Parents	^{0}Brix (as Total Soluble solids, g/L)	Use	Carpometrical analysis berry			
				Weight of berry (g)	Shape	Color of skin	Flavour
Chasselas de Băneasa, 2480	Chasselas doré	17.6	Table grape middle ripening -10	3.5	globose-ovoid	B	none
Silvania, 11808	Bicane x Chasselas doré	19.3		3.2	globose	B	none
Napoca, 8348	Alphonse Lavallée x (Regina viilor x Muscat Hamburg)	16.5		4.4	ovoid	N	muscat discreet
Splendid, 11980	Black rose x Regina viilor	17.6		5.47	ovoid	Rs	muscat discreet
Transilvania, 12613	Black rose x Cardinal	17.3		7.4	ovoid	N	muscat discreet
Istrița, 21145	Tămâioasă românească x Maria Priovano	18.0		3.6	globose	B	muscat
Triumf, 12655	Lignan x Afuz Ali	16.8		4.3	ovoid	B	none
Azur, 833	Coarnă neagră x Cardinal	16.5		2.9	ovoid	N	muscat discreet
Milcov, 21363	Coarnă neagră x Muscat de Hamburg	19.4		2.7	ovoid	N	muscat discreet
Someșan	Muscat de Hamburg x Regina viilor	17.4		3.8	ovoid	Rs	muscat discreet
Muscat Hamburg Control, 8226	Muscat de Alexandria x Frankental	23.1		2.8	globose	n	muscat intense

Table 3. Table grape varieties with late ripening - qualitative parameters

Varieties Code VIVC	Parents	^{0}Brix (as Total Soluble solids, g/L)	Use	Carpometrical analysis berry			
				Weight of berry (g)	Shape	Color of skin	Flavour
Select - 11471	Bicane x Afuz Ali	16.4	Table grape tardive ripening 6	5.1	ovoid	B	none
Greaca, 4959	Bicane x Afuz Ali	16.9		5.8	ovoid	B	none
Xenia, 13274	Bicane x Muscat de Hamburg	17.8		5.2	ovoid	B	muscat intense
Tamina, 12244	Bicane x Muscat de Hamburg	16.9		5.8	globose-ovoid	Rs	muscat discreet
Roz românesc, 10290	Bicane x Afuz-Ali roz	16.1		3.4	globose	Rs	none
Coarnă neagră selecționată, 2729	Coarnă neagră	18.1		4.2	ovoidal	N	none
Control Afuz Ali, 122	-	16.2			cylindric		none

In regard to the average weight of the berry, it varied between 2.18 g in ‘Bujore’ and 7.8 g in ‘Victoria’ cultivar. Average berry weights higher than 5 g were recorded in ‘Transilvania’ - 7.4 g, ‘Augusta’ - 6.28 g, ‘Splendid’ - 5.47 g, ‘Xenia’ - 5.2 g, ‘Select’ - 5.1 g, etc. For the three seedless grape cultivars (Table 4) used for raw consumption and industrialization, the limits of sugar accumulation were between 20-23.5°Brix, in the following order: ‘Centenar de Pietroasa’, ‘Otilia’, ‘Călina’, which exceeds the ‘Kişmis alb’ control variety and the cultivar ‘Otilia’ also exceeded the control in terms of berry size and uniformity.

Table 4. Seedless grape varieties - qualitative parameters

Varieties Code VIVC	Parents	^{0}Brix (as Total Soluble solids, g/L)	Use	Carpometrical analysis berry			
				Weight of berry (g)	Shape	Color of skin	Flavour
Călina,1996	Braghină x Sultanină	23.5	Seedless variety -3	1.6	globose	Rs	none
Otilia, 15712	Alphonse Lavalleé x Perlette	20.9		2.7	globose	N	none
Centenar de Pietroasa, 21006	Tămâioasă românească x Perlette	20.0		1.4	globose	B	muscat discreet
Kişmiș alb-Control, 12051	-	19.4		2.67	ovoid	B	none

Table 5. Qualitative parameters of grape varieties for white table wines

Varieties Code VIVC	Parents	^{0}Brix (as Total Soluble solids, g/L)	Use	Carpometrical analysis berry			
				Weight of berry (g	Shape	Color of skin	Flavour
Astra, 20876	Fetească regală x Pinot gris	17,6	white table wines 4	2,4	globose	B	none
Băbească gris, 842	Băbească neagră	20.32		2.4	obloid	Rs	none
Miorița, 7845	Coarnă albă	18.9		2.3	ovoid	B	none
Roz de Miniş, 10289	Selecție Bacator roz	18.1		2.7	globose	Rs	none
Crâmpoşie Control, 3237	-	19.8		2.7	globose	B	none

According to the data shown in Table 5, in the case of the varieties used for obtaining white table wines, the sugar accumulations are fairly high. The variety ‘Băbească gris’ exceeds the values recorded in the control variety, but generally higher than the values found in the specialised literature, as a result of the effects of climate change determined by the increased thermal resources.

Table 6. Qualitative parameters of grape varieties for quality white wines

Varieties Code VIVC	Parents	^{0}Brix (as Total Soluble solids, g/L)	Use	Carpometrical analysis berry			
				Weight of berry (g)	Shape	Color of skin	Flavour
Alb aromat, 23101	Tămâioasă românească	22.1	quality white wines 4	3.1	gobose	B	muscat
*Aromat de Iaşi, 632	Tămâioasă românească	20.8		2.2	globose	B	
Blasius, 20959	(Iordană x Traminer roz) x (R.Saint Pierre x Muscat Perlă de Csaba)	20.4		2.4	globose	B	none
Columna, 2787	Pinot gris x Grasă de Cotnari	21.6		2.3	globose	B	none
Crâmpoşie selecţionată, 3238	Crâmpoşie	21.0		2.6	globose	B	none
*Donaris, 3642	Bicane x Muscat de Hamburg	20.7		2.4	globose	B	muscat discreet
Furmint de Miniş, 16940	Furmint - mutație	19.8		1.9	ovoid	B	none
Selena, 21558	(Iordană x Traminer roz) x (Raisin de Saint Pierre x M. P. Csaba)	20.6		1.6	globose	Rs	muscat discreet
Șarba, 10738	Riesling Italian x Muscat de Hamburg	22.0		2.32	globose	B	muscat
Fetească regală Control, 4121	Frâncușă x Fetească albă	21.5		2.05	globose	B	none

Table 7. Qualitative parameters of grape varieties for red wines

Varieties Code VIVC	Parents	^{0}Brix (as Total Soluble solids, g/L)	Use	Carpometrical analysis berry			
				Weight of berry (g)	Shape	Color of skin	Flavour
Arcaş, 566	Cabernet Sauvignon x Băbească neagră	18.5	red table wines 6	1.35	ovoid	N	none
Balada, 20920	Băbească neagră x Pinot noir	20.7		1.7	globose	N	none
Codană, 2740	Băbească neagră x Cabernet Sauvignon	19.0		2.3	obloid	N	none
Cristina, 21045	Chardonnay x Băbească neagră	18.9		1.62	narrow ellipsoid	N	none
Haiduc, 17687	Roşioară x Cabernet Sauvignon	19.8		1.55	obtuse-ovoid	N	none
Pandur, 17706	Roşioară x Cabernet Sauvignon	19.4		1.9	obloid	N	none
Băbească neagră Control, 843		20.9		2.3	obloid	N	none
Mamaia, 21348	Merlot x (Băbească neagră x Muscat Ottonel)	20.6	quality red wines 4	1.9	globose	N	none
Negru Aromat, 15711	Cabernet Sauvignon	23.0		1.65	globose	N	none
Negru de Drăgășani,23178	Negru vârtos x Saperavi	22.4		2.4	globose	N	none
Novac, 16933	Negru vârtos x Saperavi	21.5		2.4	ovoidal	N	none
Cabernet Sauvignon Control, 1929		23.1		2.0	globose	N	none

Regarding the qualitative compositional panel of the varieties used for obtaining white wines, according to the data entered in Table 6, it is observed that the sugar accumulations are quite high, even exceed the control, as is the variety 'Șarba', 22.0°Brix and the variety 'Columna' with 21.6°Brix, exceeding the values of the control variety 'Fetească regală'. Some of these varieties exceed the values specified in literature: 'Alb aromat', 'Aromat de Iași' and 'Crâmpoșie selecționată'. These accumulations that can be a result of the effects of the thermal resources during the vegetation period, especially during the period of full maturation of the varieties. Broadly, it can be observed that the sugar accumulations, in most of the varieties, exceed the value of 20°Brix, foreshadowing the obtaining of wines with an alcoholic potential of over 11.5% alcohol.

The data entered in Table 7 shows that the varieties used for obtaining red table wines accumulate high quantities of sugar, as a result of the increased thermal resources of the year 2019, and their values exceed the ones found in literature (Haiduc, Pandur). The limits of sugar accumulation for the quality red wine varieties are between 20.6°Brix in 'Mamaia' variety and 23.0°Brix in 'Negru aromat' variety. Broadly, the sugar accumulations are predictable for obtaining wines with an alcoholic potential of over 11.5% alcohol and it is known that the modern consumer prefers wines with a moderate alcohol content (approximately 11-12% vol. alcohol), slightly extractive, obtained from varieties with moderate accumulations in sugars.

CONCLUSIONS

As it results from the study, the particularities of the new varieties obtained in Romania, regardless of the method of obtaining, show that they are by far some valuable creations, and these are all the more obvious, especially when they qualitatively exceed the control of the maturation group they are part of, on the one hand, and parents on the other hand.

From the category of early ripening varieties, we recommend 'Victoria', 'Augusta', 'Timpuriu de Cluj' (mixed characteristics), defined by special qualities, given the size of the grapes, the colour and uniformity of the berries, but also the fact that they were distinguished by a great ecological plasticity. From the category of varieties with medium to late maturation, we recommend the varieties 'Napoca', 'Transilvania', 'Istrița', and from those with late maturation 'Select', 'Xenia', 'Tamina', 'Coarnă neagră selecționată'.

These varieties meet the requirements of the modern, cosmopolitan consumer, who wants table grapes with large berries 3.5-4-6 g, uniform, with a moderate sugar content (16-18°Brix) against a balanced glucoacidometric index and a discreet bite flavor. In other words, even if it does not equal the control of soluble sugars, it compensates with the visual qualities that overcome the organoleptic barrier.

Among the varieties used for white wines, the distinguished varieties are 'Șarba', 'Aromat de Iași', 'Alb aromat', 'Crâmpoșie selecționată', 'Băbească gri' and in case of the ones for red quality wines, 'Negru de Drăgășani', 'Negru Aromat', 'Novac' and 'Mamaia' are distingiuished.

Regarding the potential of wine varieties, the modern consumer prefers wines with a moderate alcohol content (approximately 11-12% vol. alcohol), slightly extractive, obtained from varieties with moderate sugar accumulations and late maturation, to avoid this process in periods with excessive temperatures, qualities that are fully found in some varieties from the list of this collection, from the list of new varieties obtained in Romania

Currently, these newly obtained varieties, although very valuable, both productively and qualitatively, are not known to the general public and will soon be destined for anonymity, if they are not publicized, because, for a new variety, it is difficult to cross the borders of the area where it was created, even in the context of over-mediated globalization.

It is imperative that the marketing and promotion activity be more consistent and more aggressive, as this would be a gain for both growers (producers) and consumers.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE ASSESSMENT OF SOME HOT PEPPERS ACCESSIONS BRED AT VEGETABLE RESEARCH DEVELOPMENT STATION BUZĂU

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Abstract

The Genetic, Breeding and Biodiversity Laboratory from Vegetable Research and Development Station Buzau has a valuable germplasm collection of hot peppers consisting in 287 genotypes, structured on 3 groups according to their genetic stability. The aim of this study was to assess 15 accessions with distinct phenotypic expressivity in terms of fruit shape, weight and colour. A great variability was observed at physiologically maturity in fruit colour passing from yellow, orange, different shades of red or brown. The fruits have distinct shapes like cherry type, elongated, angular, campanulate or blocky type. The fruit mass varies between 0.42 g (A270) to 65.29 g (A282). In order to establish the quality of fruit, the firmness, dry matter and total soluble solids content were measured at both maturity stages. The studied accessions represent valuable genotypes and will be subject to intensive breeding work. In this context, each genotype will be structured for different direction of use such as: chilli powder, fresh consumption, ornamental purposes or for pharmaceutical use.

Key words: *Capsicum sp., germplasm collection, phenotype, Romania.*

INTRODUCTION

Hot pepper (*Capsicum* spp.) is a Solanaceous crop cultivated and appreciated all over the world (Mushtaq et al., 2018; Virga et al., 2020). *Capsicum* species are used for culinary purposes but it can also be eaten fresh or used as spice or food colorant. Also, they are used to obtain cosmetic and pharmaceutical products. In addition to the medicinal importance and culinary uses, hot peppers have been widely used as decorative vegetables (Taychasinpitak and Taywiya, 2003; do Rêgo et al., 2012a; do Rêgo et al., 2012b; dos Pessoa et al., 2015; Ari et al., 2016; Laguovschi et al., 2016; Lagunovschi and Vînătoru, 2016; Vînătoru et al., 2019; Virga et al., 2020; Agapie et al., 2021).

Capsicum species are traditionally identified by morphological descriptors or related traits. For taxonomic description, flower morphology, including number of flowers per axil, flower colour, and calyx constriction is the most used. Anyway, other descriptors are considered essential for a more accurate germplasm characterization, such as the ones indicated by the UPOV and IPGRI Guidelines (Sudré et al., 2010; Mushtaq, 2018).

The characterization and the evaluation of the *Capsicum* spp. is a powerful tool in developing varieties, identifying traits in germplasm and utilizing it in research and development work (Sudré et al., 2010; Mushtaq, 2018).

Several authors have highlighted the importance of studies on the characterization of *Capsicum* germplasm, as well as its diversity, in order to make them available to researchers (do Rêgo et al., 2010; Sudré et al., 2010; Cvikić et al., 2011; Zakia et al., 2013; Vînătoru et al., 2014; dos Pessoa et al., 2015; Quresh et al., 2015; Lagunovschi et al., 2016; Xiao-min et al., 2016; Rahman et al., 2017; Mushtaq, 2018; Agapie et al., 2020a; Agapie et al., 2020b; Virga et al., 2020; Barcanu et al., 2021).

In this context, it is necessary to carry out studies aiming the understanding of the local landraces, the increase of production, disease and pests resistance and tolerance to adverse environments, among other abiotic and biotic factors that can affect the yield.

The Genetic, Breeding and Biodiversity Laboratory from Vegetable Research Development Station Buzau (VRDS) has a valuable germplasm collection of hot peppers consisting in 287 genotypes, structured on 3 groups according to their genetic stability. The assessment of those genotypes is necessary in order to provide better utilization of these genetic resources in breeding programs.

The aim of this study was to assess 15 accessions from the germplasm collection of VRDS Buzau with distinct phenotypic expressivity in terms of fruit shape, weight and colour. The researches will be helpful for future breeding programs and for establish the directions of use: chili powder, fresh consumption, ornamental purposes or for pharmaceutical use.

MATERIALS AND METHODS

The researches were carried out at the Genetic, Breeding and Biodiversity Laboratory from VRDS Buzau. The study aimed the assessment of 15 accessions of hot peppers noted A93, A138, A241, A243, A245, A246, A250, A257, A261, A268, A269, A270, A272, A281, A282. The breeding method used was repeated individual selection. The crop technology applied was the one described by us in our previous studies (Barcanu et al., 2019; Agapie et al., 2020a; Agapie et al., 2020b). The descriptors used were proposed for Capsicum by International Plant Genetic Resources Institute, renamed Biodiversity International (IPGRI, 1995) and by International Union For The Protection of New Varieties OF Plants (UPOV 2006, 2015).

The qualitative traits (Table 1) were noted based on visual evaluation. The quantitative traits targeted in this study were fruit length (FL), fruit width (FW), base fruit diameter (BFD), middle fruit diameter (MFD) and blossom end fruit diameter (BED), fruit weight (W), weight of fruit pulp (WP), weight of fruit receptacle (WR), pedicel length (PDL), pedicel diameter (PD), pericarp thickness (PT), these were counted, measured using metric rulers, calliper and weighed using.

For statistical analysis, ANOVA one way was used followed by the Duncan test.

In order to establish the quality of fruit, the firmness, dry matter and total soluble solids content were measured at both maturity stages. Fruit firmness was determined by measuring the penetration force using a fruit pressure tester FT011, equipped with a piston of 3 mm diameter. The dry matter content (DM) was determined with KERN DBS60-3 thermo balance and the soluble solids (TSS) were measured with digital refractometer KERN OPTICS ORF 1RS (Agapie et al., 2020c).

Table 1. The qualitative characters targeted in this study

Descriptors	Polymorphism
Calyx margin (CM)	1. entire 2. intermediate 3. dentate 4. other
Calyx annular constriction (CC)	0. absent 1. present
Fruit: anthocyanin coloration (FAC)	1. absent 9. present
Fruit:colour before maturity (FCBM)	1. white 2. yellow 3. green 4. orange 5. purple 6. dark purple 7. other 8. light green 9. dark green
Fruit: colour at maturity (FCAM)	1. white 2. lemon yellow 3. pale orange-yellow 4. yellow orange 5. pale orange 6. orange 7. light red 8. red 9. dark red 10. purple 11. brown 12. black 13. other
Fruit: shape (FS)	1. elongate 2. almost round (cherry tyoe) 3. angular 4. campanulate 5. blocky 6. other
Fruit: shape at pedicel attachment (FSP)	1. sharp 2. obtuse 3. truncated 4. cordate 5. lobed
Fruit: neck at base (FNB)	0. absent 1. present
Fruit: shape of blossom end (FSB)	1. sharp 2. bont 3. deep 4. deep and sharp 5. other
Fruit: blossom end appendage (FA)	0. absent 1. present
Fruit: sinuation of pericarp at basal part (FSPB)	1.absent or very weak 3.weak 5.medium 7.strong 9.very strong
Fruit: sinuation of pericarp excluding basal part (FSPEB)	1.absent or very weak 3.weak 5.medium 7.strong 9.very strong
Fruit: glossiness (FG)	1. absent or very weak 3. Weak 5. medium 7. strong 9. very strong
Fruit: shape in cross section (FSCS)	3. eliptic 5. angular 7. circular
Fruit: shape in longitudinal section (FSLS)	1. oblate 2. circular 3. cordate 4. square 5. rectangular 6. trapezoidal 7. moderately triangular 8. narrowly triangula r9. hornshaped
Fruit: number of locules (FNL)	
Fruit: texture of surface (FTS)	1. smooth or very slightly wrinkled 2. slightly wrinkled 3. strongly wrinkled
Placenta: length (PL)	1. <1/4 fruit 2. ¼-1/2 fruit 3. >1/2 fruit

RESULTS AND DISCUSSIONS

The qualitative traits and their descriptor values used in this study are found in Table 2 and they have been used to establish the variability among the studied germplasm. The greatest variability was recorded by the fruit shape (Figure 1).

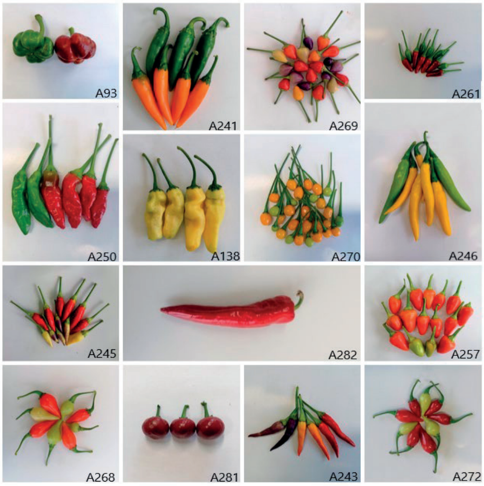

Figure 1. Fruit shape of studied accessions

The studied genotypes have distinct shapes like elongated for 53.3% of genotypes (A138, A241, A243, A245, A246, A250, A261, A282), angular for 13.3% genotypes (A257, A269), cherry type for 13.3% of genotypes (A270, A281). The accessions A270 and A281 present campanulate fruit shape (13.3%), while A93 are blocky type (7.1%).

Fruit shape at the pedicel attachment was obtuse (60%), sharp (13.3%), truncate (13.3%) cordate (6.7%) and lobate (6.7%). A neck at the base of fruit was present in 20% of accessions. A blossom end appendage was present at 26.6%.

The accessions presented between two and four locules per fruit and the number was related to fruit shape. A wide variability can also be found in the colour of fruit. The colours of fruit at harvest maturity were yellow, green, dark purple, light green and dark green. The yellow colour of unripe fruits was observed in accessions A246, A268, A269 and A272 (26.7%).

The accessions A93, A138, A281, A282 present green fruit (26.7%). Also 26.7% of genotypes present dark green colour, while 13.2% of genotypes were light green and 6.7% were dark purple. At the physiological maturity fruit colour was lemon (20%), orange (20%), red (33.3%) and dark red (26.7%).

Table 2. The qualitative descriptors and their value on studied accessions

Accession	A93	A138	A241	A243	A245	A246	A250	A257	A261	A268	A269	A270	A272	A281	A282
CM	2	3	2	3	3	3	3	3	2	2	2	3	2	3	3
CC	1	0	1	0	1	1	1	1	0	0	0	1	1	0	1
FAC	1	1	1	9	9	1	1	1	1	1	9	1	1	1	1
FCBM	3	3	9	6	2	9	9	8	9	2	2	8	2	3	3
FCAM	9	2	6	8	8	2	8	6	8	6	9	2	8	9	9
FS	5	1	1	1	1	1	1	3	1	4	3	2	4	2	1
FSP	5	2	2	2	2	2	1	3	2	2	3	2	2	1	4
FNB	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
FSB	4	4	1	1	1	1	3	2	2	1	1	5	1	3	4
FA	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0
FSPB	9	7	1	2	1	1	5	2	1	1	1	1	1	1	2
FSPEB	9	7	1	5	1	3	7	1	1	1	1	1	1	1	1
FTS	3	3	1	2	1	1	3	2	1	1	1	1	1	1	1
FG	5	3	5	5	7	5	5	7	5	9	7	9	9	7	9
FSLS	1	7	8	9	8	9	7	7	7	7	7	3	7	2	7
FSCS	7	5	3	3	3	3	3	3	3	3	3	3	3	7	7
FNL	4	3	3	2	2	2	2	2	2	3	3	3	3	2	2
PL	1	3	3	3	3	3	3	2	3	3	3	3	3	3	3

Note: The abbreviations are explained in Table 1.

The quantitative traits were the subject to ANOVA followed by Duncan test and their results can be found in Table 3.

A very highly significant difference ($p<0.01$) was found among the studied quantitative parameters. It reflected the existing of large diversity among studied genotypes and this variability can be further utilized in the chilli pepper improvement program.

The Duncan multiple range test was applied to know the variation between accessions for all the traits. Performance of all the genotypes using the Duncan multiple range test indicated that A282 accession attained maximum fruit length (19.50 cm), fruit weight (65.29 g), weight of fruit pulp (50.25 g), weight of fruit receptacle (15.03 g) and pedicel length (3.53 cm). The accession A270 recorded the smallest values for fruit length (1.37 cm), fruit base diameter (0.48 cm), fruit weight (0.42 g) weight of fruit pulp (0.29 g), weight of fruit receptacle (0.13 g). The fruit wall thickness values ranged from 0.96 to 4.55 cm and the accession A93 showed the highest value, while the accession A270 showed the smallest value followed very close by A245 with 1.01 cm. Pedicel length varied between 1.79 (A270) and 3.53 cm (A282).

Table 3. Quantitative traits of the studied genotypes

Accessions	A93	A138	A241	A243	A245	A246	A250	A257	A261	A268	A269	A270	A272	A281	A282
FL	3.10± 0.12ef	4.8± 0.43d	8.14± 0.28c	4.75± 0.61d	3.02± 0.15ef	10.29± 2.50b	5.07± 0.21d	3.59± 0.30de	2.2± 0.42ef	2.73± 0.23ef	1.82± 0.22f	**1.37± 0.42f**	2.53± 0.27ef	1.79± 0.42f	**19.50± 0.73a**
BFS	**6.03**± 0.12a	1.2± 0.07def	1.64± 0.25d	0.56± 0.27g	0.69± 0.03g	1.17± 0.06ef	1.10± 0.11ef	2.44± 0.28c	0.66± 0.06g	0.86± 0.21 fg	1.31± 0.21de	**0.48**± 0.04 g	0.67± 0.04g	2.55± 0.14c	3.54± 0.39b
MFS	**6.18**± 0.05a	1.6± 0.12d	1.65± 0.13 d	**0.55±** 0.31 g	0.82± 0.14fg	1.25± 0.20e	1.34 ± 0.08de	2.08± 0.23c	0.62± 0.07 g	1.04± 0.14ef	1.01± 0.25ef	0.82± 0.09fg	0.74± 0.16fg	2.63± 0.12b	2.91± 0.09b
BED	**5.98±** 0.06a	0.52± 0.11de	0.25± 0.06fgh	0.60± 0.3cd	**0.20**± 0.06gh	0.21± 0.08fgh	0.79± 0.04c	0.57± 0.07cde	0.23± 0.05fgh	0.22± 0.04fgh	0.35± 0.09efg	0.18± 0.06gh	0.07± 0.02h	2.55± 2.37b	0.45± 0.03def
W	44.0± 1.32b	3.86± 0.87fgh	11.37± 2.67c	2.17± 0.13gh	1.63± 0.29gh	9.82± 0.96cd	4.59± 0.28efg	7.50± 2.04de	0.82± 0.26gh	1.56± 0.26gh	1.65± 0.41gh	**0.42**± 0.02h	1.18± 018gh	7.08± 1.61def	**65.29**± 4.81 a
WP	36.58 ±1.47b	3.28± 0.74 de	8.11± 1.59 c	1.64± 0.14 ef	0.93± 0.17ef	7.59± 0.59 c	3.24± 0.30 de	5.30± 1.64 d	0.57± 0.19f	1.3± 0.24 ef	1.09 ±0.36ef	**0.29**± 0.01f	1.04± 0.18ef	4.68± 1.46d	**50.25**± 2.73 a
WR	7.47± 0.16b	0.57± 0.17ef	3.26± 1.14c	0.53± 0.02ef	0.70± 0.12def	2.22± 0.55cde	1.35± 0.04def	2.20± 0.40cde	0.24± 0.07f	0.16± 0.02f	0.55± 0.05ef	**0.13**± 0.02f	0,14± 0.01f	2.39± 0.50cd	**15.03**± 2.52a
PDL	3.09± 0.12bcde	3.01± 0.13cde	3.25± 0.21bcd	3.44± 0.20bc	2.85± 0.08de	3.27± 0.10bcd	4.49± 0.12 a	2.76± 0.30e	2.08± 0.05f	2.20± 0.02f	3.11± 0.08bcde	3.07± 0.53bcde	2.01± 0.08f	**1.79**± 0.78f	**3.53**± 0.25b
PD	**0.96**± 0.01a	0.15± 0.02de	0.34± 0.09b	0.16± 0.02d	0.130± 0.01defg	0.35± 0.02b	0.36± 0.02b	0.35± 0.02b	0.10± 0.01efg	0.06± 0.01g	0.14± 0.02def	**0.08**± 0.01g	**0.08**± 0.01fg	0.24± 1.35c	0.39± 0.03b
PT	**4.55**± 0.28a	1.13± 0.08fgh	2.20± 0.44de	1.40± 0.07fgh	1.01± 0.09h	1.48± 0.21fg	1.54± 0.09f	2.13± 0.27de	1.09± 0.11gh	2.51± 0.24cd	1.28± 0.15fgh	**0.96**± 1.03h	2.04± 0.18e	2.94± 0.16b	2.76± 0.13bc

Note: Values are mean ± SD. Means within the same row carrying different superscript letter were significantly different at $p < 0.01$ according to a Duncan`s multiple range test; FL - fruit length, BFS - base fruit diameter MFS - middle fruit diameter, BED-blossom end fruit diameter, W-fruit weight, WP-weight of fruit pulp, WR-weight of fruit receptacle, PDL-pedicel length, PD-pedicel diameter, PT-pericarp thickness.

Fruit firmness (kgf/cm^2) was measured for both unripe and fully ripen fruit (Figure 2). In this study, a decrease of fruit firmness during fruit ripening has been observed for the accessions A93, A241, A246, A268, A269, A270, A281, A282 while for the other accessions an increase of fruit firmness was observed (Figure 2).

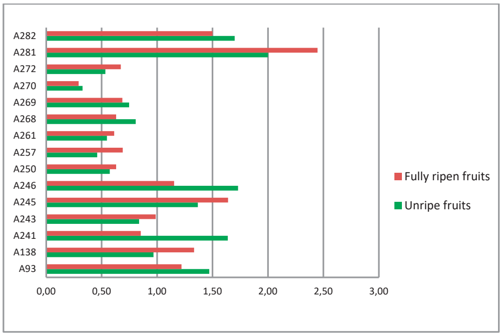

Figure 2. Fruit firmness (kgf/cm^2) of the investigated *Capsicum* accessions

According to Barcanu et al. (2021), pepper fruits with a high firmness are suitable for fresh consumption and have a long shelf life.

Throughout the vegetation period the soluble solid content (TSS) increased for all studied accessions with the exception of A241 whose content in TSS decrease from 9.75°Brix to 7.55°Brix.

The highest contents of TSS for fruits at physiological maturity were observed in the accessions A245, A261, A281 presenting 9.75°Brix, 9.99°Brix and 10.22°Brix (Figure 3).

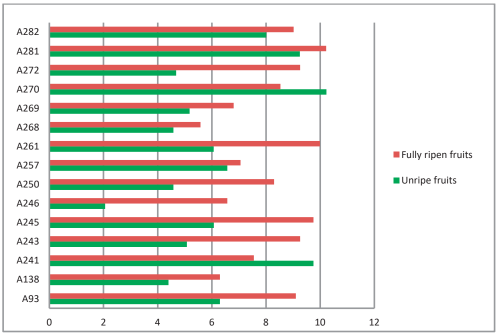

Figure 3. TSS content (°Brix) of the investigated *Capsicum* accessions

Lannes et al. (2007) states that the TSS content is inversely proportional with water content in fruit. For this reasons, the cost of dehydration of the fruit will be lower, this trait being very important in *Capsicum* breeding aiming industrial use (do Rêgo et al., 2011).

Ribes-Moya et al. (2018), in one of their study states that the high content in dry matter is an important quality descriptor in food industry for obtaining chili powder. This trait can be also used by breeders for setting the directions of use of chili peppers fruit.

According to do Rêgo et al (2011), the dry matter content is oppositely related to the fruit weight. The results of this study, regarding fruit length and dry matter content, are in agreement with these authors. In Figure 4, the accession A270 has the shortest fruit length (1.37cm), and with a dry matter content of 13.8%, while the accessions A282 has the longest fruit length

(19.20 cm) and the dry matter content (13.51%) similar to result of accession A270.

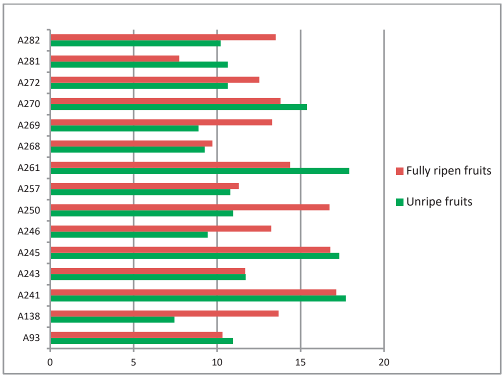

Figure 4. Dry matter content (%) of the investigated *Capsicum* accessions

CONCLUSIONS

The obtained results showed that there is a great variability among the studied accessions.
In order to obtain cultivars for fresh market accessions, A241, A246 are recommended due to the hight firmness, while the accessions A93, A281, A282 are recommended for obtaining chili powder, since they recorded high total soluble solids and dry matter contents and showed the highest values for pericarp thickness.
VRDS Buzau has a large variety of genotypes in chili peppers and the research will continue by enriching the germplasm collection in order to add new genotypes for different directions of use. Also the diversity found can be used in the next *Capsicum* breeding programs.

AKNOWLEDGEMNETS

The work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARATIVE STUDY ON LETTUCE GROWING IN NFT AND EBB AND FLOW SYSTEM

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Abstract

The study was carried out in the Hortinvest greenhouses, within the Research Center for the Quality of Horticultural Products, on five varieties of lettuce ('Alanis'; 'Aleppo' - 'Lollo Bionda' type; 'Carmessi' - 'Lollo Rosa' type; 'Kristine' and 'Saturday' - 'Red salad bowl' type) grown in two types of unconventional systems, Nutrient Film Technology and Ebb and Flow. The varieties of lettuce were grown, and data on their growth were analysed. We determined the mass of the plants and found differences between them. The aim of the study was to follow the production differences for the analysed varieties grown in the two systems, NFT and Ebb and Flow.

Key words*: lettuce, cultivars. NFT, Ebb and Flow.*

INTRODUCTION

Lactuca sativa (Lettuce) is one of the most popular green vegetables. The species originates in the eastern Mediterranean basin, Turkey, the Caucasus and the Middle East. The wild form of lettuce (*Lactuca serriola*) was known about 4500 BC. The name *Lactuca* comes from the Latin word "lactis", meaning milk, due to the white sap and milky consistency contained in the wild forms of lettuce plants (Kesseli et al., 1991; Drăghici, 2015; Draghici, 2018; Wei et al., 2014; Wei et al., 2017). The first descriptions of the use of lettuce are given by the ancient Egyptians and from whose seeds an oil similar to oil was extracted and then began to be used for consumption.

Lettuce is an annual plant, from the group of green vegetables, highly appreciated for its qualities. It is cultivated on fairly large areas, both in the field and in greenhouses or solariums, in all systems. Lately, the areas cultivated with lettuce, in a soilless system, have increased a lot. Thus, in Romania this culture system has been extended, because several crop cycles can be achieved per year, so high yields, high economic efficiency, but also the security of offering guaranteed products on the market (Oliveira et al, 2010; Drăghici, 2016; Drăghici et al., 2018).

Any protected area can be adapted for NFT and Ebb and flow cropping systems, which are much more efficient for growing lettuce or other small vegetable species as they allow better plant monitoring, (Giacomelli, 1998; Jones and Tardieu, 1998; Kang Jeong, 2014; Chidiac 2017). Efficiency consists in using a smaller and more efficient amount of water (Gent and McAvoy. 2011; Chidiac, 2017).

In Europe, the NFT system is often practiced over large areas. In Romania the system is practiced only on areas under 2 ha, in specialized farms. The largest areas of about 281 ha (FAO 2017) are grown with lettuce in conventional system, on the soil.

In order to obtain a high salad production, it is necessary to ensure the optimal conditions of temperature, light, atmospheric humidity, quality of the nutrient solution, but also the choice of a suitable assortment. These factors can influence the growth rate of the plants, the time until the formation of the edible part, the appearance of the floral stem or the bitter taste of the leaves. All varieties of salad are unpretentious to temperature. The seeds germinate in optimal temperature conditions between 18°C and 20°C. For some varieties of lettuce, temperatures above 25°C lead to decreased seed germination or even inhibition.

For varieties grown in protected areas, the minimum plant growth temperature is 16°C, and the optimum between 18-24°C. In culture, temperatures below 18°C cause a slight prolongation of the vegetation period, and those above 18°C an acceleration of the formation of the edible part. In many situations, at temperatures above 18°C in some varieties it forms only a rosette of leaves but without forming the head. In conditions of very high temperature and insufficient light, the leaves are etiolated, the head is no longer formed or it is too loose.

During the day, temperatures of 20-24°C in sunny weather and between 17-19°C in cloudy weather, and during the night 19°C will be ensured. Some varieties are very sensitive to temperatures above 22-25°C, causing the premature appearance of flower stalks. Also, in some varieties of 'Lollo rosa' type or oak leaf type, the very high temperatures associated with high light intensities influence the coloration of these leaves, no longer having a dark red colour but a light red to green colour.

It is recommended that the temperature of the nutrient solution be kept constant at 20°C, as the dissolved oxygen content may change. The oxygen content of the nutrient solution is a very important factor in the growth and development of lettuce plants. The increase of the temperature of the nutrient solution over 20°C determines a decrease of the oxygen content, at the same time it is stimulated, the development of anaerobic pathogens, in particular, of the root rot favored by *Pythium*. If the temperature of the nutrient solution falls below the normal limit, the amount of dissolved oxygen increases, but the absorption of nutrients decreases.

Depending on the variety, the salad needs 8-10 hours of direct sunlight daily, especially during the vegetative growth period. Appropriate productions are obtained if a light intensity in the range of 12-17 mol/m^2 is ensured correlated with a good ventilation. Increasing light intensity can lead to improper plant development (Kang et al., 2014). Some varieties require higher light intensity, but grown in low light conditions the plants grow with difficulty. That is why it is good to choose the variety assortment depending on the season and destination, for the greenhouse, solarium or only for the field. Additional use of light over 16 hours to 24 hours, at an intensity of 100-200 μmol/m^2/s (17 mol/m^2/day) during the winter leads to an increase in plant biomass so the crop cycle is reduced by about 25%.

Also, increasing the duration of lighting reduces the nitrate content of salad by 10-26% (Gaudreau et al., 1994). Lettuce is a major consumer of nitrogen. The administration of an insufficient amount of nitrogen influences the vegetative growth of the plants. The concentration of the nutrient solution must be increased progressively, depending on the stage of development of the salad plants. The electrical conductivity (EC) will be maintained between: 1.0-1.2 mS in the first week after planting; 1.4-1.6 mS from the second week after planting; and 1.8-2.0 mS from the third week until the salad is harvested.

Throughout the vegetation period, the pH of the solution will remain constant at 5.6-6.0 (Morgan et al., 2012). In case of pH fluctuations, problems of blocking the absorption of nutrients may occur.

The cultivation of lettuce in NFT system at EC values of 3.2 dS m^{-1} - 5.2 dS m^{-1} led to the production of unsuitable lettuce plants (Jung et al., 2016; Hammady et al. 2015) as well as at high salinity levels. Studying the performance of lettuce cultivars subjected to different salinity levels of the irrigation water, Oliveira et al. (2010) observed that growth parameters decreased linearly with the increase in salinity (Soares et al., 2010; Santos et al., 2011; Santos, 2017; Alves et al., 2011). The quality of lettuce seedlings largely depends on the substrate and the management of the crop. In modern technology the nutritive substrates are processed in specialized farms.

In the case of lettuce cultivation in an unconventional system, the mass of the plants but also the dry matter content are high (Barbosa, 2015; Han et al., 2016).

The main objective of the study was to characterize some varieties of lettuce grown in the NFT system in terms of yields.

MATERIALS AND METHODS

The study was carried out within the Hortinvest research greenhouses, which belong to the Research Center for quality control of horticultural products, Faculty of Horticulture,. USAMV Bucharest.

During the research we followed the behaviour of some varieties of lettuce grown in the NFT system in terms of production. We followed the development of lettuce plants in NFT and Ebb and Flow systems.

Biological material used in the experiment was 5 varieties of lettuce. **V1 - 'Alanis'** is a variety of head lettuce, intended to be grown in autumn, winter and early spring, in protected areas (greenhouses or high tunnels). The leaves are green, the average mass of the head can reach about 400-600 g. **V2 - 'Aleppo'** variety ('Lollo Bionda' type). It is a variety of lettuce (*Lactuca sativa* var. c*rispa*) with light green leaves. The leaves are wrinkled at the edge and lacy. The average mass of the plant reaches about 300 g.

V3 - 'Carmessi' ('Lollo Rossa' type) is an early variety of leaf lettuce, red, with a vegetation period of 65-68 days. It is recommended for spring crops, summer and autumn both in the field and in protected areas. The leaves are dark green with a reddish-brown tinge and at maturity the average mass can have values between 250 and 300 g. **V4 - 'Kristine RZ'** is an oak leaf type. It can be grown all year round. It forms a large rosette with many green leaves. The variety weighs 500-600 g and is resistant to flowering.

V5 - 'Saturday' ('Red salad bowl' is oak leaf type). The rosette has cherry-green leaves. It can be harvested either whole or regularly in the form of individual leaves, and thus will continue to produce until the end of the season.

During the experience we followed and determined:

- dynamics of seedling leaf formation;
- dynamics of lettuce leaf growth;
- dynamics of salad rosette growth;
- total and edible mass of salads obtained

Lettuce seedlings were produced in the greenhouse. The seeds were sown on 23.10. 2019. The seedling were 22 days old at planting.

RESULTS AND DISCUSSIONS

During the growth of lettuce plants we maintained in the greenhouse temperature conditions of 21ºC during the day and 19ºC at night. Data on lettuce growing conditions are recorded in the Table 1.

Table 1. Parameters of growing lettuce plants

Parameters	Number of days after planting				
	5	10	15	20	25
Temperature (ºC) days	21	21	21	21	21
Temperature (ºC) in the night	19	19	19	19	19
CO_2 content	300	300	300	300	300
EC (micromoli/cm)	0.8	1.2	1.8	2.0	2.2
pH	6.0	6.0	6.0	6.0	6.0
The flow (l/min)	2	2	2	2	2

We found that the seeds sprouted after 4 days from sowing in a percentage of 84.61% for the 'Carmessi' ('Lollo Rosa' type) and 93.45% for the 'Aleppo' variety ('Lollo Bionda' type), Figure 1.

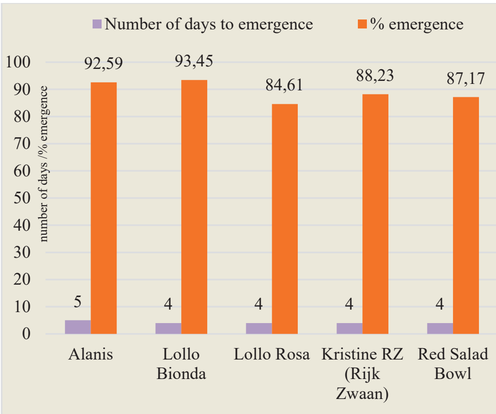

Figure 1. Number of days until emergence and percentage of salad emergence

In the Table 2, data are presented, from which it can be observed, the formation of the number of leaves in the analyzed varieties, from emergence to the end of cultivation. On 28.10.2019, for the 'Alanis', 'Aleppo' ('Lollo Bionda' type), 'Carmessi' ('Lollo Rossa' type) and 'Kristine' varieties, on the plant, on average, 2 leaves were formed and for the 'Saturday' variety ('Red Salad Bowl' type), 2.25 leaves. On November 10, 2019, the

'Alanis', 'Kristine' and 'Saturday' varieties had a number of leaves between 4 and 4.5 leaves, and the 'Lollo' type varieties had a number of 3.5 leaves. On 15.11.2019 at the time of planting in the culture troughs of the NFT system the seedlings had between 4 leaves for the varieties 'Aleppo' ('Lollo Bionda' type) and 'Carmessi' ('Lollo Rossa' type) and 4.5 leaves for the varieties 'Alanis'. 'Kristine' and 'Saturday' ('Red Salad Bowl' type), Table 2.

Table 2. Dynamics of leaf formation in lettuce seedlings

Date of determination	Number of leaves formed per plant - nr.				
	V1 Alanis	V2 Aleppo	V3 Carmesi	V4 Kristine	V5 Saturday
28.10. 2019	2	2	2	2.0	2.25
05.11.2019	3	3	3	2.8	3
10. 11.2019	4.0	3.5	3.5	4.15	4.25
15.11.2019	4.5	4	4	4.5	4.5
30.11.2019	10.67	8.25	8.0	9.25	11.25
15.12.2019	20.25	11.25	15.15	13.33	17
20.12.2019	23.55	18.25	21.33	16.25	18.55

Analyzing the data in the Table 3, on plant height at the end of the crop we could see that lettuce grown in the NFT system showed a higher height in all cultivated varieties compared to lettuce grown in the Flux Reflux system. The 'Alanis' variety obtained 4.5 leaves more than the cultivated variant in the NFT system. Also, for the 'Lollo' type salad varieties, we obtained a plant height of 16.66 for the 'Aleppo' variety ('Lollo Bionda' type) and 15.25 cm for the 'Carmessi' variety ('Lollo Rossa' type). The difference was 3.95 cm and 15.25, respectively. The variant in the Flux Reflux system was with 80.73%, respectively 77.22% under the variant from the UASMV Bucharest greenhouses - growing in the NFT system.

Table 3. Height of lettuce plants

Specification	V1 Alanis	V2 Aleppo	V3 Carmesi	V4 Kristine	V5 Saturday
	cm	cm	cm	cm	cm
NFT	**21.75**	**20.5**	**19.75**	**25.5**	**21.25**
Ebb and Flow	17.25	16.55	15.25	20	17
Diference between NFT and Ebb and Flow	4.50	3.95	4.50	5.50	4.25
Diference in percent to NFT (%)	79.31	80.73	77.22	78.43	80.00

The diameter of the lettuce plants was at the end of the crop of 27.16 cm for the (V1) 'Alanis' (head lettuce variety), 18.55 cm for (V2) 'Aleppo' (Table 4).

Table 4. The dynamics of increase in the diameter of lettuce plants grown in NFT system

Date	V1 Alanis	V2 Aleppo	V3 Carmessi	V4 Kristine	V5 Saturday
	cm	cm	cm	cm	cm
15.11.2019	8.5	8.5	9.25	10.25	10.25
30.11.2019	13.4	12.25	14	15.25	18.67
15.11.2019	20.75	15.5	16.75	18.55	22.15
20.11.2019	27.16	18.55	19.33	23.25	24.25

By comparison, in the Reflux Flux system with floodable tables, although the culture conditions and the quality of the nutrient solution were similar, the plants showed a weaker growth. The diameter of the plants is between 14 cm for the 'Aleppo' variety ('Lollo Bionda') and 16.5 cm for the 'Alanis' variety (Figure 2).

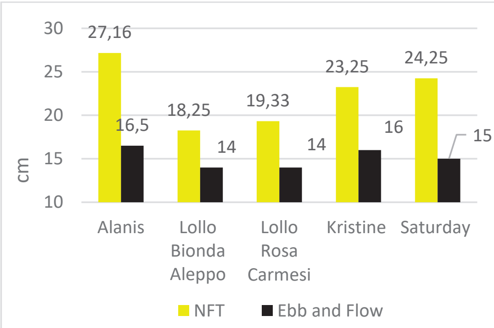

Figure 2. The diameter of the lettuce plants in NFT and Ebb and Flow systems

Regarding the diameter of the plants, in the Ebb and Flow system the lettuce plants were 10.66 cm smaller for the 'Alanis variety' and with 4.25 cm for the 'Aleppo' variety type 'Lollo Bionda' (Figure 3).

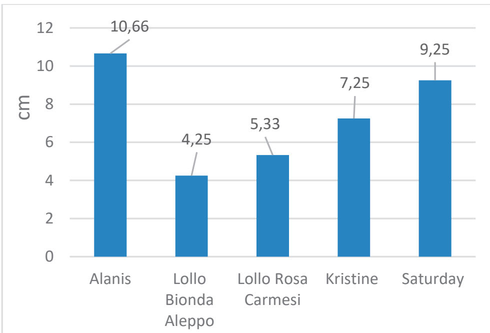

Figure 3. The difference between the two systems NFT and Ebb and Flow

The data on the quality of the salad complied with the marketing standard - Regulation (EC) no. 1221/2008 of the European Commission amending Regulation (EC) no. 1580/2007, which establishes the rules for the application of Regulations (EC) no. 2200/96. (EC) no. 2201/96 and (EC) no. Council Regulation (EC) No 1182/2007 on marketing standards in the fruit and vegetables sector (Table 5).

Table 5. The quality Standard of lettuce

Quality requirements	Category	For Head lettuce	For Leaf lettuce
Standard	I	300-450 g	150-200 g
	II	under the 150 g	under the 150 g

In the ‘Alanis’ variety, we noticed that only one plant out of the 10 examined had a very high average mass of 387 g, 87 g more than the quality standard I.

In the rest of the variant, the average mass varied between 315 g and 345 g. The lowest average mass was 257 g. We can also note that only 10% of the harvested plants had an average mass below the standard of 300 g but with an insignificant weight of only 3 g below the standard. In the case of cultivation in the Ebb and Flow system the mass of the plants was much lower (Figures 4, 5).

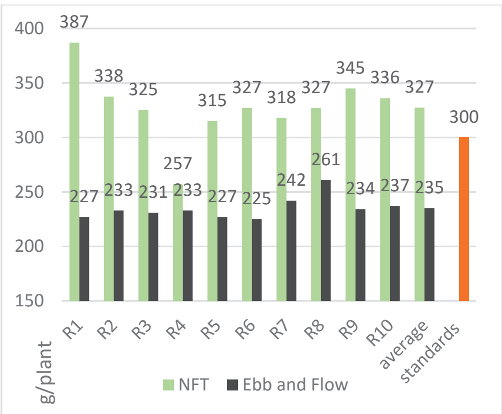

Figure 4. Lettuce plants mass, variety ‘Alanis’

In the case of the ‘Aleppo’ variety, for the 10 plants examined, we recorded average masses between 127 g and 180 g and with an average of 160 g. We found that at the time of harvest, 30% of the plants had average masses, below the standard of 150 g. In the case of cultivation in the Ebb and Flow system, the average mass of the plants was 122 g (Figures 6, 7).

Figure 5. The appearance of head lettuce variety ‘Alanis’. NFT; Ebb and Flow

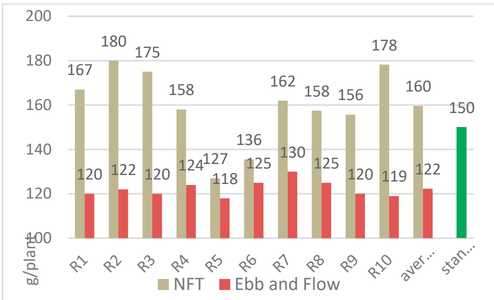

Figure 6. Lettuce plants mass, variety ‘Aleppo’ (‘Lollo Bionda’ type)

Figure 7. The appearance of the lettuce plant variety ‘Aleppo’ (‘Lollo Bionda’ type)

For the ‘Carmessi’ variety (‘Lollo Rosa’ type) we obtained average masses of over 150 g for most plants. In only 3 plants we found average masses under 150 g. In the Ebb and Flow system, the average plant mass was 128 g (Figures 8, 9).

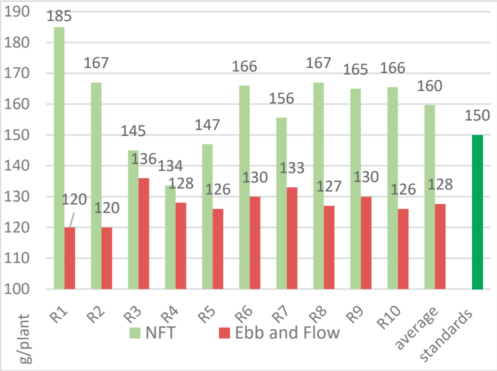

Figure 8. The mass of the lettuce - variety 'Carmessi' ('Lollo Rosa' type)

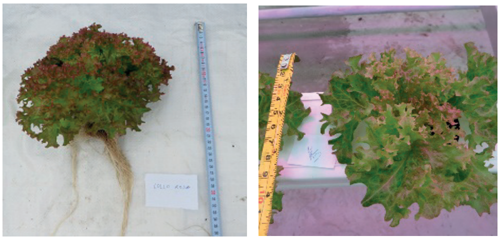

Figure 9. The appearance of the lettuce plant variety 'Carmessi' ('Lollo Rossa' type)

In the 'Kristine' variety the average mass of the plants was on average 317 g, this varied between 211 g 365 g. In the case of this variety 20% of the plants had average masses below 300 g. In the case of cultivation in the Ebb and Flow system, the average mass of the plants was 253 g (Figures 10, 11).

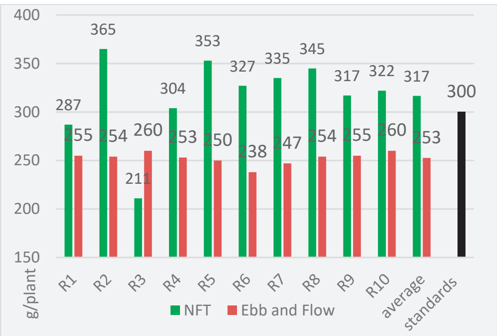

Figure 10. The total mass of plants in the 'Kristine' variety

Figure 11. The appearance of lettuce 'Kristine' variety

In the 'Saturday' variety ('Red Salad Bowl' type) 30% of the plants had average masses under 300 g. The mass of the plants varied between 305 g and 334 g. On average, the plants had a mass of 282 g, below the Quality I of Standard (300 g). In the case of cultivation in the Ebb and Flow system, the average mass of the plants was 185 g (Figures 12, 13).

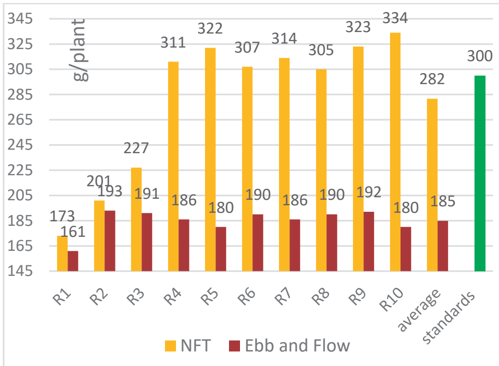

Figure 12. The total mass of plants in the 'Saturday' variety

Figure 13. The appearance of salad plants in the 'Saturday' variety ('Red Salad Bowl' type)

In summary, the average data on the mass of lettuce plants obtaine'd in the two cultivation variants are presented graphically in Figure 14.

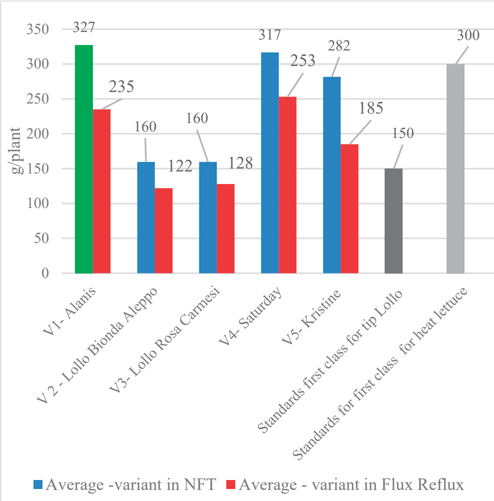

Figure 14. The average weight of plants in NFT and Ebb and Flow

CONCLUSIONS

Lettuce seedlings were uniform at planting. They had an average number of leaves between 4 and 4.5 leaves.

During the growth of the plants a greater number of leaves were formed in the variant cultivated in the NFT system because the temperature at the root system was constant of 20-21°C. In the Ebb and Flow system as the interval between watering’s is longer the nutrient substrate transmits roots a temperature lower than 20°C which leads to an extension of the growing season.

The formation time of the edible part was shorter in the variant cultivated in NFT compared to Ebb and Flow.

In the NFT system we obtained average masses which met the marketing standard in 25 days and in the Ebb and Flow system the plants reached the commercialization standard after 35 and 40 days, respectively.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

TRICHOSANTHES CUCUMERINA L. A NEW SPECIES ACCLIMATIZED AND BRED IN ROMANIA

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Abstract

Vegetable Research Development Station Buzau is known as an important research centre for acclimatizing and breeding vegetables in Romania. The climate is changing due to numerous factors and is crucial to adapt new species in different areas in order to explore the wonderful world of plants. Trichosanthes cucumerina L. also known as snake gourd and long tomato is grown for consumption and also for various medicinal uses, but some other aspects are still unexplored. Snake guard is studied at VRDS Buzau from 2010 and is a monoecious annual herb climbing with over 5-6 meters high. The plant is vigorous, with many (12-16) slender stems. The male flowers are arranged in raceme and the female are solitary. The fruits are long, over 2 m, at physiologically maturity, but usually for fresh consumption the fruits are harvested at 50-100 cm long. The fruits can be consumed when immature, as it gets a bitter taste with age. The studies have completed so far with patenting of a new cultivar according DUS test, suitable for growing in greenhouse and field, conventional and organic farming.

Key words: *breeding, Cucurbitaceae, phenotype, snake gourd.*

INTRODUCTION

The climate is changing due to numerous factors and is crucial to adapt new species in different areas in order to explore and value the wonderful world of plants. Acclimation involves physiological, anatomical, or morphological adjustments within a single organism that improve performance or survival in response to environmental change (Demmig-Adams et al., 2008). During acclimation the environment has a strong influence on the phenotype of a plant. This phenomenon is defined as phenotype plasticity and plays a key role in plant acclimatization processes. Phenotype plasticity has an enormous importance for plants, since they are sessile organism and cannot escape from unfavourable environmental conditions (Lucini et al., 2020). As a response to acclimation process, new genotypes may appear. For over 60 years, Vegetable Research Development Station (VRDS) Buzau is an important research centre for acclimation and breeding vegetable species in Romania. From year 2010, *Trichosanthes cucumerina* (L.) is studied by the Laboratory of Breeding and Biodiversity from VRDS Buzau. *T. cucumerina* also known as snake gourd, long tomato, snake tomato or viper gourd belongs to *Cucurbitaceae* family. *Trichosanthes* is one of the major genera under this family with 100 species. Only two species *T. anguina* and *T. cucumerina* are monoecious while others are dioecious (Swarup, 2012). Snake gourd has the centre of origin in India or in Indian Archipelago, but the *Trichosanthes* genus is native to Southern and Eastern Asia, Australia and Islands of the western Pacific. *T. cucumerina* can be found wild throughout these areas. The plant is grown as a minor vegetable in many countries of tropical Asia. Recently has been introduced as a new crop of increasing importance in several parts of Africa, including Ghana and Nigeria (Khare, 2007). The fruit is usually consumed as vegetable due to its good nutritional value. The plant has rich content in chemical constituents as flavonoids, carotenoid, phenolic acids which makes the plant pharmacologically and therapeutically active (Busuioc et al., 2020). Scientific studies have shown that is has anti-inflammatory effects (Kolte et al., 1997), antibacterial activity (Redy et al., 2010), anti-dandruff activity (Vishal and Prasahant, 2014), anti-diabetic activity (Arawwawala et al., 2009a; 2009b; Kirana and Srinivasan, 2008; Dias and Imai, 2017), anti-fertility activity (Devendra et al., 2009), antioxidant properties (Stellus and Nair, 2005; Ademosun et al., 2013), cytotoxic activity (Kongtun et al., 1999), gastro protective activity (Shweta et al., 2012), hypoglycaemic activity (Kar et al., 2003), hepatoprotective activity (Sathesh et al., 2009) and larvicidal efficacy (Rahuman and Venkatesan, 2008). In order to enrich the vegetable assortment and also adapt new species in Romania, the Breeding Laboratory from VRDS Buzau has started an acclimation process with *T. cucumerina*. The results of this work are presented in this article.

MATERIALS AND METHODS

From year 2010, the Breeding and Biodiversity Laboratory from VRDS Buzau has taken into seven genotypes of *T. cucumerina*. Following their evaluation, a single valuable genotype was chosen, in order to facilitate the breeding and acclimatization process and also to prevent the impurity of the genotypes by cross-polination, knowing that the species is, by excellence, allogamous. Throughout the vegetation period, biometric and phenological observations were made with an emphasis on the main characters of the plants. The descriptors used were the one from IPGRI guidelines. Morpho-agronomical descriptors used were: growth habit, plant height (PH), tendrils presence, colour of leaves, leaf margin, length (LL) and width (LW) of leaves, petiole length (PeL), dorsal leaf pubescence, number of secondary shoots (NSS), stem thickness (ST), flower colour, male female flower ratio, flower diameter (FD), peduncle shape, peduncle length (LP), peduncle separation from fruit, blossom end fruit shape, stem-end fruit shape, fruit shape, fruit length (FL), fruit diameter (FrD), fruit ribs, fruit colour, fruit skin texture, flesh colour, number of fruits per plant (NFP), seed colour and surface. The qualitative characters were noted based on visual evaluation while the quantitative traits were counted, measured using metric rulers, caliper and weighed using weighing balance. For quantitative traits, Pearson correlation coefficient was calculated. Means comparison was performed by Duncan test. The period analysed in this study was 2010-2020. Regarding crop management, the planting scheme used was 150 cm between rows and 60-75 cm between plants in a row. The snake gourd is a climbing vine and needs support in order to fully develop. Throughout all the studied years, no serious pathogens were reported who could damage the crop.

RESULTS AND DISCUSSIONS

Trichosanthes cucumerina is a monoecious annual herb climbing with growth of over 5 meters high. The plant is vigorous, with many (12-16) slender stems and tendrils. The leaves are green, simple, alternate and without stipules. The leaf margin is smooth and the degree of pubescence is low on the dorsal leaf. The leaves have 3-5 lobes (Figure 1) with a length varying from 17.5 cm to 34.2 cm and the width also range from 19.3 cm to 23.4 cm. The petiole length varies from 6.4 cm to 17.6 cm.

Figure 1. Types of leaves

The stem thickness was measured as diameter of the main stem from 10^{th}-15^{th} nodes at the time of 20^{th} expanding and it was 7.56 mm. The stems and leaves have a pungent, unpleasant smell, a plant repellent for a number of pests. Being a monoecious plant the flowers are unisexual, regular, white coloured with green and hairy calyx. Corolla is tubular in white lobes fringed and hair like outgrowths. The ratio male and female flower was medium. The flower diameter has an average 4.46 cm. The male flowers are arranged in raceme and the female are solitary. The flowers are open in the afternoon and early in the morning. Pollination is performed by insects, bees, wasp, ants, butterflies and various moths.

If may I say not even the most skilful hands could crochet such a beautiful flower (Figure 2).

Figure 2. Male flower of *T .cucumerina*

Peduncle shape has a sharply angle. The peduncle length varies from 2.1-2.6 cm. Peduncle separation from the fruit is easy. The blossom end fruit and stem end fruit is pointed. The fruit has an elongate slim shape. The fruit is straight and slightly twisted towards the top, like a snake's tail. If they are not directed properly, they take various forms, depending on the growing environment (Figure 3).

Figure 3. Different fruit shapes and maturity stage of fruit

The fruits are long, which can exceed 2 meters at physiological maturity, but usually are harvested when measure 50-100 cm to be consumed in various culinary preparations; it can replace successfully zucchini. The fruit can be consumed when immature, as it gets a bitter taste with age and inside the fruit it creates an empty interior (Figure 4).
The fruit ribs are intermediate and the colour of fruit is green with dark green stripes when young and dark-red at maturity.

Figure 4. Fruit with seeds

The fruit skin texture is shallowly wavy and the flesh colour cream. The number of fruit per plant is varying from 16-22, but if harvested at the consumption stage, their number increases significantly. If the fruits are harvest when young the yield is around 8-10 t/ha, but the yield may increase to 30 t/ha, if the fruits are harvested when they weight around 1 kg. The seed colour changes from white, grey when immature to brown when mature (Figure 5). In Figure 6 is presented a macro exposure of the seed. It can be noted that the seed surface is slightly wrinkled.

Figure 5. Macro exposure of seed

Regarding plant phenology it was noted, that snake gourd has a long vegetation period over 170 days. The male flowers appear, in general, 30 days after planting. The female flowers bear miniature fruit and appear later, after 40-45 days. The fruit reach physiological maturity after 100 days. The young fruits can be consumed approximately two weeks after fruit setting process.

Table 1. Correlation matrix of the main traits

Traits	LL	ST	PeL	LP	LW	FL	PH	NFP	FrD	FD	NSS
LL	**1**										
ST	**0.987**	**1**									
PeL	0.675	0.786	**1**								
LP	-0.118	-0.277	**-0.812**	**1**							
LW	0.098	0.258	**0.801**	**-0.998**	**1**						
FL	-0.303	-0.145	0.499	**-0.911**	**0.919**	**1**					
PH	-0.373	-0.217	0.434	**-0.878**	**0.887**	**0.997**	**1**				
NFP	-0.395	-0.240	0.412	**-0.866**	**0.876**	**0.995**	**0.998**	**1**			
FrD	0.702	0.577	-0.052	0.625	-0.640	**-0.892**	**-0.923**	**-0.932**	**1**		
FD	**0.852**	0.756	0.189	0.419	-0.437	-0.757	**-0.803**	**-0.817**	**0.971**	**1**	
NSS	**-0.852**	-0.756	-0.189	-0.419	0.437	0.757	**0.803**	**0.817**	**-0.971**	**-0.998**	**1**

Values in bold are significant at $p \leq 0.05$, according to Duncan test.

A correlation matrix of the main characters was made in order to establish significant correlations between traits (Table 1).

The significant positive correlation between number of fruits per plant, fruit length and plant height indicates that these traits are efficient in yield determination. The plant height and number of fruits per plant are a complex of desirable traits inherited in a quantitative fashion (Yuan et al., 2002; Manickavelu et al., 2006; Derera et al., 2007; Kassem et al., 2007; Jacobson et al., 2007; Alcivar et al., 2007). In this study it was shown that plant height is significant positive correlated with number of secondary shoots and number of fruits per plant, but negatively correlated with fruit diameter and flower diameter.

Peduncle length is strongly negatively correlated with leaf width, fruit length, plant height and number of fruits per plant. Other studies, on different crops, report a strong negative correlation between peduncle length and number of fruits per plant. The path analysis indicates that peduncle length had the highest direct effect on yield (Khan et al., 2010). Farooq et al. (2018) suggests that strong association of peduncle length with other yield contributing traits may be utilized as an indirect selection criterion for yield improvement.

During the vegetation period, the plant characteristics were measured and synthetic indicators of variation are presented in Table 2. In terms of yield (number of fruit per plant, fruit length and diameter) the coefficient of variation has a value below 35% which represents a stable characteristic, but the petiole length has the coefficient of variation over 35% meaning a high variation between this traits.

Table 2. Mean values of plant characteristics

Quantitative descriptors	Unit	Value ± standard deviation	CV%
Plant height	m	5.36 ± 0.66	12.40
Leaf length	cm	24.03 ± 8.92	37.12
Leaf width	cm	21.66 ± 2.12	9.79
Petiole length	cm	12.46 ± 5.65	45.38
Number of secondary shoots	pcs	14.33 ± 1.52	10.65
Stem thickness	mm	0.73 ± 0.05	7.87
Flower diameter	cm	4.46 ± 0.15	3.41
Peduncle length	cm	2.36 ± 0.21	8.79
Fruit length	cm	114.81 ± 8.38	7.30
Fruit diameter	cm	5.53 ± 0.55	9.95
Number of fruits/plant	pcs	19.2 ± 3.61	18.97

Fruit weight varies from 200 g to 1500 g. Young fruits can be kept in a room with high humidity and temperature of 15°C for 10-14 days (Vînătoru et al., 2019).

CONCLUSIONS

T. cucumerina has met favourable conditions for growing and fruit development in Romania, in field and greenhouse conditions, and it can be recommended to be cultivated in all areas favourable for cucurbit species in the country.

The studies have been completed so far with patenting of a new cultivar that can be successfully cultivated in protected areas and in the field, in a trellis system. The new cultivar will be registered in the Official Catalogue of Romanian Crop Plants. The seeds will be available to the sold from year 2022.

Because during years of study it was not noted any dangerous pathogens that could harm the crop, we recommend to be grown in both conventional and ecological systems.

ACKNOWLEDGEMENTS

This work was supported by one founding source a grant of the Romanian Ministry of Research and Innovation, CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF BIOFERTILIZING AND BIOSTIMULATING PRODUCTS ON THE PRODUCTION OF CORNICHON CUCUMBER HYBRIDS CULTIVATED IN HEATED SOLARIUMS

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Abstract

The aim of this study was to determine the influence of foliar fertilizers, applied individually or in combination, on production characteristics and the production of pickled cucumbers grown in solarium, but also the combined effect of the two factors studied. The biological material studied was represented by two cucumber hybrids, SV 9007 and Kybria, and 3 fertilizers, Cropmax, Fury and Auxym. The study was placed according to the experimental technique for bifactorial experiments. The observations highlighted that the Kybria hybrid achieved the highest total production (80.2 t/ha) while the SV-9007 hybrid had the best production of 1st quality fruit, i.e. 65.6 t/ha of the total production of 72.8 t/ha. The fertilizer product with the strongest influence on production and production parameters was Auxym, followed by Furia. However, the combination of the two products (Auxym + Furia) had the greatest influence on production in terms of quantity and quality. The results support the cultivation of cornichon cucumbers, the hybrid SV-9007 F1 and the application of the fertilization products Furia and Auxym in combination, during vegetation

Key words: *hybrid, phasal fertilization, production parameters, solarium culture.*

INTRODUCTION

The beginning of a new crop in the assortment of vegetable crops in Romanian greenhouses occurred in the immediate period of the 1980s, namely the cultivation of cucumbers, which revolutionized the cultivation of cucumbers on the whole (Becherescu et al., 2016). Horgoş et al. (2007) identified at least three main reasons for obtaining productions of this new variety of greenhouse cucumbers in the years '83-'85 (cucumbers with small cornichon-type fruits). These imply, first of all, solving an extremely acute problem at the time, namely the replacement of an unsellable production of cucumbers with long fruits in given periods of time (in the second half of May and June, but especially in cycle II). The second reason implies the increasingly pressuring requirements of the canning industry internally, and especially externally. Thirdly, the urgent need to reduce energy consumption for the second production cycle, on the background of the worsening energy crisis, had severe repercussions on the greenhouse industry, the heat requirements of cornichon crops in the second cycle being much lower or completely absent.

Cornichon cucumber cultures in the last two decades has practically replaced, almost entirely, that of long cucumbers, both in greenhouses, but especially in protected spaces, both heated or unheated. Cornichon cucumber hybrids have a lower genetic yield potential than cucumbers with long or semi-long fruits (Hoza, 2003).

The destination of the cornichon production for pickling by industrial facilities requires the harvesting of much smaller fruits (4-6 cm, 6-9 cm, sometimes 9-12 cm), with the effect of obtaining lower yields from a quantitative point of view (Ciofu et al., 2003).

New cucumber hybrids with very high yield potential have appeared, the value of which depends on the cultivation technology applied (Dinu et al., 2009). The technological links with major impact on the level of production obtained are those on root and foliar fertilization, as well as phytosanitary protection, and last, but not least, the architecture of plant conducting in vegetation, in terms of pinching shoots and balancing the number of fruit on the plant.

In conclusion, high-performance cultivation technologies and modern cultivation spaces have made cornichon cultivation an extremely profitable crop due primarily to the demand for production obtained mainly for industrialization, but also for fresh consumption, in slightly smaller proportion.

MATERIALS AND METHODS

The research regarding the influence of fertilizers on production and quality has been conducted in heated protected spaces in a traditional area for vegetable crops in the locality of Olari from Arad county, Romania.

The hybrids used in the experiment were SV 9007 F1 from the Seminis company and Kybria F1 from Rijk Zwaan. The crop was established on March 14-15, 2019, at a density of 18,000 pl/ha, and the clearing of the land was made on August 1, 2019.

A two-fold experiment was carried out, which included the following experimental factors:

Factor A - The hybrid

a_1 - SV-9007 F1; a_2 - Kybria F1;

Factor B - Foliar fertilizers applied in vegetation

b_1 - Unfertilized control;

b_2 - Cropmax - foliar fertilizer;

b_3 - Furia - foliar fertilizer and biostimulant;

b_4 - Auxym - natural compound of vegetal extracts;

b_5 - Furia + Auxym

Fertilization and irrigation were carried out through the drip irrigation system (fertigation). The applied technology was specific to cornichon crops from heated protected spaces.

Cornichon cultivation was established in a heated protected space covered with double thermal foil and equipped with a heating source. In the previous autumn, the fertilization was carried out with well-fermented manure (8 kg/m^2), followed by soil mobilization. The soil was partially mulched with black mulch film, thus ensuring weed control. The planting was done using seedlings obtained on the farm, aged 35 days. The maintenance works of the cultivation consisted in trellising the plants using strings and trellis clips, guiding them on strings, and defoliation consisting in removing old or diseased leaves.

Statistical analysis. The statistical significance of the differences between variants was obtained by determining the mean, the standard deviation of the mean and the coefficient of variability. To determine the significance of differences between the factors and their gradations, the processing of statistical data was performed by analyzing the variance and the "t" test for the experiments with two factors in the subdivided graphs (Ciulca, 2006). The significance of differences was expressed both by symbols (*; **; ***; 0; 00; 000) and in letter format. The differences between the variants marked with different letters were considered significant.

RESULTS AND DISCUSSIONS

An important component of production, with a decisive impact on the plant, and consequently on the unit area, is the number of fruits per plant, which can be influenced by the applied cultivation technology. This consists in practicing a proper density in the crop according to the plant habitus, in the correct management of the microclimate in the crop space, in the use of stimulating products with the role of intensifying the plant metabolism, etc (Luchian et al., 2002; Jurian et al., 2003; Tiwari & Sharma 1999; Çakir et al., 2017).

Table 1 shows edifying data on the level of production per hectare, depending on the interaction of the two experimental factors (hybrid and fertilizing products). The production elements that determined the quantitative levels of production are the number of fruits per plant and the average weight of the fruits. The average weight of the fruit displays variation limits, as well as the number of fruits/plant depending on the interaction between the hybrid and the foliar product used for each hybrid.

In SV-9007 F1 the variation limit is very accentuated, of 13.3 fruits/plant and 8.97 g and in Kybria F1 it reaches even higher values, of 14.6 fruits/plant and 9.70 g.

Table 1. Results regarding the influence of fertilizers on the cucumber yield

Factor A (Hybrid)	Factor B (Fertilizer)	No. fruit/plant (piece)	Average weight /fruit (g/piece)	Average yield /plant (kg/plant)	Fruit length (cm)	Average production compared with the factor B: t/ha	B: %	A: t/ha	A: %
a_1 - SV-9007 F1	b_1 - Unfertilized control	54.1	58.11	3.144	6.2-7.3	56.6	100.0	72.8	100.0
	b_2 - Cropmax	63.2	61.36	3.878	6.4-8.2	69.8	123.3		
	b_3 - Furia	67.4	64.86	4.372	6.9-9.1	78.7	139.0		
	b_4 - Auxym	65.8	65.85	4.333	7.0-9.4	78.0	137.8		
	b_5 - Furia + Auxym	67.0	67.08	4.494	7.1-9.8	80.9	142.9		
a_2 - Kybria F1	b_1 - Unfertilized control	56.0	60.41	3.383	6.4-7.4	60.9	100.0	80.2	110.2
	b_2 - Cropmax	65.8	63.73	4.194	6.7-8.5	75.5	124.0		
	b_3 -Furia	71.2	69.37	4.939	7.1-9.5	88.9	146.0		
	b_4 - Auxym	68.9	69.82	4.811	7.2-9.8	86.6	142.2		
	b_5 - Furia + Auxym	70.6	70.11	4.950	7.4-10.0	89.1	146.3		
Experimental mean (Mx)	b_1 - Unfertilized control	55.1	59.23	3.264	6.3-7.3	58.8	100.0	76.5	105.1
	b_2 - Cropmax	64.5	62.57	4.036	6.5-8.4	72.7	123.6		
	b_3 - Furia	69.3	67.18	4.656	7.0-9.3	83.8	142.5		
	b_4 - Auxym	67.4	67.83	4.572	7.1-9.6	82.3	139.9		
	b_5 - Furia + Auxym	68.8	68.63	4.722	7.3-9.9	85.0	144.6		
Experimental mean (Mx)		**65,0**	**65.38**	**4.250**	**6.8-8.9**	**76.5**	*	**76.5**	**105.1**

Table 2. Experimental results regarding the influence of fertilizers on production and quality in cornichon cucumber hybrids grown in heated protected spaces

Factor A (Hybrid)	Factor B (Fertilizer)	No. fruit/plant (piece)	Average weight/ fruit (g/piece)	Factor B: kg/ plant	Factor B: t/ha	Factor B: % comp. to b_1	Factor B: Of which Quality I t/ha	Factor B: Of which Quality I %	Factor A: kg/ plant	Factor A: t/ha	Factor A: % comp. to a_1	Factor A: Of which Quality I t/ha	Factor A: Of which Quality I %	Factor A: Of which Quality I % comp. to a_1
a_1 – SV-9007 F1	b_1 - Unfertilized control	54.1	58.11	3.144	56.6	100.0	45.0	79.5	4.044	72.8	100.0	65.6	90.1	100.0
	b_2 - Cropmax	63.2	61.36	3.878	69.8	123.3	59.8	85.7						
	b_3 - Furia	67.4	64.86	4.372	78.7	139.0	72.8	92.5						
	b_4 - Auxym	65.8	65.85	4.333	78.0	137.8	73.2	93.9						
	b_5 - Furia + Auxym	67.0	67.08	4.494	80.9	142.9	77.4	95.7						
a_2 – Kybria F1	b_1 - Unfertilized control	56.0	60.41	3.383	60.9	100.0	34.3	56.3	4.455	80.2	110.2	56.2	70.1	85.7
	b_2 - Cropmax	65.8	63.73	4.194	75.5	124.0	50.7	67.1						
	b_3 - Furia	71.2	69.37	4.939	88.9	146.0	64.5	72.5						
	b_4 - Auxym	68.9	69.82	4.811	86.6	142.2	64.1	74.0						
	b_5 - Furia + Auxym	70.6	70.11	4.950	89.1	146.3	67.4	75.7						
Experimental mean (Mx)	b_1 - Unfertilized control	55.1	59.23	3.264	58.8	100.0	39.7	67.4	4.250	76.5	105.1	61.0	79.7	92.9
	b_2 - Cropmax	64.5	62.57	4.036	72.7	123.6	55.3	76.1						
	b_3 - Furia	69.3	67.18	4.656	83.8	142.5	68.7	82.0						
	b_4 - Auxym	67.4	67.83	4.572	82.3	139.9	68.7	83.5						
	b_5 - Furia + Auxym	68.8	68.63	4.722	85.0	144.6	72.4	85.2						
Experimental mean (Mx)		**65,0**	**65.38**	**4.250**	**76.5**	*	**61.0**	**79.7**	**4.250**	**76.5**	**105.1**	**61.0**	**79.7**	**92.9**

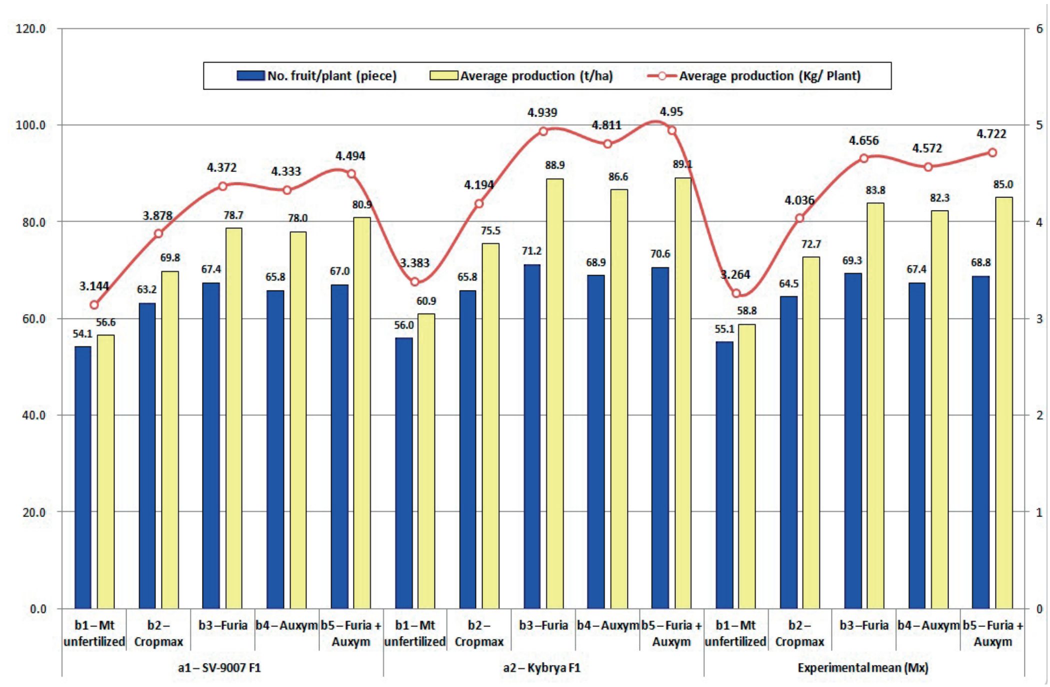

Figure 1. Experimental results regarding the influence of fertilizers on production and quality in cornichon cucumber hybrids grown in heated protected spaces

Since the level of the average production per plant is directly correlated to the product between the two production elements, an average production per hectare is determined depending on the average density per unit area.
This ranges between the limits of 56.6 t/ha (a_1b_1 - Unfertilized control) and 80.9 t/ha (a_1b_5 - Furia + Auxym) at SV-9007 F1, and between 60.9 t/ha (a_2b_1 - Unfertilized control) and 89.1 t/ha (a_2b_5 - Furia + Auxym) at Kybria F_1.
The production growth, according to the fertilizer used, in both hybrids gets to 142.9% (a_1b_5 - Furia + Auxym) compared to 100.0% (a_1b_1 - Unfertilized control) at SV-9007 F1, and to 146.3% (a_2b_5 - Furia + Auxym) compared to 100.0% (a_2b_1 - Unfertilized control) at Kybria F_1. The same Table 1 shows an average production of 72.8 t/ha for the hybrid SV-9007 F1, and 80.2 t/ha for the hybrid Kybria F1, the increase in production by comparison being 110.2%, namely 7.4 t/ha.
Table 2 and Figure 1, in addition to the data contained in the previous table, indicate conclusive elements in terms of production quality, namely 1st production quality. In the hybrid SV-9007 F1 (a_1), the 1st quality production is 65.6 t/ha - 90.1% of the total production of 72.8 t/ha; in the hybrid Kybria F1 (a_2) it is 56.2 t/ha - 70.1% of the total production of 80.2 t/ha. The percentage difference of 1st quality production in favor of the a_1-SV-9007 F1 hybrid is 20.0%, namely 9.4 t/ha of 1st quality production more than in a_2 - Kybria F1.
As a first conclusion, although in Kybria F1 (a_2) a production of 80.2 t/ha is obtained, that is 7.4 t/ha or 10.2% higher than in SV-9007 F1 (a1) - 72.8 t/ha, the 1st quality production of SV-9007 F1 (a_1) is higher both in percentage and in absolute numbers, namely 20.0%, representing 9.4 t/ha.
Under the influence of factor B fertilising products, there are obvious percentage differences in the 1st quality production between b_3 - Fury, b_4 - Auxym and b_5 - Fury + Auxym compared to b1 - unfertilized Control and b_2 - Cropmax, in the case of both hybrids, within the limits of 6.7 - 19.4%.
The highest amount of 1st quality production in the total production per unit area is recorded for b_5 - Fury + Auxym, 95.7% for a_1-SV-9007 F1,

75.7% for a_2 - Kybria F1, and 85.2% on the average of the two hybrids.

Between the individual influences of the two products, b_3 - Fury and b_4 - Auxym, there are also small differences.

Tables 3-6 and Figure 2 display the results of the research, which are interpreted based on the mathematical statistics and the variance analysis method.

Table 3. Analysis of variance for the effect of hybrid and fertilizer on cucumber yield

Source of variation	SS	DF	MS	F value
Total	7439.66	44		
Replications	11.08	2	5.54	2.22
Hybrid	568.36	2	284.18	113.79**
Residual hybrid	9.99	4	2.50	
Fertilizer	6523.12	4	1630.78	320.91**
Hybrid x fertilizer	205.15	8	25.64	5.05**
Residual fertilizer	121.96	24	5.08	

According to the results of the variance analysis method presented in Table 3 it is observed that both the hybrid and the fertilisation treatments, respectively the interaction of these two factors had considerable influences, ensured statistically on the production of cucumbers. The different fertilisation treatments had the highest contribution to the variability of production. The combined effect of the two factors also showed a significant influence on production, but considerably less than the separate effects of the factors. At the level of experience, the results obtained were influenced to a lesser extent by only 2% by other sources of variation not included in the experiment.

Given the unilateral effect of the hybrid (Table 4), the production recorded an amplitude of 9.44 t/ha with values ranging between the limits of 56.20 t/ha for Kybria hybrid and 65.64 t/ha for hybrid SV9007, having a reduced variability (7.75%) between hybrids.

Table 4. The effect of hybrid on cucumber yield

Hybrid	Yield (t/ha)		Relative yield (%)	Significance of difference
Kybria - SV9007	56.20	65.64	85.62	-9.44^{000}
Mean - SV9007	60.94	65.64	92.84	-4.70^{00}
Mean - Kybrya	60.94	56.20	108.43	4.74**

$LSD_{5\%}$=1.60 t/ha $LSD_{1\%}$=2.65 t/ha $LSD_{0.1\%}$=4.97 t/ha

Regarding the differences between the hybrids, it is observed that SV9007 showed a significantly higher production potential by about 15% compared to Kybria and by 7% compared to the average of the two hybrids.

The average values of production under the influence of different fertilisation treatments (table 5) showed an amplitude of 32.74 t/ha, with the limits between 39.66 t/ha in case of the untreated variant up to 72.40 t/ha for the associated application of Furia and Auxym, with a high variability (22.26%) between the five treatments. Compared to the untreated control, it is found that the application of fertilisation treatments allowed to obtain production increases between 39.34% for Cropmax and 82.57% for Furia + Auxym. It is also found that the individual or associated application of Furia and Auxym treatments resulted in significant increases in production by 24-31% compared to Cropmax. The associated application of Furia and Auxym showed a higher production efficiency of about 5.5% compared to the separate effects of the two fertilizers.

Table 5. The effect of fertilizer on cucumber yield

Fertilizer	Yield (t/ha)		Relative yield (%)	Significance of difference
Cropmax - Control	55.26	39.66	139.34	15.60***
Furia - Control	68.67	39.66	173.16	29.01***
Auxym - Control	68.66	39.66	173.13	29.00***
(Furia+Auxym) - Control	72.40	39.66	182.57	32.74***
Furia - Cropmax	68.67	55.26	124.27	13.41***
Auxym - Cropmax	68.66	55.26	124.25	13.40***
(Furia+Auxym) - Cropmax	72.40	55.26	131.03	17.14***
Auxym - Furia	68.66	68.67	99.98	-0.01
(Furia+Auxym) - Furia	72.40	68.67	105.44	3.73**
(Furia+Auxym) - Auxym	72.40	68.66	105.45	3.74**

$LSD_{5\%}$=2,19 t/ha $LSD_{1\%}$=2,98 t/ha $LSD_{0,1\%}$=3,99 t/ha

Considering the combined effect of the hybrid and fertilisation on the production (Table 6 and Figure 2.) it is observed that the fertilisation showed a higher influence on obtaining the production for Kybria hybrid. The hybrid had the highest effect on the production of cucumbers in the non-fertilised variant.

Table 6. The combined effect of hybrid and fertilizer on cucumber yield

Fertilizer	Hybrid				
	SV-9007	Kybria	Mean	$\bar{x} \pm s_{\bar{x}}$	s%
Control	x 45.00 d	z 34.30 c	y 39.67 d	39.66 $\pm$1.67	12.66
Cropmax	x 59.80 c	z 50.70 b	y 55.27 c	55.26 $\pm$1.41	7.67
Furia	x 72.80 b	z 64.50 a	y 68.70 b	68.67 $\pm$1.33	5.81
Auxyn	x 73.20 b	z 64.10 a	y 68.67 b	68.66 $\pm$1.50	6.53
Furia+ Auxym	x 77.40 a	z 67.40 a	y 72.40 a	72.40 $\pm$1.54	6.40
$\bar{x} \pm s_{\bar{x}}$	65.64 $\pm$3.22	56.20 $\pm$3.35	60.94 $\pm$3.26	60.93 $\pm$1.94	
s%	18.98	23.10	21.72	21.33	

Hybrid - $LSD_{5\%}$=3.74 t/ha $LSD_{1\%}$=5.23 t/ha $LSD_{0,1\%}$=7.47 t/ha
Fertilizer - $LSD_{5\%}$=3.80 t/ha $LSD_{1\%}$=5.15 t/ha $LSD_{0,1\%}$=6,90 t/ha
Note: Different letters within the same row indicate significant differences between hibryds or between fertilizers

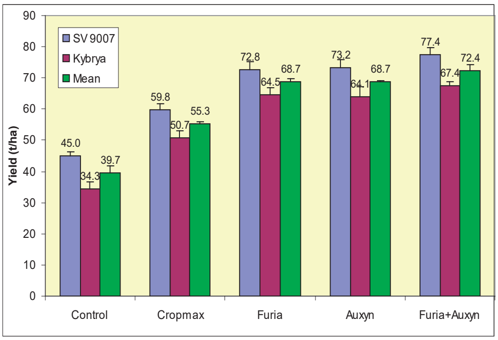

Figure 2. The yield of cucumber hybrids under the effect of different fertilizers

In the case of SV-9007 hybrid, the fertilisation treatment generated an amplitude of 32.4 t/ha with limits between 45 t/ha for the untreated control to 77.4 t/ha for Furia + Auxym variant. As such, this hybrid capitalized at a significantly higher level the treatment associated with Furia + Auxym, registering significant increases in production (5.7-29.4%) both compared to separate treatments with these products and compared to Cropmax. Treatments with Furia and Auxym had significantly equal effects on the production of this hybrid, but approximately 22% higher than Cropmax, on the basis of production increases of approximately 62% compared to the untreated control. Under the effect of Cropmax treatment, the plants of this hybrid recorded a significant increase in production of about 33%.

Regarding the influence of different fertilisation treatments, the plants of the Kybria hybrid registered a variation of the production between 34.3 and 67.4 t/ha, having as limits the untreated control and the one based on the association between Furia and Auxym. The plants of this hybrid capitalized at a similar level the separate and associated effects of the treatments based on Furia and Auxym, achieving production increases of 27.21-32.94% compared to Cropmax and 88.04-96.50% compared to untreated control. Cropmax treatment allowed an increase in plant production of this hybrid by 47.81%.

Considering the productions of hybrids under the effect of the same fertilisation treatment, it is found that regardless of the applied treatment, the plants of the hybrid SV9007 capitalized at a significantly higher level of fertilisation, registering production increases compared to the hybrid Kybria between 8.3 t/ha on the application of Fury and 10.7 t/ha on the unfertilized control.

CONCLUSIONS

Despite the fact that the hybrid with the highest yield proved to be Kybria F1, the hybrid that excels in terms of quality is SV-9007 F1, which exhibited the highest quality production. Although the production of the Kybria F1 hybrid exceeded that of the SV-9007 F1, its 1st quality production proved to be lower.

The research has concluded that Auxym is the fertilizer product with the highest influence on production, both in terms of quantity and quality, followed by Furia, which displays insignificant differences in production. However, the combination of the two products (Furia + Auxym) proved to have the greatest influence on production in terms of both quantity and quality.

Based on these results, it is recommended to cultivate the cornichon hybrid SV-9007 F1 as best suited to heated protected spaces, and to apply the combination of Furia and Auxym fertilizers.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE ROLE OF FOOD LEGUME SPECIES IN THE CONTEXT OF SUSTAINABLE AGRICULTURE, FOOD SECURITY, AGROBIODIVERSITY, CONSERVATION AND HUMAN HEALTH

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Abstract

The study addresses the major role of grain legumes in relation to the main challenges of agricultural sector as biodiversity conservation, soil fertility, use of resources on agro food chain, human and environment health. The focus was on species Phaseolus vulgaris, Ph. coccineus, Lathyrus sativus, Vigna radiata, Pisum sativum, Vicia faba, Cicer arietinum, Lens culinaris. The study included ample documentation related to recently developed EU new protein plan and H2020 currently developed projects focused on the paramount potential of food legume species in (1) sustainable agriculture - modern (inter)cropping schemes designed to reduce use of external inputs (2) agrobiodiversity and conservation - a vast amount of leguminous genetic resources hosted by different institutes in frame of major collections and strategies to make the resources available and useful for different end user categories (3) food security and human health - human plant protein intake is on the rise in many EU regions, presently is taking place a re-evaluation for useful effects of consumption in the diet, which is the basis for various health

Key words: *biodiversity, Fabaceae, nutritional profile, quality traits, use.*

INTRODUCTION

The world's population is expected to increase. Many people will face global challenges among which achieving food security, lowering the risk of climate change by reducing the net release of greenhouse gases into the atmosphere and solving in a friendly environmental manner the increasing demand for energy.

The global food production request new solutions, natural based as much as possible, to solve the sever impact of different categories of stressors, and to develop the best solutions to ensure enough and safety food and to limit the negative impact of agriculture on environment.

Moreover, since the coronavirus became a pandemic, consumers started buying much more convenient and healthy products, such as beans, chickpea, lentil, rice, and pasta, trying to stay healthy. During look down due to Covid 19 in many places along Europe, the demand for grain products and pulses was increased thanks to their suitability to long term conservation and nutritional profile. The moisture contents of all the dry legumes are in the range of 9-13% making them favorable for long storage. Thanks to their traits, content and characteristics, food legumes and their production systems can play important roles being used for their capacity to deliver multiple services in line with sustainability principles (Stagnari et al., 2017). At global level, grain legumes are introduced in different research programs, being considered the most relevant source of plant protein. These species are largely exploited in many countries of Africa and Latin America, but despite their nutritional and environmental benefits there are some constraints such as a poor adaptation, low resistance to pathogen attack, which severely influenced yield stability and limit species extinction in production system (De Ron, 2015). Despite the benefits of legumes, their status in terms of consumption is different among countries. There are countries where grain legumes are not popular, or consumption is residual. Opposite, there are countries where the consumption and cultivation are extended and encouraged. EU developed a new protein plan, and its implementation will be largely based on traditional and innovative uses of food legumes. The plan highlights the need and the interest of the agri-food sector for development of new products and modern technologies to ensure food security by providing affordable and healthy food, under the pressure of changing climate and socioeconomic conditions. Recently, major advances have been made in the development of single-seed descent (SSD) purified accessions (e.g., AGILE, BEAN_ADAPT, BRIDGE, BRESOV projects), with the possibility to associate phenotypes to reliable genotypic information by few EU projects funded for this aim: LEGATO, EUROLEGUME, LEGVALUE, PROTEINLIFE, PROTEIN2FOOD, BRESOV, ECOBREED. Grain legumes have the potential to contribute more to European agricultural systems by improving the agronomic performance of cropping systems and providing protein-rich food and feed and helping to reduce European dependence on imported protein (Watson et al., 2017). Diet is important, not only for nutrition, but for the prevention and treatment of diseases, especially when these diseases are caused by insufficient, inadequate or unbalanced dietary intake (Muzquiz et al., 2012). Legume seeds contain a great number of bioactive compounds (proteins, tannins, glycosides, saponins, dietary fiber, vitamins). Recent investigations suggest that grain legume may contribute to human health and wellbeing, mostly through prevention of chronic diseases, hypertension, diabetes, cancer, and obesity. Pulses represent an important source of protein for vegetarians, are low glycemic index food and recognized as food choice with significant potential health benefits. They are excellent foods for people managing their diabetes, heart disease or celiac disease, and additionally can help people concerned with weight control. To improve the nutrition of many developing countries, or to combat the incidence of various chronic diseases worldwide, food technologists have developed products based on pulses, adding value thereby contributing to increase in the consumption of legumes (Kamboj & Nanda, 2017; Khang et al., 2016)

MATERIALS AND METHODS

The study represents an ample literature review of the food legumes as key agriculture-related to societal challenges and supports the development of a smart tool aimed to optimize legumes cultivation and their integral use on agri-food chain. The study is developed in frame of nationally and internationally research projects that deals with the urgent need to provide climate-resilient cultivars technics and methods addressed to organic and conventional vegetable production systems. These new resources will benefit growers, seed industry, providing much needed security both under current and future scenarios of climate change. The study is focused on exploit of the genetic variation of legumes species for enhanced productivity, by exploiting up-to-date knowledge of genome structure and function for use in different directions as conservation, human health, sustainable agriculture.

RESULTS AND DISCUSSIONS

Grain legumes represent almost 27% of the world crop production and provide about 33% of the dietary protein consumed by humans. (Smykal et al., 2014). A selected set of food legumes genetic resources representing the diversity available in Europe and world-wide, have been chosen as target species to improve the knowledge related value of the germplasm along the entire value chain of genetic resources, which ranges from conservation and research to breeding and cultivation. These species represent a cross-section in terms of their potential value for sustainable food production, and they are all well linked to the European food tradition, with significant options for EU agriculture. At the molecular level, the species represent extremes regarding their genome sizes and comprehensive panoplies of genomic resources have been developed recently.

Grain legumes and sustainable agriculture - Legumes are adaptable to cultivation under unfavorable ecological conditions, nutritious and stress tolerant, possessing characteristics for enhancing the sustainability of deferent agricultural systems. Despite their high adaptability to semi-arid climatic conditions, pea and faba bean may occasionally be faced with drought stress, depending on the yearly variations in climatic factors, when they are grown as non-irrigated crops. Reduced watering application in faba bean increases the concentrations of crude protein and carbohydrates in seeds. Pea responds to water deficit mainly by immediate abortion of reproductive organs which results in reduced number of seeds per plant rather than by adverse efects on the mean seed weight or on other quality characteristics (Ntatsi et al., 2018). The modern cropping schemes designed to reduce use of external inputs and to exploit legumes thanks to their capacity to fix the atmospheric nitrogen, release in the soil high-quality organic matter and facilitate soil nutrients' circulation and water retention are developed to reduce the negative impact of agriculture on environment; Currently, the European Union devotes only 3% of its arable land to protein crops, and imports more than 75% of its plant protein (EC Report COM (2018) 757 final). The main reasons for this fact are low yield capacity and lack of breeding efforts for adaptation of legumes to European agro-ecosystems. The low level of European plant protein self-sufficiency is due to the late development and adaptation of protein plants in Europe (COPA-COGECA report, GOL (18)585). Better use of genetic resources represents a precondition to increase sustainability. Until about 1970, common bean (*Phaseolus vulgaris* L.) was the most widely cultivated grain legume in Europe. Following the introduction of policy support for soybean and protein feed crops in the 1970s, field pea and soybean became the most widely grown grain legumes (Zander et al., 2016). The main pulses are field peas and faba beans, whereas lentils and chickpeas are only grown on limited areas (EC Report COM, 2018). Pea is the most widely grown grain legume in Europe, but it suffers from poor standing ability, poor ground coverage, and low competitive ability against weeds, along with relatively low protein (20%–24%) and, on many soils, low productivity. Faba bean is the second in area, the first in yield per hectare, and on account of its higher protein content (28-32%), the highest in protein yield, but is adapted to heavy or clay-rich soils and too sensitive to water deficit on sandy soils. Lentil, chickpea, and common bean all have protein contents in the same range as that of pea and have relatively low yields, but high values as they are primarily food rather than feed crops (Watson et al., 2017). Since 2013 production has almost tripled in the EU and reached 6 million t (2.6 million ha) in 2018. Thanks to their capacity to fix nitrogen in soils by synergic relationships with *Rhizobium* and mycorrhizal fungi, legumes help to reduce the need for fertilizers and avoid economic inputs and environmental impacts. The amount of nitrogen fixed by legumes depends not only on species and cultivar but also on environmental factors such as temperature, water availability (Watson et al., 2017). This nitrogen is being utilized through the plant to compose protein which becomes available to humans (Kamboj & Nanda, 2017; Kouris & Belski, 2016). Other benefits of legumes, which should not be ignored, include reduced pest and weed occurrence, and improved soil quality (Kessel, & Hartley, 2000). To cover the increasing amounts of nitrogen requirements during formation and filling, pods attract nitrogen from the nodules. If the nodules cannot cover their N requirements, pods attract nitrogen from older leaves, thereby reducing the photosynthetic capacity of the plants and determining rapidity of ripening. Different studies showed the paramount role of *Rhizobium* strains selection. An increased nodulation capacity may improve N availability to pods, and the effect can be quantified in pod quality by increasing pod size. Selection of efficient bacteria requires specific selection processes based on efficiency and competitiveness for nodulation of the associations (Ntatsi et al., 2018). Salinity is a common and most severe environmental stressor in agriculture, which is dramatically exacerbated by irrigation. Additionally, erroneous fertilization schemes contribute to salt accumulation in plant rooting zone. Under these circumstances the use of commercial inoculants containing arbuscular mycorrhizal fungi is quickly expanding, rewarded as an environmentally friendly technology which contributes to the alleviation of the negative effects of soil irrigation water salinity (Meca et al., 2017). For a while intercropping was ignored by modern techniques focused on standardized products and remained widely applied in low-input and low-yield farming systems and in developing countries. Despite several recognized beneficial aspects of intercropping such as better pest control, competitive yields with reduced inputs, pollution mitigation, reducing the need for fertilizer-N, increasing the utilization efficiency of available nutrients and water, more stable aggregate food or forage yields per unit area, there are a number of constrains that make intercropping not common in modern agriculture, such as example the request of a single and standardized product and the suitability for mechanization or use of other inputs as a prerogative in intensive farming system (Kessel & Hartley, 2000; Aziz et al 2015). It is therefore necessary to optimize intercropping systems to enhance resource-use efficiency and crop yield simultaneously, while also promoting multiple ecosystem services. Most recent research has focalized on the potential of intercropping in sustainable productions and on grain legumes that can fix nitrogen through biological mechanisms. Legumes (top 10 most frequently used intercrop species, seven are legumes) can contribute up to 15% of the N in an intercropped cereal, thus increasing biomass production and carry-over effects, reducing synthetic mineral N-fertilizer use and mitigating N_2O fluxes (Stagnari et al., 2017.) When maize and beans are intercropped, their yields are generally lower than those of maize or beans grown in monoculture. Studies have found that maize yielded 5.3 t ha^{-1} when monocropped, 5.2 t ha^{-1} when intercropped with bush beans, and 3.7 t ha^{-1} when intercropped with climbing beans. Maize-legume rotations also help to maintain soil fertility (FAO, 2016). Different studies developed in H2020 projects showed that some intercropping schemes are not competitive, species as onion and faba bean, being in competition for moisture and nutrients. The detrimental effect of this competition impairs mainly onion, especially when grown under unfavorable conditions (high temperature and low precipitation). Other conclusions highlighted the fact that carrot and faba bean plants compete and share available soil resources. A comparative study showed that carrot yield in intercropping variant was 2.28 kg m^{-2} and did not differ significantly from the control variant developed without N fertilization. Complex studies developed by researchers in frame of EUROLEGUME project showed the fact that cabbage is suitable for intercropping with faba bean without yield losses. (Lepse et al., 2017). Cereal–legume intercrops can be used for forage or grain depending on growing conditions and farm management and using them for whole-crop silage is a way of boosting the forage protein content of livestock diets. The cereals are generally better than legumes at taking up mineral N. Legume root exudates liberate phosphate and several cation species, and the cereal roots liberate other nutrients, leading to increases in the P uptake of cereals and Fe and Zn uptake of legumes compared to sole crops. The advantage of cereal-legume mixtures over pure cereals is generally greater in systems with little or no usage of nitrogen fertilizer. Chemical weed control is difficult or impossible in intercrops, as few herbicides are tolerated by both a cereal and a legume (Watson et al., 2017). Intercropping grain legumes and cereals has demonstrated multiple agronomic and environmental benefits, all listed in the final report of LEGATO project.

One significant effect of intercropping versus grain legume sole crops consists in increased capacity to reduce weed abundance. This, together to decreased severity of pest or disease problems, leaded to accomplishment of stable grain and cereal yields, increased biodiversity able to support pollinating insects, according to data reported and retrieved form http://www.legato-fp7.eu/, in frame of LEGATO project.

Grain legumes are weak suppressors of weeds but mixing species in the same cropping system could represent a valid way to improve the ability of the crop itself to suppress weeds (Stagnari et al., 2017). A mixture of the two varieties of pea can reduce weeds compared to sole crop of semi-leafless pea, and whether this benefit can be obtained in synergy with reduced lodging compared to sole crop of normal leafed according to data reported and retrieved form http://www.legato-fp7.eu/, in frame of LEGATO project.

Grain legumes significantly reduced their emission factors suggesting that legume-fixed N is a less-emissive form of N input to the soil than fertilizer N. Legumes have a proven role in reducing GHG in accord to the management of agro-ecosystems in which they are included. Results reported by (Senbayram, 2016; Jensen,

2012) showed that faba bean grown as mono cropping, led to threefold higher cumulative N_2O emissions than that of unfertilized wheat; when faba bean was intercropping with wheat, cumulative N_2O emissions fluxes were 31% lower than that of N-fertilized wheat. There is also some interest in intercropping legumes with oil crops, such as pea with linseed (*Linum usitatissimum* L.) or faba bean with either safflower (*Carthamus tinctorius* L.) or mustard (*Sinapis alba* L.) (Watson et al., 2017). Direct mutual benefits in cereal-legumes intercropping involve below-ground processes in which cereals while benefiting of legumes-fixed N, increase Fe and Zn bioavailability to the companion legumes. Higher yields are therefore observed for crops following legumes e.g. yields of wheat, maize or rapeseed can be up by 10 % compared to following a cereal. The quality of cereals is also improved (e.g. higher protein contents or lower mycotoxins contamination) when following a legume (EC Report COM, 2018). Studies and results related large insertion of legumes in farm rotations, crop management practices (planting time, densities, fertilization, soil management, weeding, irrigation), need to be implemented and harmonized as standard techniques to be applied by farmers.

Agrobiodiversity and conservation - In Europe, the Common Agricultural Policy (CAP) has driven the intensification of agriculture, promoting the simplification and specialization of agroecosystems through the decline in landscape heterogeneity, the increased use of chemicals per unit area, and the abandonment of less fertile areas (Emmerson et al., 2016). High-input farming practices directly affecting biodiversity, such as herbicide application or monocultures, may disrupt potential pest control services (Pappagallo et al., 2018). A significant challenge is to solve the impact of erosion of agricultural genetic diversity which is reported at global level. Numerous publications and debates over several decades have been devoted to the impact of genetic erosion on resilience to climatic, economic, or societal context. There are several cases where lack of within crop diversity has resulted in substantial production losses. Crop's diversification can ensure benefits for environment and along agri-food chain. Legumes have an impact on breeding - pre breeding, screening of a rich genetic material, investigating genetic resources will ensure the creation of a valuable breeding material, used to improve the productive and qualitative potential, resistance to biotic and abiotic stress; nutritional profile; antioxidant capacity; The characterization and maintenance of food-legume genetic resources, and their exploitation in pre-breeding, can play a significant role not only in a sustainable agriculture but also in entire agri-food chain, by providing healthier food products. Need for improvement of food legume genetic resources: to date, exploitation of genetic resources in crop breeding is limited in comparison to availability of materials, and the potential impact of their use is far from optimal (i.e., lack of comprehensive information regarding passport data and descriptors useful for users, accession heterogeneity, unharmonized data), which also affects ability to attract funds for genetic-resources conservation. These issues are more critical in food legumes, as breeding investment and research activities remain modest. Efficient genetic-resources management is required to attract additional private and public investment to boost food legumes breeding. From this perspective, the availability and access to well-described and well-managed genetic-resource collections of food legume species that capture the full diversity range will be paramount to advance legume crops and to reach a competitive level in the EU regarding agronomic performance and sustainability. Indeed, without correct handling of EU legume genetic resources, the European Commission's goal of achieving the nine CAP objectives (i.e., economic, environmental, climatic and socio-economic, including healthier diets) will be unattainable (EC Report COM, 2018). According to INCREASE an H2020 ongoing project, the actual utilization of grain legumes GenRes is limited in comparison to the availability of materials and the potential impact of their use, due to several concurrent factors: *a) genetic structure of accessions* - in most cases, accessions have unknown genetic structure and are heterogeneous, which impedes the projection of the phenotypic information to the genotype and vice versa. *b) limited information availability on GenRes*:

large numbers of accessions have only minimal, if any, information regarding biological status and geographic origin; information regarding traits of interest for breeders and users is very low and mostly limited to morphological descriptors; *c) limited access to available information* (*) the heterogeneous nature and non-standardized way of data collection and integration causes that a huge amount of information is heavily under-used; (**) databases are centralized and not designed to integrate data obtained by external users strongly limiting the access to available information; (***) the available information is not easily accessible to users due to unfriendly searching and visualization tools. Collections are assembled and are maintained on an accession basis, where each accession usually comprises a mixture of genotypes that represent a population. The conservation of the population represents substantial challenges that arise from genetic drift and/or selection, which are difficult to fully address in conventional conservation management, and from the lack of knowledge of their diversity. A survey accomplished in INCREASE proposal presented the actual status of some grain legumes collections, according to different sources. Based on GENESYS data retrieved from https://www.genesys-pgr.org the largest collections of chickpeas are maintained at ICARDA and ICRISAT, centers of CGIAR with unique accessions estimated at more than 15,000 and 20,000, respectively. The largest collection of wild materials and derived introgression lines is maintained at UC Davis in California. Regarding the availability of common bean GenRes, based on GENESYS data the most important *P. vulgaris* collections are maintained at Centro Internacional de Agricultura Tropical (CIAT) and USDA-ARS, at Washington State University (USA), with more than 24,000 and 18,500 accessions, respectively. In Europe large collections are conserved at Leibniz Institute of Plant Genetics and Crop Plant Research-IPK (~8,500 accessions). There are currently over 58,000 lentil accessions held in various genebanks worldwide. Genesys displays information for about 70% of these (https://www.genesys-pgr.org). ICARDA, with 12,463 accessions, is the center with the largest lentil collection. Genetic improvement of nutritive value can modify the content of bioactive compounds. For this reason, it has been possible to genetically eliminate the alkaloids in different species of *Lupinu*s and decrease vicine and convicine in *V. faba* by breeding.

Legumes a bridge between food and health **-** Global diets have considerably changed in the last decades and more attention is paid by majority of countries to these implications. More calories are being consumed per person, and the proportion of fat and animal protein consumed has increased significantly with wealth (Williams, 2020). Diet is now recognized as important, not only for nutrition, but for the prevention and treatment of diseases, especially when these diseases are caused by insufficient, excessive or unbalanced dietary intake. One of the most controversial subjects of discussion is the establishment of an optimum human diet. Grain legumes are featured by superior quantity of protein comparing with other plant foods and have twice the dietary protein content of cereal grains (Kouris-Blazos & Belski, 2016), strongly having perspective to exploited against malnutrition and generally in food sector. Recent investigations suggest that grain legumes, beside their beneficial role on environment may contribute to human health and wellbeing, mostly through prevention of chronic diseases. The most frequent diseases linked with unhealthy diets are the coronary heart disease, obesity, hypertension, diabetes, and cancer. Consumption of legumes reduces the risk of cardiovascular disease, some cancers and helps to manage body weight due to its satiety value (Kamboj, 2017). Consumption of common beans contributes to the prevention and/or treatment of degenerative-chronic diseases, such as obesity, diabetes, cancer, cardiovascular diseases, partly due to the influence of micronutrients (mainly folic acid and magnesium) and high fiber content, condensed tannins, phytoestrogens, and non-essential amino acids. Common beans are a good source of aromatic amino acids, lysine, leucine, and isoleucine, but deficient in sulfur amino acids (methionine and cysteine), valine, tryptophan and threonine (De Ron et al., 2015). Vicine and convicine are natural pyrimidine glucosides found in the faba bean plant and are likely to be involved in plant defense mechanisms against pathogens. Recent papers suggest that faba bean and derivatives could represent a suitable food in treatment of diabetics, in hypertension and may help to prevent cardiovascular disease (Turco et al 2016). Among micronutrients, peas have high ascorbic acid, β-carotene, thiamine, and riboflavin contents and compared with other vegetables, they are rich in iron (20.2 μg^{-1} of the edible portion). Peas, like beans, lentils, and chickpeas, are also good sources of folate, the vitamin that lowers the blood level of homocysteine. Legumes seeds contain a great number of bioactive compounds that vary considerably and can be proteins (protease inhibitors, a-amylases, lectins), glycosides, tannins, saponins or alkaloids (Muzquiz et al, 2012). Their physiological effects are diverse: pyrimidine glycosides present in *Vicia faba* are the causative agents of favism; the non-protein amino acid b-N-oxalyl-L-a,b-diaminopropionic acid (b-ODAP) contained in the seeds of *Lathyrus* spp. can cause neurolathyrism. Some other bioactive compounds detected in grain legumes, as for example a-galactosides are associated with capacity to induce flatulence. Despite the negative role in consumption, inducing digestive discomfort, some of these bioactive compounds have a positive and supportive role for plants in their defense mechanisms. Published studies pointed out the role to strengthen plants behavior against predators or unfavorable environmental pressure. Pulses represent an important source of protein for vegetarians, are low glycemic index food and recognized as food choice with significant potential health benefits. They are excellent foods for people managing their diabetes, heart disease or celiac disease, and additionally can help people concerned with weight control. To improve the nutrition of many developing countries, or to combat the incidence of various chronic diseases worldwide, food technologists have developed products based on pulses, adding value thereby contributing to increase in the consumption of legumes (Khang et al., 2016). A plenty of studies highlight the essential amino acids in legumes fact that make them available for different combination with cereals in daily intake. The presence of calcium, magnesium, potassium, phosphorus, and iron was also detected in legume seeds. Bioavailability of nutrients can be increased by soaking, sprouting and fermentation (Kamboj & Nanda, 2017). Grain legumes contain 20-45% protein compared with 7-17% in cereals. The protein content ranges from 20% to 25% in common bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medik.), and pea, to over 40% in soybean and yellow lupin (*Lupinus luteus* L.) (Watson et al., 2017). On the other hand, legumes are considered to have incomplete proteins (except soy) because they contain relatively low quantities of the essential sulphur containing amino acids cystine, methionine and cysteine (which are found in higher quantity in grains). However, grains contain relatively low quantities of lysine, whereas legumes contain appreciable quantity (Kamboj & Nanda, 2017). Studies to investigate the variation of protein content in different organs of same species showed that the highest protein content was detected in seeds, and lower in pods and immature seeds. The nutritional value of legume vegetables as protein sources depends not only on their protein content but also on the amino acid composition and the protein digestibility (Ntatsi et al., 2018). Adequate dietary fiber is vital for proper working of the gut, which is related to reduce risk of several chronic diseases including certain cancers, heart disease and diabetes. Fiber comprises pectin, mucilage, cellulose, gum, hemicelluloses and lignin. Most of the legume grains which are consumed as pulses by humans, their fiber content ranges from 0.9-5.3%. Legumes are mainly rich in resistant starch (RS), have low glycaemic index carbohydrates. The oligosaccharides (mainly raffinose and resistant starch) and fiber pass through the stomach and small intestine in the undigested form until they reach the colon, where they act as food (prebiotics) for the probiotic or beneficial bacteria which resides there. This bacterial fermentation leads to the development of short-chain fatty acids, such as butyrate, which possibly will improve colon health through promoting a healthier gut micro biome and reducing colon cancer risk. They also play a positive role in weight reduction due to its satiety value. In addition, they are capable to help in moderating blood sugar levels after meals and improve insulin sensitivity (Trinidad et al., 2009). Commonly consumed legumes having carbohydrate content in the range of 20.9-60.9%. In legume seeds, starch is the main source of accessible carbohydrate and most plentiful 22-45% along with 1.8-18% oligosaccharides and 4.3-25% dietary fiber (Kamboj & Nanda, 2017). Legumes are excellent source of iron, calcium, zinc, selenium, magnesium, phosphorus, copper, and potassium. Cereals grains generally supply the higher energy and make up the volume of diets. As sources of micronutrients legumes are superior to cereals because legumes have higher initial minerals content. Most legumes, including common beans are consumed whole, resulting in conserving their mineral contents (Trevor et al., 2003). Micronutrient deficiencies have become more common, even in developed countries. Legumes are superior source of vitamin B-complex but are a poor source of vitamin C and fat-soluble vitamins. Legumes are normally low in fat and have no cholesterol, with soybeans and peanuts exception. Mono and poly unsaturated fatty acids decrease the possibility of coronary heart diseases (Kamboj & Nanda, 2017). Legumes have anti-nutritional factors which affect its nutritional quality. Anti-nutritional factors can decrease palatability and diminish protein digestibility and bioavailability of nutrients. Several usually considered antinutritional compounds like phytic acid, phenols and tannins are currently considered as potential antioxidants containing health promoting effects. Phytochemicals reduce the digestion and absorption of nutrients or interfere with their action. The bioactive phytochemicals including enzyme inhibitors are mainly represented as phytoestrogens, oligosaccharides, phytosterols, phytates, saponins, flavanoids and phenolic acids (Kouris & Belski, 2016). Grain legumes are the main sources of lectins in human food. Lectins from some of the pulses can reduce the digestibility and biological value of dietary proteins. (Muzquiz et al., 2012) demonstrates that lectins may be beneficial by stimulating gut function, limiting tumor growth, and ameliorating obesity. The significance of phenolic compounds was gradually recognized, and several studies have now reported that phenolics offer many health benefits and are vital in human nutrition. Some studies investigated and reported correlations between phenolic compositions and antioxidant activities. Pulses with highest total phenolic content (lentil, red kidney, and black bean) exert the highest antioxidant capacity (Singh & Basu, 2012). Saponins have been reported in many pulses, lupin, lentil, and chickpea, as well various beans, and pea (Kamboj & Nanda, 2017). Recent evidence suggests that saponins possess hypocholesterolemic, anticarcinogenic and immune stimulatory properties. The antioxidant properties of food have been extensively studied since excessive production of free radicals/reactive oxygen species (ROS) and lipid peroxidation are widely believed to be involved in the pathogenesis of many diseases. The most common diseases are cardiovascular diseases, cancers, autoimmune disorders, rheumatoid arthritis, various respiratory diseases, cataract, Parkinson's or Alzhemier's diseases and ageing. Sprouts (including *Vigna* sprouts) are assumed to be good sources of these natural antioxidants and hence can be exploited in food, pharmaceutical and cosmetic industry (Tomar et al., 2018). The market for pulses for food in the EU is benefitting from innovations in pre-cooking processes, inclusion of pulses in prepared convenience foods and the development of new pulses such as 'edamame' (European Commission, 2018). In the case of extruded legumes, which have high protein content, could be the base material for the development of high protein snack bars, since taste is sufficiently neutral, and so they can be used for salty or sweet preparation. An added value can be given by adding functional supplements such as hemp seeds, goji berries, ginger, and others as reported by EUROLEGUME project. The introduction of legume flour into cereal products can therefore increase the protein, insoluble fiber, vitamin, and mineral contents. Considering the results of ongoing and or already developed projects and also screening the market we many innovative products based on legumes can be easily identified: immature seeds of peas and beans with extended shelf-life, pesto sauce made from fresh faba bean seeds, cowpea fresh pods as a novel legume vegetable, faba bean 'cheese, ready-to-eat-pulse spreads, extruded snacks made from dry pea and bean seeds, and protein and fiber rich legume bars with various flavors, according to new developed products in frame of EUROLEGUME project. Faba bean can be an ingredient for beer. In 2016, the new Bean Beer was introduced as a beer made with 40 % whole faba beans and 60% malted barley. The beer is marketed as a sustainable drink made as it is made from a crop that contributes to more sustainable farming practices (Hamann, 2019). There are new opportunities of using legumes for food products of improved nutritional value.

CONCLUSIONS

Legumes represent a valuable source of food proteins, and their exploitation is expected to increase in relation of a growing world's food need. The actual context of the need for available, healthy, long self-life food, new opportunities and challenges for the agriculture and food sector open. The value chain needs a strong improvement with new varieties with higher adaptation to different environments, better yield, and improved qualities with a particular concern in the development of new products with high organoleptic and nutritional value. The availability of novel varieties will facilitate the adoption of food legumes in the agroecosystem improving the agrobiodiversity with all its related positive consequences associated to the inclusion of legumes in the cropping systems (e.g. sustainability, food security, economic returns, stable farming systems, increase of soil fertility, diversify products, improve human nutrition, etc.).

For the sustainable use of genetic resources, a coordinated, interdisciplinary, and multi-sectorial effort is needed to exploit the recent scientific and technological ground-breaking advances. Future strategies need to focus on the sustainable re-introduction of grain legumes into crop rotations, based on their positive effects on yield and quality characteristics on subsequent crops.

ACKNOWLEDGEMENTS

This work was supported by BRESOV project which has received funding from European Union's HORIZON 2020 research and innovation programm under GA no 7742 44. a grant of the Romanian Ministery of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III, and ADER 2020 projects developed by VRDS Bacau.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

FARMING FOR TOMORROW - ENSURING ENVIRONMENT'S SUSTAINABILITY BY INCREASING COMPETITIVENESS FOR ORGANIC SYSTEM

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Abstract

The current strategies and programs encourage the urgent need to develop and exploit climate-resilient cultivars addressed to organic vegetable production systems and the imperative need to use friendly environmental methods for plant cultivation to decrease the negative impact of agriculture on human health and environment. The research's purpose was to develop a study in organic versus conventional system to detect and select the most productive and qualitative vegetable genotypes, able to perform under application of environmental friendly inputs. The rationale choice of species portfolio was based on our area market request for organic vegetables. The main differences between experimental plots were the cultural practices as soil preparation and its management during vegetation period, the applied products for fertilization, pest and disease control, according to each species characteristics.

***Key words**: vegetables, biodiversity, friendly practices, yield, quality.*

INTRODUCTION

A comprehensive screening of literature, polices, and funded projects have emphasized the need for major changes in the global food system: agriculture must meet the twin challenge of feeding a growing population, with rising demand for meat and high-calorie diets, while simultaneously minimizing its global environmental impacts (Charles et al., 2010; Foley et al., 2011). Conventional agriculture produces about one third of global greenhouse gases using chemical inputs, machinery, and livestock (FAO, 2011a). It results in wind and water erosion from soil surface, loss of soil fertility, water holding capacity and desertification (FAO, 2011b). Pesticides and herbicides are accumulated in groundwater, pest and disease become resistant to one or more pesticides. It has been reported that harmful pesticides spray applied by the farming community contaminated nearly 10-15 percent of stored food during storage (Ali et al., 2015). Salinity affected million hectares land and caused potential yield reduction. Organic agriculture can act as an important tool in sustainable food production. Despite the efforts there are still some limitative factors which affect the organic yields and surfaces extinction. For this reason, a strong need for fully understood, alongside assessments of the many social, environmental, and economic benefits of organic farming systems is still requested. Organic yields are typically lower than conventional yields. But these yield differences are highly contextual, depending on system and site characteristics, and range from 5% lower organic yields (rain-fed legumes and perennials on weak-acidic to weak-alkaline soils), 13% lower yields (when best organic practices are used), to 34% lower yields (when the conventional and organic systems are most comparable).

Some vegetable species are more suitable to organic cultivation, than other. The yield is under the severe incidence of pests and pathogens which is more aggressive in organic system. Organic system enhanced optimal production level but with higher cost of cultivation (certification procedures, higher cost per unit of fertilizer, phytosanitary treatments applied, more labor etc.), compared with conventional farming (Ilić et al., 2014).

Organic tomatoes achieve higher prices and a guaranteed placement compared to conventional tomatoes (Kapoulas et al., 2011), because these products are often linked to better quality and low footprint on environment during cultivation. Ilić S. Z. et al., in 2014, conducted a study about the quality of tomato fruits from organic and conventional production which concluded that in general, the significant differences between tomatoes grown in organic or conventional production systems are that organic tomatoes contain more carotenoids, more minerals (P, K, Mg, Ca), contain far less heavy metals (Pb, Zn, Cu, Ni), less nitrates, about 30-40% less, and do not contain any pesticide residues. Depending of the cultivar and growing conditions ascorbic acid content is around 14.6 mg per 100 g of fresh red tomato (Abushita et al., 2000). A lot of intrinsic and extrinsic factors act commonly to imprint tomato quality as for example: choice of cultivar, seed quality, cultural practices, harvest time and method, storage, and handling procedures. Increased interest in organic tomato production imposed the need to evaluate the quality and nutritional value of organic tomato (Ilić S. Z. et al., 2014). Some studies have shown higher levels of bioactive compounds in organically produced tomato fruits compared to conventional ones, but not all studies have been consistent (Ordonez-Santos et al., 2011; Chassy et al., 2006).

A comparative study about morphological characteristics of some pepper genotypes in organic and conventional system was conducted by Bicikliski et al. (2018). In this experiment, in the organic system for plant nutrition were applied organic fertilizers, foliar organic fertilizer from sea algae (*Ascophyllum nodosum*) and 12% humic acid. In the conventional system were applied foliar plant nutrition PK (30:20) and NPK (15:15:15) fertilizers in combination with soil fertilization with NPK magnesium pellets.

Organic food is increasingly attracting the interest of consumers, as it is perceived to be healthier than food produced by conventional agriculture, and to be more sustainable for the environment. (Gomiero, 2018).

MATERIALS AND METHODS

The experiments were conducted at Vegetable Research and Development Station, Bacau. (2016, 2017, 2018). Laboratory and field experiments were set up. The open field experiments were conducted in 2015, 2016, 2017 and 2018. Location is placed at an elevation of 91 m, latitude 46.521946 N, longitude 26.910278 E., average annual temperatures during the experimental period averaged between 9 to 11°C. During the summer temperature reached 42°C. Precipitation ranged from 500 and 1100 mm per year.

The biological material was represented by genotypes of *Solanum lycopersicum, Capsicum annum, Cucumis melo, Zea mays* var. *sacharata, Cucurbita moschata*. The species and genotypes selection was based on market request for organic vegetables. The aim was to select the most appropriate varieties suitable to organic cultivation (yield and quality).

The experimental fields were established using genotypes which were investigated comparatively in both systems: conventional and certified organic farm (conformant to European laws). The main differences between cultivation systems were cultural practices, such as soil preparation and its management during vegetation period, the plant densities and the applied products for fertilization, pest and diseases control, according to each species characteristics. The differences between cultivation systems are highlighted in the table 1.

For each species, randomized plots were placed in organic and conventional conditions. The investigations related to the yield potential (t ha^{-1}) included the number of fruits/plants, weight of fruits/ plant, fruit shape and size, total yield. Pest and disease tolerance/resistance was accomplished to all experimental variants. To investigate the pathogens attack, a conventional scale was applied, giving the notes according to the degree of attack.

The laboratory experiment was accomplished to assess the quality. In terms of quality, we investigated the items related to taste acceptability and visual quality: the total dry matter content (%), total soluble matter, (°Brix), pigments content (mg 100 g^{-1}) carotene and lycopene, vitamin C (mg 100 g^{-1}).

The determination of total dry matter substance was carried out gravimetrically, by weighing the fresh vegetal material, drying it for 24 hours at 105°C, cooling it outside and then weighing again the dry vegetal material. The results were

expressed in percentage. The difference till 100% represents the water content.
The soluble dry matter content was determined using refractometry (°Brix).
The pigment content, lycopene and β-carotene, were extracted in petrol ether and spectrometrically determined. The content was expressed in mg/100 g.
Ascorbic acid was extracted in oxalic acid and titration with 2.6 dichlorophenolindophenol to a light pink color. The quantity of ascorbic acid was expressed in mg/100 g.

Table 1. Applied technologies in both cultivation systems: organic and conventional

Item	Organic system	Conventional system
Biological material	Same material (seeds obtained from organic) in both systems.	
Field preparation	No tillage	Tillage
Weed control	Organic mulch (dried legumes)	Herbicide application
Fertilizer requirement	Organic fertilizer	NPK fertilizer
Irrigation requirement	3-5/ vegetation period	3-5/ vegetation period
Pest management	Biological control	Pesticides application

The biological material used in our investigations was represented by lines from breeding programs, featured by heat and specific pathogen tolerance. The genotypes were: tomatoes (*L 161, L 162, L 167, L 168, L 213*), sweet pepper (*L 335, L 336, L 337, L 339, L 340*), sweet corn (*L 1, L 2, L 12, L18, L 29*), melon (*L 555, L 556, L 557, L 558, L 559*), pumpkin (*L 535, L 536*).

RESULTS AND DISCUSSIONS

Usually, the consumers evaluate tomato fruit quality using visually appreciation of size, color and firmness. Color evolution during fruit ripening is mainly related to the breakdown of chlorophyll and synthesis of lycopene, which is responsible for the red color and constitutes 75-83% of the total pigment content at full ripeness (Radzevičius et al., 2016; Schouten et al., 2014).
The taste is appreciated according to the fruit sweetness given by the report between soluble solids and titratable acidity. The total content of water is also important in taste appreciation. In our study five lines were investigated to detect basic quality and their suitability to ecological cultivation. The growth was determinated, having a total height between 101 and 109 cm. The total number of shoots was 3 to 6. The foliage varied from 28 to 55 leaves. The fruits were big, round (Figure 2), and the index shape was around 1 to all lines. The fruits were red to dark red at physiological maturity, with strong firmness and good resistance to cracking. Figure 1 and Figure 2 present other morphological features of the investigated material. The total number of fruits varied from 31 to 38, and the fruit weight ranged in small limits, between 110 and 130 g. The lines developed 195 to 280 seeds in one fruit (figure 1). The total weight of fruits per plant varied from 4.07 to 4.48 kg plant^{-1} (Figure 2).

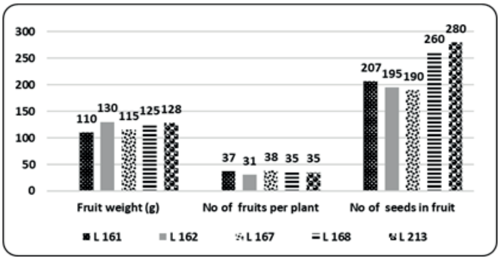

Figure 1. Number of fruits per plant, and seeds in fruit, fruit weight

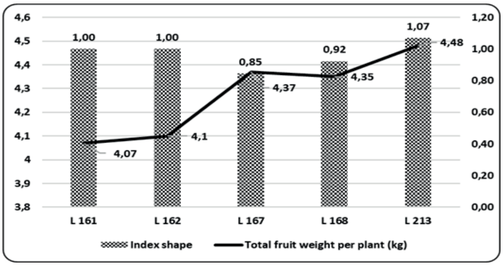

Figure 2. Index shape and total weight of fruit per plant

Table 2 presents the data related to yield, accomplished in conventional and ecological system. All investigated lines obtained higher yields in conventional system, L213 being the most productive in both systems. All lines registered superior yields compared to control.
The main differences between organic and conventional tomato cultivation were represented by applied strategies for fertilization, disease and pest's management. According to conventional practices, insecticides, fungicides and miticides were applied for plant protection, while in organic system, were applied extracts of garlic, nettle;

Bordeaux mixture, and biofertilizer for control the disease and pests and were applied. Our results related yield in conventional versus organic, confirming the previous results published by Bettiol et al., 2004 and Kapoulas et al. 2011.

Table 2. Tomatoes yield in both cultivation systems

Genotype	Yield in conventional system			
	Yield t ha^{-1}	Yield %	Difference compared to control	Signifi-cance
L 161	122	103,4	+4,0	-
L 162	123	104,2	+5,0	***
L 167	131	111,0	+13,0	***
L 168	130	110,0	+12,0	***
L 213	134	113,6	+16,0	***
Control	118	100	-	-
			DL (p 5 %)= 2,90 t ha^{-1} DL (p 1%) = 3,19 t ha^{-1} DL (p 0.1%) = 4,12 t ha^{-1}	

Genotype	Yield in ecologic system			
	Yield t ha^{-1}	Yield %	Difference compared to control	Signifi-cance
L 161	84,4	102,2	+ 1,8	-
L 162	85,1	103,0	+ 2,5	*
L 167	90,7	109,8	+ 8,1	***
L 168	90,0	109,0	+ 7,4	***
L 213	92,8	112,3	+10,2	***
Control	82,6	100,0	-	-
			DL (p 5%) = 2,22 t ha^{-1} DL (p 1%) = 3,19 t ha^{-1} DL (p 0.1%) = 4,10 t ha^{-1}	

Other investigations related to quality from this study confirms data of Toor et al. (2006), Caris-Veyrat et al. (2004): the mean ascorbic acid content, soluble solids, total dry matter, and pigments registered in organically fertilized tomatoes higher values than the results obtained from tomatoes that were fertilized with mineral solutions. As in Figures 3 and 4, all investigated items registered slightly superior values in ecological, compared to conventional. The medicinal value is attributed to the high content of vitamins and minerals; peppers are recognized for their beneficial effects on the human body, for maintaining health and hygiene. They are an excellent source of vitamin A and C, which are two powerful antioxidants that together neutralize free radicals. Red pepper contains lycopene, with antitumor effects in prostate, breast, pancreatic, skin, uterine, intestinal cancer. According to literature, many studies that investigate the effects of organic system on *Capsicum annuum* plants, in terms of growth, yield, fruit quality or morphological characteristics and also the differences between the organic and conventional production system were developed thanks to pepper biodiversity, economic importance, suitability to be exploited in different industries (food, agriculture, pharmacy).

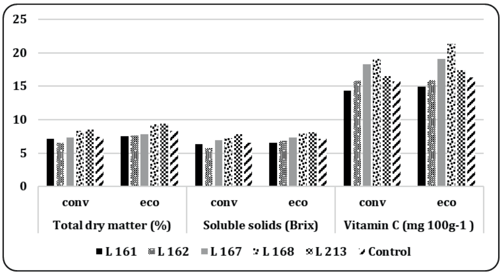

Figure 3. Dry and soluble matter, vitamin C

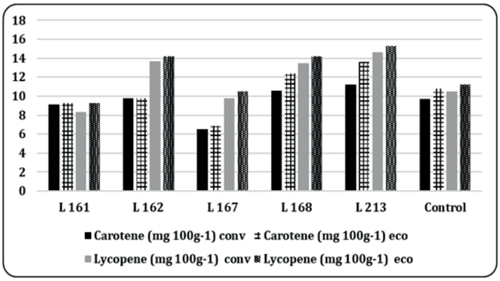

Figure 4. Pigments content in tomatoes fruits

Our study included five genotypes and one control variant of sweet pepper. The fruits are distinguished by red color, exception L340 which was orange. Figure 5 presents the variation of height, diameter, and fruit weight as it was registered for each cultivation system. As a general remark, fruits obtained in conventional cultivation system developed bigger size being heavier than those obtained in ecological conditions.

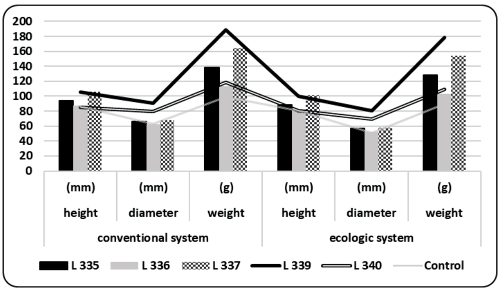

Figure 5. Sweet pepper fruit characteristics (height, diameter, weight)

Comparing the yield (Table 3), all genotypes were more productive in conventional system; L 337 has accomplished the highest level of production in both systems: 50.3 t ha^{-1} (conventional) and 35.23 t ha^{-1} (organic).

Table 3. Pepper yield in conventional and ecological cultivation systems

Genotype	Yield in conventional system			
	Yield t ha^{-1}	%	Difference compared to control	Signifi-cance
L 335	48,5	126	+ 10,0	***
L 336	46,2	120	+7,7	***
L 337	50,3	131	+11,8	***
L 339	51,0	132	+12,5	***
L 340	49,6	129	+11,1	***
Control	38,5	100	-	-

DL (p 5%) = 1,90 t ha^{-1}
DL (p 1%) = 2,49 t ha^{-1}
DL (p 0.1%) = 3,72 t ha^{-1}

Genotype	Yield in ecologic system			
	Yield t ha^{-1}	%	Difference compared to control	Signifi-cance
L 335	34,0	137	+7,0	***
L 336	32,3	120	+5,3	***
L 337	35,2	130	+8,2	***
L 339	35,7	132	+8,7	***
L 340	34,7	129	+7,7	***
Control	27,0	100	-	-

DL (p 5%) = 1,82 t ha^{-1}
DL (p 1%) = 2,11 t ha^{-1}
DL (p 0.1%) = 3,22 t ha^{-1}

All investigated germplasm was suitable to ecological cultivation, no significant attach of pest and pathogens was registered. The differences between the total conventional yield and ecological one can be explained by the influence of fertilization schemes, with allowed and specified products for each system. Our results confirm previous published results of Bicikliski et al. (2018), who developed a comparative study about morphological characteristics of some pepper genotypes in organic and conventional system. The values of the primary statistical estimators corresponding to the data characterizing the degree of dispersion of the results obtained for the total dry matter content of the 6 pepper genotypes (*Capsicum annuus*) were reduced. Thus, the standard error of the mean was within the limits of 0.06 ÷ 1.18. The analysis regarding the homogeneity of the results for the studied parameters obtained from the 6 genotypes showed a very good homogeneity for dry matter (%), and a relative homogeneity for the values for the soluble dry matter content, carotene and lycopene.

Figure 6 presents the variation of dry matter and soluble solid content in condition of conventional and ecological cultivation. Figure 7 presents variation of carotene and lycopene accumulation in both cultivation system.

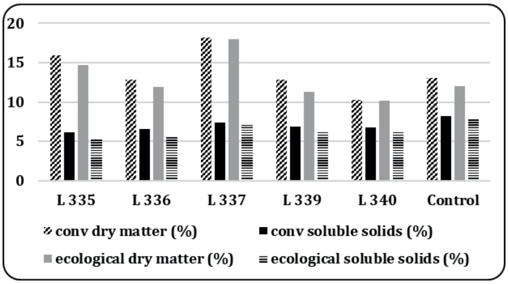

Figure 6. Dry and soluble matter variation

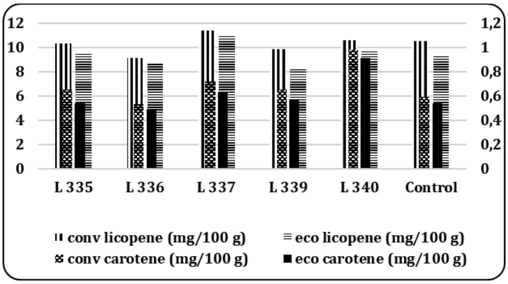

Figure 7. Carotene and lycopene variation

As a general conclusion, sensitive higher level of accumulation was detected in conventional system. All items reflect the accumulation at physiological maturity.
Sweet corn is an appreciated horticultural product, being continuously demanded by farmers.
One of the main reasons can be its short production life and a relatively high price, being considered profitable to be cultivated. Our unit produces seeds for farming and develops different breeding programs to answer farmers request to decrease the cultural inputs and to have available resources for ecological cultivation. The collection under investigation included five lines featured by plant following traits: height ranging from 179 to 193 cm, a total need of 551.3 to 558.3°C till silk appearance, cold test value between 90 and 92, and 15.2 to 21.4% broken plants (Figure 8). Traits related yield varied in quite small limits, cob length from 20 to 23 cm, seed weight per

cob from 93.2 to 99.8 g, and MMB from 241 to 255 g (Figure 9).

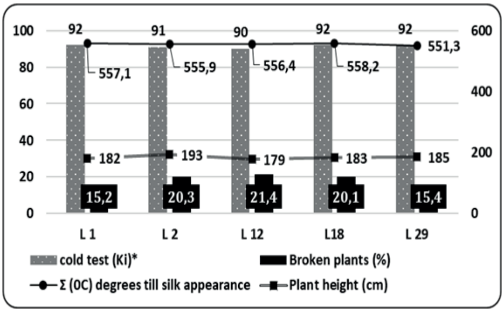

Figure 8. Parameters for plant development

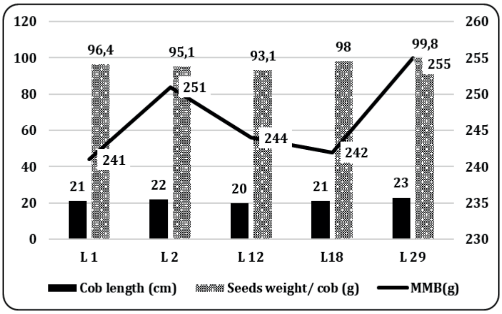

Figure 9. Traits related to yield capacity

Table 4. Sweet corn yield in both cultivation systems

Line	**Yield in conventional system**			
	Yield t ha^{-1}	**%**	**Difference compared to control**	**Signifi-cance**
L 1	12,2	152,5	+4,5	*
L 2	14,1	176,2	+6,1	***
L 12	13,8	172,5	+5,8	***
L18	14,5	181,2	+6,5	***
L 29	15,2	190,0	+7,2	***
control	8,0	100	-	-

DL (p 5%) = 2,10 t ha^{-1}
DL (p 1%) = 3,60 t ha^{-1}
DL (p 0.1%) = 4,40 t ha^{-1}

Line	**Yield in ecologic system**			
	Yield t ha^{-1}	**%**	**Difference comparing control**	**Signifi-cance**
L 1	8,5	151,8	+2,9	**
L 2	9,9	176,8	+4,3	***
L 12	9,7	173,2	+4,1	***
L18	10,2	182,1	+4,6	***
L 29	10,7	191,1	5,1	***
control	5,6	100	-	-

DL (p 5%) = 1,95 t ha^{-1}
DL (p 1%) = 2,60 t ha^{-1}
DL (p 0.1%) = 3,50 t ha^{-1}

Table 4 presents a comparative synthesis for organic and conventional yield. Ecological seed yield registered inferior values. L29 had the best behavior in both system, 15.2 t ha^{-1}, 10.7 t ha^{-1}, respectively.

Table 5. Melon yield in both cultivation systems

Line	**Yield in conventional system**			
	Yield t ha^{-1}	**%**	**Difference comparing control**	**Signifi-cance**
L 555	155	158,2	+57	***
L 556	158	161,2	+60	***
L 557	175	178,6	+77	***
L 558	170	173,5	+72	***
L 559	185	188,8	+87	***
Control	98	100,0	-	

DL (p 5%) = 2,10 t ha^{-1}
DL (p 1%) = 3,60 t ha^{-1}
DL (p 0.1%) = 4,40 t ha^{-1}

Line	**Yield in ecologic system**			
	Yield t ha^{-1}	**%**	**Difference comparing control**	**Signifi-cance**
L 555	8,5	151,8	+2,9	**
L 556	9,9	176,8	+4,3	***
L 557	9,7	173,2	+4,1	***
L 558	10,2	182,1	+4,6	***
L 559	10,7	191,1	5,1	***
Control	5,6	100	-	-

DL (p 5%) = 1,95 t ha^{-1}
DL (p 1%) = 2,60 t ha^{-1}
DL (p 0.1%) = 3,50 t ha^{-1}

The *Cucurbitaceae* species, represented by melon and pumpkin, exhibit proper behavior in ecological cultivation. Melon is highly requested on Romanian market thanks to its exceptional flavor and its application in traditional medicine (especially organic melons). All melon genotypes registered superior yields compared to control in ecological system. Conventional system was slightly more productive. L 559 registered the highest level of yield in both systems (Table 5).

Table 6. Pumpkin yield in both cultivation systems

Line	**Yield in conventional system**			
	Yield t ha^{-1}	**%**	**Difference compared to control**	**Signifi-cance**
L 535	190	190	+90	***
L 536	185	185	+85	***
Control	100	100	-	***

DL (p 5%) = 5,1 t ha^{-1}
DL (p 1%) = 7,6 t ha^{-1}
DL (p 0.1%) = 14,4 t ha^{-1}

Line	**Yield in ecologic system**			
	Yield t ha^{-1}	**%**	**Difference compared to control**	**Signifi-cance**
L 535	133	190	+63	***
L 536	129	184	+59	***
Control	70	100	-	-

DL (p 5%) = 5,85 t ha^{-1}
DL (p 1%) = 7,80 t ha^{-1}
DL (p 0.1%) = 13,80 t ha^{-1}

In the case of pumpkin, two different genotypes were comparatively investigated. The highest value of yield was obtained in conventional system (Table 6).

CONCLUSIONS

The study investigated yield potential (t ha^{-1}) and quality as taste acceptability and visual quality: the total dry matter content (%), total soluble matter, (°Brix), pigments content (mg 100 g^{-1}) carotene and lycopene, vitamin C (mg 100 g^{-1}), shape index. The most productive genotypes obtained highest yield in conventional system against ecological system in case of all species: tomatoes 134 versus 92.8 t ha^{-1}, sweet pepper 50.3 versus 35.23 t ha^{-1}, pumpkin 190 respectively 133 t ha^{-1}, 185 against 129 t ha^{-1}melon and sweet corn 15.2 and 10.7 t ha^{-1}, respectively. Under certain conditions, which includes proper varieties, good management practices, growing conditions, ecological systems can ensure the obtaining of yields, comparable to those achieved in conventional. Considering the multiple benefits of organic, it is expected that agricultural policies will pay more attention to organic, agroecological and low-input agricultural practices, and invest in research and innovation.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III, and ADER 2020 projects developed by VRDS

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE MOST SUITABLE IRRIGATION METHODS IN CABBAGE CROPS (*BRASSICA OLERACEA* L. VAR. *CAPITATA*): A REVIEW

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Abstract

To achieve high horticultural productivity, it is known that an optimal supply of irrigation water is required. Cabbage (Brassica oleracea var. capitata) has been classified as an intermediate sensitive to water stress. Also, the development of plants under drought stress can have effects on quality and productivity, so it is necessary to use water efficiently when it comes to cabbage crops. Also, many studies show that the early effects of stress against plants can be compensated by an optimal supply of water in the growth stages. This review paper collected the latest information from the literature on cabbage water requirements, listing the most effective irrigation methods that lead to water efficiency in crops and irrigation programming to have the best cabbage production in the field.

Key words: *irrigation, cabbage, water efficiency, production.*

INTRODUCTION

Water is the dominant factor for crop diversification and production. More than 80% of water assets have been used for agricultural irrigation (Sitta., 2011). To handle the water-shortage, it is fundamental to adopt water saving agriculture counter measures as adequate use of irrigation water is becoming increasingly crucial.

Irrigated crop production is important for global food security for the reason that it contributes around 40% to the total food supply for the entire world (Garces-Restrepo et al., 2007). Irrigated agriculture covers only 20% of total arable land, however, it consumes more than two-thirds of the total available freshwater (Chai et al., 2016). The world population is growing at an alarming rate, and the demand for good quality, non-saline water is rising considerable (Evans and Sadler., 2008).

Cabbage is one of the most important brassica vegetable cultivated in Romania and is known for its diversity and culinary properties, and for its adaptability to divers environmental conditions (Voican et al., 2002). According to Romanian Ministry of Agriculture and Rural Development the total production of cabbage in 2018 was 1065.5 thousand tons harvested from an area of 17.9 thousand ha.

Irrigation is one of the major uses of water throughout the world. Is very important for every vegetable crop and depending on planting and growing season, cultivated variety and should not exceed the optimal limits because excess of moisture leads to cracking of the heads, disease of the plants and suffocation of the roots (Munteanu et al., 2001).

Cabbage yield is mostly affected by different factors like as irrigation and soil fertility. This vegetable requires high amounts of N, K, S and also needs an optimum soil moisture of 75-80% and even 90% during the growth and maturation of the heads and a relative air humidity of 85-90% (Abdrabbo et al., 2015; Voican et al., 2002).

Increasing the volume of water administrated, head size and weight, leaf weight and, yield can be improved. Cabbage watering methods are numerous, sprinkler irrigation is mostly used and is followed by furrow, border, basin and trickle irrigation methods (Bilal Acar et al., 2006).

Sprinkle irrigation is similar to rainfall, water is distributed through a system of pipes usually by pumping and then sprayed into the air and irrigated entire soil surface through spray heads so that it breaks up into small water drops which fall to the ground (Christiansen., 1942).

Furrow irrigation is one of the most common method of surface irrigation and is mostly associated with low application efficiency and high labour requirements for land levelling.

(Sayari et al., 2019). Moreover, this system greatly reduces the amount of surface wetted, leading to less evapotranspiration (Jemal Nurr, 2018) and even if it has some advantages, it does not ensure a high humidification of the air (Munteanu et al., 2001).

Trickle irrigation consists in a slow process of delivering water to the soil surface through a plastic pipe that is provided with several emitters. (Bucks et al., 1974). A lot of proponents sustain that the most important advantage of trickle irrigation refers to its ability to apply small quantities of water at frequent intervals and leads to improvement of quality and quantity of production, reduce labour cost and increase fertilizer efficiency. (Goldberg et al., 1970)

The purpose of this review investigation was to identify and combine different information on the most suitable irrigation methods in cabbage crops and to evaluate the cabbage water requirements (quantity and frequency of water application) to achieve an increase of production by triangulating different literatures on the topic.

MATERIALS AND METHODS

Cabbage (*Brassica oleracea* L. var. *capitata*) is one of the most widely grown, traded, and consumed horticultural crops worldwide and according to FAOSTAT in 2019 the area harvested of cabbage and other brassicas at monial level was 2 446 294 ha and the production was 70 150 406 tons. In the same year, in Europe the production of cabbage and other brassicas was 9 719 903 tons compare to an area of 325 804 ha. In Romania the total area harvested of cabbage and other brassicas, in 2019, was 28 790 ha and yield were 214 286 kg ha^{-1}. This vegetable is known and preferred by consumers due to its high nutritional value beneficial to human health which is rich in vitamins, fibres, polyphenols and flavonoids (Nawaz et al., 2018).

Tiwari et al. and Stan et al. claim that the nutritional value of 100 g of edible portion of cabbage contains 1.8 g protein, 0.1 g fat, 4.6 g carbohydrate, 0.6 g mineral, 29 mg Ca, 0.8 mg Fe, 14.1 mg Na, and in addition contains the vitamins C in proportion of 50 mg 100 $g^{-1}$and B in proportion of 1.1-1.2 mg 100 g^{-1} and vitamin A. *Brassica oleracea* var. *capitata* is a rich mine of bioactive phytochemicals. The main compounds that can be found in cabbages are carotenoids, alkaloids and phenolic compounds (Ramirez et al., 2020).

Phenolic compounds are considered to grant to the health benefits associated with dietary consumption of *Brassicaceae* species such as anticarcinogenic power, antioxidant capacity, anti-aggregation activity and activation of detoxification enzymes, along others. The main phenolic compounds that can be found in cabbages are kaempferol glucosides and epicatechins (Li et al., 2018; Fusari et al., 2019).

Carotenoids are highly pigmented phytochemicals, some of them as β-carotene and α-carotene and β-cryptoxanthin have provitamin A activity since they act as precursors of vitamin A and, therefore, acquires an important function as a human health promoter. The main carotenoids that can be found in cabbages are luteolin, followed by β-carotene, zeaxanthin, and α-carotene (Kaulmann et al., 2016).

Alkaloids are secondary metabolites of plants synthesized from amino acids. These compounds have also been reported in cabbage cultivars and also in cabbage seeds (Guriya et al., 2015; Khalid et al., 2014).

Cabbage is consumed throughout the year and is a species that easily fits into vegetable crops and, in the same time, can be grown in different systems and types of crops. During transport, temporary storage and recovery, the harvest is less perishable and the expenses per unit area are reduced due to the complete technology of the works (Stan et al., 2001).

Among various factors affecting crop growth, water and fertilizer are the leading components that can be adjusted and controlled. In certain agricultural production, to obtain greater yields, redundant water use has become standard practices, and these habits not only leach nutrients from surface soil to deep soil, reducing water and nutrient use capability (Quiñones et al., 2007; Li et al., 2017), but may also cause soil environmental degradation (Zhang et al., 2011; Kuscu et al., 2014).

RESULTS AND DISCUSSIONS

CABBAGE WATER REQUIREMENTS

Cabbage has been classified as intermediately vulnerable to water stress, with the head formation cycle being more sensitive than the cycle before (Adeniran et al., 2010). The trials of these authors were in line with (Thomas et al., 1970) which reported that the most demanding irrigation period for cabbage appeared during the last 3 to 4 weeks before harvest.

Cabbages need frequently irrigation to ensure hasty growth and evenness of maturity. They can be irrigated by moveable spray lines, travelling irrigators or solid set, or, if the soil is suitable and water available, flood irrigation might be more productive. Likewise, cabbages grown in beds will require more irrigation than those grown on the flat. On the other hand, soil type and weather will also influence the frequency of irrigation. Hence, the use of tensiometers or other measuring equipment will improve yields and reduce water costs (Murison., 2006).

Doorenbos and Pruitt (1977) define the crop water requirements as "the depth of water needed to meet the water loss through evapotranspiration (ET crop) of a disease-free crop, growing in large fields under non-restricting soil conditions including soil water and fertility and achieving full production potential under the given growing environment". Crops are categorized according to their yield response to water namely: crops sensitive to water stress and those tolerant to drought. Cabbage has been classified as an intermediate sensitive to water stress, and the highest requirements for water are manifested in the formation phase of the head, but also during the planting and formation of leaf rosette even if the quantitative requirements are lower (Stan et al., 2001).

Cabbage should be irrigated to maintain available soil water above 50% because an insufficient amount of water leads to a decrease in quantitative and qualitative production. (Vittum et al., 1967). The critical period for irrigation in humid and semi humid regions is usually during the last 3 to 4 weeks of head formation and development. Irrigation not only increases the acre yield of marketable cabbage but also increases the average weight per head (Nettles et al., 1952; Vittum and Peck, 1956).

Cabbage has been made known as intermediately susceptible to water stress, with the most critical irrigation period occurred during the last 3 to 4 weeks before harvest (Smittle et al., 1994). Efficient water management is a prerequisite to successive cabbage production. Water requirements vary from 380 to 500 mm depending on climate and length of growing season after (FAO water development and management unit, 2012).

Cabbage does not tolerate drought and prefers cultivation on a modeled field so that it can be irrigated on furrows. Usually, early cabbage is less irrigated due to the water reserve in the soil in winter-spring and the short vegetation period, the rest of the cabbage species are irrigated abundantly during the vegetation period depending on the climatic conditions of that year. Muddling should be avoided because it promotes the appearance of diseases, especially cabbage hernia (*Plasmodiophora brassicae*) (Stoian., 2006).

In a research of Smittle et al. (1994) was studied the yield and water-use responses of cabbage to three irrigation regimes. Water was applied with a hand-held sprinkler nozzle and irrigation was scheduled by measured soil water deficits. Total production of cabbage and marketable cabbage yield were highest with irrigation application when the soil water tension at 10 cm was less than 25 kPa.

According to Mihalache et al. (1985), at a precipitation amount of 400-500 mm, the irrigation regime and scheme for early cabbage, summer cabbage and autumn cabbage is presented in Table 1.

Irrigation depending on climate crop development, and soil type and the frequency of irrigation varies between 3-12 days. If available water supply is limited, early irrigation should not be practiced unless these can be continued until the end of the crop growing period. Water saving could be made in the beginning of the crop growing period (Maynard and Hochmuth, 2007).

Table 1. Irrigation regime and watering scheme o cabbage

Crop	The amount of precipitation: 400-500 mm			
	Watering norm m^3 h^{-1}			Watering scheme
	Irrigation	Watering	No. of watering	
Early cabbage	2400-2600	400-500	5-6	I-II: after 10-12 days: 300-350 m^3 ha^{-1}; III-IV: after 8-10 days: 400 m^3 ha^{-1}; V – 400-500 m^3 ha^{-1};
Summer cabbage	3000-3500	400-600	4-5	I-II: after 10 -12 days 400-450 m^3 ha^{-1}; III-V: 400-500 m^3 ha^{-1};
Autumn cabbage	4000-4500	500-600	7-8	I-IV: frequently, after 6-7 days: 400-450 m^3 ha^{-1}; V-VII: after 8-10 days: 500-600 m^3 ha^{-1};

IRRIGATION METHODS

To ensure the optimal use of water resources and the distribution uniformity of water is at an acceptable level, the condition of sprinkler packages, the pressure variation within the system, the strength and direction of wind, needs to be correctly manage. (Ascough., 2002).

Sprinkler irrigation have some benefits like as: produces a favourable effect on the microclimate, eliminates the division of the land through a dense network of canals, allows watering of crops located on lands with high permeability and allows accurate dosing of irrigation water (Stan., 2010).

Sanchez et al. (1994) found a significant yield increase when a sprinkler irrigation is applied and sprinkler-applied N fertilizer on a coarse-textured soil. In the same study it is also argued that the deficit and excess irrigation reduce cabbage production.

In a study carried out in Malawi on dry season of 2006-2007 it was evaluated yield response of cabbage to irrigation frequency. The cabbage was subjected to three irrigation frequencies: F1 – irrigated twice a week, F2 – irrigated once a week and F3 – irrigated once a fortnight. The irrigation system used in this experiment was furrow irrigation and the water was applied through PCV pipes with a diameter of 10 cm, in furrows 5 m long. F1 resulted in highest yield and WUE (water-use efficiency) while F3 yielded lowest. Comparing all the irrigation frequencies, F3 turns out to be the most effective water saving irrigation frequency (Kadyampakeni., 2013).

On the other hand, Singh and Alderfer have demonstrated that when applying an irrigation at 100 kPa soil water tension during growth and 1000 kPa before head formation and 100 kPa after head formation, marketable yields of cabbage are similar (Singh et al., 1966). In Texas, when cabbage was produced during the winter and was applied an irrigation at 360 kPa soil water tension, the yield was reduced (Thomas et al., 1970).

In a study release in 2016 in Romania about water use efficacy on cabbage and cauliflower treated with a new biostimulator composition, total irrigation for cabbage vegetation period was 334 mm and 250mm for 100% and 75%, respectively and was demonstrated that a percent of 75% ETc (evapotranspiration crop) deficit irrigation significantly reduce total and marketable yield.

Optimum yield of cabbage the soil water deficiency in most climates should not exceed 35% to 40% of the total available soil water. Due to this light irrigation administration are recommended. Therefore, studies show the effects of varying water supply on the water regime and productivity of head cabbage. Field trials with mid-early and mid-late cabbage cultivars using sprinkler irrigation to maintain field water capacity at 80 per cent resulted in highest yield (Gancharyk and Paulenka., 1975).

For the cabbage crops it is necessary to know the quantity of water and when it should be applied for enhancing its productivity. Drip irrigation is one of the irrigation methods that will help to apply water evenly to the field in the area of the roots and at the same time at the desired intervals. In a field experiment conducted in India indicate that a water use efficiency was registered in treatment with drip irrigation at 60% ET (evapotranspiration) scheduling at once in two days in the first year. For higher productivity of cabbage head and higher water use efficiency, drip irrigation is very effective at 80% ET compared to irrigation at 100% ET scheduling at once in two days (Harris et al., 2014).

Xianbing et al. performed a study in 2020 on fertigation on cabbages grown in a greenhouse and as result, they recommend that when cabbage is planted in greenhouses, the irrigation application of drip irrigation under mulch should be approximately 114.7-125.0 mm and the N fertilization should be 200 kg ha^{-1}.

In a study conducted by Fasika Terefa in 2017 the results obtained from the experimentation showed that sprinkle irrigation scheduling at six days and nitrogen fertilizer at an amount of 92 kg ha^{-1} were optimum for cabbage growth. This optimum treatment combination resulted in higher nitrogen content (3.35%), higher nitrogen recovery (89.9%) and higher water use efficiency (152.47 kg ha^{-1} mm^{-1}) for cabbage crop. Comparing of farmer practice with 6 days irrigation scheduling and 92 kg of nitrogen fertilizer and with 3 days and 9 days irrigation scheduling by 92 kg ha^{-1} nitrogen fertilizer all the above variables were greater in this optimum treatment combination than in farmers usual management.

It is essential to develop irrigation scheduling strategies under local climatic conditions to utilize limited water resources efficiently and effectively.

CONCLUSIONS

Water requirement of cabbage varies with climate, crop variety, soil type, and agricultural practices. On sandy soil frequent cultivation of cabbage is highly productive in comparison to clay soil. This is because of down word and lateral movement of soil water is prevailed in sandy and clay soil respectively. As follows, yield and water use efficiency of irrigated cabbage generally increased with increasing levels of management options. As a result, crop water requirement is comprehensive to crop modelling strategies which are dedicated to sustainable agriculture particularly in the view of climate change. Thus, crop production should be supported by prediction data.

In summary, the optimum yield was achieved by providing an amount of irrigation water of not less than 400m^3 ha^{-1} sprinkle and furrow irrigation. On the other hand, organic acid content was higher in cabbage grown under low soil water content. Likewise, the amount of water used for drip irrigation should be approximately 125-150 m^3 ha^{-1} without exceeding six days between irrigation moments for any type of irrigation method.

Also, it is important to keep into account the amount of precipitations throughout the vegetation period and the climate of the area where the crop is being established. It is critical to not skip the most precarious moments of watering, and these stages are when the seedling has 6-7 leaves and at the beginning of the head formation.

These results can be considered as a strategy for water management in white cabbage (*Brassica oleracea* L. cv. *capitata*).

ACKNOWLEDGEMENTS

This research work was carried out with the support of MADR and ASAS Project no. 529/2018. For the doctoral thesis entitled “Research for improving and developing new technological links in the production of white cabbage seeds (*Brassica oleracea*, var. *capitata*, f. *alba*)”, contract no. 11834/2020.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VITALITY DYNAMICS OF POPULATIONS OF SOME LEGUME SPECIES IN FLOODPLAIN MEADOWS OF THE PSEL RIVER BASIN UNDER GRAZING AND HAYMAKING (UKRAINE)

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Abstract

The results of the study of the vitality structure and its dynamics in the three species of the Fabaceae family (Trifolium repens L., Medicago falcata L. and Vicia cracca L.) in the floodplain meadows of the northeastern Forest-Steppe of Ukraine under grazing and haymaking are presented. The analysis of the vitality structure of the populations of the three studied species of legumes in the control areas of the pascual and fenisicial gradients has shown that they are well adapted for existence in conditions without anthropogenic loads. It has been established that Trifolium repens is more resistant to both grazing and haymaking loads. This is evidenced by the preservation of the equilibrium status of the populations until the last stages of both gradients, as well as the obtained indices of vitality dynamics (IVD, in most cases less than 1, with the positive and negative signs). The Vicia cracca populations are characterized by considerable vulnerability to pasture loads, which is manifested in the sharp transition of populations to the category of depressed at the stages of PD1. This species completely falls out of the composition of the meadow phytocoenosis at the stage of the gradient PD2. In relation to the haymaking load, the species is quite resistant and can withstand both one-time (FD1 stage) and two-time (FD2 stage) haymaking per season, where the populations have the status of equilibrium, and IVD is less than 1. Haphazard haymaking causes significant changes in the vitality composition (IVD is greater than 1 with the negative sign) and the transition of populations to the category of depressed. The populations of Medicago falcata are well adapted to moderate grazing loads (stages PD1 and PD2), as evidenced by IVD less than 1 and maintaining the status of populations at the level of prosperous and equilibrium. Starting with the stage PD3, the populations undergo significant changes in vitality composition (IVD = -1.5060) and transfer to the category of depressed. On the fenisicial gradient, the Medicago falcata populations demonstrate resistance at the FD1 and FD2 stages. Only under haphazard haymaking (FD3), vitality composition is significantly transformed, as evidenced by the depressed status of their populations. The data obtained indicate the need to regulate both grazing and haymaking loads in order to preserve the integrity of meadow ecosystems and ensure the sustainable, long-term and effective provision of a range of ecosystem services by them.

***Key words**: index of vitality dynamics, Medicago falcata, population quality index, Trifolium repens, Vicia cracca, vitality structure.*

INTRODUCTION

The conservation of biodiversity as a primary and critical indicator of the efficient operation of ecosystems is the centerpiece of a list of global environmental problems, the solution of which is the objective of a significant amount of research (Kaur, 2018; Rahman, 2018; Sui et al., 2018; Cardinale et al., 2011; Wilsey, 2018; Segelbacher, 2018).

Floodplain meadow ecosystems, the formation of which is the result of both natural processes and human economic activities, act as a reserve of unique biodiversity, and are also stabilizers of the natural environment and valuable forage lands. The conservation of their population-species integrity is the basis for the sustainable operation of the biosphere as a whole (Reine et al., 2014; Tilman and Downing, 1994; Wilsey, 2018; Huhta and Rautio, 2014; Hofman et al., 2001; Harvolk et al., 2015). Floodplain meadows are in a state of constant dynamic development - edaphogenic changes, which occur primarily under the influence of river flooding (alluvium sediments, groundwater level, surface sediment washing-off, etc.) (Hölzel and Otte, 2004). This process is also affected by the mode of use of meadows, the main forms of which are grazing and haymaking (Rui et al., 2010; Sizykh, 2016; Biró, 2014; Rusev et al., 2007; Kahmen and Poschlod, 2008; Harvolk et al., 2015; Mathar et al., 2016; Díaz et al., 2007). The result of a significant anthropogenic impact is the marked transformation of meadow ecosystems and a decrease in their productivity (Huhta and Rautio, 2014; Wellstein et al., 2007). In this regard, the problem of conservation of floodplain meadows has both regional and global significance (Liira et al., 2009; Rui et al., 2010; Biró, 2014; Mathar et al., 2014; Mathar et al., 2016). This is precisely why the parameters of meadow areas and their condition are used as the indicators for agricultural greening, and some countries are actively developing different approaches to meadow restoration and conservation (Magda et al., 2014; Trnka et al., 2016).

It is known that the main form of species existence is the population, at the level of which all the processes and mechanisms associated with the response of organisms to various environmental impacts are implemented (Zlobin, 2009). That is why one of the ways to organize scientifically grounded natural resource management, including floodplain meadows, is the widespread use of population analysis (Begon et al., 1986; Zlobin, 2009; Gibson, 2014; McCall, 2017). The most important component of population monitoring is the assessment of the life status of individuals and plant populations. One of the tools of this assessment is a vitality analysis (Zlobin, 2018). Recently, it has become widespread in population studies due to its informative value (Sherstyuk, 2017; Skliar, 2013; Tikhonova, 2011; Vöge, 2015; Thazaplizheva and Chadaeva, 2010; Gavrilova, 2008). In particular, part of works is devoted to the study of the vitality structure of meadow species (Kyrylchuk, 2007; Bondarieva and Bjelan, 2010; Kyrylchuk and Bashtovyi, 2018). Legumes, as one of the most important components of meadow phytocenoses, along with cereals and herbs, ensure the integrity of a meadow ecosystem, and the conservation of the full floristic, population and cenotic diversity of floodplain meadows is an urgent scientific problem. Within this framework, the goal of this study is to assess the features of operation of the populations of some species of legumes, which are part of the floodplain meadow phytocenoses of North-Eastern Ukraine by establishing patterns of change in their vitality parameters in terms of grazing and haymaking.

MATERIALS AND METHODS

The analysis of vitality dynamics of populations of meadow legume species was carried out in the pascual and fenisicial gradients in floodplain meadows of the Psel River (forest-steppe zone of North-Eastern Ukraine). The objects of the study were the populations of the three species of legumes typical for meadow stands of the study region: *Trifolium repens* L., *Medicago falcata* L. and *Vicia cracca* L. In two of the studied species (*T. repens* and *V. cracca*) the accounting units were ramets, and in *M. falcata* - genets.

Areas corresponding to different stages of pasture and haymaking digressions were chosen to study the dynamics of changes in vitality parameters of individuals and populations under increasing pasture and haymaking loads. Pasture or pascual digression (PD) is all changes in vegetation and soils that occur under the influence of increasing grazing intensity. Pascual digression gradient (PD) included the five stages chosen on the basis of a set of features, among which the main place was occupied by data from farms on the actual load on pasture, as well as the floristic analysis of species, the ratio of projective cover of dominant and minor species, a decrease in the height of grass stand, the transformation of population parameters of individuals of indigenous species towards their oppression, the appearance of synanthropic species as part of the plant population, etc.: PD0 - control area (CA) - areas without anthropogenic impact; PD1 - the initial stage of grazing (moderate grazing with a load of 2-3 heads of cattle per 1 ha; PD2 - intensive grazing; PD3 - the stage of heavy grazing, where the number of cattle heads increases to 10-12 per 1 ha; PD4 - outrageous haphazard grazing (failure).

Haymaking or fenisicial digression (FD) represents all changes in floristic composition and structure of meadow coenosis phytopopulations occurring as a response to the growth of haymaking loads. The fenisicial gradient included four stages: FD0, or control area (CA); FD1 - the initial stage (haymaking once a year); FD2 - moderate haymaking (twice

a year); FD3 - excessive haymaking (multiple haphazard haymaking).
The vitality analysis was made by the three stages provided for by the algorithm developed by Yu. A. Zlobin (Zlobin, 2018). The first stage included the morphometric analysis of individuals of legume meadow plant populations (Zlobin et al., 2009). A set and number of morphoparameters were determined by the peculiarities of the species morphology (they were about 10 dimensional parameters in the studied species). The key parameters, which provided an integral assessment of the vitality of individuals, were selected from the obtained number of quantitative indicators characterizing the life state of individuals (Zlobin, 2009). For the studied species such parameters were: W - total aboveground phytomass of an individual (g), A - leaf surface area (cm^2) and Re -reproductive effort (%). The assessment of the vitality of some individuals was carried out at the second stage. According to the methodology of vitality analysis, individuals of populations of the studied species at each stage of pascual and fenisicial gradients were referred to one of the three classes of vitality: “a” - high, “b” - medium and “c” - low. The integral assessment of vitality of populations is a third stage of vitality analysis, on which each population receives a certain vitality status - prosperous, balanced and depressed, based on the generalized indexes of population quality (Q).
The comprehensive assessment of dynamics of vitality parameters of the studied species populations on the pascual and fenisicial gradients and the study of vitality flexibility were carried out on the basis of the index of vitality dynamics (IVD), which was calculated by the following formula (Skliar, 2013):

$$IVD = (Q_n – Q_p) / 0.166,$$

Where: Q_n is the value of the population quality index at the next stage of the gradient;

Q_p is the value of the population quality index at the previous stage of the gradient;

0.166 is the value of the quality index, at which there is the transition of populations from one qualitative type to another (according to the classical vitality analysis, when the value of the quality index Q ranging from 0 to 0.166 the population is depressed; at Q ranging from 0.167 to 0.332 this population is equilibrium; at Q ranging from 0.333 to 0.50 it is prosperous).
The value of the index of vitality dynamics (IVD) lies in the range from -3.012 to + 3.012. At IVD = 0, there are no changes in the value of the quality index Q by pascual and fenisicial gradients in populations. If IVD (in magnitude) is less than 1, changes are insignificant. If IVD (in magnitude) is between 1 and 2, changes are significant. If IVD (in magnitude) is greater than 2, changes are progressive. If values of IVD are negative, there is some deterioration in the population state, if values of IVD are positive, there is some improvement (Skliar 2013).

RESULTS AND DISCUSSIONS

The obtained research results have shown a more or less pronounced transformation of the vitality structure of populations of the studied legume species on the pascual and fenisicial gradients. The analysis of vitality structure of such populations as *T. repens, M. falcata* and *V. cracca* in the control areas has shown that they belong to the category of prosperous populations with the population quality index of 0.48; 0.48 and 0.45, respectively. This indicates a good adaptation of species to existence in meadow areas without anthropogenic impact (Figure 1).
Subsequently, on both gradients the population quality index is gradually decreasing that is connected with change in a ratio of individuals of different vitality classes, and the populations change their status (Figures 2-4). Figure 2 shows the dynamics of changes in the ratio of individuals of different vitality classes in the populations of *T. repens* both on the pascual and fenisicial gradients. The proportion of individuals of “a” and “b” classes in the populations of this species remains quite high even at the last stages of the gradient. Thus, at the stage of the gradient PD4 the proportion of individuals of “a” and “b” classes are respectively 0.28 and 0.24. On the fenisicial gradient, qualitative changes in the species populations are associated with a gradual decrease in the proportion of individuals of “a” class to 0 on FD3 with a sufficiently high proportion of individuals of “b” class - 0.50 (Figure 2). According to this, the populations of

T. repens on both gradients change their status to a minor degree, and transfer from the category of prosperous to equilibrium and retain it until the last stages.

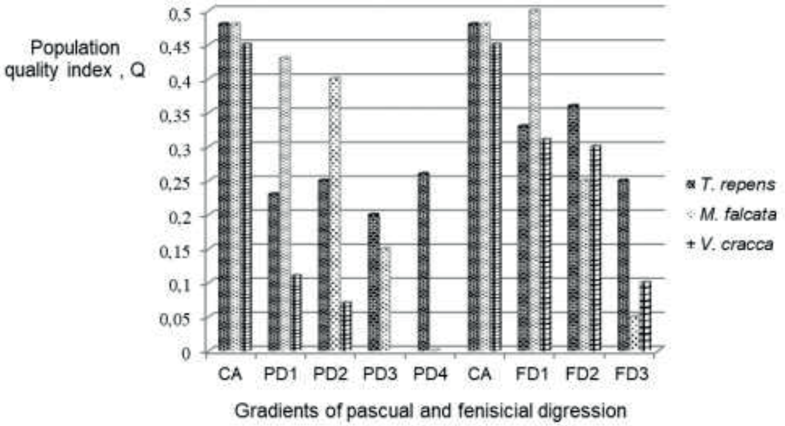

Figure 1. Quality indices (Q) of populations of *Trifolium repens, Medicago falcata* and *Vicia cracca* on the gradients of pascual and fenisicial digression

The vitality structure of the *M. falcata* populations varies in a greater degree, in contrast with *T. repens* (Figure 3). By the pascual digression gradient, the population quality indices are gradually decreasing, and the most significant decrease is observed at the stage of PD3, where the population quality index is 0.15, and at PD4 it is already equal to 0 (Figure 1). By the gradient there is a gradual decrease in the proportion of individuals of “a” and “b” classes (up to 0 per PD4) against the background of an increase in the proportion of individuals of “c” class. As a result, the populations transfer to the category of depressed at the stage of PD3. On the fenisicial gradient, an increase in haymaking load leads to a natural decrease in the quality index to 0.05 on FD3 (Figure 1). This is also due to an increase in the populations of individuals of the lower vitality class. It should be noted that at the initial stage of grazing PD1 and the first stage of fenisicial gradient FD1, the populations retain the status of prosperous that indicates the efficient operation of the populations of the studied species under moderate economic loads.

The greatest vulnerability to grazing is observed in the populations of *V. cracca* - this species split from the meadow grass stand at the stages of PD3 and PD4. The vitality structure of this species is significantly transformed on the grazing gradient - the quality indices are reduced by the gradient from 0.45 per TA to 0.07 per PD2 (Figure 1). At the stage of PD1, the population *V. cracca* already belongs to the category of depressed. They are characterized by a significant decrease in the proportion of individuals as “a” and “b” classes (Figure 4). In contrast to grazing loads, the population of *V. cracca* is more tolerant of haymaking loads. At the FD1 and FD2 stages, the populations retain the status of equilibrium and only at the FD3 stage become depressed, as the proportion of individuals of “a” and “b” classes decreases (Figure 4).

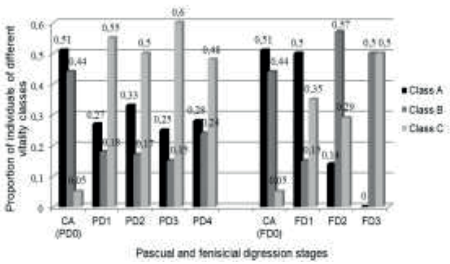

Figure 2. Vitality spectrum of *Trifolium repens* populations on the pascual and fenisicial gradients

The results obtained on the vitality structure of legumes reflect their adaptation to different types of economic loading, which is manifested in a change in the proportion of individuals of different vitality classes (“a”, “b” and “c”) and demonstrates the implementation of vitality variability. In general, with outrageous grazing and haymaking loads, there is an increase in the total proportion of individuals of the middle and lower classes of vitality, while reducing the proportion of individuals of the higher class, sometimes to their complete disappearance.

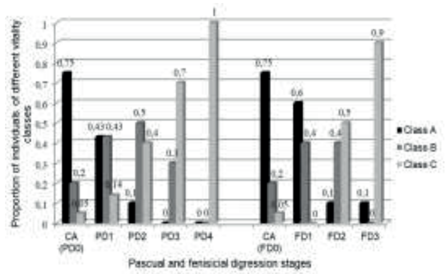

Figure 3. Vitality spectrum of *Medicago falcata* populations on the pascual and fenisicial gradients

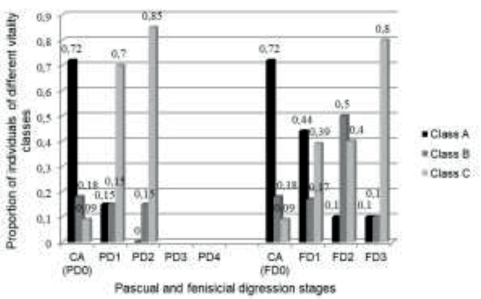

Figure 4. Vitality spectrum of *Vicia cracca* populations on the pascual and fenisicial gradients

The vitality variability of the studied species of legumes on the pascual gradient manifests itself in the form of three variants (Figures 2-4). The first of them demonstrates the populations *T. repens*, in which there is a sharp increase in the total proportion of individuals “b” and “c” at the stage of the gradient PD1 and further by the gradient this parameter is almost unchanged: 0.49 (PD0); 0.73 (PD1); 0.67 (PD2); 0.75 (PD3); 0.72 (PD4). In the *T. repens* populations, the proportion of individuals of “a” class remains unchanged until the last stage of the gradient (PD4), where their proportion is 0.28. The second variant is characteristic of *M. falcata,* in the populations of which this parameter naturally increases by the gradient. This trend has the following form: 0.25 (PD0); 0.57 (PD1); 0.9 (PD2); 1 (PD3); 1 (PD4). In the populations of this species, individuals of “a” class disappear at the stage of PD3. The last variant of manifestation of vitality variability is presented by the populations *V. cracca,* in which there is the sharpest increase in a total proportion of individuals “b” and “c” at the stage of PD1. At the stage of PD2, this sum is already equal to 1 that indicates the absence of individuals of class “a” in the population. The changes are as follows: 0.27 (PD0); 0.85 (PD1); 1 (PD2). Subsequently, by the gradient the populations of this species completely fall out of the grass stand, demonstrating a significant vulnerability to grazing.

The acute similarity is observed in the response of all three species of legumes to haymaking load that is manifested in a gradual natural increase in the total proportion of individuals of the middle and lower classes of vitality. This trend is as follows: in *T. repens* – 0.49 (FD0); 0.5 (FD1); 0.86 (FD2); 1 (FD3); in *M. falcata* – 0.25 (FD0) – 0.4 (FD1) – 0.9 (FD2) – 0.9 (FD3); in V. *cracca* – 0.27 (FD0) – 0.56 (FD1) – 0.9 (FD2) – 0.9 (FD3). In the populations of *M. falcata* and *V. cracca*, individuals of “a” class remain even at the last stage of the FD3 gradient (Figures 2-4).

In addition to vitality variability, the operation of legume populations on the pascual and fenisicial gradients is accompanied by the manifestation of vitality flexibility, which is associated with a change in the quality index Q by the stages of gradient. The index of vital dynamics (IVD) and change in the status of populations during their transition from one stage to another have been used for its evaluation (Tables 1, 2).

Table 1. Values of the index of vitality dynamics (IVD) and change in the qualitative type of legume populations on the pascual gradient

Vitality parameters	Transition by the stages of gradient			
	CA (PD0) → PD1	PD1 → PD2	PD2 → PD3	PD3 → PD4
Trifolium repens				
IVD value	-1.5060	0.12048	-0.3012	0.3614
Change in population type	P→B	B-B	B-B	B-B
Medicago falcata				
IVD value	-0.3012	-0.1807	-1.5060	-0.9036
Change in population type	P-P	P→B	P→D	D-D
Vicia cracca				
IVD value	-1.8072	-0.2409	-	-
Change in population type	P→D	D-D	-	-

Table 2. Values of the index of vitality dynamics (IVD) and change in the qualitative type of legume populations on the fenisicial gradient

Vitality parameters	Transition by the stages of gradient		
	CA (FD0) → FD1	FD1 → FD2	FD2 → FD3
Trifolium repens			
IVD value	-0.9036	0.1807	-0.6626
Change in population type	P→B	B → P	P→B
Medicago falcata			
IVD value	0.1205	-1.5060	-1.2048
Change in population type	P-P	P→B	B→D
Vicia cracca			
IVD value	-0.8434	-0.0602	-1.2048
Change in population type	P→B	B-B	B→D

Depending on trends in the vitality dynamics indices by the pascual digression gradient, legumes implement two response tactics (Table 1). The first of them belongs to *T. repens* and *V. cracca*, in the populations of which there is a significant decrease in IVD at the first stage of the PD1 gradient. It should be noted that a sharper decline is typical for *V. cracca*, the populations of which change their status immediately from prosperous to depressed. The populations *T. repens* transfer to the category of equilibrium. Further, IVD by the gradient changes slightly. In this case, in *T. repens* during the transition PD1 → PD2 → PD3 → PD4, the index of vitality dynamics has the positive sign that indicates even a slight improvement in the state of individuals of the populations. *M. falcata* shows the second variant of response to the increase in pasture loads, which manifests itself in the form of minor changes during the transitions PD0→PD1 and PD1→ PD2 (in the first variant, the status does not change and remains prosperous, and in the second one it changes to equilibrium). Significant changes in the qualitative vitality composition of the populations of this species are observed during the transition of PD2 → PD3, when the populations change their status to depressed. The transition to the last stage of the gradient is accompanied by minor negative changes in the vitality structure of the populations at maintaining the depressed type (Table 1).

The analysis of the indices of vitality dynamics on the pascual gradient has enabled to distinguish the two groups by the features of response of the studied legume species (Table 2). The first group is represented by the populations of *T. repens*, the IVD value of which indicates minor changes in vitality structure until the last stage of gradient, mostly with the negative sign. At the same time, the status of populations after its change to PD1 remains unchanged - equilibrium. The second group is represented by *M. falcata* and *V. cracca*, in the populations of which there are significant changes in the index of vitality dynamics at different stages of gradient. Thus, in *M. falcata*, these abrupt changes occur at the transitions of FD1 → FD2 (the population changes its status to equilibrium) and FD2 → FD3, whereupon the population turns into depressed. In the populations *V. cracca*, significant changes are characteristic during the transition to the last stage of the gradient FD2 → FD3, where the population changes its category to depressed (Table 2).

The analysis of the indices of vitality dynamics and transformation of the quality type of legume populations by both gradients has shown that most indices have the negative sign (78.9% of the total number that indicates the marked deterioration in the populations state under the influence of economic loading (primarily outrageous). The share of indices with the positive sign was 21.1%. Most of the indices of vitality dynamics (68.4%) were less than 1 that, according to the IVD scale, corresponds to minor changes in the vitality structure of populations. Indices with significant changes in the vitality structure of populations (from 1 to 2) account for 31.6%. The type of populations changed in 57.0% of the cases. The most common changes were P→B (31.6%), B-B (21.0%) and B→D (15.8%). The types of changes D-D (10.5 %), P-P (10.5 %), P→D (5.3%) and B→P (5.3%) rarely occurred.

Haymaking and grazing at the present stage in many regions are indeed powerful factors in the transformation of meadow communities and populations of coenosis forming species (Shushpannikova, 2014; Biró, 2014). The obtained results of study on the dynamics of the vitality structure of legume populations in floodplain meadows under the influence of the above economic loads indicate the availability of certain adaptive mechanisms that are involved in the populations to ensure their survival. Pronounced tolerance to various environmental factors was also observed in other species of the *Fabaceae* family. Thus, *Hedysarum alpinum* L. (the territory of Baikal Siberia) can grow both in favorable conditions of floodplain meadows and extreme permafrost conditions due to significant anthropopressing (Karnauhova and Sandanov, 2015; Karnauhova, 2018). The transition of populations from the category of prosperous to depressed with the increase of anthropogenic loads is typical not only for the populations of *M. falcata* and *V. cracca*. Similar changes in the conditions of floodplain meadows of the Forest-Steppe of Ukraine under the influence of excessive grazing and haymaking are typical for other legumes as well (Zlobin and Kirilchuk, 2005; Kyrylchuk, 2007; Kyrylchuk and Bashtovyi, 2018; Bondarieva et al., 2019), and for meadow grasses, in particular for the populations of *Dactylis glomerate* L. (Bondarieva and Bjelan, 2010, Zlobin et al., 2010). Moreover, a significant transformation of vitality spectra under the influence of pasture loads with the transition of populations from the status of prosperous to depressed was observed in the populations of such legume species as *Gueldenstaedtia monophylla* Fisch in the desert steppes of northwestern Mongolia (Seljutina et al., 2017) and *Oxytropis sulphurea* (Fisch. ex DC.) Ledeb. (Ore Altai and Saur Ridge) (Seljutina and Zibzeev, 2016). The populations of *T. repens* are characterized by pronounced stability in relation to economic loads that is manifested in the fact that the vitality status has changed only from prosperous to equilibrium and remained the same until the last stage of the gradient. Some meadow grasses, in particular *Festuca pratensis* Huds and *Phleum pratense* L. show similar resistance (Bondarieva and Bjelan, 2010). The ability of populations of coenosis forming species, including *T. repens, M. falcata* and *V. cracca*, to implement certain adaptation mechanisms and to show a certain level of resistance to anthropogenic impacts, are the factors that contribute to the sustainable provision of ecosystem services by meadow phytocenoses. The assessment of this aspect of meadow operation is also a relevant scientific problem, especially provided that the anthropocentric approach to assessing services is increasingly changing to an ecological one in modern conditions (Didukh, 2018).

CONCLUSIONS

The analysis of the vitality structure of the populations of the studied legume species in the control areas of the pascual and fenisicial gradients has shown that they are well adapted to existence in conditions without anthropogenic loads. *T. repens* is the most resistant species both to grazing and haymaking loads, as evidenced by the preservation of the equilibrium status of populations by the species until the last stages of both gradients, as well as the obtained indices of vitality dynamics (IVD, in most cases, less than 1, both with the negative and positive signs). The populations of *V. cracca* are characterized by significant vulnerability to grazing loads, which is manifested in a sharp transition of populations to the category of depressed at the stage of PD1. This species completely falls out of the meadow phytocoenosis at the stage of the gradient PD2. In relation to haymaking loads, the species is quite resistant and can withstand both one-time (FD1 stage) and two-time (FD2 stage) haymaking per season, where the populations have the status of equilibrium, and IVD is less than 1. Haphazard haymaking causes significant changes in the vitality composition (IVD is greater than 1 with the negative sign) and the transition of populations to the category of depressed. The populations of *M. falcata* are well adapted to moderate grazing loads (stages PD1 and PD2), as evidenced by IVD less than 1 and maintaining the status of populations at the level of prosperous and equilibrium. Starting with the stage PD3, the populations undergo significant changes in vitality composition (IVD = -1.5060) and transfer to the category of depressed. On the fenisicial gradient, the *M. falcata* populations demonstrate resistance at the FD1 and FD2 stages. Only under haphazard haymaking (FD3) vitality composition is significantly transformed, as evidenced by the depressed status of their populations. The data obtained indicate the need to regulate both grazing and haymaking loads in order to preserve the integrity of meadow ecosystems and ensure the sustainable, long-term and effective provision of a range of ecosystem services by them.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

BIOACTIVE COMPOUNDS CONTENT AND ANTIOXIDANT ACTIVITY IN THE LEAVES OF SOME SWEET POTATO CULTIVARS (*IPOMOEA BATATAS* L.)

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Abstract

This study was aimed to determine the content of bioactive compounds and the antioxidant activity of sweet potato leaves, to encourage the consumption of the vegetative organs of this plant (leaves) and to identify new sources of natural antioxidants. The studied biological material was represented by six sweet potato cultivars cultivated in the Didactic Field (2018) of the Faculty of Horticulture in Craiova. The total dry matter content (DMC), the content of phenolic compounds and flavonoids, the enzymatic activity of catalase and peroxidase and the antioxidant activity (DPPH and ABTS) were determined. Cultivar 3 with the highest enzymatic activity of catalase (6.6 mM H_2O_2/g/min fm) and peroxidase (615.8 ΔA/min/g fm) and cultivar 6 with the highest content of phenolic compounds (10.237 mg GAE/g fm) were noted and flavonoids (9.73 mg QE/g fm) as well as the highest antioxidant activity (72.18 µM TE/g fm by DPPH method and 75.22 µM TE/g fm by ABTS method). The results suggest that the leaves of this species should be used as valuable food to cope with future changes in food supply and demand, especially in developing countries.

Key words: *peroxidase, catalase, phenols, flavonoids, antioxidant activity.*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) has become a research topic in recent years due to its unique nutraceutical and functional properties. It is the sixth most important food crop in the world, with an annual production of tuberous roots of over 178 million tons in 2014 (FAOSTAT, 2017). This culture has tolerance to diseases and pests, to water and heat stress (Dinu et al., 2015). The unique composition of the species has various benefits for human health such as: antioxidant, anti-inflammatory, antitumor, antidiabetic, antimicrobial, anti-obesity effects. Sweet potato is characterized by a production represented by the tuberous root, shoots, petiole and leaf. The daily consumption of tuberous roots was estimated at 17.70 g/day in the Korean population, and that of petiole was represented at a small amount of 0.42 g/day (KHIDI, 2014). Recent studies have shown that sweet potatoes contain many functional components such as phenols, flavonoids, dietary fiber that improve the health of the population.

It should be noted that in addition to the significant amount of tuberous roots, this species annually produces over 70 million tons of biomass, which is mostly discarded (Yang, et al., 2019). Young shoots and leaves are rich in nutrients and are widely used as healthy plant foods (Dinu et al., 2018; Sun et al., 2014), but mature shoots and leaves that account for most of the sweet potato biomass are partly ignored or end up, in the happiest case, fodder for animals. Truong et al. (2007) also found that the leaves have the highest phenolic acid content compared to the peel and root of three varieties of American sweet potatoes. These indicate that sweet potato leaves can be a valuable source of antioxidants. However, a large amount of leaves generated by the production of sweet potatoes of 3 million tons in 2014 (FAOSTAT, 2017) was discarded in South Korea, a large consumer of this species. It is very important to know the value and use of the shoots and ripe sweet potato leaves, to improve the economic value of the crop and reduce environmental waste.

Ishida et al. (2000) showed that the content of total phenolic compounds in leaves, petioles, shoots and roots is 90, 45, 90 and 180 mg/100 g in the 'Koganesengan' cultivar and 356, 126, 197 and 154 mg/100 g in the 'Beniazuma' cultivar. Islam (2006) and Jeng et al. (2015) identified several anthocyanins, flavonoids, phenolic extracts from sweet potato stems and leaves. Extracts of sweet potato stems and leaves have been shown to have multiple physiological and health functions (Jeng et al., 2015) and potential uses in the food and medicine industries (Yuan et al., 2015; Matins et al., 2017).

Peroxidase (Hamid, 2009) has been widely used in several industries. It is said to be the key enzyme in the enzyme-linked immunosorbent assay and has been used as a biosensor to accurately detect physical, chemical, and biological signals (Zhang et al., 2018; Fojtíková et al., 2017; Fatibello-Filho et al., 2007). Peroxidase can be used in many manufacturing processes, such as adhesives, computer chips, auto parts and drum and box liners. In the food industry, peroxidase is an excellent food additive for whitening flour. Like polyphenol oxidase, peroxidases can catalyze the transformation of catechins into theaflavins and can improve the quality of black tea (Kusano et al., 2015; Stodt et al., 2014). Peroxidase can also be used to treat industrial wastewater and remove toxic pollutants by oxidation and polymerization catalyzed by enzymes or by converting toxic materials into less harmful substances such as phenols, polycyclic aromatic hydrocarbons and aromatic amines (Kotchey et al., 2013; Durán and Esposito, 2000).

Enzymatic systems may combat oxidative stress in the human body by maintaining a balance between oxidants and antioxidants. This is particularly important because under oxidative stress excessive formation of reactive oxygen species (ROS) can damage biomolecules, such as DNA, proteins, lipids and carbohydrates, and lead to numerous disease conditions. The enzyme superoxide dismutase (SOD) catalyzes the scavenging of (O−2) to H_2O_2, whereas peroxidase (POX) and catalase (CAT) are involved in reduction of H_2O_2 from cells (Băbeanu et al., 2017).

The screening carried out in this study aimed to highlight the major content of bioactive compounds and the antioxidant activity existing in leaves of six sweet potato cultivars cultivated in south-western Romania.

MATERIALS AND METHODS

The research was located in the teaching field of the Faculty of Horticulture in Craiova, in southwestern Romania. Six sweet potato genotypes were studied. The culture was established by cuttings obtained by forcing the tuberous roots. The cuttings were planted in the first decade of May 2018, on 30 cm high layers. The distance between rows was 70 cm, and between plants in a row of 40 cm, resulting in 35,714 pl/ha. Two months after planting, leaves were harvested from all 6 cultivars and brought to the laboratory for chemical analysis.

Biochemical analysis. Methanol used for the extraction was from Sigma-Aldrich. Gallic acid, ascorbic acid, 1.1-diphenyl-2-picrylhydrazyl, 6-hydroxy -2,5,7,8 - tetramethylchromon 2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich, Germany. Quercetin was purchased from Carl Roth. Folin-Ciocalteu reagent was obtained from Merck, Germany. All the other used chemicals were of analytical grade.

Dry matter content (DMC) (%) was determined gravimetrically by drying 25 g leaf fresh tissue to a constant weight at 105°C.

For antioxidant enzymes extraction, fresh tissue was homogenised with 0,1M phosphate buffer, pH 7.5 (1:20 w/v),) containing 0,1mM EDTA. The homogenates were centrifuged for 20 min at 6000 rpm and the supernatants were used for enzyme assays.

Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) activity (POX) was assayed by measuring the increase in A_{470} due to guaiacol oxidation to tetraguaiacol on addition of H_2O_2 (Dinu et al., 2018) and their activity was expressed as ΔA/min/g fm (fresh matter).

Catalase activity (E.C.1.11.1.6) CAT activity was assayed through the colorimetric method at 570 nm using $K_2Cr_2O_7$/acetic acid reagent, and the results were expressed as mmol H_2O_2/min/g fm at 25°C (Dinu et al., 2018).
The extracts for the determination of total phenolic content, total flavonoids content and antioxidant activity were prepared into 80% aqueous methanol (1:20 w/v) in "Fungilab" ultrasonic bath at 24°C for 70 minutes. The resulting slurries were centrifuged at 4000 g for 5 min and the supernatants were collected.
Determination of total phenolic content (TPC). Each extract was mixed with Folin-Ciocalteu reagent and saturated sodium carbonate (Na_2CO_3) solution (Băbeanu et al., 2020). The mixture was allowed to stand at room temperature for 60 min and then the absorbance was recorded at 765 nm. The total phenolic content (TPC) was calculated using a standard curve prepared using gallic acid and expressed as mg of gallic acid equivalents (GAE) per gram.
The total flavonoids content was determined by colorimetric method with 10% $Al(NO_3)_3$ and 5% sodium nitrite ($NaNO_2$) in alkaline medium (Băbeanu et al., 2020). The absorbance was measured at 500 nm and the results were calculated from quercetin calibration curve and expressed as mg QE/100 g fm.
*DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay***:** A 0.075 mM (final concentration) DPPH solution in methanol was mixed with sample extracts and vortexed thoroughly. The absorbance of the mixtures was recorded after 20 min. The absorbance of the remaining DPPH radicals was measured at 517 nm (Dinu et al., 2018). A blank reagent was used to study stability of DPPH over the test time. The scavenging activity of extracts was evaluated according to the formula: % scavenging = $[A_0-(A_1–A_S)]/ A_0]100$, where A_0 is the absorbance of DPPH alone, A_1 is the absorbance of DPPH + extract and A_S is the absorbance of the extract only.
The standards calibration curves (Trolox-T and ascorbic acid- AsA) were plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as µmol TE/g fm and µmol AsA/g fm.
ABTS radical cation scavenging activity: ABTS was dissolved in water to a 7 mmol/l concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/l potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS radical cation solution was diluted with methanol to an absorbance of 0.70 at 734 nm. 0.1 ml sample extract was mixed with 2.9 ml of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The standards calibration curves (Trolox and ascorbic acid) were plotted as a function of the percentage of *ABTS radical cation* scavenging activity. The final results were expressed as µmol TE/g fm and µmol AsA/g fm.
All the spectrophotometric measurements were carried out with Evolution 600 UV-Vis spectrophotometer, Thermo Scientific, England, with VISION PRO software.
Statistical analysis. The statistical significance of differences betwee variants was determined with variance analysis using ANOVA and the Statgraphics Centurion XVI program (Statpoint Technologies, Warrenton, VA, USA) and by calculating the limit differences, LSD≤0.05% (LSD = least significant differrence). In addition, the correlation coefficient (R^2) between the antioxidant activity (expressed Trolox and ascorbic acid) and the total polyphenol content was calculated.

RESULTS AND DISCUSSIONS

The results of this study, on the total *dry matter content* (DMC) demonstrate a variation from 18.42% for cultivar 6 to 14.3% for cultivar 2 (Table 1). High content was recorded in cultivar 5 (18.20%) but also in cultivars 1, 3 and 4 with values over 16%. In a previous study conducted by us in 2018, on two sweet potato cultivars grown in the same area, DMC values were obtained in the leaf blade of 16.00% in Pumpkin and 18.5% in Chestnut (Dinu et al., 2018). The DMC content recorded in the sweet potato leaf is higher than that reported by Asaolu (2012) of 10.0-12.8% in different vegetable species.
Phenolic compounds showed cultivar 6 with 10.237 mg GAE/g fm, followed by cultivar 1 with 7.188 mg GAE/g fm and cultivar 3 with 6.071 mg GAE/g fm (Table 1). The lowest content was recorded in cultivar 4 with only

1.747 mg GAE/g fm, 5.8 times lower than in cultivar 6. Our results are consistent with those reported by (Sun et al., 2014) in a study on 40 sweet potato cultivars, and in some cultivars, they are even higher than these values (values from 2.73 g ChA/100 g DW and 12.46 g ChA/ 100 g DW, equivalent to 1.579 mg GAE/g fm – 7.286 mg GAE/g fm). Hue et al. (2012) determined a content of total polyphenols in sweet potato leaves between 2.78 and 5.35 g GAE/100 g DW, and Islam et al. (2002) reported a content in phenols ranging from 1.42 to 17.1 g/100 g dry matter. Values similar to our results have been reported in other studies evaluating the content of phenolic compounds, one in 116 varieties of sweet potatoes grown in China (Xu et al., 2010) or another study on three sweet potato cultivars grown in Kansas State University (Su et al., 2019).

The content of polyphenols in leaves is influenced by the interaction of several factors: genotype, environmental conditions, applied crop technology, harvest period. In our study the investigated cultivars being grown in the same environmental conditions and respecting the same technology differences observed in content in total polyphenols are due to the cultivar.

Truong et al. (2007) stated that leaf polyphenols recorded much higher values than those from tuberous root, pulp or bark as well as from other common vegetable species. Over 20 phenolic acids have been identified in sweet potato leaves, of which chlorogenic acid is the majority, followed by caffeic, quinic and protocatechuic acids (Islam et al., 2002).

The values for *flavonoid compounds* ranged from 2.71 mg QE/100 g fm in cultivar 2 to 9.73 mg QE/100 g fm in cultivar 6 (Table 1). Cultivars 1, 3, 5 and 6 are also highlighted for this biocompound, cultivars that also had a high content of phenolic compounds. The presence of these flavonoid compounds in sweet potato leaves has been investigated in several studies: Hue et al., (2012) on six sweet potato varieties identified a content that ranged from 96 µg catechin equivalents/g to 263.5 catechin equivalents µg/g (determined by the vanillin-HCl test), and Fu et al. (2016) reported a value of 3.4 mg QE/g dry matter, in ethanol extract of 70%.

The data reported in the literature are difficult to compare due to differences in extraction methods, solvents used and test methods. Two important flavonoids have been identified in the composition of sweet potato leaves: quercetin and isoquercetin (Suarez et al., 2020). Flavonoid compounds exert a strong biological activity with beneficial effects on human health: it suppresses the reactive formation of oxygen, chelates trace elements involved in the production of free radicals and regulates and protects antioxidant defense.

Table 1. Biochemical compounds and enzymatic activity in sweet potato cultivars

The cultivar	DMC (%)	Phenolic compounds (mg GAE/g fm)	Flavonoid compounds (mg QE/100 g fm)	Peroxidase (ΔA/min/g fm)	Catalase (mM H_2O_2/g/min fm)
1	16.97^b	7.188^b	7.66^c	615.8^a	3^c
2	14.3^c	2.395^e	2.71^e	212.2^f	4.9^b
3	16.8^b	6.071^c	8.23b	320.0^c	6.6^a
4	16.38^b	1.747^f	3.18^d	346.4^b	3.0^c
5	18.2^a	4.369^d	8.64^b	296.0^d	6.2^a
6	18.42^a	10.237^a	9.73^a	246.6^e	4.9^b
LSD 5%	**1.01**	**0.21**	**0.41**	**19.83**	**0.47**

Note: Different letters within the same row indicate significant differences ($P \leq 0.05$) between cultivars

Enzymatic antioxidant activity

Catalase catalyzes the dismutation of hydrogen peroxide generated during photosynthesis in water and molecular oxygen and peroxidase breaks down H_2O_2 by oxidizing substrates. In addition to their role in treatment systems, peroxidases have been recognized as being involved in several cellular processes, including growth control, lignification, pathogen defense, and growth regulator catabolism. The enzymatic activities and the antioxidant activity vary depending on the analyzed species and cultivar (Băbeanu et al., 2017; Soare et al., 2017; Korus, 2011). In a study of different species of *Brassica*, superoxide dismutase activity recorded high levels in kale, peroxidase recorded high values in red cabbage, highest value for catalase activity was determined in broccoli, and highest value for antioxidant activity in kale (Soare et al., 2017).

Peroxidase activity ranged from 615.8 ΔA/min/g fm at cultivar 3 to 212.2 ΔA/min/g fm at cultivar 1 while catalase activity ranged from 6.6 mM H_2O_2/g/min fm at cultivar 3 and 3.0 mM H_2O_2/g/min fm in cultivars 1 and 4. Cultivator 3 with high enzymatic antioxidant activity is highlighted followed by cultivar 5, 6 and 2.

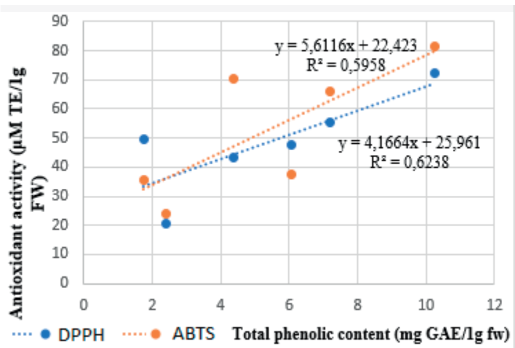
Kim et al. (2009) investigating the activity of antioxidant enzymes during the development of sweet potato leaves found significant increases in peroxidase activity during the late stage of development while catalase activity increases during the early stage of leaf development. It was observed that in sweet potato there is a major isoform of catalase that has the highest protein content and the highest enzymatic activity in mature leaves compared to young and completely yellow leaves at senescence (Afiyanti and Chen., 2014; Chen et al., 2011).

The antioxidant activ*ity* of leaf extracts was determined by DPPH and ABTS cation radical removal test, which are the most accepted and used methods of evaluating antioxidant activity. The results were calculated using two standards: Trolox and ascobic acid. Antioxidant activity varies depending on the cultivar studied (Table 2).

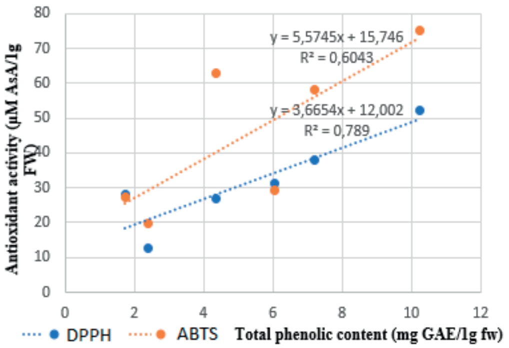
Table 2. Antioxidant activity in sweet potato cultivars

The cultivar	Antioxidant activity DPPH (μM TE/g fm)	Antioxidant activity ABTS (μM TE/g fm)	Antioxidant activity DPPH (μM AsA/g fm)	Antioxidant activity ABTS (μM AsA/g fm)
1	55.16 ^{b}	65.93 ^{c}	37.94 ^{b}	19.92 ^{f}
2	20.7 ^{f}	23.8 ^{f}	12.66 ^{f}	27.33 ^{e}
3	47.88 ^{d}	37.4 ^{d}	31.26 ^{c}	29.29 ^{d}
4	49.7 ^{c}	35.46 ^{e}	28.28 ^{d}	58.35 ^{c}
5	43.5 ^{e}	70.29 ^{b}	26.92 ^{e}	62.79 ^{b}
6	72.18 ^{a}	81.27 ^{a}	52.27 ^{a}	75.22 ^{a}
LSD 5%	**1.07**	**1.04**	**0.84**	**0.82**

Note: Different letters within the same row indicate significant differences ($P \leq 0.05$) between cultivars



a.



b.

Figure 1. Correlation between antioxidant capacity (expressed as Trolox and ascorbic acid) and polyphenol content

The values of *DPPH* radical scavenging activity compared to standard Trolox ranged from 20.70 μM TE/g fm (for cultivar 2) to 72.18 μM TE/g fm (for cultivar 6). High value is also found in cultivar 1 with 55.16 μM TE/g fm. The results obtained expressed in ascorbic acid ranged from 12.66 μM AsA/g fm (in cultivar 2) to 52.27 μM AsA/g fm (in cultivar 6) followed by cultivar 1 by 37.94 μM AsA/g fm. The free radical scavenging activity expressed in Trolox or ascorbic acid highlights cultivar 6 with the highest values, followed by cultivar 1 and cultivar 2 with the lowest values. The results obtained in our study are similar to those reported by Sun et al. (2014); Suarez et al., (2020); Dinu et al. (2018). Suarez et al. (2020) in a study that determines and compares the content of phenolic compounds and antioxidant activity in sweet potato leaves determined in three different periods recommends harvesting leaves in late September when they determined the highest content of total polyphenols.

The values of the *ABTS* radical scavenging activity compared to the standard Trolox show cultivar 6 with 81.27 μM TE/g fm, followed by cultivar 5 with 70.29 μM TE/g fm, cultivar 1 with 65.93 μM TE/g fm and the lower value in cultivar 2 - 23.80 μM TE/g fm. The same order is found for the activity of free radicals expressed in ascorbic acid (cultivar 6 - 75.22 μM TE/g fm, cultivar 5-62.79 μM TE/g fm, cultivar 1 with 58.35 μM TE/g fm and the smallest value in cultivar 2 with 19.22 μM TE/g fm.

The values for antioxidant activity recorded in this study are higher than those obtained by Truong et al. (2007) and Ghasemzadeh et al. (2012). The high level of activity of capturing sweet potato leaves was also demonstrated in the study by Yang et al. (2005), in which *Ipomea batatas* had the highest DPPH radical scavenging activity of 23 vegetable species consumed in Taiwan. The content in total polyphenols correlates linearly positively with the antioxidant activity expressed Trolox or ascorbic acid (Figure 1 a and b). These correlations can be explained by the fact that antioxidant activity is largely due to the content in phenols and flavonoids that have the property to scavenge free radicals being powerful antioxidants.

CONCLUSIONS

The high content of phenolic compounds and flavonoids in 4 sweet potato cultivars.
suggests that the leaves of this species should be used as valuable food to cope with future changes in food supply and demand, especially in developing countries. The antioxidant activity determined in the extract from sweet potato leaves, explained by the presence of compounds with antioxidant properties (phenols, flavonoids, ascorbic acid, antioxidant enzymes) is responsible for the beneficial effects of eating sweet potato leaves. These antioxidants act as chemo-preventive agents against oxidative stress, caused by the excess of reactive oxygen species and which is associated with the appearance of many diseases.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IDENTIFICATION OF SOME ROOTSTOCKS FOR TOMATO CULTURES FROM ROMANIA

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Abstract

The research was conducted at the Horting Institute - Bucharest, on a grafted and non-grafted tomato collection cultivated in a greenhouse. The Solanum lycopersicon cultivars used to obtain of grafted seedlings were the Abellus F1 Alamina F1 and Siriana F1 hybrid scions and the Emperador F1, He Man F1 and L568 rootstocks. The biometric measurements on the fruit yield were made in 2020 year. The experience were aimed the identification of some rootstocks for the tomato cultures from Romania. The research show that the rootstocks has influenced the fruit yield and some grafting combinations researched may be recommended for the cropping in Romania.

Key words*: grafted culture, rootstock, scion, Solanum lycopersicon, yield.*

INTRODUCTION

Tomatoes are valuable vegetables, with the highest share of Romanian crops in protected spaces (Sora et al., 2019). Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crops in the world and the grafting on the *Solanaceae* is a similar approach to crop rotation (Sora et al., 2020).

Tomato is one of the most economically important vegetables in the world. Grafting in tomato has grown for various purposes including the increase of yield. An alternative use of native tomato genotypes is as rootstocks for grafting improved tomato (Velasco-Alvarado et al., 2017).

Abd El-Wanis et al., 2013, show that among the objectives of grafting are plant growth, yield and fruit quality.

Tomato grafting is becoming a well-developed practice worldwide with many horticultural advantages. The primary motivation for grafting tomato has been to prevent the damage caused by the soilborne pathogens under the intensive production system. However, recent reports suggest that grafting onto suitable rootstocks can also alleviate the adverse effects of abiotic stresses. This review gives an overview of the scientific literatures on the various aspects of tomato grafting including important steps of grafting, grafting methods, scion–rootstock interaction, and rootstock-derived changes in vegetative growth, fruit yield, and quality in grafted plants under different growing conditions (Singh et al., 2017).

Velasco-Alvarado et al., 2017, show that some grafted tomatoes used in the study (27,000 plants/ha) had a significant increase in yield up to 54 t/ha. They believe that tomato grafting has developed for various purposes, including to increase yield, and have researched some tomato cultivars as rootstocks to identify of some remarkable genotypes for their potential in tomato production.

Gelan and Waraka, 2019, have shown that the yield of tomatoes can increase up to 98.3% by grafting.

The results concerning the grafting influence on vegetable crops require more researches in this domain for to highlight the grafting effect on the some aspects regarding the grafted vegetable production.

This research were aimed the identification of some rootstocks which positively influences fruit yield in the tomato cultures from Romania.
Research concerning the influence of rootstocks on the quantitative and qualitative properties of tomatoes is an important research activity at ICDIMPH - Horting Bucharest.
Here, the research activity in the domain of the tomato grafting started since 2000 year.

MATERIALS AND METHODS

The experience has been realized at the Horting Institute in some vegetable greenhouses.
The vegetal material used in experience has consisted from tomato scions and rootstocks commonly used in Romania for tomato grafting.
The scions were tomato F1 hybrids, Alamina, Abellus (Rijk Zwaan, US) and Siriana (Vegetable Research and Development Station Buzău, Romania). The rootstocks were tomato F1 hybrids, Emperador (Rijk Zwaan, US) He Man (Syngenta, Switzerland), L685 (Vegetable Research and Development Station Buzău, Romania).
The non-grafted and grafted tomato seedlings have been obtained into a specialized greenhouse for research & production of grafted seedlings.
The non-grafted and grafted tomato plants had been cultivated on soil into a glass greenhouse for vegetable production.
The research variants were made up from lots of non-grafted (control) and grafted tomatoes:

- non-grafted plants (control):
  - Alamina
  - Siriana
  - Abellus
- grafted plants (scion x rootstock):
  - Alamina x Emperador,
  - Alamina x L685,
  - Alamina x He Man,
  - Siriana x Emperador,
  - Siriana x L685,
  - Siriana x He Man,
  - Abellus x Emperador,
  - Abellus x L685,
  - Abellus x He Man,

All the scion F1 hybrids are indeterminate tomatoes with spherical and red fruit and a weight more than 150 g/fruit.

The experimental lots with non-grafted and grafted tomatoes were set up at May 6, 2020 (Figure 1).

a) planting day

b) vegetative period

c) harvest period

Figure 1. Experimental lots with grafted tomatoes Horting Institute

The experience with 27,000 non-grafted tomatoes/ha and 18,000 grafted tomatoes/ha) was made up by the randomized block method: 12 variants (90 plants/variant) with 3 repetions (30 plants/repetion). The soil from greenhouse where the experiment was created has had the chemical and organic characteristics listed in the Table 1.

Table 1. Chemical and organic characteristics of soil

Analyze	Resultat	MU	Interpretation
pH (20±2˚C)	8.04	-	
EC (25±1˚C)	0.475	mS/cm	
CATIONI			
N-NH_4 (λ=660 nm)	12.1	mg/kg	
K (λ=766.490 nm)	32.9	mg/kg	
Na (λ=589.592 nm)	117.3	mg/kg	
Ca (λ=317.933 nm)	186.4	mg/kg	
Mg (λ=279.077 nm)	65.1	mg/kg	
ANIONI			
N-NO_2^- + N-NO_3^- (λ=540 nm)	163.4	mg/kg	
Cl^- (λ=470 nm)	123.1	mg/kg	
SO_4^{2-} (λ=420 nm)	144.9	mg/kg	
P (λ=213.617 nm)	218.0	mg/kg	
MICROELEMENTS			
Fe (λ=238.204 nm)	19.6	mg/kg	
Mn (λ=257.610 nm)	8.2	mg/kg	
Zn (λ=213.857 nm)	10.4	mg/kg	
B (λ=249.677 nm)	0.3	mg/kg	
Cu (λ=327.393 nm)	22.5	mg/kg	
HUMUS	2.48	%	

MU – measure unit   big   medium   small

The fertilization program used in experience: at soil preparation was administered Complex 16–16-16 (300 kg/ha) and then (kg/100 m^2/day), as follows:

- *the 5th day after planting - the 15th day:* 0.1 kg potassium sulphate + 0.1 kg ammonium nitrate,
- *the 16th day - the 25th day:* 0.15 kg potassium sulphate + 0.1 kg ammonium nitrate,
- *the 26th day zi - the 35th day* 0.05 kg GL fruit 18-11-59 + microelements + 0.05 kg azotat amoniu,
- *the 36th day - the 45th day:* 0.1 kg GL fruit 18-11-59 + microelements,
- *the 46th day - the 55th day*: 0.1 kg potassium sulphate + 0.1 kg ammonium nitrate,
- *the 56th day - the 60th day*: 0.1 kg potassium sulphate,
- *in vegetation, at 10 days intervals*: 4 treatments with Folimax 0.3%.

The Delta-T meteorological station with sensors for temperature, atmospheric humidity, precipitations and solar radiation was used for the monitorizing of climatic factors, programmed to record the climatic data at one hour intervals; its there are in the Table 2.

Table 2. Climatic data from greenhouse (May - August, 2020)

Month	Temperature (°C)			Atmospheric humidity (%)			Soil temperature (°C)	Soil humidity (%)
	minimum	maximum	average	minimum	maximum	average		
May	15.6	31.7	20.9	30.1	68.5	50.7	20.2	39.1
June	14.9	39.2	25.5	28.1	86.3	61.3	21.4	30.8
July	16.5	40.5	27.3	25.6	85.2	53.3	22.1	40.7
August	16.2	40.1	27.4	22.5	81.8	49.4	25.1	50.6

The Observations and the biometric determinations on the tomato fruits were performed in the research laboratory of the Horting Institute on biological samples harvested at the consumption maturity.

The statistical calculation consisted in the processing of results using the Duncan test (p = 5%). The regression equations and the correlation coefficients were been calculated through the analysis of variance (ANOVA) for highlighting a correlation between production per plant and per hectare for working variants.

RESULTS AND DISCUSSIONS

It was observed that rootstocks have influenced precocity. Thus, the non-grafted plants in the all researched variants were earlier (July 15 - first harvest) comparative to the grafted plants (July 20 - first harvest). The difference consisted in 5 days between the first harvest of non-grafted variants and the first harvest of grafted variants. In a study on grafted and non-grafted tomatoes, researchers Peil and Gálvez, 2004, have showed that the rootstocks had a negative effect on the precocity, but did not affect the total fruit production.

There were differences on the tomato production in the experimental variants.

The fruit yield per plant obtained in this research is shown in the Table 3; the results are presented as an average/variant.

Table 3. Fruit yield on tomato variants

Variants	Number of fruit/plant	Average weight/fruit (kg)	Production/plant (kg)
Alamina (control)	9.66	0.175	1.69
Alamina x Emperador	9.4	0.184	1.73
Alamina x L685	9.43	0.175	1.65
Alamina x He Man	9.57	0.184	1.76
Siriana (control)	7.93	0.145	1.15
Siriana x Emperador	8.14	0.156	1.27
Siriana x L685	7.4	0.15	1.11
Siriana x He Man	8.18	0.159	1.3
Abellus	10.3	0.165	1.7
Abellus x Emperador	11.64	0.165	1.92
Abellus x L685	10.58	0.155	1.64
Abellus x He Man	11.86	0.167	1.98

The fruit numbers per plant had been relatively similar between the control and grafted plants conducted in the all 3 tomato hybrids, Alamina (9.66/control plants comparative with 9.4-9.57/grafted plants); Siriana (7.93/control plants comparative with 7.4-8.18/grafted plants), Alamina (10.3/control plants comparative with 10.58-11.86/grafted plants).

There were differences between variants at average weight per fruit: the He Man rootstock has influenced most positively this production parameter, in all grafting combinations it had fruits with the highest weight (159-184 g/fruit) compared to the fruits from the plants grafted on the Emperador rootstock (156-184 g/fruit), the control plants (145-175 g/fruit) and those grafted on the L685 rootstock (150-175 g/fruit).

There were small differences between the control variants and the grafted variants at the average weight per fruit (0-0.009 kg for Alamina, -0.005-0.014 kg for Siriana and -0.010-0.002 kg for Abellus); the He Man rootstock had had the most positive influence on this production parameter.

Total production per plant was positively influenced by the Emperador and He Man rootstocks and negatively by the L685 rootstock; there were significant differences.

At all grafting combinations, the fruit yield per hectare had been negative comparative with control plants because the planting had been made with 18,000 grafted plants/ha and 27,000 non-grafted plants/ha (Table 4).

Table 4. Fruit yield per hectare

Variants	Production (t/ha)	Diferența (%)
Alamina	45.63 a	100
Alamina x Emperador	31.14 c	-14.59
Alamina x L685	29.7 d	-16.03
Alamina x He Man	31.68 b	-14.05
Siriana	31.05 a	100
Siriana x Emperador	22.86 c	-8.08
Siriana x L685	19.98 d	-10.96
Siriana x He Man	23.4 b	-7.54
Abellus	45.9 a	100
Abellus x Emperador	34.56 c	-11.26
Abellus x L685	29.52 d	-16.3
Abellus x He Man	35.64 b	-10.18

a, b, c, d - significant differences (Duncan test, p = 5%).

The total production per at the non-grafted plants was 45.9 t/ha (Abellus), 45.63 t/ha (Alamina), 31.05 t/ha (Siriana), between 23.4 – 35.64 t/ha by grafting on the He Man rootstock, 22.84-34.56 t/ha by grafting on the Emperador rootstock and 19.98-29.7 t/ha by grafting on the L685 rootstock. The production differences from the control were 14.05-16.03% (Alamina), 10.18-16.3% (Abellus) and 7.54-10.96% (L685).

Milles et al., 2016, found that at some genotypic combinations (Rita variety grafted on the ES 99-265, Line 9242, PG 99, Robusta rootstocks) obtained an equal yield with the yield of the non-grafted variety and some rootstocks produced a lower yield (Energy, Firefly, Line 9243, Nico) and others produced a higher yield (Beaufort, He-Man, Joint, P1614, RS 1427).

Researchers Severino et al., 2017, showed that the yield increased from 81 to 103 t/ha comparing the lowest (20,000) and the highest (29,000) stem density/ha, but the difference between the systems was only 4 t/ha.

Doltu et al.2019, have obtained at the Siriana tomatoes grafted on the Emperador rootstock (20,000 plants/ha) a fruit production per plant more with 54.76% and a production per hectare more with 14.64% comparative to the non-grafted Siriana tomatoes.

The grafting researchers have experimented and they recommend different planting densities for the cultivation of some grafted tomatoes:

- 10,000 plants/ha (Torres et al., 2015),
- 12,500-15,000 plants/ha (5,060-6,070 plants/acre) (Milles and et al., 2016),
- 12,800 plants/ha (Khah et al., 2006),
- 15,000 plants/ha (Bogoescu et al., 2011),
- 20,000 plants/ha (Doltu et al., 2019)
- 26,000 plants/ha (Mohammed et al., 2009),
- 27,000 plants/ha (Velasco-Alvarado et al., 2017).

The indirect linear correlations were obtained between production per plant and per hectare for all grafting combinations.

The value of determination coefficients shows that at production per hectare, the significance of the correlation is distinctly significant (r^2 = 0.5639; r^2 = 0.4071; r^2 = 0.4532) (Figures 2, 3 and 4).

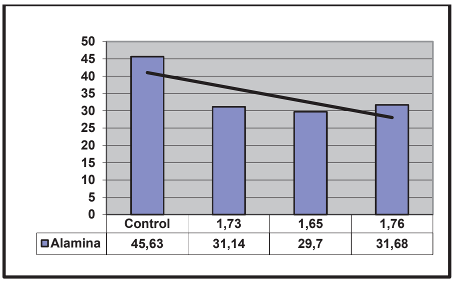

Figure 2. Indirect and distinctly significant linear correlation at Alamina tomatoes

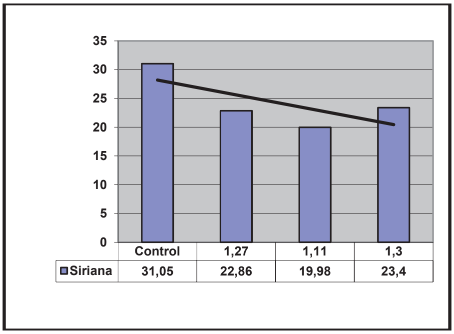

Figure 3. Indirect and distinctly significant linear correlation at Siriana tomatoes

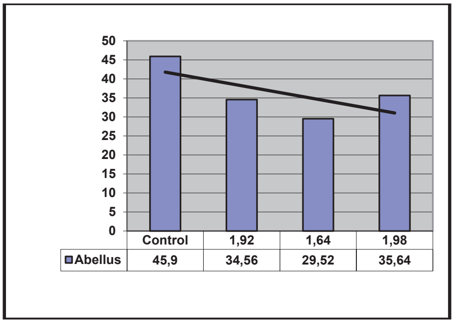

Figure 4. Indirect and distinctly significant linear correlation at Abellus tomatoes

CONCLUSIONS

The rootstock has influenced the fruit production per plant; He Man and Emperador rootstocks had have a higher production and L685 rootstock has have a lower production compared to the control plants.

The plant densities have influenced the fruit production per hectare: the non-grafted tomatoes (27,000 plants/ha) had a bigger production comparative to the grafted tomatoes (18,000 plants/ha); there were significant differences.

Indirect linear and distinctly significant correlations were obtained between production per plant and per hectare for all grafting combinations due to planting density.

The use grafted Alamina, Siriana, Abellus tomatoes, the grafting combinations (scions x rootstocks) researched in this paper, may be recommended for growing in Romania.

Based on these results, it can be said that rootstocks played an important role in fruit production and the use of other planting densities is recommended (20,000-27,000 plants/ha) or other culture systems (2-3-4 stems/plants).

The results showed that grafting on the appropriate rootstocks (He Man, Emperador) has positive effects on fruit production per plant compared to non-grafted tomatoes and tomatoes grafted on other rootstock (L685).

ACKNOWLEDGEMENTS

This research was conducted within the Sectorial Plan, ADER 7.3.1 project of the Horting Institute Bucharest on the 2019-2022 period, project supported from the funds of the Agriculture Ministery.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDY REGARDING THE EVOLUTION OF HIGH-PERFORMANCE CULTIVATION TECHNOLOGIES IN GREENHOUSES AND HIGHT TUNNELS IN ROMANIA

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Abstract

The present study on the evolution of high-performance technologies as well as the evolution of areas with greenhouses and solariums in Romania aimed, in particular, to identify farmers who have adopted high-performance technologies in order to increase tomato production. We identified the high-performance greenhouses in Romania, especially those that grow tomatoes in them. They also identified the areas occupied by greenhouses and high tunnels, cultivated in a soilless system, on substrates or on the ground in some areas of Romania.

Key words*: Romania, greenhouses, tomatoes, soilless, substrate.*

INTRODUCTION

Greenhouse vegetables are a viable alternative to the constant supply of fresh vegetables, demonstrating their effectiveness by using technologies that have proven to meet the challenges of the greenhouse effect and ensure the sustainability of the system.

Numerous studies argue that the protection of vegetable crops is not a new concept, it existed since the Roman Empire during the reign of Emperor Tiberius (14-37 BC) when he consumed cucumbers all year round using a greenhouse-like protection system (Leoni, 1994; Tognoni and Serra, 1994), The first information about heating greenhouses appeared during the Joseon dynasty in Korea in the 1450s, they arrived in America in the 1700s and then in the Netherlands around the 1800s.

After 1960, when polyethylene foil appeared, protected cultures (areas) began to develop and evolve. At present, significant performs have been ensured regarding UV resistance and service life.

Integrated control guarantees the best economic result, and provides a systematic way to design control systems for the innovative greenhouses of the future (Straten and Hente, 2010; Kolokotsa et al., 2010; Doltu et al., 2020; Sora et al., 2019; Sora and Doltu, 2018).

Romania, due to its geographical position has beneficial conditions for obtaining extra-early vegetable crops in greenhouses because during the winter, December - February, it has a light radiation superior to the countries of Western Europe.

Through the studies carried out within the project "Development of the administrative capacity of MCI to implement some actions established in the National Strategy for Research, Technological Development and Innovation 2014-2020", SIPOCA code 27, financed by the Operational Program Administrative Capacity (POCA) it is mentioned that In terms of market performance, the South Muntenia Region achieves 20% of national vegetable production (Tran et al., 2018).

In order to take advantage of this advantage, it is necessary to streamline the technology of protected vegetable crops in Romania, regardless of how they are heated.

If before 1989 the vegetable farms were merged and the properties belonged to the State after this period, the areas were taken over by their owners, thus leading to their fragmentation. Thus, due to the uncertainty of an association, the surfaces remained in fragmented ownership, and it was not possible to conceive the construction of large greenhouse complexes by associating the landowners.

However, recently land has been leased or purchased, which has made it possible to build high-performance greenhouse complexes.

Regarding the areas occupied by greenhouses before 1989, there were 2,460 ha of heated greenhouses, built in all areas of the country that ensured both the domestic market and the export of fresh vegetables (tomatoes, cucumbers, bell peppers, lettuce, greens). Many of them were located both in large, heavily populated urban areas (Bucharest) and near large cities (Codlea - Brașov, Ișalnița - Dolj), Scornicești - Olt, etc.).

Analyzing the territorial distribution of greenhouses, before 1990, they were found in each county, and their areas and number depended on the population of the area but also on the degree of suitability for climatic conditions. The largest areas of greenhouses but also the mode of distribution and specialization of production were found in Bucharest (Popești greenhouses 142 ha) followed by Arad, Timiș, Bihor and Dolj. Also, the favorable areas for the protected cultures (areas) covered with different types of plastics were found, in the largest area, in zone I, (Bihor, Arad and Timiș counties) as well as in the entire southern part of Romania. Thus, the largest areas were found in the counties of Dolj (Ișalnița 200 ha) (Popa et al., 2021), Olt, Teleorman, Giurgiu, Ilfov, Arad, Bihor, Timiș and Galați.

Regarding the production of seedlings intended for covered areas there were sectors in greenhouses, specialized for seedling production but also certain farms specialized in this regard. The ratio between the area of the for seedling greenhouses and that of the heated covered spaces was 1: 5.

If in 1971 in Romania there were only 2 ha of seedling greenhouses and 10 ha of heated solariums in 1976 the surface of seelding greenhouses reached 90 ha and that of heated solariums reached 270 ha (Ceaușescu, 1979).

The evolution of the surfaces occupied by greenhouses or heated solariums according to the recorded statistical data varied from one year to another. Thus, if in 2007 the total area of greenhouses in Romania was 418 ha in 2008 it increased to 668 ha and it will fluctuate year by year in 2015 the area of greenhouses in Romania was 323 ha. In 2016, there was an increase to 431 ha and then to decrease continuously to 250 ha in 2017 to 233 ha in 2018 reaching that in 2019 only 206 ha of greenhouses will be registered (Statistical Yearbook 2020). The motivation for the continuous decrease of heated surfaces is due to both the high costs of heating and their lack of technology. The vegetables obtained in greenhouses were grown in a conventional system, on the ground, being destined for foreign markets.

According to data from the National Institute of Statistics, areas built with greenhouses decreased after 1990 due to both high heating costs.

If we follow the distribution of greenhouses on large cultivating units, according to data recorded in 2012 Hortifruct Association owned an area of 180 ha with greenhouses of which 137 ha located around Bucharest through three production points: Popesti - Leordeni with 90 ha, Berceni with 30 ha and Pipera with 13.5 ha. Another 3.5 ha in Ișalnita are currently fully compromised due to the fact that during the winter they were no longer heated.

An area of about 2000 ha of modern greenhouses would be needed to provide the necessary with vegetables during the year. In present, according to statistical data, 80% of vegetables come from imports.

From 1951-1956 there were conventions for the export of fresh vegetables with some states such as Austria, Czechoslovakia, R.D. Germany also with R.F. Germany (1955-1956) USSR (1956), Hungary (1952), Switzerland (1953-1956) (Stefan et al., 2008).

If in Romania, in 1989, the value of global production was 196,920 million lei, of which 54.4% owned vegetable production (Statistical Yearbook of Romania, 1990, p. 398-399).

According to data centralized by the National Institute of Statistics, in 2019, over 740,000 tons of vegetables were imported, worth 516 million euros. In 2018, imports were 678,000 tons, 62,000 tons lower compared to 2019 with an average price per ton of product of 697.3 euros/ton compared to 625.81 euros/ton in 2018.

The studies regarding the zoning of the vegetable culture started since 1954 and in the period 1956-1961 the Zoning Commission within the Ministry of Agriculture established on the basis of the competent studies the favorable areas for the vegetable cultivation. Currently, Law no. 312/2003 establishes three areas for vegetable production.

There is also the concern for obtaining and marketing quality vegetables, so by Law no. 312/2003, Article 14 prohibits the marketing of both fresh and processed vegetables for human consumption, which contain pesticide residues and heavy metals, nitrates, nitrites or other products, which exceed the maximum permitted levels established by the legislation in force.

If before 1989 the greenhouses were built according to a standard technology, with a height of 4-6 m at the ridge, now in Romania there are companies specialized in the construction of greenhouses or solariums with high-performance professional structures, with or without endowments, heating equipment and installations, drip irrigation, hydroponic systems, fertigation. The constructive variants of the tunnel greenhouses are varied, with vertical side walls and side vents, with vertical side walls, with large volumes and with side vents, with single or double foil, 2.5-3.0 m or 4 m high, with opening of more than 5 m.

As there is also a global demand for vegetables throughout the year, new technologies are being identified to increase the efficiency of vegetable production in greenhouses or solariums.

Worldwide, the area occupied by greenhouses and solariums in 2017 was 489214 ha, of which 173561 ha in Europe, 224974 in Asia, 19790 in America, 36993 ha in Africa (Cuesta Roble Consulting, 2017).

In 2019, the vegetable area in the greenhouse worldwide was 496,800 ha Cuesta Roble World Greenhouse Vegetable Statistics - 2019 https://www.producegrower.com/article/cuesta-roble-2019-global-greenhouse-statistics/

A solution for the efficient use of space is also the construction of vertical greenhouses in all areas of the world, mainly in arid areas and where the requirements for vegetables are high. Although the initial investment and energy costs are high, it is still a challenge to create a viable business based on a vertical farm, where LEDs are used for lighting (Publication date: Fri 26 Feb 2021 Source: freshPlaza.com)

In Turkey, the area occupied by greenhouses in an unconventional system, on substrates was 700 ha (Gruda, 2017).

The prospect of implementing new technologies as well as advanced artificial intelligence and machine learning are the future of maintaining the climate in the greenhouse. Greenhouse automation leads to better economic efficiency and reduced product cost.

In China, a high-tech greenhouse was recently built (Shanghai in eastern China), covering an area of about 56 hectares, using additional artificial LED lighting but also intelligent technologies to control the plant growth environment. it is possible for the huge plant factory to operate non-stop in all seasons (www.news.cgtn.com.).

In the Netherlands, over 90% of the area cultivated with vegetables is represented by substrate crops.

Taking into account that in Romania the areas occupied by solariums in crops in conventional system, on soil, are currently registered 1600 ha, with a view to increasing their areas, in this study we will make an analysis and identification of areas occupied by protected areas in some areas southern Romania as well as the identification of high-performance greenhouses in unconventional system, on different types of substrate or in NFT system.

MATERIALS AND METHODS

The study aimed to identify the areas occupied by greenhouses and solariums in some zones of Romania, especially in the south but also in the center of the country. Some data were taken from the County Agricultural Directorates, APIA, but also from the field analysis. We aimed to:

- identify the areas and the size of the surfaces with heated and unheated protected spaces, by counties;

- identification of the type of construction;
- degree of technologicalization;
- the cultivation system practiced conventionnally or unconventional;
- type of substrate used;
- culture structure and culture cycle;
- the productions obtained.

RESULTS AND DISCUSSIONS

According to data recorded by the Research Institute for Agricultural Economics and Rural Development in 2018, the average annual consumption of tomatoes per capita was 41.4 kg. Starting from the reality that in 2019, 1420 ha of protected surface (areas) were declared in Romania, in order to ensure the Romanian market with fresh vegetables, it is necessary to increase the protected surfaces areas so that according to its program it will reach in 827 to about 8-10 thousand hectares.

In Romania most greenhouses were between 4 and 7 m high (Figure 1).

Figure 1. Appearance of low greenhouses 4 m high and 7 m high in România (original)

Data on the situation of areas declared with protected, by farmers, and beneficiaries of payments managed by APIA (Agency for Payments and Intervention in Agriculture) show that in 2020 there were a total surfaces 2889.2 ha of which 160.76 ha in greenhouse, 1750.28 ha in high tunnels, and 978.08 ha represented other types of protected cultures. By counties the situation is presented in the Figures 2, 3 and 4).

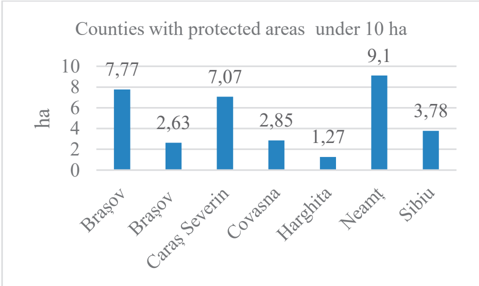

Figure 2. Counties with protected areas under 10 ha from Romania

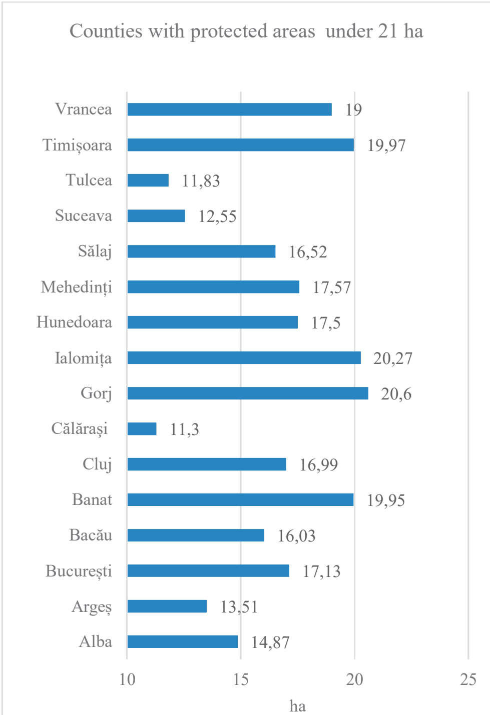

Figure 3. Counties with protected areas over 10 ha up to 21 ha in Romania

In the study we identified a total of 29,548 ha occupied by modern greenhouses that use unconventional technology, on nutrient substrates and 11.4 ha modern greenhouses that use conventional technology, some on the ground being ecologically accredited (Figure 5).

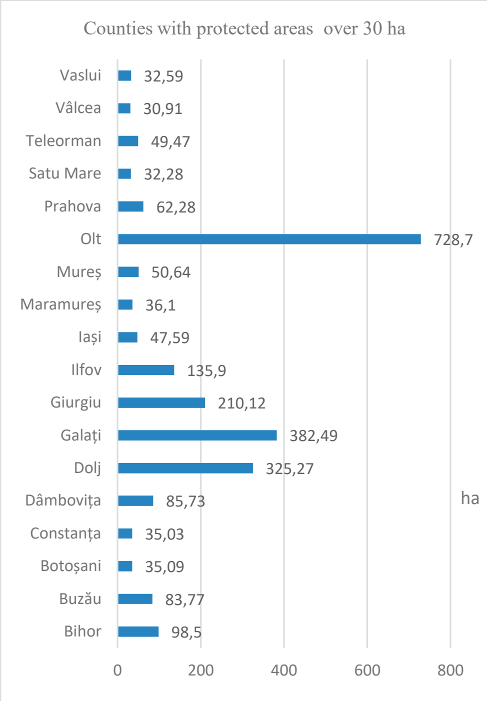

Figure 4. Counties with protected areas over 30 ha

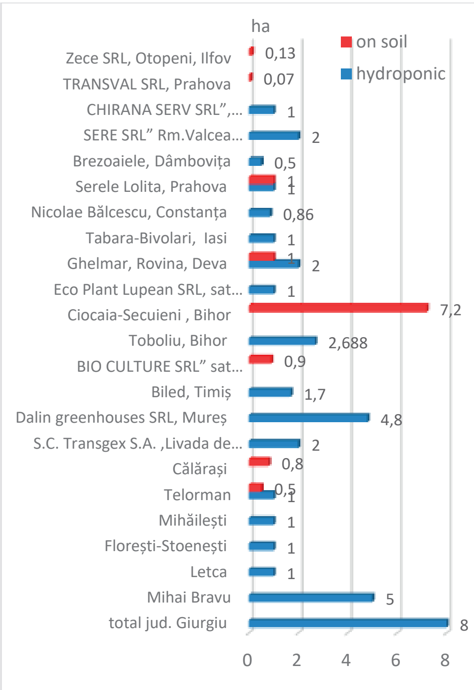

Figure 5. Situation of areas occupied by greenhouses and high tunnels, cultivated in a system without soil, on substrates, or on the ground in some areas of Romania

From the data registered in the documents of the Agricultural Directorates we found that in Giurgiu county the area occupied with unheated and heated solariums was in 2020 of 513 ha of which only 15 ha have a heating system. Of the heated greenhouses, only one has the largest surface of 5 ha, in Mihai Bravu. This is a modern greenhouse that has all the specialized equipment for a greenhouse on nutritive substrates. They grow tomatoes using coconut mattresses as the growing medium.

In three other localities in Giurgiu county, Letca, Mihăilești and Floresti-Stoenești, we had indentify modern greenhouses are built on an area of one ha each. Of these, only the greenhouses built in Floresti-Stoenești are intended for flower crops on substrates.

In the greenhouse from Letca Nouă commune, S.C. Gradina Letca SRL is grown in an unconventional system, on the ground, tomatoes but also other vegetable species, all of which are certified GlobalGAP. These were built through a Project financed with non-reimbursable European funds through the National Rural Development Program (PNDR). (https://letca.ro/produse/).

The greenhouses from Mihăilești distict - *Oxigen Agro Product Company* - from Girgiu county are modern greenhouses, in cultivation system on coconut substrate. The obtained products have GlobalGAP certification (Figure 6). As for the type of construction, they are tall greenhouses, covered with polyethylene foil.

Figure 6. Greenhouses Oxigen Agro Product Company, Mihăilești, Giurgiu county (original)

The applied technologies at this greenhouses are of the latest generation this being endowed with specific equipment for crops on nutritive substrates. Coconut mattresses are used as culture substrates. The constructive system is closed, the nutrient solution being recovered and reused. Tomatoes, cucumbers and peppers are mainly grown in the greenhouse. Using modern technologies, the productions exceed the level of the conventional ones on the ground, eeaching over 500 t/ha.

In Teleorman county, we identified as protected surfaces areas on the surface of 57.9 ha, of which only 0.5 ha are heated greenhouses. In these, the classic soil cultivation system is used.

According of data from agriculture department (2020) in Călărași county 10.5 ha with solariums are registered, of which only 0.8 ha are heated. And in their case, the technology of unconventional cultivation is practiced, on the soil.

The belief of some private entrepreneurs that, by adopting modern greenhouse cultivation technologies both their production and their quality is much higher compared to the unconventional system led to their adoption on the ground. In the unconventional system, the quality of the products can vary from one week to another, due to the variations of the environmental factors in the culture space. Starting from the fact that in the unconventional system, in Romania, the obtained tomato productions reach only 120-250 t/ha, in extended cycle, but, using the unconventional cultivation system, on nutritive substrates (mineral wool, coconut or perlite), some farmers also obtained yields of 500-650 t/ha.

In Romania, in all areas of the country, there are constantly new surfaces built with modern, heated greenhouses or solariums, which have high-performance equipment. Although some farmers start with constructions on small areas, of about 2500 m^2 or 5000 m^2 but convinced of the efficiency of modern technologies they have expanded their areas from 10,000 m^2 to about 50,000 m^2. According to economic analyzes, modern greenhouse areas under 1 ha are considered family businesses for small farmers. The maximum economic efficiency is felt in the case of greenhouses over 2 ha.

In Ialomița county, in 2020, the company "Green Houses Andrei", built in Sinești commune, a modern greenhouse on an area of 1.2 ha, specific for tomato cultivation, in an unconventional system, without soil, using grodan as a substrate. The seedlings were purchased from Greece (Figure 7).

Figure 7. Greenhouses "Green Houses Andrei", from Sinești, Ialomița county (original)

The greenhouses of the "AnnaBella Group" of Companies "SERE SRL" Râmnicu Valcea, Gradinari have a surface of protected space, covered with polyethylene foil, in an area of 2 ha. In particular, it cultivates cucumbers in an unconventional system, on Grodan substrates (Figure 8**).**

Figure 8. Appearance from the Greenhouses of the AnnaBella Group of Companies "SERE SRL" Râmnicu Valcea (original)

The greenhouse complex "CHIRANA SERV SRL", from Mihail Kogălniceanu commune, Ialomita county, has an area of 1 ha, fully automated block greenhouse. It specializes in growing tomatoes on nutritive grodan substrates (Figure 9).

Figure 9. Aspect from "CHIRANA SERV SRL" greenhouse, Ialomita county (original)

One of the first modern greenhouses with the largest area in the Bucharest area was built during 2007-2008, in Pipera, on an area of 13.2 ha, and the establishment of the tomato crop, was made in January 2019 on substrate of grodan, This greenhouses construction was made by "Sere com Ro SRL" (Figure 10).

Figure 10. Appearance inside Pipera greenhouses (original)

The Greenhouse Complex of S.C. Transgex S.A. Oradea, from Livada de Bihor, with an area of 2 ha of crops on mineral wool grodan substrates, has been producing tomatoes since 2006 with very good results using geothermal energy, this being unique in Romania.

The yields obtained are over 400 tons of tomatoes per hectare, per production cycle, which meansa harvest three times higher compared to the yield of the classic greenhouse system.

In Reghin, Breaza commune, Mureș country, Dalin greenhouses, initially the built surface was 2 ha but now it has expanded to 4.8 ha. The greenhouse complex was built using Dutch technology. Greenhouses are specific for growing tomatoes on a nutritious grodan substrate. In 2012, yields of 500 t/ha were obtained, but currently they are over 610 t/ha. The greenhouse is equipped with specific technological equipment and the quality of the products is high because they use cultivars with resistance to diseases but also pollination with the help of bumblebees.

For a short time in the Dalin complex was grown lettuce in NFT system on an area of only 2200 m^2 but the farmer thought it better to specialize in one or two species grown on larger areas, as control and efficiency were higher. In the case of salad, the nitrite / nitrate concentration was below the standard maximum limits.

Figure 11. Aspects from Dalin greenhouses, Mureș county (original)

In 2015, a greenhouse was built in a soilless system, covered with plastic foil in Brezoaiele commune, Dambovița county. Initially he used the mineral wool substrate and then other perlite substrates, respectively coconut. The farmer found that the perlite substrate influenced the early growth of the tomato crop, obtaining yields two weeks earlier compared to the use of other types of substrate. The yields obtained were much higher than those of conventional culture, moreover the quality of the fruit was superior. It obtained productions from March to July of 280 t/ha and 372 t/ha, respectively, of which only about 7% was under STAS. Because in the summer the cost price was low, he set up successive crops of cornichon cucumbers in an unconventional system (Figure 12).

Figure 12. Greenhouse aspect - Brezoaiele, Dambovița county: a. the establishment of culture; b. after 40 days from planting; c. culture on perlite substrate at 70 days; d. culture on coconut substrate at 90 days (original)

In Biled, Timiș county, in 2017 the construction of a greenhouse on an area of 1.7 ha was completed, a greenhouse that has state-of-the-art technologies. In this, tomatoes will be grown on a coconut substrate. The average productions obtained in 2020 were about 550 tons.

In Bihor county, 7.2 ha of greenhouses are built in Ciocaia-Secuieni for tomatoes and cucumbers. The heat source is partially provided by the use of pellets (Figure 13).

Figure 13. Greenhouses Ciocaia-Secuieni, Bihor county (original)

Also, in 2018, in the Bihor commune Toboliu, through a project financed by the Agency for Financing Rural Investments, through the National Rural Development Program (PNDR) L, an investment of over 700,000 euros was built a hydroponic greenhouse for cherry tomatoes on an area of over 2,688 sqm.

The greenhouses "ECO PLANT LUPEAN SRL" located in Sanbenedic village from Alba county have an area of 1 ha, were built in 2010. Tomatoes are grown in a soilless system using nutritive substrates.

In Cluj county, Capusu Mare village, there are "BIO CULTURE SRL" greenhouses with an area of 0.9 ha. The greenhouses are covered with glass, and tomatoes are grown in a conventional system, the soil being organically certified crops (Figure 14).

Figure 14. Appearance inside the greenhouse, "BIO CULTURE SRL", Cluj county (original)

The Corvinia Producers Group has been producing vegetables since 2008 and the “big greenhouses” from Rovina, in an unconventional system, on coconut, perlite and grodan substrates with production over 500 t / ha tomatoes and about 260 t/ha cucumbers. The use as a substrate of mattresses filled with perlite from SC Perlit SA led to an earlier production compared to other types of substrate (Figure 15).

Figure 15. Rovina greenhouses, Deva (original)

Through the National Rural Development Program (PNDR) 2007-2013, Measure 121 - “Modernization of agricultural holdings”, SC SERRA-IVAS SRL implemented between December 2009-December 2013, a project through which a greenhouse was built with heating from sources renewable. Located in Constanța County in Nicolae Bălcescu locality, the greenhouse is built on an area of 0.86 ha,

using mineral wool Grodan as a culture substrate. The obtained production is capitalized in the area starting with March.
Another modern greenhouse on the surface of 1 ha, in system on nutritive substrates is located in Tabara-Bivolari commune, Iași county (Figure 16).

Figura 16. Tabara-Bivolari, Iasi (image from https://www.youtube.com/watch?v=fsIRdbBuRyc)

"Max Central Deposit SRL", is a firm from Brăila county, Village Muchea, county Braila, where is built high tunnel on an area of 0.4 ha, where are grown tomatoes, in a soilless system.
One of the largest hydroponic greenhouse areas, on cultivation tables in the Ebb and Flow system, in the area of 1 ha is found in Prahova County. Lolita Company greenhouses were built in 2011, in Poienarii Burchii commune, Prahova county, with own resources. In addition to the hydroponic greenhouse, cultivated throughout the year with lettuce, the farm has expanded with another 1 ha of solar high in which tomatoes, peppers and other successive vegetables are grown. The greenhouse, due to the specifics of construction and technology, can also be used for the production of seedlings for vegetable farms. The structure of the complex was made by a specialized Dutch company, it is equipped with the most modern heating, climate control, ventilation and shading systems, and the water management is ensured by an irrigation unit with perfect precision which helps to reduce water consumption necessary for irrigation (Figure 17).
And as technologies evolve, in favor of farmers, they invest in the permanent adaptation of the greenhouse.

Figure 17. Appearance of greenhouses Lolita (original)

In Romania there are no farms specialized in producing specific seedlings for hydroponic crops. Grafted or non-grafted seedlings are purchased from Greece or Hungary. In Romania there is a seedling production complex at Fetești S.C. Cox Agricol Farms, S.R.L., which produces about 60 million seedlings, produced in 21 greenhouses.
The interest in securing the market with fresh fruit led to the intensive cultivation of cherries in a glass-covered greenhouse. The surface of the greenhouse is only 0.13 ha belongs to the company "ZECE SRL" and is located in Otopeni, Ilfov County.
The continuous expansion of the new high-performance greenhouses also involves the provision and continuous training of specialists who, by using high-performance technologies to ensure the market with fresh vegetables all year round but also safe in terms of product safety. That is why in the big university centres, high-performance greenhouses have been built through various research programs in which students acquire skills for the use and application of modern technologies. At the same time to ensure a high level research. Among the great university centers we mention:
Greenhouses within USAMV Bucharest, belonging to the Research Center for the Study of Agri-Food Quality with an area of 2752 m^2 (Figures 18 a and b).

Figure 18 a. Appearance from the research greenhouse - tomatoes, pepper, eggplants from USAMV Bucharest

Figure 18 b. Appearance from the research greenhouse - cucumbers, melons and gerbera on perlite substrates also lettuce in NFT from USAMV Bucharest

Research greenhouses within the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" of Iasi - Greenhouse 1 with an area: about 1200 m^2 for research. With the help of European funds and its own resources, the university has modernized its practice bases so that it is able to face the current challenges of contemporary society. (Figure 19).

Figure 19. The greenhouses from USAMV Iași (original)

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca has 3 research greenhouses: Research greenhouse 1 with an area of 448 m^2; Research greenhouse 2 with surface of 1413.92 m^2; and Research greenhouse 3 with an area of 636.73 m^2 (Figure 20).

Figure 20. Research greenhouses from USAMV Cluj-Napoca (original)

Figure 21. Greenhouses from SCDL Buzău (original)

Research institutes in Romania also have heated greenhouses or tall solariums for carrying out improvement works. Thus, in Buzau there are research greenhouses on the surface of around 1 ha as well as a vertical greenhouse (Figure 21).

At SCDL Bacău, there are research greenhouses low, covered with glass, with a height of 3.4 m and a width of 3.2 m. There is also a research greenhouse of 3000 sqm and two greenhouses with a total area of 8000 square meter. They also have a multi-tunnel greenhouse, covered with double foil, with an area of 1050 sqm and 6.5 m high and tunnels with a height of 3.8 m. They also have a semi-buried greenhouse with an area of 100 sqm intended for organic farming research, 4.5 m high (Figure 22).

Figure 22. Research greenhouses from SCDL Buzău (original)

CONCLUSIONS

Modern shelters, greenhouses or high tunnels are an alternative to the constant supply of fresh vegetables. High-performance technologies demonstrate their efficiency in obtaining high yields per unit area but also by reducing the greenhouse effect.

In the study I made an analysis on the evolution of protected areas over time and after 1989. I came to the conclusion that in Romania the greenhouse areas covered the country's vegetable needs while also being exported.

After 1989 the areas have decreased from one year to another so that the need for vegetables, especially tomatoes, is covered by imports.
In present, a return to production is being attempted to cover consumption needs. Through the new constructions and high-performance technologies, although the areas with built greenhouses are smaller, the efficiency being much better with the productions high of over 700 t/ha.

ACKNOWLEDGEMENTS

This research work was carried out with the research leading to these results has funding from the Norway Grants 2014, under Project contract no. 40/2021

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF ORGANIC FERTILIZATION ON ORGANOLEPTIC INDICATORS AND NITRATE CONTENT IN GREENHOUSE SALAD

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Abstract

In an experiment with a greenhouse lettuce in the period 2018-2020, the influence of biological fertilization on the visual characteristics, taste indicators and nitrate content were studied. The biological fertilizers were tested: Arcobaleno, Italpolina, Lumbrical and the microbiological fertilizer Ecoprop NX with control of non-fertilized and mineral fertilization (MF) - NPK. The study was conducted with three varieties of salads: 'Maritima' - 'Batavia' type, 'Tuska' variety - 'Lolo rosa' type and 'Winter Butterhead' - 'Butterhead' type. An organoleptic assessment was performed on a five-point scale. A differentiated variety reaction was established. The 'Maritima' variety is the most responsive to the applied organic fertilizer. The variants with organic fertilization have a complex score from 3.52 for Ecoprop NX to 3.62 for Arcobaleno compared to 3.30 for the control of non-fertilized and 3.49 for the control of mineral fertilization. For the variety 'Tuska' only the variant fertilized with Italpolina exceeds the control and has the highest complex score of 3.82. Biological fertilization has improved the quality of the 'Winter ButterHead' variety and the complex assessment is from 3.14 in Italpolina to 3.41 in Lumbrical compared to the control variants non-fertilized 3.07 and MF 3.10. Fertilization with the tested biological fertilizers does not increase the amount of nitrates in lettuce leaves above the maximum permissible amounts set by the European Commission.

Key words: *lettuce, greenhouse, organic fertilizers, sensory evaluation, nitrates.*

INTRODUCTION

Salad is one of the most grown and marketed vegetables in Bulgaria. Although the production is year-round consumption is mainly in the autumn-winter and winter-spring period. Consumers are interested in both the price and the quality of the product. More and more attention is paid not only to the taste, but also to the health of the food. Interest in organically produced vegetables, including salads, is growing. Visual and taste characteristics are important in the choice of consumers. In this direction, a number of studies have been conducted on salad varieties and growing dates for the selection of those with an attractive appearance and high taste. Thicoipe et al. (2004) performed sensory analysis of 9 varieties of salads of the types: Batavia, Butterhead, Lollo, Romaine, Iceberg. They point out that the varieties: Iceberg and Romaine have the greatest crunchiness, juiciness and sweetness. Evaluation of taste and sensory qualities in 50 varieties of *Lactuca sativa* is conducted by Liu et al. (2016). They found significant differences between the different groups and different colored varieties. The head lettuce has a better sensory evaluation compared to the leaf lettuce and the lettuces with green coloring than those with purple. The taste qualities are mainly determined by the variety and the variety type (Bunning et al., 2010). This conclusion is the result of the evaluation of sensory qualities and total phenols in 5 varieties of lettuce (Butterhead, Crisphead, Green leaf, Red leaf, Romaine). Harvesting is done at the beginning, middle and end of the season. They found that taste and bitterness had nothing to do with environmental factors (temperature and light) and the content of phenolic compounds. Akand et al. (2010) set up an experiment with 6 genotypes of lettuce to study the organoleptic characteristics 15, 30, 45 days after planting. After the analysis with the most points (1027) is the genotype Green waves on day 30 and with the least (927) Okayama salad 45 days after planting. Fertilization and the applied cultivation technology have a significant influence on the taste and visual characteristics. Great interest is shown in organically produced salads and their organoleptic and visual evaluation. Some studies indicate that there are no significant differences in appearance and taste after treatment with organic and non-organic fertilizers (Masarirambi et al., 2010). They use three organic fertilizers: 1. bounce back compost; 2. cattle manure; 3. chiken manure; 2 and 3 40 t/ha, 1 - 2.5 t/ha, control - mineral fertilizer 1055 kg/ha. The best results in growth, development and yield are obtained after fertilization with chicken manure, followed by beef and compost, and the last is inorganic manure. Similar results were obtained by Sajjad et al. (2020) in a study of the influence of organic fertilizers on the morphological characteristics and organoleptic characteristics of Iceberg and Batavia type salads. The best results are obtained after fertilizing with chicken manure. The highest value for flavor 4.5 was found in leaf lettuce. From a practical and scientific point of view, it is of interest to determine whether there is an organoleptic difference between organically and conventionally grown vegetables. Zhao et al. (2007) conducted experiments with organically and conventionally grown salads (red and green), spinach, tomatoes, cucumbers and onions. The consumer test does not show significant differences in liking and perception of sensory quality. However, consumers believe that organic products are healthier (72%) and more environmentally friendly (51%), and 28% consider organic vegetables to be tastier. Similar study for salads grown organically, conventionally, and hydroponically was conducted by Murphy et al. (2011). They analyzed 5 varieties of types: Romaine, Green leaf, Red leaf, Butter and Common lettuce. They used 5 and 3 degree scales to assess taste, smell, visual quality and texture. The obtained results did not show a significant difference in the sensory evaluation of hydroponic, conventionally and organically produced salads. The content of nitrates also has a significant impact on the quality assessment of salads. In a field experiment conducted in Greece, the influence of the date of sowing and fertilization on the growth and development of Great Lake and Nabuco lettuce varieties was studied. The effect on physical and organoleptic characteristics has been determined. They found that the heads had a lower content of nitrates than the leaves. The values of the sensory parameters are inversely proportional to the amount of fertilizers used (Khah & Arvanitoyannis, 2003). In another experiment, Boros et al. (2020) investigated the influence of technology and the term (season) of cultivation on the content of nitrates in variety tipes salads Batavia, Butterhead, Lollo, Oak leaf. They found that when Oak Leaf lettuce was harvested in the fall, they accumulated less nitrate than the red ones and the Lollo type. During spring harvest, Batavia accumulates more nitrates than Butterhead.

In summary, it can be stated that the results of the studies are not one-way. They vary depending on the variety and type, as well as the fertilizers used. The specific consumer requirements, tastes and preferences have a significant influence. Based on this, we set ourselves the goal of conducting an experiment to assess the visual and organoleptic characteristics after application of different organic fertilizers in salad varieties of different types for the conditions of Bulgaria.

MATERIALS AND METHODS

The experiment was conducted in the period 2018/2020 in an unheated polyethylene greenhouse of the Agricultural University - Plovdiv. The influence of biological fertilization on the visual characteristics, taste indicators and nitrate content of lettuce was studied. The biological fertilizers were tested: Arcobaleno, Italpolina, Lumbrical and the microbial fertilizer Ecoprop NX. The following variants were set: 1. Unfertilized; 2. Mineral fertilization; 3. Italpolina; 4. Arcobaleno; 5. Lumbrical; 6. Ecoprop NX. The study was conducted with three varieties of lettuce: variety ‘Maritima’ (type ‘Batavia’), variety ‘Tuska’ (type ‘Lollo rosa’) and variety ‘Winter Butterhead’ (type ‘Butterhead’). The experiment was based on 4 repetitions of 28 plants in a plot. Fertilizers were applied during the last soil preparation in the following norms: Italpollina - 250 kg/ha, Arkobaleno - 1000 kg/ha, and Lumbricompost - 4000 L/ha. Soluble microbial fertilizer Ekoprop NX was applied twice at a dose of 1 kg/ha before planting in phase 4-5 leaf seedlings and 10 days after planting. Mineral fertilization was done with potassium sulfate, triple super phosphate and ammonium nitrate N- 125 kg/ha,

P_2O_5- 12.5 kg/ha, K_2O - 47.5 kg/ha. The lettuce was planted in phase 4-5 leaves at the beginning of November according to the scheme 70 + 30 + 30 + 30/30 cm. During the vegetation the appropriate care was taken, as the watering was done with a drip system.

The harvesting took place from the second decade of March to the end of March. In the middle of the harvest period, an organoleptic evaluation was performed by an 11-member jury on a five-point scale using the following methodology. The visual indicators of the rosette were evaluated: 1. Coloring - separately for the green and red colored varieties with application of 5 degree scale according to the color intensity; 2. Habitus type - on a three-point scale (dense, semi-loose, loose); 3. Size - on a three-point scale (very large, large, medium); 4. General appearance - on a five-point scale (highly attractive, attractive, impressive, normal, unattractive). A tasting evaluation of the indicators was conducted: 1. Taste - on a five-point scale (sweet, neutral, sweet bitter, slightly bitter, bitter); 2. Consistency - on a five-point scale (tender, brittle, juicy, coarse, fibrous); 3. Aroma - on a five-point scale (strong pleasant, moderately pleasant, weakly pleasant, typical, unpleasant). A overall organoleptic assessment was made as a sum of the values of all indicators divided by their number.

During the harvest, the amount of nitrates was determined in an average sample of 4 plants from each variant (1 plant from each replication) in all three varieties. The analysis was performed by VVL (internal laboratory methodology), created and verified on the basis of BDS EN 12014-3: 2005

A one-way analysis of variance and an LSD test were performed to assess the differences in each of the traits resulting from the application of the tested fertilizers. The mathematical processing of the experimental data was performed using the software product IBM SPSS Statistics 24 (Ganeva, 2016).

RESULTS AND DISCUSSIONS

1. Sensory evaluation of 'Maritima' lettuce.

The Maritima variety has reacted positively to the applied fertilization with the used organic fertilizers. The values for most indicators are higher than the mineral fertilization and significantly exceed the unfertilized control.

1.1. Visual indicators (Table 1)

After fertilization with organic fertilizers, the color, density and size are improved. Coloring becomes more intensive, with values in all bio-variants exceeding unfertilized control and mineral fertilization. The highest score of 2.73 was given to variants 4, 5 and 6 (with biological fertilizer). Density has improved in all variants with bio-fertilization and is superior to mineral fertilization and non-fertilization control. The highest values for this indicator have the variants 4 Arkobaleno and 5 Lumbrical, respectively 2.64 and 2.82 at 1.91 for mineral fertilization. Biological fertilization had the strongest effect on the size of the rosette. Variants 3, 4 and 6 are superior to mineral fertilization and unfertilized control. The highest score of 4.20 was given to option 4 Arkobaleno . The differences in the visual indicators of the indicated variants are statistically significant both in the control of non-fertilization (Table 2) and in the control of mineral fertilization (Table 3).

Table 1. Sensory analysis in lettuce variety 'Maritima'

Variant	Visual indicators				Tasting evaluation			Overall assessment
	Leaf rosette							
	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma	
1	4.18	2.55	2.27	3.18	4.36	3.00	3.55	3.30
2	4.18	2.55	1.91	3.73	4.18	4.09	3.82	3.49
3	3.82	2.60	2.45	4.09	4.64	3.82	3.91	3.62
4	4.09	2.73	2.64	4.20	4.09	3.45	3.91	3.59
5	4.18	2.73	2.82	3.55	4.27	3.45	3.82	3.55
6	3.91	2.73	2.45	3.91	3.91	4.00	3.73	3.52

Table 2. Proof of the differences in the variety 'Maritima' after fertilization with biofertilizers compared to control 1 (unfertilized) with a significance level ≤ of 0.5

Variant	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma
3	*..	n.s.	n.s	*	*	*	*
4	n.s.	*	*	*	n.s.	*	*
5	n.s.	*	*	*	n.s.	*	*
6	n.s.	*	n.s	*	. *	*	n.s.

Table 3. Proof of the differences in the variety 'Maritima' after fertilization with biofertilizers compared to control 2 (mineral fertilization) with a significance level ≤ of 0.5

Variant	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma
3	*.	n.s.	*	*.	*	n.s	*
4	n.s.	*	*	*	*	*	*
5	n.s.	*	*	n.s.	n.s.	*	n.s.
6	n.s.	*	*	.n.s.	*.	n.s	n.s.

1.2. Organoleptic indicators (Table 1)
The performed tasting evaluation also gives an advantage to most variants with biological fertilization. The taste of the 3 Italpolina and 5 Lumbrical variants is 4.64 and 4.27, respect-tively, superior to the mineral fertilizer 4.18 as the mathematical differences for variant 3 are proved in the non-fertilized and fertilized controls (Tables 2 and 3).The consistency was less affected by the application of organic fertilizer. All variants have higher scores than the control - unfertilized with proof of differences, but inferior to mineral fertilization. Only the variant fertilized with Ecoprop NX has a score of 4.00 close to mineral fertilization - 4.09.
The fertilizers used have enhanced the aroma of the salads. All salads fertilized with organic fertilizers exceed the control - unfertilized with proof of differences, as options 3, 4 and 5, respectively, with scores of 3.91; 3.91 and 3.82 are equalized and exceed the mineral fertilization by 3.82.
The complex assessment from the sensory analysis gives, although a slight advantage of the variants with biological fertilization over the mineral fertilization 3.49 and the control - non-fertilization 3.30. With the highest scores of 3.62 and 3.59 are options 3 and 4 fertilizers with Italpolina and Arkobaleno.

2. Sensory evaluation of 'Tuska' leaf lettuce (Table 4)
The 'Tuska' variety reacted less well to fertilization with organic fertilizers. The effect is insignificant in the visual indicators of the leaf rosette: color, density, size. There is a more pronounced positive effect on the indicators of the tasting evaluation: taste and consistency.

2.1. Visual indicators (Table 4)
After the application of the tested biological fertilizers, the salads have a more intense red color compared to the control - unfertilized, but are inferior to the mineral fertilization with a score of 4.70. Only option 5 fertilization with Lumbrical is equal to mineral fertilization. Statistically, differences were shown in the coloration compared to the control - not fertilized for variants 3, 4 and 5 (Table 5) and in comparison with the mineral fertilization for variants 4 and 5 (Table 6).The same trend is observed in the density of the leaf rosette. Only variant 5 with 3.14 approaches the mineral fertilization 3.18. This trend is maintained in terms of rosette size. Only the Italpolina variant has a higher value of 3.40 than the mineral fertilizer 3.20. In terms of density and size, only option 4 has a proven difference in terms of mineral fertilization (Table 6).

2.2. Organoleptic indicators (Table 4)
The taste has improved and all variants with organic fertilization exceed the control - unfertilized. The highest score of 4.14 was given to the fertilizer variant with Arkobaleno. The fertilizer variant with Lumbrical has a score of 3.00 and slightly exceeds the mineral fertilizer 2.82. The differences were proved for variants 3, 4 and 5 compared to unfertilized (Table 5) and for variants 3 and 5 compared to mineral fertilization (Table 6). The consistency has improved only after the application of the organic fertilizers Arkobaleno and Italpolina

with a value of 4.09 and 4.64 and exceeds the mineral fertilizer with a score of 3.91 and the differences are mathematically proven. The aroma is best in the variants with mineral fertilization. The applied biological fertilizers in variants 3, 4 and 5 are superior to the control - not fertilized, but inferior to the variants with mineral fertilization. The complex assessment from the sensory analysis only in variant 3 fertilization with Italpolina 3.82 is superior to the mineral fertilization 3.71. All variants with biological fertilization are superior to the control - non-fertilized.

Table 4. Sensory analysis in lettuce variety 'Tuska'

Variant.	Visual indicators				Tasting evaluation			Overall assessment
	Leaf Rosette							
	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma	
1	4.70	4.55	3.00	3.00	2.00	3.64	3.09	3.42
2	4.50	4.70	3.18	3.20	2.82	3.91	3.64	3.71
3	4.80	4.64	3.00	2.82	4.14	4.09	3.27	3.82
4	4.60	3.80	2.82	3.40	2.73	4.64	3.45	3.63
5	4.94	4.70	3.14	3.00	3.00	3.55	3.00	3.62
6	4.88	4.55	2.82	2.82	2.40	3.82	3.18	3.49

Table 5. Proof of differences in the variety 'Tuska' after fertilization with biofertilizers suspended control 1 (unfertilized) with a significance level ≤ of 0.5

Variant	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma
3	*	*	n.s.	n.s.	*	*	*
4	n.s.	*	n.s.	*	*	*	*
5	*	*	*	n.s.	*	.*	n.s.
6	*	n.s.	n.s.	n.s.	n.s	*	n.s.

Table 6. Proof of the differences in the variety 'Tuska' after fertilization with biofertilizers compared to control 2 (mineral fertilization) with a significance level ≤ of 0.5

Variant	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma
3	*	n.s.	n.s.	n.s.	*	*	n.s.
4	n.s.	*	n.s.	*	n.s.	*	n.s.
5	*	n.s.	n.s.	n.s.	*	n.s.	. *
6	*	. *	n.s.	n.s.	n.s.	n.s.	. *

3. Sensory evaluation of head lettuce 'Winter Butterhead'

The 'Winter Butterhead' variety, like the 'Tusca' variety, was less affected by the applied organic fertilizers. From the visual indicators the coloring and the size of the leaf rosette were more strongly influenced.

In the indicators of the tasting evaluation, the strongest positive effect was given by the used biological fertilizers in terms of consistency.

3.1. Visual indicators (Table 7)

Coloring only in variant 5 with Lumrical has a score of 2.79, which exceeds the mineral fertilization 2.36 and the control - non-fertilization 2.55 with proven differences in both controls (Tables 8 and 9).

The density of the leaf rosette is also slightly affected, as options 4 and 5 are equal to the mineral fertilizer 2.82, and only option 6 exceeds it by 3.00 and the difference is proven (Table 9).

The size of the leaf rosette is better in all variants with biofertilization and significantly exceeds the mineral fertilizer 1.73. The highest score of 3.18 is the fertilizer variant with Lumrikal.

3.2. Tasting evaluation (Table 7)

The taste did not improve after the biofertilizers were used. Only variant 5 with Lumrical has a score of 4.40, equal to the mineral fertilizer 4.40 and surpasses the control - unfertilized. The consistency is most strongly influenced by the biofertilizers used. All variants are superior to mineral fertilization 3.73 and control - unfertilized 3.25. Option 6 with Ecoprop NX received the highest score - 4.36 .Statistical differences were proved in all variants compared to the control not fertilized (Table 8) and for variants 4 and 6 compared to mineral fertilization (Table 9).

In terms of flavor, option 4 is equal to mineral fertilizer 3.45, and options 5 Lumrical and Ecoprop NX 6 surpass it with grades of 3.55 and 3.65, respectively.

In general, however, the complex assessment from the sensory analysis gives preference to the variants fertilized with biofertilizers. The highest score of 3.41 is in the variant fertilization with Lumrical, which is superior to mineral fertilization with a score of 3.10.

Table 7. Sensory analysis in lettuce variety 'Winter Butterhead'

Variant	Visual indicators				Tasting evaluation			Overall assessment
	Leaf Rosette							
	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma	
1	3.22	2.55	3.00	1.55	4.30	3.25	3.64	3.07
2	3.22	2.36	2.82	1.73	4.40	3.73	3.45	3.10
3	3.44	2.36	2.09	2.82	4.10	3.91	3.27	3.14
4	3.67	2.36	2.82	1.91	4.00	4.27	3.45	3.21
5	3.11	2.79	2.82	3.18	4.40	4.00	3.55	3.41
6	3.44	2.45	3.00	2.82	3.90	4.36	3.64	3.37

Table 8. Proof of the differences in the variety 'Winter oil head' after fertilization with biofertilizers compared to control (unfertilized) with significance level ≤ of 0.5

Variant	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma
3	n.s.	n.s.	.*	*	. *	*	n.s.
4	*	n.s.	n.s.	*	n.s.	*	n.s.
5	n.s.	*	n.s.	*	*	*	n.s.
6	n.s.	n.s.	n.s.	*	*	*	n.s.

Table 9. Proof of differences in the variety 'Winter oil head' after fertilization with biofertilizers compared to control 2 (mineral fertilization) with significance level ≤ of 0.5

Variant	Appearance	Coloring	Density	Size	Taste	Consistence	Aroma
3	n.s.	n.s.	. *	*	n.s.	n.s.	*
4	*	n.s.	n.s.	n.s.	. *	*	n.s.
5	n.s.	*	n.s.	*	n.s.	n.s	n.s.
6	n.s.	n.s.	n.s.	*	*	*	*

4. Content of nitrates in salad varieties fertilized with mineral and biofertilizers

4.1. Nitrate content in 'Maritima' variety (Table 10)

The values measured in the individual variants by years vary from 945 mg/kg in the control - not fertilized to and 2450 mg/kg in mineral fertilization. These are levels below the maximum levels established by Regulation (EU) № 1258/2011 as regards maximum levels for nitrates in foodstuffs. For salads (*Lactuca sativa*) greenhouses they are for the period from 1.X. to 31.III. up to 5000 mg/kg, and for the period from 1.IV to 30.IX to 4000 mg/kg.

The lowest amount of nitrates was measured after fertilization with organic fertilizers Lumrical and Arkobaleno, respectively for 2019 - 1615 mg/kg and 1775 mg/kg, and for 2020 are 1449 mg/kg and 1382 mg/kg. These values are significantly lower than the variants with mineral fertilization, but higher than the control - non-fertilized.

Nitrates are the highest in variant 3 - fertilization with Italpolina and are close to mineral fertilization.

Table 10. Nitrate content in salad variety ‘Maritima’ fertilized with mineral and biofertilizers

Variant	Nitrates				
	2019	%	2020	%	average
1. Control-unfertilized	1265	51.6	945	60.3	1105
2. Control-MF (mineral fertilization)	2450	100	1582	100	2016
3. Italpolina	2320	94.7	1519	96.0	1919.5
4. Arkobaleno	1775	74.4	1382	87.4	1578.5
5. Lumrical	1615	65.9	1449	91.6	1532
6. Ekoprop NX	2235	91.2	1414	89.5	1824.5

The applied fertilization with the studied organic fertilizers in Maritima variety does not form nitrates above the maximum allowable concentration and is lower in value than the mineral fertilization.

4.2. Nitrate content in ‘Tuska’ variety (Table 11)
This variety (representative of the ‘Lollo rosa’ type) tends to accumulate more nitrates. A similar finding was made in his study Boros et al. (2020).
Minimum values were measured during the control - unfertilized 1180 mg/kg.

4.3. Nitrate content in the ‘Winter oil head’ variety (Table 12)
maximum for mineral fertilization 2800 mg/kg. In 2019, the variants fertilized with biofertilizers have lower values of nitrates than mineral fertilization. This trend continues in the second year. The lowest amount of nitrates in both experimental years was reported in variant 4 - Arkobaleno, 1885 mg/kg and 1300 mg/kg, respectively.
The highest levels were measured after fertilization with Italpolina, but lower than the control - mineral fertilization.

Table 11. Nitrate content in ‘Tuska’ salad fertilized with mineral and biofertilizers

Variant	Nitrates				
	2019	%	2020	%	average
1. Control-unfertilized	2030	57.3	1790	59.7	1910
2. Control-MF (mineral fertilization)	3545	100	3005	100	3275
3. Italpolina	2985	84.2	2586	86.1	2785.5
4. Arkobaleno	2615	73.8	2303	76.6	2459
5. Lumrical	2325	65.6	1822	60.6	2073.5
6. Ekoprop NX	2875	81.1	2100	69.9	2487.5

The nitrate levels of this ‘Butterhead’ variety are comparable to those of the ‘Maritima’ variety (‘Batavia’ type). Nitrates in both years range from 1180 mg/kg in the control - fertilized to 2800 mg/kg in mineral fertilization and are below the maximum allowable concentration. The applied fertilization with the mentioned biofertilizers has formed plants with lower content of nitrates compared to the mineral fertilization.
The lowest values of 1885 mg/kg and 1300 mg/kg were measured after fertilization with Arkobaleno - option 4. The lowest values are option 5 - fertilizer with Lumbrical and option 6 fertilizer with Ekoprop NX, significantly below the levels of mineral fertilization. The highest values close to mineral fertilization are reported for fertilization with Italpolina.
The varieties included in the experiment reacted differently to the tested biofertilizers. Variety ‘Tuska’ (‘Lollo rosa’ type) accumulates more nitrates than the ‘Maritima’ variety (‘Batavia’ type) and the ‘Winter Butterhead’ variety (‘Butterhead’ type). The applied biofertilizers form plants with a lower amount of nitrates compared to mineral fertilization. The lowest values for all three types of salads (Lollo, Batavia, Butterhead) are obtained after fertilizing with Arkobaleno and Lumbrical.

Table 12. Nitrate content in lettuce variety 'Winter Butterhead' fertilized with mineral and biofertilizers

Variant	Nitrates				
	2019	%	2020	%	average
1. Control-unfertilized	1205	43	1180	59.5	1192.5
2. Control-MF (mineral fertilization)	2800	100	1983	100	2391.5
3. Italpolina	2505	89.5	1742	87.8	2123.5
4. Arkobaleno	1885	66.3	1300	65.6	1592.5
5. Lumrical	2270	81.1	1651	83.3	1960.5
6. Ekoprop NX	2295	82	1573	79.3	1934

CONCLUSIONS

The varieties lettuce 'Maritima' (type 'Batavia'), red lettuce 'Tuska' (type 'Lollo rosa') and head lettuce 'Winter oil head' (type 'Butterhead') fertilized with organic fertilizers Italpolina, Arkobaleno, Lumbrikal and Ekoprop NX accumulate less nitrates of 5.3% to 39.4% compared to mineral fertilization. The 'Tuska' variety accumulates more nitrates than the 'Maritima' and 'Winter Butterhead' varieties. Salads fertilized with organic fertilizer Arkobaleno and Lumbrikal have the lowest amount of nitrates, respectively: 'Maritima' 1578.5 mg/kg and 1532 mg/kg; 'Tuska' 2459 mg/kg and 2073 mg/kg and 'Winter oil head' 1592.5 mg/kg.

Highest complex sensory evaluation in the case of the 'Maritima' variety it is obtained after the application of the organic fertilizers Italpolina and Arkobaleno, respectively 3.62 and 3.59, and in the case of the mineral fertilizer it is 3.49. The 'Tuska' variety reacted less to the applied organic fertilizers.

The organoleptic indicators of taste and consistency are improved. After fertilization with Italpolina and Lumbrikal, the estimates are 4.14 and 3.00, respectively, and exceed the mineral fertilization by 2.82.

High scores on the consistency are reported when fertilizing with Italpolina - 4.09 and Arkobaleno - 4.64, exceeding the mineral fertilization - 3.91.

The complex assessment from the sensory analysis in 'Winter Butterhead' gives a slight advantage to the variants with biological fertilization. The highest score of 3.41 is after fertilization with Lumbrikal and exceeds mineral fertilization by 10%.

ACKNOWLEDGEMENTS

This study was conducted with funding provided by the Center for Research, Intellectual Property Protection and Technology Transfer at the Agricultural University - Plovdiv.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRELIMINARY RESULTS ON THE BEHAVIOUR OF SOME GARDEN PEA ACCESSIONS BRED AT VEGETABLE RESEARCH DEVELOPMENT STATION BUZAU, ROMANIA

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Abstract

Pisum sativum belongs to Fabaceae family and is especially appreciated for its nutritional qualities. According to FAO statistics from 2019, in the world green peas are cultivated on relatively large areas. The highest yield of world production was obtained in Asia followed by Europe and USA with lower percentage. In Romania, only 2430 ha of garden peas are cultivated and the assortment of cultivars is quite limited. At VRDS Buzău, a breeding program has started and currently 15 stable accessions are being studied. This study presents preliminary results on the behaviour of improved accession. According to UPOV descriptors, it was observed that the accessions show distinct phenotypic expressiveness, with types of flowers varying from white to pink, with pods having a length ranging from 6.43 to 13.4 cm and weight of 10 pods differs from 41.8 to 84.8 g. The coefficient of variations exceeds 35%, which indicates that there is a large variability among studied accessions. The study will continue with selection work of genotypes that have distinct results and high ecological plasticity.

Key words: *genotype, improved, phenotype, Pisum sativum, selection.*

INTRODUCTION

According to FAO studies and statistics, peas have long been recognised as an inexpensive, readily available source of protein, complex carbohydrates, vitamins and minerals.

The high nutrient density of peas makes them a valuable food commodity, capable of meeting the dietary needs of the estimated 800-900 million undernourished individuals worldwide (FAO Statistics, 2011).

Based on the studies performed, it can be appreciated that the importance of legumes is due, first of all, to the high protein content of the seeds giving a significant nutritional value. The protein content of legumes exceeds 2-3 times more than cereals (Samuil, 2007).

In the context of current concerns about the future of human nutrition the protein content is an important issue. Proteins are basic, essential components of food rations so that any deficiency of these substances can lead to more or less severe food deficiencies (Mincu, 1978).

Many studies have identified potential health benefits of peas, as mentioned: cardiovascular health (Sandström et al., 1994; Trinidad et al., 2010), gastrointestinal health (Dahl et al., 2003; Flogan and Dahl, 2010) antioxidant activity (Dueňas et al., 2004), high content in complex carbohydrate (Hoover et al, 2010; Tosh and Yada, 2010) and glycaemic response and insulin resistance (Marinangeli et al., 2009).

From an agronomic point of view, peas are valuable for: helps to enriches the soil with nitrogen, having the ability to fix molecular nitrogen in the atmosphere, through nodules resulting from symbiosis with *Rhizobium* sp. bacteria, thus contributing to improving soil fertility (Popescu and Roman, 2008).

Also, the pea root system has the capacity to solubilize phosphorus compounds, making this element available to the successor plant in a more accessible form.

The pea root system is well developed in depth, ensuring a good stability of the soil.

The beneficial intake of pea root in land is based on the improvement of nitrogen, one of the key factors for ensuring soil fertility, supporting the production of cereals in dry or developing areas (Jacobsen et al., 2012).
World production of peas in 2019 (according to FAOSTAT) was more than 19 million tonnes, with a harvested area of over 2 million ha. The major producers being in Asia (China, India) with a percentage of 85.1%, in Europe with just 8.7% and USA with 6.2%. In Romania, according to the same statistics, green peas occupy just 2430 ha, with a yield of 5589 t, but the assortment of cultivars is quite limited.
Currently, in the Official Romanian Crop Plant Catalogue, has a number of 9 cultivars of garden peas. Because of the low number of valuable cultivars, at Vegetable Research Station (VRDS) Buzau has started a breeding program (Barcanu et al., 2019). In this work, 15 accessions of *Pisum sativum* have been evaluated from the agro-morphological point of view, in order to establish the important traits of the studied accessions.

MATERIALS AND METHODS

The germplasm pea collection from Breeding and Biodiversity Laboratory of VRDS Buzau had as main sources of genetic material: local landrace; cultivars collected from the main vegetable basins in Romania; cultivars and hybrids from Romania and abroad. VRDS Buzau has a valuable germplasm collection of *Pisum sativum* and in the present study were selected 15 stable accessions.
The experiments were carried out at VRDS Buzau in the research field. The sowing was made in the first decade of March. The Research Centre is located in the Buzău riverside and has sandy-loam soil.
The area is characterized by a dry climate, with hot summers, with temperatures exceeding an average of 22°C in the warmest month and with a low rainfall distributed unevenly throughout the year. The annual average rainfall being 538 mm. The maximum temperature, minimum temperature and rainfalls in the studied years are shown in Table 1.

Table 1. Mean of temperature and rainfall during studied years (°C, mm)

2019	**March**	**April**	**May**	**June**	**July**
Temp. max	15.93	16.5	22.51	29.3	29.35
Temp. min	2.74	5.43	11.74	17.9	16.87
Rainfall	48.1	39.4	221.6	33.2	64
2020	**March**	**April**	**May**	**June**	**July**
Temp. max	14.35	19.93	22.77	28.3	30.77
Temp. min	3.32	4.8	10.7	16.16	18.09
Rainfall	29.3	6.9	102.5	78.1	75

The morphological characteristics are divided in two groups: qualitative and quantitative traits according to UPOV guidelines.
The qualitative traits used in this study are presented in Table 2.

Table 2. Qualitative traits

Plant: anthocyanin coloration AC	1. absent, 9. present.
Foliage: colour FC	1. yellow green, 2. green, 3. blue green.
Leaf: leaflets F	1. absent, 9, present.
Flower: colour of wing CW	1. white with pink blush, 2. pink, 3. reddish purple.
Flower: shape of base of standard BS	1. strongly raised, 3. moderately raised, 5. level, 7. moderately arched, 9. strongly arched.
Pod: shape of distal part SP	1. pointed, 2. blunt.
Pod: curvature C	1 absent or very weak. 3 weak. 5. medium 7 strong. 9 very strong.
Pod: colour PC	1. yellow, 2. green, 3. blue green, 4. purple.
Intensity of green colour IGC	3. light, 5. medium, 7. dark.
Pod: suture PS	1. absent, 2. present.
Immature seed: intensity of green colour GC	3. light, 5. medium, 7. dark.
Seed: shape S	1. ellipsoid, 2. cylindrical, 3. rhomboid, 4. irregular.
Seed: type of starch grains SG	1. simple, 2. compound
Seed: intensity of wrinkling of cotyledon IC	3. weak, 5. medium, 7. strong, 9. very strong.
Seed: colour of cotyledon CC	1. green, 2. yellow, 3. orange.
Seed: hilum colour HC	1. same colour as test, 2. darker than testa

Quantitative traits used were: plant length (PL), stem length (SL), number of nodes including first fertile node (NN), number maxim leaflet (NML), leaflet length (LL), leaflet width (LW), stipule length (SL), stipule width (SW), Stipule length from axil to tip (ST), petiole length measure from axil to first leaflet or tendril (PLA), flower width (FW), width of upper sepal (WS), length of spur (LS), length from stem to first pod (LFP), length between first and second pods (LSP), pod length (PL), pod width (PW). The production characters used were: number of ovules per pod (PNO), number of pods/m^2(NP), number of pods per plant (NPP), weight of 10 pods (WP), weight of 10 seeds (W), diameter seeds (D), total soluble content (°Brix).

RESULTS AND DISCUSSIONS

The studied accessions were described using UPOV guidelines. During vegetation period qualitative traits were noted and their distribution are found in Table 3. The qualitative traits were statistically distributed using histograms, and it was noted that 12 accessions did not have anthocyanin coloration, while 3 accession (A6, A7, A9) had. The colour of the foliage varied from green-yellow on accession (A8), to green to 3 accessions (A5, A7, A9) and green-blue to 11 accessions.

Figure 1. Type of flowers on studied accessions

The wing color of the anthocyanin-colored varieties was pink on one accession (A7) and red-purple on two accession (A6) (Figure 1). The standard colour of the flower in the varieties without anthocyanin coloration was white in all studied accessions (Figure 1). The shape of distal part of the pod was pointed at 80% of the accessions, and blunt at accessions A7, L8 and A11.

The curvature pod was absent or very weak in 40% of the accessions and weak in 8 accession, and medium in one accession, A8. The colour of the pod varied from green (A1, A6, A8, A9, A10), to green-blue (A2, A3, A4, A5, A11, A13, A15), to yellow (A12) and purple (A7).

Table 3. Qualitative traits

Traits	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
AC	1	1	1	1	1	9	9	1	9	1	1	1	1	1	1
FC	3	3	3	3	2	3	2	1	2	3	3	3	3	3	3
F	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
CW	1	1	1	1	1	3	3	1	1	1	1	1	1	1	1
BS	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1
SP	1	1	1	1	1	1	2	1	2	1	1	2	1	1	1
C	3	3	1	1	3	1	1	5	3	3	1	3	3	3	1
PC	2	3	3	3	3	2	3	2	2	2	2	1	3	2	3
IGC	3	3	3	5	3	5	5	3	5	5	3	3	5	7	3
PS	9	1	9	9	9	1	1	9	9	1	1	1	1	1	1
GC	3	3	3	3	3	3	3	3	3	3	3	3	3	5	5
S	1	2	1	1	2	1	2	2	2	2	2	2	2	2	2
SG	1	1	2	1	1	1	2	1	1	1	1	1	1	1	1
IC	1	2	1	1	2	1	2	2	2	2	2	2	2	2	2
CC	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1
HC	1	1	2	1	2	1	1	2	2	1	1	2	2	1	1

The intensity of the green colour also varied from light to 53.3% of accessions, to medium for 30% of accessions and dark for accession A14. In Figure 2 are presented the type of pods studied.

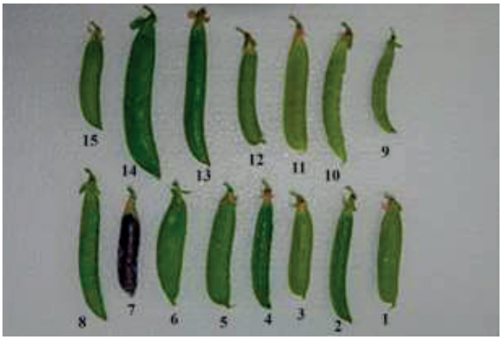

Figure 2. Phenotypic expression of pea pods

The pod suture was present for 6 accessions and absent for accessions: A2, A6, A7, A10, A11, A12, A13, A14 and A15. The grain shape of was ellipsoidal for 26.7% of accessions and cylindrical for the rest of 73.3%. The type of starch grain was simple for 7 accessions and compound for 8 accession. The intensity of wrinkling of cotyledon seed was weak for all the studied accessions. The colour of cotyledons was green for 13 accessions and yellow for 2 accessions (A8, A9). The hilum colour was the same for 10 accessions and darker than the skin for the remaining 5 accessions (A3, A5, A8, A12, A13). In Figure 3 are presented the crop detail and a pod grain aspect.

Figure 3. Crop details and pod development

The quantitative traits were measured and the mean results were noted in Tables 4 and 5. The coefficient of variation had a wide variability especially in terms of stem length, number of pods/m^2 and weight of 10 pods.

The plant height had varied from 27.36 cm to (A1) to 75.55 cm (A6). According to standards, cultivars are considered dwarf or semi-tall with a height between 30-70 cm, and tall, with a height over 100 cm (Drăghici, 2015). The number of nodes including first fertile node had variations from 15.5 to A7 to just 6.5 nodes at A5. The pea leaf has a pair of basal stipules and a rachis supporting opposite pairs of leaflet. Leaflets and tendrils occupy predictable positions on the rachis (Gould, 1986); tendrils are normally formed distal to leaflets. The leaflet length on studied accessions varied from 2.46 cm on A10 to 4.45 cm to A14. Leaflet width had some variations between the same accessions, from 3.2 cm to A10 and 1.55 cm to A14. Stipule length was diverse from 4.65 (A10) to 7.07 cm (A14). The pea flower is self-fertile.

The pea pod is dehiscent, but in "edible podded" (mange-tout) accessions, like A6, the pods have little or no parchment. The largest variations within studied accessions were in terms of size pods. The lowest value was recorded by A7with just 6.44 cm number and the maximum value was noted at A6 with 13.53 cm. The number of pods/m^2 is accession dependent, in this case, A3 and A4 had a number of pod varied from 556 to 597 and at the opposite pole, A8 had recorded a number of 98 pods/m^2. In terms of pods weight, the mass of 10 pods fitted between 84.85 g (A14) to 44.85 g (A4). Regarding the weight of 10 seeds, A6 had registered 15.39 g and A3 just 3.2 g.

The mean yield/m^2 was graphically represented in figure 4. It can be noted that the highest yield potential was recorded by A3 and A4 (over 2 kg/m^2), and the lowest yield were obtained by the A7 and A8 (below 900 g/m^2).

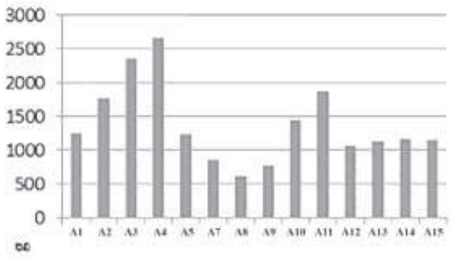

Figure 4. Yield per plant on studied accessions (grams)

Table 4. Quantitative traits the plant

Accession	(PL)	(SL)	(NN)	(NML)	(LL)	(LW)	(SL)	(SW)	(ST)	(PLA)	(FW)	(WS)
A1	27.37	3.73	6.83	6	3.96	1.82	5.33	2.86	3.86	2.36	0.85	2.06
A2	50.32	3.35	8.5	3.5	3.96	2.42	6.66	3.35	5.13	2.77	0.86	2.05
A3	49.12	3.76	9.17	5	2.85	1.75	6.04	3.34	4.51	4.02	0.75	2.35
A4	64.43	6.26	12.67	4.5	3.74	1.65	6.75	4.97	4.74	4.42	1.06	2.13
A5	52.3	3.25	6.5	5	3.33	2.37	5.84	2.8	4.42	2.67	0.78	1.96
A6	75.55	2.06	8	5	3.23	2.44	6.55	3.46	4.85	3.2	0.52	2.23
A7	38.75	3.68	15.5	5	2.94	1.84	5.74	5.4	3.89	5.2	0.66	1.34
A8	57.08	1.76	14.5	4	3.25	1.79	5.72	4.71	4.15	4.07	0.98	2.53
A9	30.34	1.2	12.67	5	3.07	1.88	5.06	4.82	3.83	3.72	0.98	2.56
A10	44.5	2.26	7	4.88	2.47	1.56	4.65	2.25	3.54	2.9	0.85	2.65
A11	50.94	2.3	7	5	3.8	2.22	4.75	2.46	3.93	3.72	0.92	1.96
A12	55.17	5.44	9	6	3.77	1.85	5.1	2.45	4.25	4.4	0.45	2.45
A13	45.77	2.36	8	4	4.2	2.3	5.78	2.65	4.65	4.45	0.85	2.25
A14	65.7	1.6	8	6	4.45	3.2	7.08	4.5	6.44	6.04	1.03	2.55
A15	56.3	2.4	7	4	4.2	2.25	5.92	2.65	4.5	4.45	0.85	2.3
Means	50.9	3	9.4	4.9	3.5	2.1	5.8	3.5	4.4	3.9	0.8	2.2
SD	12.36	1.36	2.87	0.74	0.56	0.41	0.71	1.04	0.68	0.97	0.17	0.32
CV%	24.28	44.92	30.7	15.23	15.68	19.75	12.28	29.53	15.33	24.94	20.22	14.48

Table 5**.** Quantitative traits the pods

Accession	(LS)	(LFP)	(LSP)	(PL)	(PW)	(PNO)	(NP)	(NPP)	(WP)	(W)	(D)	(G)
A1	0.71	18.82	3.96	8.49	1.01	7.37	238	4.53	53.22	2.48	8	54.5
A2	0.86	28.66	2.85	11.45	0.93	6.5	326	10.82	54.53	3.37	5.83	61
A3	0.46	27.75	3.05	7.12	0.8	5.75	556	14.5	42.46	3.2	5.71	76
A4	0.72	21.25	4.07	8.15	1.05	8.13	597	11.2	44.86	2.66	5.07	64.5
A5	1.09	19.84	2.84	7.64	0.97	6.65	250	4.83	49.65	2.94	5.07	85.5
A6	2.77	33.32	3.86	13.53	0.36	6.13	114	4	47.69	15.39	7.78	51
A7	0.86	31.75	3.7	6.44	2.2	9	171	7.7	50.75	5.09	6.29	66
A8	0.73	17.78	3.13	7.82	1.27	5	99	8	63.27	3.94	8.55	84
A9	0.75	23.3	5.74	7.45	1.15	8	137	8.8	56.96	4.57	5.94	67
A10	0.82	20.16	4.15	8.84	1.25	7.4	253	7	56.96	4.57	9.81	64.5
A11	0.66	25.52	3.54	9.15	0.94	4.45	376	8.17	50.04	4.2	10.13	94.5
A12	0.85	28.65	2.85	7.6	0.85	6	234	10	45.62	4.67	5.56	76
A13	1.05	26.85	2.85	9.66	0.65	8	175	8	65.11	2.87	7.97	68.5
A14	1.55	40.65	6.4	12.45	0.85	7.7	137	6	84.85	5.6	9.2	69
A15	0.8	28.55	4.85	8.84	1.1	3.68	280	8	41.19	4.54	7.55	76
Means	1	26.2	3.9	9	1	6.6	262.7	8.1	53.8	4.7	7.2	70.5
SD	0.54	6.03	1.05	1.96	0.39	1.45	144.6	2.68	10.69	3.01	1.66	11.29
CV%	54.76	23.04	27.21	21.84	37.61	21.77	55.05	33.12	19.87	64.4	22.91	16

CONCLUSIONS

The result of the study showed that there is great variability within the studied genotypes. The A7 was the only accession with purple flower and pods and A6 had light purple flowers and green pods.

These accessions could also be used as decorative plants due to the pleasant appearance of the flowers.

Following the assessment of accessions, it was highlighted that A3 and A4 had the highest yield. A6 is recommended as mange-tout peas. The research will continue through more detailed study.

ACKNOWLEDGEMENTS

The work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI/2018, within PNCDI III.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

URBAN AND PERI-URBAN VEGETABLE GARDENS COMPOSITION

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Abstract

The design of a small urban vegetable garden is very important because in this way the site area allocated to it will be used at maximum capacity to provide decor and sufficient production for family use. In this paper, we studied the way of designing an ornamental vegetable garden on raised beds with different heights and the appearance offered by them. The environment, the way of preparing the site and creating the raised beds will play an important role on the vegetable garden composition spectrum. According to the information gathered from preliminary research some visual results regarding the development of the plants will be evident from the first week of planting due to the different heights of the raised beds. Vegetable and medicinal plants such as curly kale, mangold, patisson, lavender, rosemary, basil and other ornamental species such as French marigold, silver ragwort, fountain grass, will offer the ornamental value. Most species had a good production and development in V1 (40 cm above ground level). Instead, mint and leeks had higher values in V3 (at ground level) and V2 (20 cm above ground level), respectively.

***Key words**: urban garden, raised beds, vegetable garden, design.*

INTRODUCTION

Vegetables must occupy an important place in human food, due to the high content of nutrients and especially vitamins, whose lack or deficiency in nutrition can cause serious disorders in human metabolism (Hangan et al., 2018). The favourable effects of vegetable consumption are due to the high-water content, which leads to hydration of the body, as well as the high content of hydrocarbons, which is crucial in stimulating the activity of the muscular system (Valnet, 1982).

The technology cultivation of vegetable species allows their different associations for an intense, good and calculated use of the lands on which they are cultivated, allowing its use for a long period, by practicing vegetable crop rotation, whether it is a household garden or by a vegetable farm (Creasy, 1984; Liebman et al., 1993).

The biggest challenge for urban growers is the soil quality and in order to have a good soil quality and to lessen the exposure to soil contaminants is to plant in raised beds with an appropriate growing substrate (Megan et al., 2016). Another way to reduce contamination is taking into consideration companion planting and to use various plant species that can rapidly absorb soil contaminants or air particles (Hangan et al., 2020). The raised beds vegetable cultivation system in the urban and peri-urban area represents a valuable method in terms of contamination with various heavy metals or pesticides that can come from the soil by accumulation over time (Stoleru et al., 2015).

The gardens near houses in peri-urban and urban areas, represents a case in which consumers' preference is to associate various horticultural species with different functional roles: fruit trees, medicinal plants, fruit shrubs, vegetable species and vines (Rotaru et al., 2010).

In order to ensure the staggered production and for the most intensive use of the space, successive and intercalated cultures will be made, the latter increasing the ornamental potential of the design by adding complementary species by colour, shape and texture of the leaves or habitus.

Vegetable plants have many decorative features, starting from their size, to the colour and texture of leaves, flowers and fruits. The multitude of vegetable species currently cultivated includes species that decorate in spring by means of leaves forming vegetable

carpets, such as salad and spinach, in summer by flowers and habitus such as large beans and mangold (Kourik, 1986). Throughout the summer, all plants will provide decoration both in shape and colour. Shades of green-yellow leaves will be present in combination with yellow and red petiole of mangold plants. The green-grey leaves from leeks will contrast with the mangold leaves. Tomato fruits have a wide range of shapes and colours; from appearance to maturation, from an intense green to yellow, purple or cherry red, thus contributing to increasing the decorative effect that plants have (Mihai & Hoza, 2012).
The temperature differences existing at the beginning of 2020, respectively May, the time of establishment of the crop in the field, had a negative effect on the development of certain plants causing negative effects on their health and production. From an ornamental point of view, the plants returned to their desired shape and appearance in a relatively short time.
In this context, the aim of the study was to find out which hight of the raised beds offers the best results regarding the decoration offered by plants and the best results regarding the optimal production for family use.

MATERIALS AND METHODS

Experimental site
The research was carried out at University of Agricultural Science and Veterinary Medicine of Iași, Faculty of Horticulture, Romania, V. Adamachi farm during 2018-2020.
After a rigorous analysis of the ecological conditions offered by the area and according to the specific requirements of the plant species used the site was selected (Figure 1).
The existing soil in the experimental vegetable field is favourable for horticultural crops, showing good fertility due to the high content of organic matter (3.4-6.45%) (Stoleru et al., 2014; Munteanu et al., 2010). According to Teliban et al. (2021) and Cojocaru et al. (2019) the soil is an anthropic cambic chernozem with a pH of 7.2 and 31% clay.
Soil samples were taken to be analysed and to determine the nutritional elements in it. The Conrad probe was used to determine the pH. All measurements and samples taken were from a depth of 20 cm.

Figure 1. The plot intended for study within the experimental field of the Faculty of Horticulture, Iași (original)

The soil tests performed to the site were completed by WD-XRF S8 TIGER Sequential Spectrometer. The following values resulted for the macro and microelements and are embodied in Tables 1 and 2.

Table 1. Concentration of macroelements

	Substrate	**Soil**
N	0.39%	0.28%
P_2O_5	0.24%	0.32%
K_2O	2.05%	0.21%
CaO	3.12%	0.41%
MgO	0.60%	0.27%

Table 2. Concentration of microelements

	Substrate	**Soil**
MnO	0.10%	0.02%
Fe_2O_3	0.36%	0.74%
Na_2O	0.58%	0.39%
Zn	118 PPM	127 PPM
Cu	39 PPM	43 PPM
Mo	6 PPM	7 PPM
Cd	0 PPM	1.2 PPM
Pb	96 PPM	74 PPM
Ni	35 PPM	16 PPM
Cr	80 PPM	11 PPM
Co	8 PPM	0 PPM

The experimental farm is located at 47°10’43”N and 27°37’14”E and is part of a temperate-continental climate, pronounced in the hilly area and more moderate in the plateau area. It is characterized by differentiations of the climatological elements both in time and space. The average annual temperature is 9.6°C and with a total average rainfall of 521 mm per year (Hamburdă et al., 2016).

During the experimental year, in the vegetation period, the average temperature was 19.56°C, the precipitation was 369.01 mm and the relative air humidity was 62.55% (Table 3) (Centrul Meteorologic Regional Moldova, Iaşi, 2020).

Table 3. Meteorological conditions during April-October 2020

Month	Average air temp. (^{0}C)	Max. air temp. (^{0}C)	Min. air. Temp. (^{0}C)	Relative humidity (%)	Total duration of sunlight	Total amount of precipitation (mm)
April	11.1	26.9	-5.9	42.0	279.8	1.6
May	24.4	30.1	3.5	67.0	178.2	130.5
June	21.3	33.4	6.1	71.0	235.7	99.0
July	22.92	26.99	18.49	61.39	280.4	27.49
August	23.63	27.87	18.12	54.09	289.6	8.8
September	19.54	23.86	14.47	59.89	264.1	26.24
October	14.09	16.98	10.79	82.54	129.5	75.38

Biological material and experimental design
The biological material used is composed of seeds, seedlings and cuttings. Crops are established by sowing directly into the field (patisson, beans, carrot) and by planting seedling (kale, leek, celery, celeriac, mangold, sweet pepper, cucumber, tomatoes, eggplant, peppermint, basil, oregano, parsley, sage, thyme, onion, silver ragwort, French marigolds) and cuttings (aster, lavender, Chinese fountain grass, decorative sage, lamb's ear and rosemary).
The seeds and cuttings were purchased from various physical and online specialty stores and nurseries. The seedlings were made in the greenhouse nursery of the vegetable growing department within the University of Agricultural Sciences and Veterinary Medicine Iaşi. The crop management practices applied during the vegetation period were those recommended by the special literature and according to the plant species (Munteanu et al., 2010; Stoleru et al., 2012; Sima, 2017).
The plot intended for research has an area of 190 m^2 and is divided into three experimental variants of equal size and shape (Figure 2) as follows:
V1 - raised beds with a height of 40 cm above ground level;
V2 - raised beds with a height of 20 cm above ground level;
V3 - beds at the ground level.

The cultivation technology applied is the basic one, regarding the optimal management of the vegetation factors for the selected species, in order to obtain healthy plants, with nutritional value and with a pleasant aesthetic aspect.
Crop rotation was taken into account for better pests and disease management according to ecological agriculture principles (Figure 3).

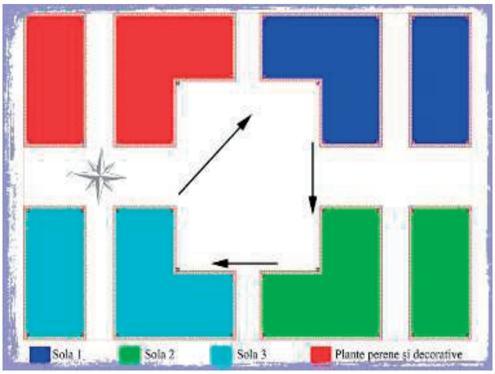

Figure 3. Crop rotation scheme (original)

Each experimental variant consists of the same plant species used in the same quantity and planted using the same design in order to evaluate which experimental variant offers the best results for crop yield and ornamental effect offered by intercropping method.

Figure 2. Representation of experimental variants (original)

The landscaping was done by basic levelling of the land. Subsequently, the site was divided into three equal parts and was marked according to the design. For the V1 and V2 variants (Figure 4), fir wood frames were built for raised beds with heights of 40 cm and 20 cm above ground level and were treated with water-based wood primer for long protection. The V3 variant was delimited with a plastic border at ground level.

Figure 4. Raised beds construction stages (original)

The substrate used in V1 and V2 is the existing soil in the experimental field improved with leaf compost and manure. Peat and Orgevit were incorporated in all three variants.

After the establishment of the raised beds, the basic soil works were carried out in order to prepare the germination and planting bed: deep soil mobilization, soil shredding and soil modelling.

The last stage of preparation of the culture substrate is represented by the installation of the irrigation system. It consists of Arctic automatic irrigation station, solenoid valve, main pipe with a diameter of 0.5 cm, taps, watering hose with built-in drippers every 20 cm and other necessary elements (elbows, connecting hose, connectors, plug end, clamps for fixing). In Figure 5 is represented the different stages of crop establishment and the functional irrigation system.

After the establishment of the raised beds, the preparation of the culture substrate and the installation of the irrigation system, the crop was established as follows.

On 29th of April 2020, the silver ragwort crops, leek, celeriac, celery, kale and purple cauliflower were established.

On 30th of April 2020, the crops of red cabbage, sweet pepper, cucumber, patisson, carrot, tomato, mint, basil, oregano and parsley were established.

On 11th of May 2020, the cultures of sage, eggplant, thyme, white onion, beans, aster, lavender, Chinese fountain grass, decorative sage, lamb's ear and rosemary were established.

The care works applied were the general and special ones depending on the species and according to the special literature.

Figure 5. Different stages of crop establishment (original)

Statistical analysis
The statistical evaluation of the data was carried out by one-way ANOVA using a SPSS ver. 21 Tukey tests were performed in order to estimate the significant difference of vegetable production between the tree experimental variants. Differences between groups were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSIONS

Results regarding the ornamental effect
The design of a small urban vegetable garden is very important because in this way the site area allocated to it will be used at maximum capacity to provide decor and enough production for family use. Its purpose is to combine the utilitarian function with the aesthetic one (Hangan et al., 2018). In this garden aromatic plants and vegetable plants are combined with flowering plants to enhance the aesthetic effect. Also, for aesthetic reasons, ornamental varieties of utilitarian plants are used (e.g.: decorative sage, mangold).
Gardens for culinary purposes look better designed in a free style, but can be organized according to a mixed or geometric scheme as the style adopted in the present study. According to Sima (2017) in order to create an ornamental vegetable garden some factors must be taken into account: contrasting hights, habitus, flowers, fruits, colours and other visual effects.
In this research, the ornamental value of the whole design is offered mainly by the colour of the leaves, petiole, flowers and flower clusters, as can be seen in Figure 6. The eye is drawn primarily by the bright orange colour of the French marigold flowers.

Figure 6. V1 overview (original)

The colour and shape of these plants is balanced in compositions by associating them with plants in cold shades - silver ragwort or lamb's ear - and by using in the background medium-sized plants (carrots, celery, basil) and creeping plants (patisson) and climbing plants (beans).
Opposed to these compositions are the plants with cold shades, having a medium size and voluminous shape. The scenery in this area is mainly offered by the shape and colour of the leaves. Kale cabbage provides the background for decorative sage flowers while mangold is used as a light green background for the leeks. Also, the mangold offers small accents of red and yellow from the petiole (Figure 7).

Figure 7. Colour detail - mangold and leek (original)

The perennial species provide the desired decoration mainly by the shape and colour of the leaves, as seen in Figure 8. Depending on the season, they also provide decoration through flowers. The colour contrast is balanced. Purple flowers offered by Russian sage and lamb's ear, in contrast to the red-pink flowers offered by the gaura. Even the Chinese fountain grass provides decoration through the floral spikes towards the end of summer.

Figure 8. Detail of the decoration offered by the leaves of the lamb's ear and the gaura (original)

With the onset of the cold season, the garden design continues to be ornamental due to kale, mangold, leek and perennial species. At low temperatures, the species of mangold and kale cabbage intensify the colour of their leaves, thus having a very high visual impact as it can be seen in Figure 9.

Figure 9. Detail on plant colouring in November (original)

The evolution of the plants is observed in Figure 10. The four images were taken at four different months to highlight how plants develop and how various their colours can be during different seasons.

Figure 10. Experimental variants in May, June, August and November (original)

Results regarding the yield

Due to temperature fluctuations from the beginning of May 2020, certain species have been affected, resulting in low yields for tomato, cucumber and carrot species. Two preventive treatments were performed based on copper and sulphur in accordance with the principles of organic farming (Stoleru et al., 2014).

Despite these temperature fluctuations, yield differences can be observed between the three variants, thus the V1 (40 cm above ground level) has the best results in terms of yield. The results of V2 (20 cm above the ground) and V3 (at ground level) vary depending on the species.

The total production values per plants from the three experimental variants are represented in Table 4.

According to the statistical analysis of the yield, both of the tomato varieties (Tigerella and Black Cherry) have no significant differences between the three variants. For both cultivars of tomatoes, the lowest yield was obtained on beds made at 20 cm above ground level. The best results of tomato were obtained on the V1 at 40 cm beds.

Sweet pepper obtained the best results in V1 compared with others version significant for $p \leq 0.05$.

The best result of onions can be obtained on raised beds with a hight at 40 cm (1,235.01 g) compared with V2 (608.66 g).

Leek has better results in both of the raised beds variants with a close production of 4,115.00 g and 4,190.00 g compared to the V3.

The different hights and substrate used in the three variants influenced the celery yield significantly. The production varied from 1,640.00 g in V1 to 1,029.00 g in V3.

On the other hand, celeriac obtained the best results at ground level compared with the other to variants significant for $p \leq 0.05$.

The best results from the entire compositions were obtained by mangold in V1 with 13,097.00 g production and with a relevant difference from the other two variants.

Table 4. Results regarding the yield of vegetable species included in composition (g)

Common name / cultivar	Total weight (g)		
	V1	V2	V3
Tomato cv. Tigerella	805.00 ± 116.00 ns	616.00 ± 93.92 ns	680.00 ± 92.17 ns
Tomato cv. Black Cherry	645.00 ± 151.32 ns	470.00 ± 45.48 ns	530.00 ± 77.37 ns
Sweet pepper cv. Barbara	1,846.42 ± 218.83 a	1,066.46 ± 90.78 b	702.00 ± 141.05 b
Onion cv. Di Parma	1,235.01 ± 259.49 a	608.66 ± 59.73 b	879.59 ± 89.71 ab
Leek cv. Blue de Solaise	4,115.00 ± 135.98 a	4,190.00 ± 44.34 a	2,920.00 ± 36.47 b
Celery cv. Gigante Dorato 2	1,640.00 ± 5.68 a	1,323.00 ± 10.58 b	1,029.00 ± 16.33 c
Celeriac cv. Giant Prague	1,715.00 ± 49.47 b	1,560.00 ± 30.09 b	3,960.00 ± 570.67 a
Mangold cv. Chard Bright Lights	13,097.00 ± 1,996.94 a	6,226.00 ± 843.89 b	5,679.00 ± 1,332.28 b
Carrot cv. Cosmic Purple	505.00 ± 48.87 a	265.00 ± 38.69 b	310.00 ± 36.75 b
Carrot cv. Royal Chantenay	3,130.00 ± 266.55 a	2,040.00 ± 409.89 b	2,475.00 ± 520.03 ab
Kale cv. Kadet	4,620.00 ± 453.89 a	2,050.00 ± 209.22 c	3,061.00 ± 101.15 b
Kale cv. Nero di Toscana	2,326.00 ± 24.61 a	1,570.00 ± 19.61 c	1,997.00 ± 6.92 b
Kale cv. Scarlet	2,608.00 ± 20.86 a	961.00 ± 15.26 c	1,104.00 ± 31.85 b
Cucumber cv. Ekol	2,784.00 ± 53.7 a	1,386.00 ± 199.73 b	1,174.00 ± 179 b
Peppermint cv. Cinderella	935.00 ± 126.73 b	670.00 ± 157.18 b	1,340.00 ± 129.67 a
Oregano cv. Kreta	565.00 ± 82.48 ns	478.00 ± 56.66 ns	449.00 ± 38.24 ns
Parsley cv. Triple Moss Curled	2,569.00 ± 516.19 ns	2,163.00 ± 454.48 ns	2,069.00 ± 203.26 ns
Thyme cv. Di Provenza	846.00 ± 86.35 a	739.00 ± 24.42 a	576.00 ± 6.09 b

Legend: cv. - cultivar; V1 - raised beds on 40 cm; V2 - raised beds on 20 cm; V3 - beds on the ground level; ns - nonsignificant; the lowercase letters represent the results of the Tukey test for $p \leq 0.05$ (a - the highest value and c - the lowest value). The test was calculated on line.

Carrot cv. Cosmic Purple obtained the best results in V1 compared with others version significant for $p \leq 0.05$.

Due to the very well-loosened and nutrient-rich substrate, the carrot cv. Royal Chantenay has good result in V1 compared to V2 and V3.

Kale cv. Kadet has significant differences in all three variants with highest production in V1 with a total of 4,620.00 g.

Kale cv. Nero di Toscana has lower production compared to cv. Kadet but despite this it has significant differences in all variants.

Kale cv. Scarlet has the lowest production compared to the other too cultivars but it has significant differences in all three variants with a maximum production in V1.

Cucumber obtained the best results in V1 compared with others version significant for $p \leq 0.05$.

Despite the well-loosened and nutrient-rich substrate in V1 and V2, peppermint has better results in V3 compared to other two version significant for $p \leq 0.05$.

Both oregano and parsley have nonsignificant results in all three variants but with high production in V1 with 565.00 g and 2,569.00 g, respectively.

Thyme has the highest values in V1 and V2 with 846.00 g and 739.00 g, respectively.

CONCLUSIONS

In the development process, the climatic conditions of the area, the type and shape of the land, the ecological requirements of the plants, the way of their association and succession, the decorative elements and the possibility to decorate for a longer period of time were taken into account.

In addition to the manure and compost, crop rotation and crop residue management are also key tools for improving soil fertility and food quality.The ornamental value of the whole design is offered mainly by the colour of the leaves, petiole, flowers and flower clusters. Other plants, such as fountain grass, decorate during the whole year through their shape and colour from green during spring and summer to yellow in autumn.

Overall, there are important yield differences between the three versions, thus the V1 version (40 cm bed) has the best results in terms of yield. The results of yield from V2 (20 cm above the ground) and V3 (at ground level) versions vary according on the species.

Most species had a good production and development in V1. Instead, peppermint and leeks had higher values in V3 and V2, respectively.

Overall, the most productive plant species are the ones for leaf consumption like mangold and kale, especially in V1.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PESTS OF THE SOLANACEOUS VEGETABLES: AN OVERVIEW OF BIOLOGICAL CONTROL

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Abstract

The Solanaceae family comprises species present on all continents except Antarctica. They are species with different biological cycle durations and adapted to a wide range of environments. Vegetables of the Solanaceae family are not only important for human nutrition, some species are grown for ornamental purposes but also pharmaceutical purposes. Pest species cause significant losses, and many are vectors of disease. Today, many scientists focus on developing new tools to control pest populations, with promising results of use in plant protection. Therefore, the quantitative and qualitative increase in the production of vegetables from the Solanaceae family is not only dependent on environmental factors, an important role is also played by pest prevention and control activities. This is why the objective of this paper was to identify, based on the publications available in the scientific databases of the last ten years, pests from solanaceous vegetable crops from Romania and to list the most important and effective methods of biological control that can be used by producers as more sustainable forms of pest management.

Key words: *solanaceous vegetables, pest prevention and control, Romania vegetables crops, pest management.*

INTRODUCTION

Vegetables of the *Solanaceae* family comprise about 98 genera and about 2,700 species, with a great diversity of habitats, morphology and ecology (Khafagi, El-Ghamery, Ghaly, & Ragab, 2018; Shilpa Kumari, Anokhe, & Kumar, 2017). They have a wide distribution worldwide, being present on all continents, except Antarctica. The greatest diversity of species is found in South and Central America (Dupin et al., 2017). They are good sources of protein, vitamins, minerals and carbohydrates (Shweta Kumari et al., 2019; Kunjwal & Srivastava, 2018). The *Solanaceae* family includes many horticultural species of economic importance as tomatoes (*Solanum lycopersicum*), peppers (*Capsicum annuum*), eggplants (*Solanum melongena*) and potatoes (*Solanum tuberosum*) (Y. Liu et al., 2018). But in this paper, we will focus on the first three species of major importance for Vegetable Research and Development Station Bacău. Also, many of these vegetables are grown for fruit, for ornamental and medical purposes (Gebhardt, 2016; Kunjwal & Srivastava, 2018; Kwon et al., 2020). Vegetable crops have been selected and breaded predominantly for production and, to a lesser extent, for resistance to diseases and pests (Bebber, Holmes, & Gurr, 2014). Crops destruction by pests represents one of the worst problems for vegetable growing, especially in developing countries (Chowański et al., 2016). Some species are vectors in the transmission of viral diseases or mycoplasmosis (Shweta Kumari et al., 2019; Navas-Castillo, Fiallo-Olivé, & Sánchez-Campos, 2011). In the last century, chemical pest control management occupied an important place among plant protection methods (Pavela, 2016), and made rapid progress since the development of DDT (Taiz, 2013). The persistence of pesticide residues causes them to accumulate in the food chain (Pavela, 2016) and, since this phenomenon has become known, the application of these products seemed no longer justified and has been already prohibited in many countries (Alavo, 2015). In the past 20 years, global public awareness has grown regarding the dangers of using chemical-synthesis pesticides (Pérez-Consuegra et al., 2018). The European agriculture is currently in transition from conventional crop protection, based on chemical pesticides, to integrated pest management (IPM). To make any IPM system successful, an alternative to conventional pesticides and plant protection measures that allow an effective management of crop pests is needed. Biological control has the potential to become one of the main pillars of IPM systems (Lamichhane et al., 2017). By biological control, we mean all plant protection (tools, methods, agents, measures) (Umpiérrez, Lagreca, Cabrera, Grille, & Rossini, 2012; C. Wang & Feng, 2014) that are based on the use of beneficial organisms and their natural mechanisms and interactions between biological species found in the natural environment (Lamichhane et al., 2017), used by humans as a biological means to control animals that have become pest (Alavo, 2015). In this review we identify, based on the publications available in the scientific databases of the last ten years (mainly Google Academic, ScienceDirect.com and Springer), pests from solanaceous vegetable crops from Romania and to underline the most important and effective methods of biological control that can be used by producers as more sustainable forms of pest management.

MATERIALS AND METHODS

Data sources and selection criteria

We have compiled data from specialized studies over the past ten years, studies that address pest management for solanaceous crops (tomatoes, peppers, and eggplant). The main pests, as well as their main pathogens, predators and parasites have been identified. We searched the databases in Google Academic, ScienceDirect, Springer using the following keywords "*Solanaceae* crop pests", "Biological pest control", "Solanaceous pests in Romania", "Pathogens for pests in solanaceous crops", "Parasites for solanaceous pests", "Predators for solanaceous pests", "Pest prevention and control" and "Integrated pest management".

We have identified a number of 623 papers that have been analyzed. The studies selected for this review had to meet the following criteria: (1) publication period, 2011-2021; (2) to present the pests of the plant species in question; (3) to present the microbial biologic control agents, parasites, and predatory species for the identified pests.

RESULTS AND DISCUSSIONS

Biological pest control

The number and importance of harmful species is constantly changing due to international tourism, trade and transport of crops (van Lenteren, Alomar, Ravensberg, & Urbaneja, 2020). From the outset, it should be noted that biological pest control does not solve all problems with a complex of pests in a crop. To some degree, populations of all living organisms are reduced by actions of their predators, parasites, antagonists and diseases. This is why this process has been called "natural control" because it includes the effects of natural enemies (Alston, 2011; Snyder, 2019; Ulloa-Ogaz, Muñoz-Castellanos, & Nevárez-Moorillón, 2015). Biological control or biocontrol differs from chemical, cultural, and mechanical controls because the biocontrol agent must survive. It is therefore necessary to maintain a certain level of food supply, such as the pest. Biological control itself is not a mean of eradicating the pests from the crops, it is only a measure to reduce the adverse effect of pest (Alston, 2011). Humans can exploit biological control in various ways to suppress pest populations (Hajek & Eilenberg, 2018), that's why biological control has been used for centuries (Barratt, Moran, Bigler, & Van Lenteren, 2018), and it is mainly defined as the introduction of a natural enemy to control a pest, making it less abundant or less harmful for crops (Kenis, Hurley, Hajek, & Cock, 2017). Integrated pest control is extremely important due to the number of vegetable species grown in our country, as well as the diversity of existing pests. Farmers should know that over the past two decades, Romanian researchers have found and developed solutions that concern the controlling of pests in vegetable crops. Therefore, there are integrated control technologies and control methods that excludes or limits the use of chemicals (Scurtu, Lăcătuș, Sbîrciog, & Buzatu, 2016).

The main groups of biocontrol agents are the microorganisms and invertebrates (van

Lenteren et al., 2020). Microbial pest control refers to viruses, bacteria and fungi. In greenhouses, mainly three species of microbial control agents are used, while several species are applied to field crops. For example, *Bacillus thuringiensis* is used for over 40 years in control of young lepidopteran caterpillars, *Beauveria bassiana* is one of the oldest known entomopathogenic organisms and is applied especially in protected greenhouses crops and nucleopolyhedrovirus is used against *Helicoverpa armigera* specie. The invertebrates use to control plant pests belongs to *Acari* subclass, *Coleoptera* orders, *Diptera, Hymenoptera, Hemiptera, Nematodes, Neuropteran* and different microorganisms (van Lenteren et al., 2020).

Biological pest control in Romania

After Spain, France, Italy and Germany, which are the main agricultural producers in the EU, Romania is currently ranked in the top sales of pesticides (Petrescu-Mag, Banatean-Dunea, Vesa, Copacinschi, & Petrescu, 2019). According to Ene et al. (2012), who studied the levels and distribution of organochlorine pesticides (CMOs), polycyclic hydrocarbons (PAH) and DDT from S-E region of Romania soils, it claims that the level of contamination is high according to Romanian legislation on soil pollution, mainly due to agricultural activities (Ene, Bogdevich, & Sion, 2012). Changes in temperatures and seasons could also affect the proliferation and spread of pests (Andrei & Cristina, 2018), which can affect solanaceous crops. The use of biocontrol agents is an important strategy for integrated pest management in many economically important plants species (Flint & Van den Bosch, 2012). Like other countries, Romania aims to reduce the use of chemical pesticides, so Romanian researchers are developing biocontrol technologies that maintain a natural balance between pests and useful fauna (Stoleru, Munteanu, Stoleru, & Rotaru, 2012).

Pests of solanaceous plants

Knowledge of pests specific to solanaceous crops is very important for establishing the most effective measures to prevent attacks and reduce crop losses (Badii, Billah, Afreh-Nuamah, Obeng-Ofori, & Nyarko, 2015; Campos, Biondi, Adiga, Guedes, & Desneux, 2017; Mazzi & Dorn, 2012). In the literature of the last ten years, the authors of this paper have found a number of 15 pests for solanaceous crops (tomatoes, peppers and eggplant) listed in Table 1.

Table 1. List of the main species of pests for tomato, pepper, and eggplant crops in Romania

Plant species	Pest	Author
Tomatoes (*Solanum lycopersicum*)	- *Meloidogyne incognita* (root-knot nematode) - *Nezara viridula* (southern green stink bugs) - *Tuta absoluta* (tomato leaf miner) - *Halyomorpha halys* (brown marmorated stink bug) - *Tetranychus urticae* (two-spotted spider mite) - *Helicoverpa armigera* (cotton bollworm) - *Liriomyza trifolii* (celery leaf miner) - *Macrosiphum euphorbiae* (potato aphid) - *Thrips tabaci* (onion thrips) - *Trialeurodes vaporariorum* (glasshouse whitefly) - *Tetranychus urticae* (two-spotted spider mite) - *Leptinotarsa decemlineata* (Colorado potato beetle)	(Calin et al., 2020; Cean, 2011; Cean & Dobrin, 2009; Ciceoi & Gutue, 2020; Gabriela Șovărel, Marcel Costache, Emilia Cenușă, & Hogea, 2020; Grozea, Ștef, Virteiu, Cărăbeț, & Molnar, 2012; Kurzeluk, Fătu, & Mihaela Monica, 2015; Macavei et al., 2015)
Peppers (*Capsicum annuum*)	- *Meloidogyne incognita* (root-knot nematode) - *Nezara viridula* (southern green stink bugs) - *Halyomorpha halys* (brown marmorated stink bug) - *Tetranychus urticae* (two-spotted spider mite) - *Helicoverpa armigera* (cotton bollworm) - *Thrips tabaci* (onion thrips) - *Trialeurodes vaporariorum* (greenhouse whitefly) - *Liriomyza trifolii* (celery leaf miner) - *Polyphagotarsonemus latus* (broad mite) - *Myzus persicae* (green peach aphid) - *Frankliniella occidentalis* (Californian thrips)	(Calin et al., 2020; Cean, 2011; Gabriela Șovărel et al., 2020; Hoza et al., 2016; Kurzeluk et al., 2015; Macavei et al., 2015)

Plant species	Pest	Author
Eggplants (*Solanum melongena*)	- *Meloidogyne incognita* (root-knot nematode) - *Halyomorpha halys* (brown marmorated stink bug) - *Tetranychus urticae* (two-spotted spider mite) - *Helicoverpa armigera* (cotton bollworm) - *Liriomyza trifolii* (celery leaf miner) - *Polyphagotarsonemus latus* (broad mite) - *Macrosiphum euphorbiae* (potato aphid) - *Thrips tabaci* (onion thrips) - *Trialeurodes vaporariorum* (glasshouse whitefly) - *Bemisia tabaci* (tabacco whitefly) - *Leptinotarsa decemlineata* (Colorado potato beetle)	(Calin et al., 2020; Cean, 2011; Gabriela Șovărel et al., 2020; Macavei et al., 2015)

Types of biological control

Four different types of biological control are known: natural, conservation, classical, and augmentative biological control (van Lenteren, Bolckmans, Köhl, Ravensberg, & Urbaneja, 2018):

- Natural biological control refers to the fact that harmful organisms are reduced by natural beneficial organisms without human intervention. This is found in all ecosystems and has a benefit especially for agrophyto-technical ecosystems (Rusch, Bommarco, & Ekbom, 2017; van Lenteren, 2012).
- Conservation biological control takes advantage of the pest's natural enemies and involves management strategies to conserve populations and the services it provides (Romeis, Naranjo, Meissle, & Shelton, 2019).
- Classical biological control refers to the intentional introduction of an exotic biological control agent for the permanent establishment and long-term pest control in an area that has been infested by pests (Heimpel & Cock, 2018; Kenis et al., 2017).
- Augmentative biological control refers to the release of an additional numbers of natural enemies when there are fewer in agrophytotechnical ecosystems to effect-tively control a pest (Barratt et al., 2018; De Clercq, Mason, & Babendreier, 2011).

Microbial biological control agents (MBCA)

MBCA contains living organisms, such as bacteria, fungi or viruses (Figure 1) (Lacey, 2017) for pest control in crops, like vegetable crops, and are regulated in the European Union (EU) at both EU and Member State(MS) levels (Frederiks & Wesseler, 2019). Compared to the chemical pesticides these agents have been considered safe for mammals (Baelum, Larsen, Doekes, & Sigsgaard, 2012). Most research are focused on the baculoviruses, important pathogens of some 34 globally important pests for which control has become difficult due to either pesticide resistance or pressure to reduce pesticide residues (Lacey et al., 2015). Usually the MBCA are isolates from local soil (Rios-Velasco et al., 2014).

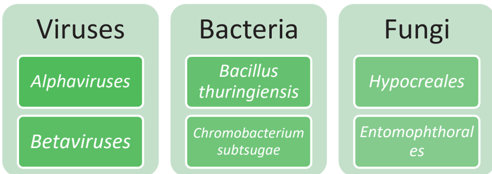

Figure 1. Entomopathogens, after Lacey, 2017

Parasite and predatory species

Farmers are facing serious plant protection issues and phytosanitary risks. The ability of some pests to use a wide range of plants as alternative hosts is the main limitation to the suppressive role (Ratnadass, Fernandes, Avelino, & Habib, 2012). Biological control can be achieved with both MBCA help, as well as by launching parasites and predators (Cornelius, Dieckhoff, Vinyard, & Hoelmer, 2016). This maintain a natural balance between pests and their predators and parasites (N.W. Yang et al., 2014). There are 1590 different terrestrial arthropod species identified in Europe, from which 513 are predators or parasites. The largest group is represented by insects (409 species), spiders (47 species), myriapods (34 species) and mites (23 species) (Roy, Roy, & Roques, 2011). All these organisms contribute to regulatory mechanisms that maintain the stability in agroecosystems (Peterson, Eviner, & Gaudin, 2018), and the success or failure of pest management strategy is mainly attributed to the ability of a few key natural enemies to suppress the pest density (Bouvet, Urbaneja, Pérez-Hedo, & Monzó, 2019). In the following we will present the microbial biologic control agents, parasites, and predatory species for pests of the main Solanaceous crops (Table 2.).

Table 2. Microbial biologic control agents, parasites, and predatory species for pests of the main Solanaceous crops

Solanaceous pest	Microbial biological control agents	Parasite and Predatory species	Authors
***Meloidogyne incognita* (root-knot nematode)**	- *Aspergillus niger* - *Hypocrea rufa* - *Beauveria bassiana* - *Bacillus megaterium* - *Trichoderma album* - *Trichoderma harzianum* - *Ascophyllum nodosum* - *Bacillus firmus T11* - *Bacillus aryabhattai A08* - *Paenibacillus barcinonensis A10* - *Paenibacillus alvei T30* - *Bacillus cereus N10w*	- Oribatid mites (*Scheloribates species, Scheloribates praeincisus, Scheloribates fimbriatus africanus*) - *Stratiolaelaps scimitus*	(Compendium, 2021h; Radwan, Farrag, Abu-Elamayem, & Ahmed, 2012; Ramakrishnan & Neravathu, 2019; Viljoen, Labuschagne, Fourie, & Sikora, 2019; S.-H. Yang, Wang, Chen, Xu, & Xie, 2020)
***Tetranychus urticae* (two-spotted spider mite)**	- *Beauveria bassiana* - *Bacillus thuringiensis* - *Aspergillus melleus* - *Aspergillus terreus* - *Emericella nidulans* - *Chaetomium globosum* - *Lecanicillium attenuatum* 4-1 - *Purpureocillium lilacinum* 2R-4-6	- *Stethorus gilvifrons* - *Orius albidipennis* - *Amblyseius swirskii* - *Phytoseiulus persimilis*	(Compendium, 2021l; Hoza et al., 2016; Osman, Elnasr, Nawar, & Hefnawy, 2019; Shin, Bae, Kim, Yun, & Woo, 2017; Taghizadeh, Haddad Iraninejad, Iranipour, & Moghaddam Vahed, 2020)
***Polyphagotarsonemus latus* (broad mite)**	- *Isaria fumosorosea* - *Beauveria bassiana*	- *Amblyseius andersoni* - *Neoseiulus californicus* - *Neoseiulus cucumeris* - *Amblyseius swirskii*	(Compendium, 2021k; LeFors, 2018; Onzo, Houedokoho, Hanna, & Liu, 2012; Satpathy, Gotyal, & Babu, 2019)
***Macrosiphum euphorbiae* (potato aphid)**	- *Lecanicillium lecanii*	- *Aphidius matricariae* - *Adalia bipunctata* - *Aphidoletes aphidimyza* - *Chrysoperla carnea* - *Coccinella septempunctata* - *Episyrphus balteatus* - *Eupeodes corollae* - *Harmonia axyridis*	(Brodeur, 2012; Compendium, 2021g; Mohammed, Kadhim, & Kamaluddin, 2018)
***Myzus persicae* (green peach aphid)**	- *Beauveria bassiana* - *Glomerella cingulate* - *Lecanicillium lecanii*	- *Aphidius matricariae* - *Ephedrus plagiator* - *Adalia bipunctata* - *Anthocoris sibiricus* - *Aphidoletes aphidimyza* - *Chrysopa formosa* - *Chrysoperla carnea* - *Coccinella septempunctata* - *Episyrphus balteatus* - *Propylea quatuordecimpunctata* - *Scaeva pyrastri*	(Abbas, Khan, & Sohail, 2015; Amnuaykanjanasin et al., 2013; Compendium, 2021i; Ikeura, 2014; Li et al., 2018; Rotari, Tălmaciu, & Tălmaciu, 2011)

Solanaceous pest	Microbial biological control agents	Parasite and Predatory species	Authors
***Thrips tabaci* (onion thrips)**	- *Beauveria bassiana* - *Lecanicillium lecanii*	- *Nabis pseudoferus* - *Amblyseius swirskii* - *Chrysoperla carnea* - *Coccinella septempunctata* - *Deraeocoris serenus* - *Eupeodes corollae* - *Nabis pseudoferus* - *Nabis rugosus*	(Annamalai, Kaushik, & Selvaraj, 2016; Compendium, 2021m; Rotari et al., 2011; Wu et al., 2013)
***Frankliniella occidentalis* (Californian thrips)**	- *Beauveria bassiana* - *Lecanicillium lecanii*	- *Amblyseius swirskii*	(Compendium, 2021b; Hoza et al., 2016; Zhang, Lei, Reitz, Wu, & Gao, 2019)
***Liriomyza trifolii* (celery leaf miner)**	- *Bacillus thuringiensis* - *Beauveria bassiana*	- *Orius dissitus*	(Compendium, 2021f)
***Tuta absoluta* (tomato leaf miner)**	- *Bacillus thuringiensis* - *Beauveria bassiana*	- *Copidosoma spp.* - *Diadegma spp.* - *Necremnus tutae* - *Trichogramma spp.* - *Nabis pseudoferus*	(Allegrucci, Velazquez, Russo, Pérez, & Scorsetti, 2017; Compendium, 2021o; Manohar, Sharma, Verma, Sharma, & Chandel, 2020)
***Helicoverpa armigera* (cotton bollworm)**	- *Bacillus thuringiensis* - *Beauveria bassiana*	- *Copidosoma spp.* - *Bracon brevicornis* - *Compsilura concinnata* - *Exorista larvarum* - *Exorista xanthaspis* - *Trichogramma spp.* - *Argiope brunnichii* - *Chrysoperla carnea* - *Chrysopa formosa* - *Chrysopa pallens* - *Coccinella septempunctata* - *Passer domesticus*	(Compendium, 2021d; B. Liu et al., 2016; Pereira, Reigada, Diniz, & Parra, 2019; Rana, Chand, & Patyal, 2014)
Bemisia tabaci *(tabacco whitefly)*	- *Bacillus thuringiensis* - *Lecanicillium lecanii* - *Beauveria bassiana*	- *Encarsia formosa* - *Amblyseius swirskii* - *Chrysoperla carnea* - *Coccinella septempunctata* - *Eupeodes corollae*	(Compendium, 2021a; Salazar-Magallon, Hernandez-Velazquez, Alvear-Garcia, Arenas-Sosa, & Peña-Chora, 2015; Q. L. Wang & Liu, 2016)
***Trialeurodes vaporariorum* (glasshouse whitefly)**	- *Bacillus thuringiensis* - *Lecanicillium lecanii* - *Beauveria bassiana*	- *Chrysopa pallens* - *Chrysoperla carnea* - *Coccinella septempunctata* - *Deraeocoris serenus* - *Propylea quatuordecimpunctata* - *Encarsia formosa*	(Compendium, 2021n; Oreste, Bubici, Poliseno, & Tarasco, 2016; Rotari et al., 2011)
***Halyomorpha halys* (brown marmorated stink bug)**	-	- *Trissolcus flavipes*	(Compendium, 2021c; Y. Yang et al., 2015)

Solanaceous pest	Microbial biological control agents	Parasite and Predatory species	Authors
***Nezara viridula* (southern green stink bugs)**	- *Bacillus thuringiensis* - *Beauveria bassiana*	- *Telenomus chloropus* - *Trissolcus grandis*	(Compendium, 2021j; Hasnah, Susanna, & Sably, 2012)
***Leptinotarsa decemlineata* (Colorado potato beetle)**	- *Bacillus thuringiensis* - *Beauveria bassiana*	- *Meigenia mutabilis* - *Ardea cinerea* - *Chrysoperla carnea* - *Nabis rugosus* - *Coccinella septempunctata* - *Hexamermis spp.* - *Formica pratensis* - *Formica rufa* - *Passer domesticus* - *Perdix perdix* - *Perillus bioculatus* - *Phasianus colchicus* - *Pica pica* - *Pterostichus cupreus*	(Compendium, 2021e; Rotari et al., 2011; Sorokan et al., 2020)

The insecticidal properties of MBCA, like *Bacillus thuringiensis, Beauveria bassiana, Lecanicillium lecanii, Glomerella cingulate, Isaria fumosorosea*, are used to provides evidence that MBCA are efficient not only to decreased the number of pests but also to reduce the spread to the next host. Plant-promoting rhizobacteria (PGPR) can increase reduction of the gall numbers of *M. incognita* up to 86% (Viljoen et al., 2019). Also, fungi are a promising source of bioactive secondary metabolites against various agricultural pests (Osman et al., 2019). Both *Isaria fumosorosea* and *Beauveria bassiana* recorded significantly higher mortality of broad mite (Satpathy et al., 2019). Plants that were treated by *L. lecanii* showed a significant reduction in the number of *M. persicae* and *A. gossypii*. Although *M. euphorbiae* is less susceptible to *L. lecanii* infection in laboratory experiments, this entomopathogen is still effective against this pest (Mohammed et al., 2018).

The use of parasites and predators in biological control programs revealed spectacular economic and ecological results (Hajek & Eilenberg, 2018). The species presented in this paper contributed to the suppression of pests from *Solanaceae* crops. The use of natural predators has led to the protection of biodiversity and the protection of vegetable crops. For example, studies on the activity of natural enemies for *H. armigera* have shown that the diversity and abundance of natural enemies tends to be higher in crops that have not been sprayed with insecticides (Downes et al., 2017). Scheloribates mites in the soil are effective predators against juvenile nematodes. Following a study by Ramakirishnan (2019) , it was shown that after the introduction of mites into the soil, juveniles of *M. incognita* not only decreased numerically, but also reduced the spread to the next host (Ramakrishnan & Neravathu, 2019). Another study tested the effectiveness of *Stratiolaelaps scimitus* in feeding on *M. incognita*. It was concluded that *S. scimitus* could develop normally and can complete its life cycle by feeding only on the harmful nematode. In fact, it is found to be an effective predator and an effective enemy of nematodes (S.H. Yang et al., 2020). The results of a study by Tagizadeh et al. (2020) on the efficacy of predation rate of *T. urticae* showed that both *Stethorus gilvifrons* and *Orius albidipennis* are promising biological control agents of the pest. However, the species *Stethorus gilvifrons* proved to be a superior predator in terms of population growth parameters, predation rate and ability to convert prey biomass into predatory offspring (Taghizadeh et al., 2020). Four predatory mites (*Amblyseius andersoni, Neoseiulus californicus, Neoseiulus cucumeris and Amblyseius swirskii)* are utilized as biocontrol agents for *P. latus*. The efficacy of these predatory mites could be affected by seasonal changes in broad mite densities caused by dispersal, but the mites would make very good predators of the broad mite (LeFors, 2018; Onzo et al., 2012). The ladybird beetle *Coccinella septempunctata* is one of the most widespread aphidophagous coccinellids and has proved to be an effective biocontrol agent for selected aphid species such as *Macrosiphum euphorbiae*, *Myzus persicae nicotianae*, etc. (Norkute, Olsson, & Ninkovic, 2020).

CONCLUSIONS

Given demand, plants of the *Solanaceae* family are the most cultivated. Whether grown in greenhouses or in open fields, pests can affect roots, leaves or fruits and therefore decrease crop yields. Thus, knowledge of harmful fauna, pathogens, parasites, and predators can enhance the results of pest management practices.

The method of biological pest control has been practiced for centuries and is a cost-effective, environmentally friendly approach to solving the problems of vegetables agroecosystems.

MBCA, predators and parasitoids are important in pest management and have proven to be an excellent alternative to chemical pesticides. The main pathogens for solanoceus pests observed in Romania are represented by *Bacillus thuringiensis*, *Beauveria bassiana* and *Lecanicillium lecanii*. Also, there are many parasites and predators described in the literature for Romania solanaceous plants, as shown in Table 2, and they need national protection programs.

This review of the major pest of *Solanaceae* family crop may serve as a base in developing recommendations for sustainable management of vegetables pests. Also, the species of pests recently entered in the Romanian fauna, such as *Nezara viridula*, suggest the importance and critical need for the permanent monitoring of pests from vegetables crops.

Future field studies may increase the list of pests but also of pathogens, parasites, and predators.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0659/ contract 11 PCCDI /2018, within PNCDI III, and ADER 735 projects developed by VRDS Bacău

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARATIVE STUDY REGARDING THE BEHAVIOR OF SOME VARIETIES OF BASIL CULTIVATED IN NFT SYSTEM (NUTRIENT FILM TECHNOLOGY)

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Abstract

The study was carried out in the greenhouses of the Research Center for the quality of Horticultural products, Hortinvest greenhouses but also the greenhouse in Nutrient Film Technology (NFT) system in the vertical greenhouse (Plant Factory) in the Greenhouses of the Faculty of Horticulture built through a project in collaboration with China. 2 varieties of basil were cultivated, Genovese and Aromat de Buzău. We found differences in vegetative mass between different crop varieties. The aim of the study was to analyze the behavior of these varieties in the unconventional culture system, on nutritive film (NFT)

Key words: *basil, cultivars, NFT, production.*

INTRODUCTION

Basil is native to India, China, Sri Lanka, where it has spread around the globe. It was introduced to Europe in the middle of the 16th century. As a spice plant, basil is grown in southern Ukraine, Armenia, Georgia and Central Asia, in many countries in Western Europe (France, Italy, Germany, etc.) and in North America (Ciofu, 2004).

The genus *Ocimum* belongs to the *Lamiaceace* family and includes about 50-150 species of plants and shrubs found in tropical regions of Asia, Africa, Central and South America. (Darrah, 1980; Tucker and DeBaggio, 2009) with a large number of varieties (Runyoro et al., 2010; Aligiannis et al., 2001). Types of this genus differ in their characteristics, leaf size, flower color, phenotypic characteristics and aroma. *O. gratissimum,* pink basil, *O. viride* dendritic, *Ocimum basilicum* basil, sweet americanus, tenuiflorum.

The name of the basil plant comes from the Latin basilisk or dragon; this etymological connection may explain the symbolic connection between basil and scorpions (Grieve, 1971; Stobart, 1982).

Basil is an annual plant, the stem has a height between 25-50 cm, and it is branched and bears elongated, fleshy leaves. It blooms from June to September and presents small, white or purple flowers. The fruits are small achenes, black in colour, 1.5-2 mm long and about 1 mm wide. The germination capacity is 60-80% and it is kept for 4-5 years. One gram contains 600-800 seeds.

Basil is grown mainly on lands with southern exposure, rich in humus and nutrients, with light or medium texture, flat, with a neutral reaction (pH between 6.5-7.5).

Temperature can affect plants in many different ways. Extremely low or high temperatures can influence the production and quality of basil Carvalho et al. (2002); Abbas (2014).

Increasing or decreasing differences between day and night air temperature ('DIF') may increase or decrease the elongation of the stem and the internode (Walters and Currey, 2016).

Basil, also called the king of herbs, is one of the species that behaves very well in the NFT culture system horizontally but also vertically, in floors. It can be grown all year round on a vertical farm where much higher yields can be obtained compared to the standard from greenhouse culture.

One of the advantages of NFT systems is the reduced volume of nutrient solution. This reduces the energy required to heat the nutrient solution in the winter months if desired (Thompson et al., 1998).
The troughs in NFT systems are usually placed at heights that are comfortable for greenhouse employees to access for transplanting and harvesting. (Walters and Currey, 2015; Enache et al., 2019).
It grows very well in moderate light conditions, but for better efficiency it can be grown in LED-lit areas, where and other growth factors, climate, CO_2, nutrients and substrates can positively influence the production of basil (Pennisi, 2019; Rahman et al., 2021).
Omer et al. show that the quantitative effects of temperature and light intensity on growth parameters have an influence on sweet basil plants grown in the greenhouse (*Ocimum basilicum* L., fam. *Lamiacae*).
Increasing air temperature to 29°C resulted in an increase in fresh and dry weight accumulation, node number, per-cent of plants with visible flower buds or flowers, plant height, internode length, branch number, and chlorophyll fluorescence for all species and cultivars evaluated (Walters and Currey, 2019).
Basil contains antioxidants (Peirce, 1999) but also vitamins A and C that have made it useful for protecting cells against disease (Romesh et al., 1993) fever and headache (Ducke, 2002). It is also used to treat stomach pain, as an expectorant, diuretic (Nyarko et al., 2002), as well as insecticides (Gill and Randhawa, 2000) and scorpion repellent and snakes (Gill et al., 1992).
It is an important source of essential oil that is used in food, perfumery and cosmetics (Tutulescu et al., 2017), and some types of *Ocimum* are used as a folk remedy for some diseases, especially in Asian and African countries (Aldjwi, Ali, 1996).

MATERIALS AND METHODS

The experiments were carried out within USAMV Bucharest, Hortinvest greenhouses, Research Center for the Quality of Horticultural Products.
The biological material used in the experiment was represented by the 'Genovese' and 'Aromat de Buzău' varieties.
The 'Genovese' basil variety (*Ocimum basilicum*) is a variety with large, green leaves that can be grown in pots, the height of the plant can reach 50 cm.
The 'Aromat de Buzău' basil variety is a semi-late variety, being very well adapted to the existing environmental conditions in Romania. The leaves have a specific aroma and have the ability to retain their properties during the conservation period. The mature plant has the shape of a globular bush, slightly spread, having a height between 40-60 cm. The average production of shoots is 12.4 t / ha.
The EC was maintained in the first week after planting at 1.6 ppm, then it was raised to 2.2 ppm. The nutrient solution had a constant pH of 6. We performed determinations on the percentage of seeds grown in the case of the analysed varieties, as well as measurements on plant height, number of leaves per plant, number of stems formed. We also performed determinations on plant mass. The duration of keeping the plants in culture was of 26 days.

RESULTS AND DISCUSSIONS

The percentage of sprouted seeds was 97% for the 'Genovese' variety and 98% for 'Aromat de Buzău' (Figure 1).

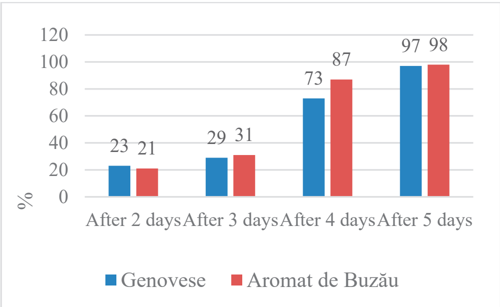

Figure 1. Percentage of sprouted seeds for the analysed varieties

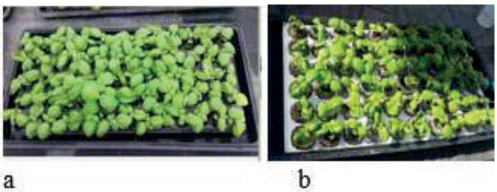

Figure 2. Basil seedlings: 'Genovese' variety (a) and 'Aromat de Buzău' (b)

We found that in the case of the 'Genovese' variety, the highest height was recorded in variant 2, grown in NFT system, with LED lighting, it was 32.0 cm, with a significance, statistically, distinctly very significant compared to the control. In the case of the variant grown in peat pots (V3), the height of the plants was 19.75 cm. From a statistical point of view, there were distinctly significant positive meanings at V1 and V2 (Table 1).

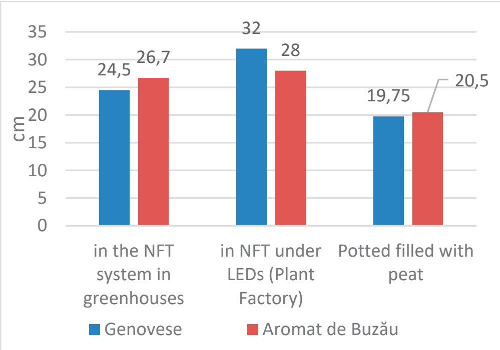

Figure 3. Plant height after 26 days after planting

Table 1. Influence of growing conditions on the height of basil plants - 'Genovese' variety

Variants	Height (cm)	Difference (cm)	Difference (%)	Significance
V(0) average	25.42	5.67	128.69	***
V(1)	24.50	4.75	124.05	***
V(2)	32.00	12.25	162.03	***
V(3)	19.75	0.00	100.00	Ct

DL5% = 1.420 DL5% in % = 7.1899
DL1% = 2.360 DL1% in % = 11.9494
DL01% = 4.420 DL01% in %= 22.3797

In the case of the 'Aromat de Buzău' variety, we found that the height of the plants was influenced by the growing conditions. In the case of variant 1, plants grown in the greenhouse in natural light conditions, the height of the plants was 26.7 cm by 6.2% above the average experience. The best variant regarding the height was registered at V2, plants cultivated in LED lighting conditions. From a statistical point of view, there were distinctly significant positive differences for all variants grown in the NFT system (Table 2).

Table 2. Influence of cultivation conditions on the height of basil plants 'Aromat de Buzău' variety

Variants	Height (cm)	Difference (cm)	Difference (%)	Significance
V(0) average	25.07	4.57	122.28	***
V(1)	26.70	6.20	130.24	***
V(2)	28.00	7.50	136.59	***
V(3)	20.50	0.00	100.00	Mt

DL5% = 0.910 DL5% in % = 4.4390
DL1% = 1.510 DL1% in % = 7.3659
DL01% = 2.840 DL01% in %=13.8537

Figure 4. Aspect of basil plants cultivated in NFT system and in potted

a. b.

Figure 5. Appearance of the greenhouse illuminated with LED (Plant Factory): a. 'Genovese' variety; b. 'Aromat de Buzău' variety

Figure 6. Plant appearance a. In NFT - natural light and b. In NFT LED lighting

Regarding the number of leaves on plant, we found that the best results were recorded for the variant grown under LED lighting (Figure 7).

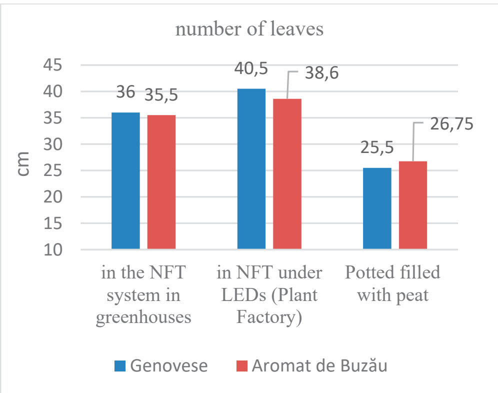

Figure 7. Number of leaves on plant

From a statistical point of view, there was a statistically significant difference in V2, where the difference regarding in the number of leaves per plant was with 15 leaves, over the control variant (V3), Table 3.

Table 3. Influence of temperature and lighting conditions on the number of leaves on plant in the 'Genovese' variety

Variants	Number of leaves (cm)	Difference (cm)	(%)	Significance
V(0) average	34.00	8.50	133.33	N
V(1)	36.00	10.50	141.18	N
V(2)	40.50	15.00	158.82	*
V(3)	25.50	0.00	100.00	Control

DL5% = 13.440 DL5% in % = 52.7059
DL1% = 22.240 DL1% in % = 87.2157
DL01% = 41.630 DL01% in %= 163.2549

In the case of the 'Aromat de Buzău' variety, the number of leaves per plant was 35.5 at V1 and 38.6 at V2. The lowest number of leaves was recorded at V2 (control). From a statistical point of view we found very distinctly significant positive differences at V1 and V2 (Table 4).

Table 4. Influence of temperature conditions on the number of leaves on plant in the 'Aromat de Buzău' variety

Variants	leafes Number (no)	Difference (no)	(%)	Significance
V(0) average	33.62	6.87	125.67	***
V(1)	35.50	8.75	132.71	***
V(2)	38.60	11.85	144.30	***
V(3)	26.75	0.00	100.00	Control

DL5% = 1.540 DL5% in % = 5.7570
DL1% = 2.560 DL1% in % = 9.5701
DL01% = 4.790 DL01% in %= 17.9065

Regarding the number of branches formed on the plant, we found that there were no significant differences between the variants (Tables 5 and 6).

Table 5. Number of branches formed on plants of the 'Genovese' variety

Variants	number (no)	Difference (no)	(%)	Significance
V(0) average	3.33	0.33	111.11	N
V(1)	3.50	0.50	116.67	N
V(2)	3.50	0.50	116.67	N
V(3)	3.00	0.00	100.00	Ct

DL5% = 0.650 DL5% in % = 21.6667
DL1% = 1.080 DL1% in % = 36.0000
DL01% = 2.020 DL01% in %= 67.3333

Table 6. Number of branches formed on plants in the 'Aromat de Buzău' variety

Variants	Number (no)	Difference (no)	(%)	Significance
V(0) average	3.57	0.07	101.90	N
V(1)	3.70	0.20	105.71	N
V(2)	3.50	0.00	100.00	N
V(3)	3.50	0.00	100.00	Mt

DL5% =	3.180	DL5% in % =	90.8571
DL1% =	5.260	DL1% in % =	150.2857
DL01% =	9.850	DL01% in %=	281.4286

In the experiment, the total mass of plants harvested was for the Genovese variety between 93.81 g at V3 and 168.75 g at V2. From a statistical point of view, we found positive differences in this variety, distinctly significant compared to variant 3 control. In the case of the 'Aromat de Buzău' variety, average masses of 97.53 g/plant were recorded at control V3 and 157.23 g / plant at V2 (Tables 7 and 8).

Table 7. Total mass of the plant in the 'Genovese' variety

Variants	Mass (g)	Diference (g)	(%)	Significance
V(0) average	138.76	44.95	147.92	***
V(1)	153.73	59.92	163.87	***
V(2)	168.75	74.94	179.88	***
V(3)	93.81	0.00	100.00	Ct

DL5% =	12.510	DL5% in % =	13.3355
DL1% =	20.710	DL1% in % =	22.0765
DL01% =	38.770	DL01% in %=	41.3282

Table 8. Total mass of the plant in the 'Aromat de Buzau' variety

Variants	Mass (g)	Diference (g)	(%)	Significance
V(0) average	132.82	35.29	136.18	***
V(1)	143.70	46.17	147.34	***
V(2)	157.23	59.70	161.21	***
V(3)	97.53	0.00	100.00	Ct

DL5% =	4.220	DL5% in % =	4.3269
DL1% =	6.990	DL1% in % =	7.1670
DL01% =	13.080	DL01% in %=	13.4113

In the case of the 'Genovese' variety, we found that the mass percentage of leaves obtained on a plant was higher in the variant cultivated under LED lighting conditions compared to the rest of the variants (Figure 8).

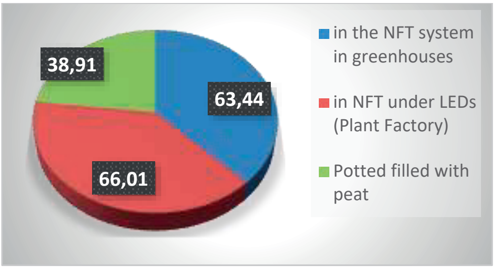

Figure 8. Percentage of average leaf mass reported to total mass of plant - 'Genovese' cultivar

Figure 9 shows that the percentage of average mass of the stems was lower in the LED lighting variant compared to the rest of the variants.

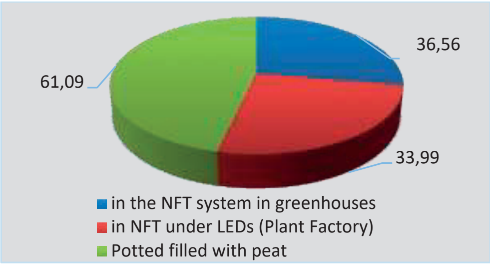

Figure 9. Percentage of the average mass of the stem reported to total mass of plant - 'Genovese' cultivar

In the case of the 'Aromat de Buzău' variety, the highest percentage of the average leaf mass was also registered for the variant cultivated in constant LED lighting conditions, the percentage being 61.17% leaf mass of the total plant (Figure 10).

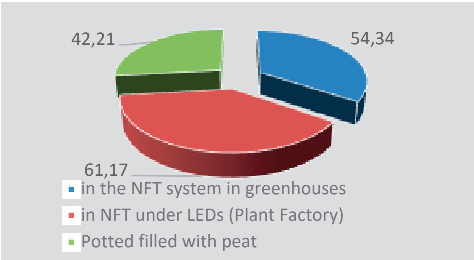

Figure 10. Percentage of average leaf mass reported to total mass of plant - 'Aromat de Buzău' cultivar

Also, in the case of the 'Aromat de Buzău' variety, the highest percentage to be registered at the mass of the stem was in the variant cultivated in pots (57.79%) (Figure 11).

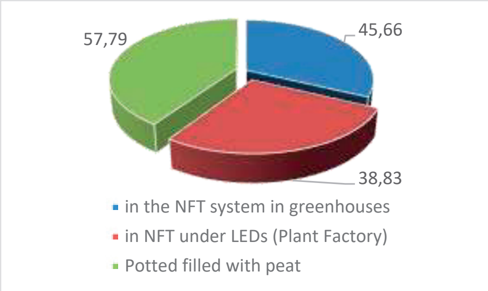

Figure 11. Percentage of the average mass of the stem reported to total mass of plant 'Aromat de Buzău' cultivar

CONCLUSIONS

In this study we cultivated two varieties of basil, Genovese and 'Aromat de Buzău' in 3 systems of growing: in NFT system in greenhouse with natural lighting conditions, in NFT system in conditions of permanent lighting with LEDs (10 hours/day) similar greenhouse conditions. As a control variant, we used the culture on peat substrate, in 10/10 cm pots.

We found that in the conditions of growing under LED lighting, plant height, number of leaves per plant and plant mass were higher compared to the control variant (in pots), but these results also appeared in greenhouse in NFT system.

Also, the leaf yield was higher under NFT growing conditions for both varieties.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDY ON THE INFLUENCE OF ENVIRONMENTAL CONDITIONS FROM GREENHOUSE ON THE ACCUMULATION OF VEGETATIVE MASS AND FRUCTIFICATION IN SOME VARIETIES OF CHERRY TOMATOES

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Abstract:

The preliminary study was carried out in Hortinvest greenhouses on two varieties of cherry tomatoes, 'Flaviola' and 'Cheramy' F. The environmental factors in the greenhouse, temperature, atmospheric humidity, CO_2 concentration inside the greenhouse during January-March were registered. Also, the obtained productions and the vegetative mass of the leaves were registered. We found that for the 'Flaviola' variety, the temperature had a greater influence on the production compared to the 'Cheramy' variety. We noticed that each variety reacted differently both in terms of temperature and CO_2 values. The accumulated vegetative mass was higher in 'Cheramy F1' compared to 'Flaviola', this being a genetic characteristic of the variety. The percentage of fruits obtained per plant was 54.75% and only 45.25% of the vegetative mass in 'Cheramy F1'. For the 'Flaviola' variety, the percentage of fruit was 60.64% and 39.36% vegetative mass. The aim of the study was to analyse the behaviour of some varieties on total production.

***Key words**: tomatoes, cerise, cultivars, substrate.*

INTRODUCTION

Currently, the production of tomatoes in the greenhouse is constantly growing due to the application of new high-performance technologies that ensure very high yields. At the same time, consumers are looking for quality fruits, of different sizes, shapes, colors and with specific flavors. In greenhouses, were grown mainly, varieties with large, spherical fruit, but now are increasingly appreciated tomatoes with cherry fruits (*Lycopersicon esculentum* var. *cerasiforme*), due to their aroma, content also balanced in carbohydrates and vitamin C and ornamental effects (Dinu et al., 2015). Another aspect regarding the extension of cherry tomatoes in the culture is their high price but also the ability to better adapt to variations in environmental factors.

In the greenhouse, one of the important objectives is to reduce the temperatures in the growing space, especially in periods of strong sunshine and to maintain values corresponding to obtaining high and quality yields. Studies have shown that high greenhouse temperatures influence productivity but also nutritional quality (Bitaand and Gerats, 2013; Adams et al., 2001).

The use of shading screens can reduce the temperature inside of greenhouses, but if they are not supported by other air conditioning systems, they reduce their efficiency, leading to the compromise of tomato cultivation.

Tomatoes are very pretentious of temperature. Temperatures below 10°C block the growth of plants and the phenomenon manifests itself in the form of short knotting of the growth tips and the size of the leaf petiole decreases.

(Gertsson, 1992; Luxhoi, et al., 2007; Grimstad, 1993; Heuvelink and Dorais, 2018).

The negative effect is even more obvious in conditions of insufficient light (Pescar Svetlana, 2013).

At temperatures above 40°C, plant turgidity decreases, followed by wilting and drying of growth tips. The optimum temperature range for growth and fruiting is 18-32°C but at temperatures above 35°C pollen viability decreases.

Hussainet et al. (2001) cited by Abdelmageed and Gruda (2009) says that the number of fruits per plant and the percentage of fruits was lower due to the high temperature.

Temperatures of 15-16°C during the formation of flowers, for long periods, determine abundant flowering, the flowers can remain open on the plant for several weeks but without forming fruit, the flowers are larger and the inflorescence branches a lot. Also, temperatures below 10 °C during flowering can affect pollination but can also cause fruit cracking and affect fruiting. Temperatures above 35 °C during the ripening period of fruits negatively influence their coloration due to the reduction of the synthesis of red pigment (lycopene) and the increase of the synthesis of yellow pigment (carotene).

Zhang et al., 2012, state that exposure of tomato plants to an extremely high temperature of 35°C for eight hours reduced the rate of photosynthesis.

Another phenomenon that can occur during the physiological maturation of fruits is due to the maintenance in the greenhouse of low temperatures of 15-18ºC day and night, below the recommended temperature of 20-24ºC during the day and 18ºC at night but also of a low level of potassium in the nutritional recipe. In this situation, the fruits have an inappropriate appearance, they give the impression of fruits that have not reached physiological maturity, but if we cut a fruit, it is found that the seeds are already sprouted.

Large differences between day and night temperatures can increase the period of fruit growth (Van der Ploeg and Heuvelink, 2005), however, a large difference results in a more generative increase (Heuvelink, 1989; Peet and Welles, 2005 quoted by Noemi et al., 2014). Temperatures below 10ºC inhibit vegetative growth, delay fruit ripening, and those above 35°C inhibit fruiting, fruit development and coloration. Zhang et al., 2012, state that exposure of tomato plants to an extremely high temperature of 35°C for eight hours reduced the rate of photosynthesis

Temperatures above 22°C correlated with high atmospheric humidity above 80% create optimal conditions for the development of the pathogen *Clodosporium fulvum*, and at temperatures below 15°C and relative air humidity above 75% create optimal conditions for the attack of *Phytophtora infestans*.

Greenhouses and hight tunnels capture solar radiation so that, especially in summer, the temperature inside the culture space increases, creating the greenhouse effect. Ventilation systems eliminate excessive heat and ensure adequate conditions for the growth and development of tomato plants. Modern greenhouses have computerized operating systems that monitor all plant growth parameters.

Even in low light conditions, increasing the CO_2 content during the reproduction phase induces early flowering and fruit formation (Grouda, 2005; Sima et al., 2011), increases the net assimilation rate, plants can photosynthesize more at concentrations higher levels of CO_2 (Heuvelink and Dorais, 2005; Grouda, 2005; Singh, et al., 2005; Sima et al., 2011). Additional CO_2 fertilizations of up to 800-100 ppm can be performed in the greenhouse. In professional greenhouses, of the latest generation, all environmental factors can be monitored, that is essential for achieving high yields. Also, the light factor can be easily controlled and we can provide the necessary in the culture space, especially in the autumn-winter or spring periods. Low light intensity, short photoperiods and high night temperatures can be important limiting factors for fruit formation (Wittwer and Honma, 1986).

Supplementing light in greenhouses is necessary especially in periods of strong cloudiness, but also in autumn when the number of lighting hours decreases (Panțer et al., 2016).

Another factor that we must take into account and which in modern greenhouses can be well controlled is atmospheric humidity.

It is known that both low and very high atmospheric humidity affect pollination. Also, the very high atmospheric humidity correlated with the lack of aerations determines the appearance of adventitious roots on the stem in some varieties.

The work of removing the leaves must be done with great caution (Buitelaar and Janse, 1987; Buitelaar, 1989; Xiao et al., 2004).

The assortment grown in the greenhouse must be chosen according to the specific requirements of the cultivars.

In terms of nutrients, they can be well monitored, they are used effectively on phenophases. Tomatoes intensively capitalize on mineral resources (Dinu et al., 2019; Dinu et al, 2015; Kostadinov, 2020).

Numerous studies on fruit yield at the end of the crop (Papadopoulos and Ormrod, 1991), De Koning, (1990b) (quoted by Heuvelink, 1996) have shown that 74.3% of the total fresh mass of the plant is the fruit and only 23.7% vegetative mass (stem and leaves). Kleinhenz et al. (2006) obtained a different plant biomass depending on the plant density, this being 16-19% with 13% higher in the case of driving on two stems.

The EC of the solution is well monitored as well as the amount of nutrient solution. Moreover, the nutrient solution resulting from the drain is recovered, rebalanced, disinfected and reused. The temperature of the nutrient solution reached in the culture substrate is distributed at values of 20ºC, with a direct effect on tomato production. Also, the pH of the nutrient solution can be controlled and maintained constantly at values in accordance with the nutritional recipe.

Regarding the culture substrates, organic substrates (coconut, peat, compost, etc.) or inorganic substrates (mineral wool, perlite, vermiculite, zeolite, etc.) can be used depending on the geographical area. When choosing the type of substrate, their characteristics and physical properties must be taken into account: total porosity; substrate density; water retention capacity; be able to drain the solution.

From a chemical point of view, the substrate must not interact with the nutrient solution, nor must it solubilize or favor the transfer process (Jerca et al., 2015; Drăghici et al., 2019).

All these aspects lead to the support of modern cultivation technologies in a soilless system. Regarding the cultivated assortment, hybrids with both large fruit of over 120 g/fruit and a cherry-type assortment are chosen, especially very appreciated by consumers.

In the present study we analysed the behaviour of some cherry tomatoes in greenhouse conditions on perlite substrate. I used these crops because they have very high plasticity although they are intended for soil crops.

MATERIALS AND METHODS

The study was carried out within the Hortinvest research greenhouses, which belong to the Research Center for quality control of horticultural products, Faculty of Horticulture, USAMV Bucharest. The greenhouses are modern constructions with high of 6.2 m, and which are equipped with all the facilities for monitoring and maintaining the climatic factors in the greenhouse. We used as biological material 2 cultivars of cherry 'Cheramy F1' (V1) type tomatoes and a Romanian variety, 'Flaviola' (V2), created at the Buzău Vegetable Research Station. The culture was established in a soilless system, on mattresses filled with perlite with a grain size of 4 mm from SC Perlit SA, Ilfov County.

The duration of the experiment was 90 days.

I watched the parameters of vegetative growth in height, number of leaves and fruiting. We determined the number of inflorescences as well as the number of fruits in the inflorescence. Fruit production per plant was weighed. We recorded throughout the experiment the values of temperature, relative humidity through sensors located at a height of 1.5 m inside the greenhouse.

'Cheramy F1' is an early-maturing hybrid with undetermined growth. The fruits are of the cherry type, around 15 g/fruit

'Flaviola' variety presented an indeterminate vegetative growing, with fruit cherry type, recommended to crops in protected area.

The temperatures from greenhouse were recorded daily during 24 hours, day and night, for 90 days also light radiation, atmospheric humidity and CO_2 concentration.

During the vegetation was recorded the total amount of vegetative mass (leaves and stem) as well as the amount of fruits harvested during the entire period under observation (1 January-30 March 2021).

The area cultivated with tomatoes in the greenhouse was 160 m^2. A number of 154 plants of ‘Cheramy F1’ and 101 plants of the ‘Flaviola’ variety were analysed.
The data were recorded and correlated to see the influence of environmental factors in the greenhouse on the production and accumulation of vegetative mass.

RESULTS AND DISCUSSIONS

The recorded data showed that in January the minimum and maximum values of light radiation were between 77.69 W/m^2 and 649.29 W/m^2. During this period, the plants were in the phenophase of vegetative growth and beginning of formation but also of fruit maturation.
In February, the value of the recorded radiation showed higher values compared to January, being between 788.78 W/m^2 and 864.07 W/m^2. During this period, tomato plants were found both in the phenophase of fruit growth as well as in that of intense maturation.
In March, the values of solar radiation also increased with the increase of the lighting duration, reaching in some days, minimum values of 127.5 W/m^2 and 162 W/m^2 and maximum values of 611 W/m^2 respectively 967.13 W/m^2, and for short periods at 1132.75 W/m^2 (Figures 1-3).

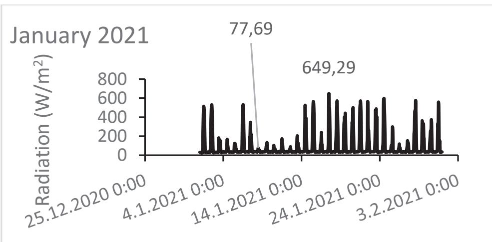


Figure 1. Solar radiation recorded in the greenhouse in January

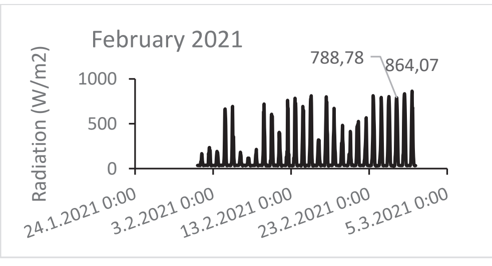


Figure 2. Solar radiation recorded in the greenhouse in February

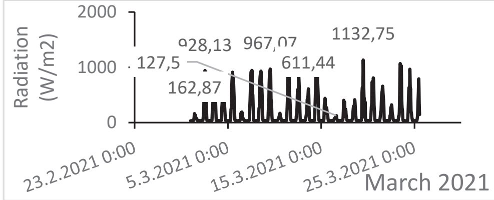


Figure 3 Solar radiation recorded in the greenhouse in March

Analysing the environmental factors in the greenhouse, between January 1 and March 30, 2021, we found that the temperature values were different both from one month to another and during one day (24 hours).
The temperature values registered outside the greenhouse, throughout the study period, reached in January minimum values of -6.91ºC and maximum values of 12.48ºC.
In February, outside the greenhouse, were recorded negative temperatures from February 10 to February 25. The lowest values were between -7.88ºC and -0.3ºC. In the rest of the period, only positive temperatures of over 20.47ºC were recorded.
In March, were recorded, only for short periods, negative outdoor temperatures of -0.5ºC or -2.1ºC and temperature maximums of 17.9ºC (Figures 4-6).

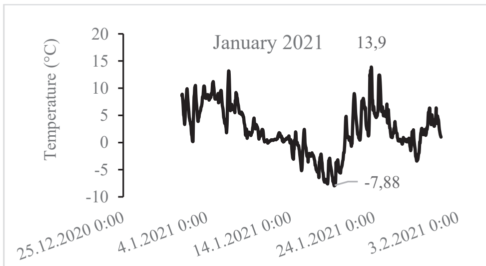


Figure 4. The temperature recorded in the greenhouse in January

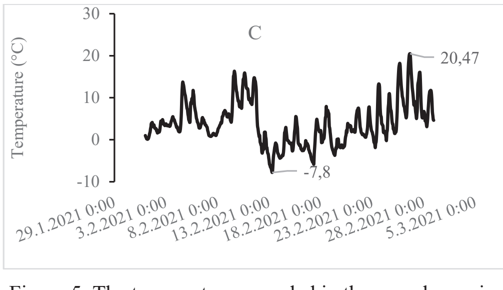


Figure 5. The temperature recorded in the greenhouse in February

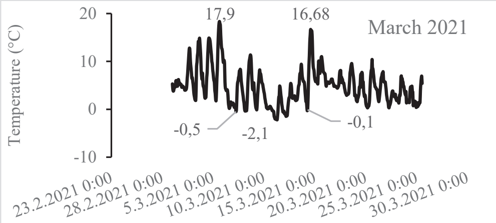


Figure 6. The temperature recorded in the greenhouse in March

În January, the lowest minimum temperatures recorded in the greenhouse were 14.8°C and 15.3°C, respectively. During the rest of the period, the temperatures ranged between 18°C and 22.5°C, depending on the temperature accumulations during the day.

The maximum of temperatures recorded in the greenhouse reached values of over 24.9°C to 33.1°C in direct correlation with solar radiation (Figure 7).

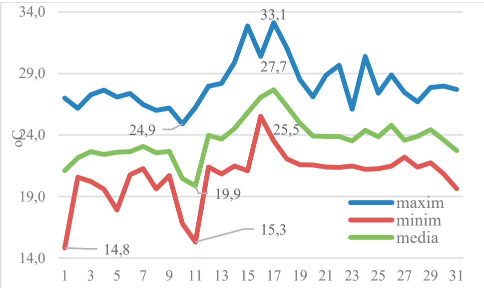


Figure 7. Minimum, maximum and average temperatures recorded daily in the greenhouse in January

In February, during a 24-hour day, average daily temperatures were recorded, between 22°C around 1 AM and 26.3°C around 6 PM. Also, during the day the temperatures reached average values of 17.02°C to 23.3°C and maximums of 32.98°C (Figure 8; Table 1).

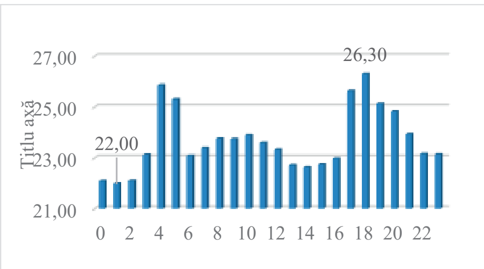


Figure 8. Average daily temperatures recorded in February 2021

Table 1. Average, minimum and maximum monthly temperatures recorded during 24 hours as well as the sum of temperatures recorded in February

Hour	The monthly average temperature degrees (°C)	Minimum temperature (°C)	Maximum temperature (°C)	The sum of the temperature degrees (°C)
0	22,10	18,72	26,86	640,85
1	**22,00**	17,32	26,84	636,89
2	22,11	**17,02**	26,7	639,14
3	23,13	20,2	27,08	647,61
4	25,86	23,3	28,39	724,13
5	25,30	20,38	27,68	708,46
6	23,09	20,99	25,9	646,45
7	23,39	20,5	29,25	654,97
8	23,77	21,22	32,28	665,42
9	23,75	20,24	**32,98**	664,95
10	23,90	20,41	33,5	669,17
11	23,59	19,87	31,77	660,63
12	23,34	20,98	27,33	653,38
13	22,72	19,95	26,65	636,18
14	22,63	20,08	25,12	633,77
15	22,75	20,6	25,78	637,07
16	22,98	19,55	25,85	643,36
17	25,63	17,93	C	717,74
18	**26,30**	20,13	31,4	736,31
19	25,13	22,97	28,02	703,76
20	24,83	21,88	28,72	695,21
21	23,94	21,16	28,2	670,29
22	23,16	20,32	27,62	648,34
23	23,14	20,29	27,2	647,87

We can remark that for the analysed period we found that the maximum temperatures recorded in the greenhouse reached values of 32.9°C in January, 33.5°C in February and 37.25°C in March. Very high values correlate with high values of external light intensity. Taking into account the optimal values regarding the requirements of the species, in our case tomatoes, were affected the physiological processes of the species determining to the acceleration of fruit ripening and stopping vegetative growth (Table 2).

Regarding the average daily temperatures recorded during January-March, we found that, in January, the temperatures varied between 19.9°C and 27.7°C, in February between 21.88°C and 26.29°C. If we compare with the average recommended temperatures for vegetative growth of 20.5°C (23°C during the day and 18°C at night) and 21°C for fruiting (24°C during the day and 18°C at night), we noticed that very high temperatures influenced the production of fruits but also the duration up to until their ripening, shortening it (Figure 9).

Table 2. Maximum temperatures recorded in January, February and March

	January		February		March	
Day	Max.	Min.	Max.	Min.	Max.	Min.
1	27,0	**14,8**	27,77	20,51	26,87	21,0
2	26,2	20,6	27,68	21,01	26,0	21,09
3	27,3	20,2	27,6	21,91	25,54	20,59
4	27,7	19,6	29,37	22	26,23	21,62
5	27,1	17,9	29,35	21,4	**37,25**	22,32
6	27,4	20,8	27,78	20,73	27,59	17,19
7	26,5	21,3	25,58	19,9	24,3	18,53
8	26,0	19,6	28,18	20,8	24,58	19,8
9	26,2	20,7	31,4	21,98	24,99	19,52
10	24,9	16,8	30,98	21,79	24,62	13,2
11	26,2	15,3	29,76	22,64	24,6	**13,7**
12	28,0	21,4	26,63	20,28	32,73	18,9
13	28,2	20,8	**33,5**	22,05	24,71	19,92
14	29,9	21,5	27,4	20,94	27,02	20,35
15	**32,9**	21,1	27,02	22,23	25,37	17,86
16	30,4	25,5	29,9	21,92	26,91	21,78
17	33,1	23,5	29,25	20,24	27,18	20,73
18	31,1	22,1	25,9	19,87	25,62	20,24
19	28,5	21,6	27,12	19,95	25,41	19,98
20	27,1	21,6	27,7	20,29	24,13	19,05
21	28,9	21,4	25,41	20,42	23,2	16
22	29,7	21,4	24,79	20,99	23,96	13,31
23	26,1	21,5	25,38	17,93	24,13	19,46
24	30,4	21,2	25,17	20,41	23,7	18,3
25	27,4	21,3	27,5	20,42	24,51	20,6
26	28,9	21,5	28,72	**17,02**		
27	27,5	22,2	27,88	20,08		
28	26,7	21,4	26,58	21,98		
29	27,9	21,7				
30	28,0	20,8				
31	27,7	19,6				

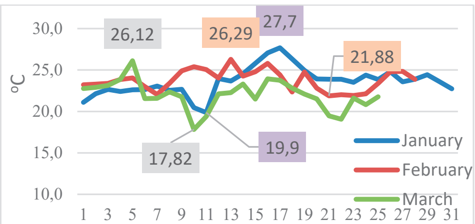


Figure 9. Average daily temperatures for January and February

Another factor that influences plant biology is atmospheric humidity. In the case of growing tomatoes in the greenhouse, we found that the atmospheric humidity in the growing space varied during the day, which was correlated with light intensity and temperature values. It ranged from 35% to 87% in January, from 30% to 78% in February and from 25 to 80% in March. In the first part of the day, in the morning, higher values were recorded (Figures 10-12).

Table 3. Difference between maximum and minimum temperature

	Month		
	January	February	March
Day	°C	°C	°C
1	**12,18**	7,26	5,87
2	5,6	6,67	4,91
3	7,05	5,69	4,95
4	8,05	7,37	4,61
5	9,17	7,95	**14,93**
6	6,62	7,05	10,4
7	5,2	5,68	5,77
8	6,38	7,38	4,78
9	5,49	9,42	5,47
10	8,1	9,19	11,42
11	10,94	7,12	10,9
12	6,57	6,35	13,83
13	7,37	**11,45**	4,79
14	8,42	6,46	6,67
15	11,77	4,79	7,51
16	4,87	7,98	5,13
17	9,61	9,01	6,45
18	9,02	6,03	5,38
19	6,92	7,17	5,43
20	5,54	7,41	5,08
21	7,46	4,99	7,2
22	8,31	3,8	10,65
23	**4,62**	7,45	4,67
24	9,17	4,76	5,4
25	6,14	7,08	**3,91**
26	7,41	11,7	
27	5,26	7,8	
28	5,3	**4,6**	
29	6,12		
30	7,14		
31	8,07		
Media	7,42	7,13	7,04

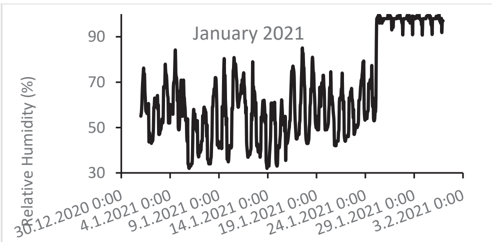


Figure 10. Relative humidity in January

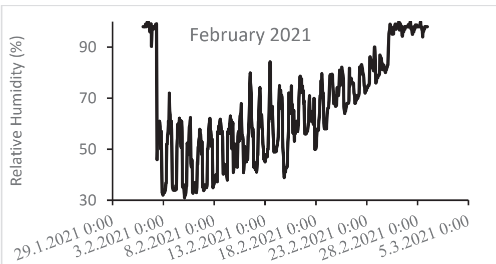


Figure 11. Relative humidity in February

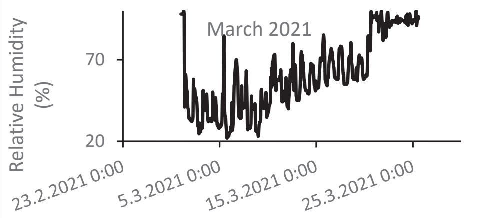


Figure 12. Relative humidity in March

Regarding CO2, its beneficial effect is known. Inside the greenhouse, in January, we found minimum values of 120.38 ppm and 1250.31 ppm in the morning, but in general the CO_2 concentration was around 400 ppm (Figure 13).

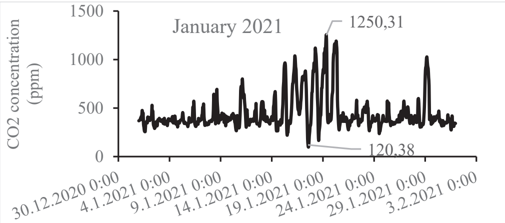


Figure 13. CO2 concentration in greenhouses in January

In February, CO_2 values ranged from 187.45 ppm to 1186.38 ppm (morning). In general, the CO2 concentration was within the physiological limits for the vegetative growth and fruiting of tomatoes (Figures 14 and 15).

In March we found normal values that ranged between 216.52 ppm and 720.06 ppm (morning).

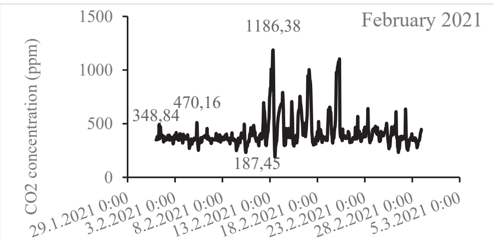


Figure 14. CO_2 concentration in greenhouses I February

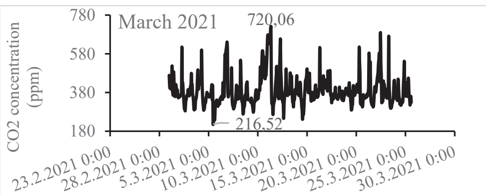


Figure 15. CO_2 concentration in greenhouses in March

If we analyse if there was any relationship between CO_2 values and production and calculating the correlation coefficient we found moderate negative relationships R^2 = 0.395 (Figure 16).

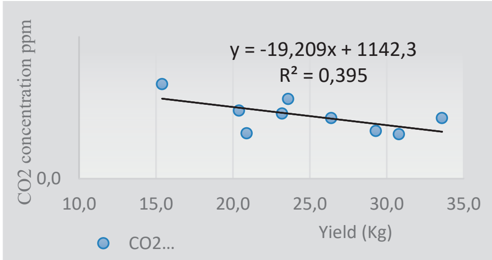


Figure 16. Relationship between the CO_2 content of the greenhouse and the production obtained for the 'Cheramy' variety

However, if we analyse how carbon dioxide influenced production, we could see a negative influence with its increase.

The Person relationship indicates a moderately negative correlation (R = -0.629). For the 'Cheramy' variety, the increase in CO_2 content did not show an increase in production (table). If we analyse how carbon dioxide influenced production, we could see a negative influence, as it increases.

The Person relationship indicates a moderately negative correlation (R = -0.629).

For the 'Cheramy' variety, the increase in CO_2 content did not show an increase in production (Table 4).

Table 4. Pearson test on the relationships between the total production obtained in the greenhouse of the 'Cheramy' variety, the concentration of CO2 and the sum of the temperatures accumulated in the greenhouse

Cheramy	Production (kg)	CO_2 concentration (ppm)	Accumulated temperature sum (°C)
Production (kg)	1		
CO_2 concentration (ppm)	-0.629	1	
Accumulated temperature sum (°C)	-0.540	0.033	1

In the ‘Flaviola’ variety there is a weak negative relationship between the CO_2 concentration and the production obtained $R^2 = 0.1695$ (Figure 17).

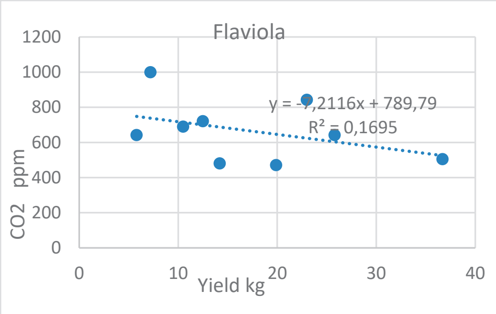


Figure 17. The influence of CO_2 on ‘Flaviola’ production

If we look how much the CO_2 value in the greenhouse influenced, we find that the ‘Flaviola’ variety reacted less to the increase in CO_2 content, the Person relationship indicates a moderately low relationship (r = - 4117) (Figure 18).

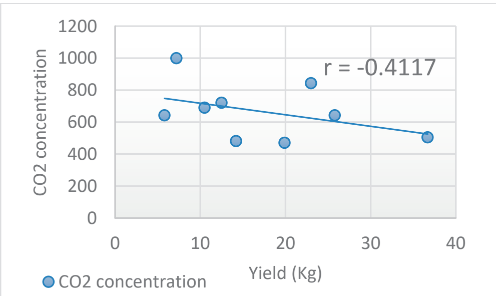


Figure 18. Influence of CO_2 on ‘Flaviola’ production

Figure 19. Aspect of culture

In ‘Cheramy F1’, the correlation made between the productions obtained during the study period and the sum of the temperatures recorded between the harvesting periods indicated a weak positive influence ($R^2 = 0.2654$) between them.
To see how much the temperature influenced the production level by the calculation performed by the Person test we found a high positive relationship r = 0.51518 (Figure 20).

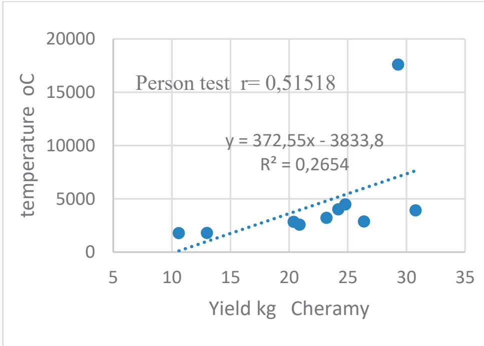


Figure 20. The influence of the accumulated temperature between harvests on the total production obtained for the ‘Cheramy’ variety

The correlation made between the yields obtained and the cumulative sum of the temperatures recorded during the harvest periods indicated a weak positive influence ($R^2 = 0.0049$) between them.
To see how much the temperature influenced the production level, the cumulative sum of the recorded temperatures and by the calculation performed using the Person test we found an insignificant positive relation (r = 0.0698) (Figure 21).

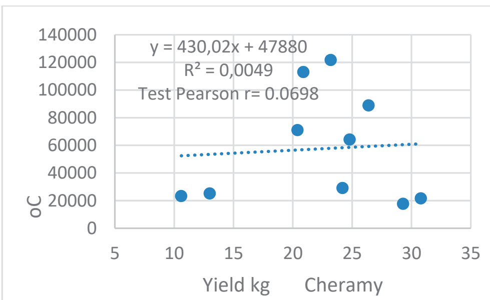


Figure 21. Influence of the sum of the cumulative temperatures between harvests on the production of the ‘Cheramy’ hybrid

Figure 22. Aspect of culture

In the case of the ‘Flaviola’ variety, we noticed that it reacted to the temperature values recorded between harvests.

The correlation made between the productions obtained on each harvest and the temperatures recorded between harvests had a positive influence ($R^2 = 0.4081$).

To see the extent to which temperature values influenced production we calculated using the Pearson test and obtained a large relationship between them r = 0.639 (Figure 23).

If we analyse the influence of the sum of the temperatures accumulated in the greenhouse between harvests we can see that there is a negative relationship $R^2 = 0.4736$.

To see how much it influences this we calculated using the Pearson test and we noticed that the value was negative (r=-0.688), the influence being average (figure 24).

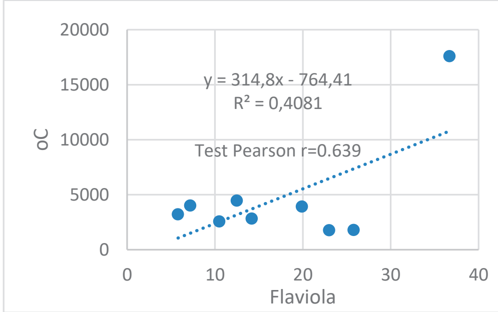


Figure 23. Relationship between yields obtained on ‘Flaviola’ varieties and the sum of temperatures between harvests

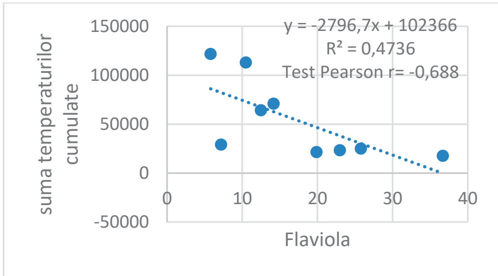


Figure 24. Relationship between yields per harvest and the sum of cumulative temperatures recorded at harvest

Table 5 summarizes the data on the influence of CO2 and the sum of the cumulative temperatures on production of the ‘Flaviola’ variety.

Table 5. Influence of CO_2 and sum of temperatures on crops on ‘Flaviola’ production

Flaviola	Production (kg)	CO_2 concentration	The sum of the accumulated temperatures
Production (kg)	1		
CO_2 concentration	-0.4117	1	
The sum of the accumulated temperatures	-0.741	0.033	1

The number of plants under observation was 154 plants in the ‘Cheramy’ hybrid and 101 plants in the ‘Flaviola’ cultivar.

The total fruit production obtained from the plants harvested from the greenhouse was 223.6 kg for ‘Cheramy F1’ and 155.6 kg for the ‘Flaviola’ variety (Figure 25).

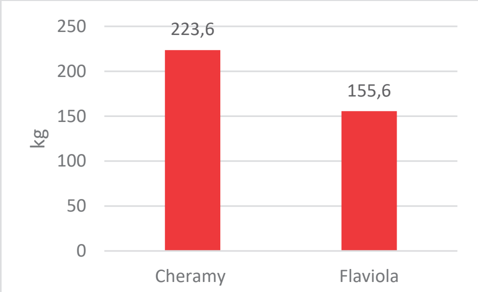


Figure 25. Total production of tomatoes obtained in the greenhouse

If we follow the relation regarding the production obtained between the analysed varieties, we found positive relations between them $R^2 = 0.4031$ but the Pearson test clearly shows that the variety reacts differently to the temperature conditions, the influence being significant (Pearson test r = 6635) (Figure 26). The influence of cumulative temperatures had a significant influence (Figure 27).

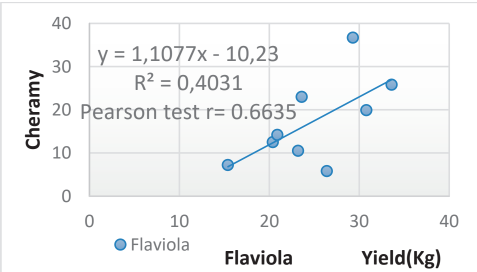


Figure 26. The interaction between the yields obtained for the analysed variety

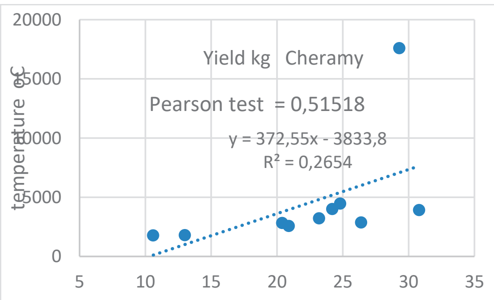
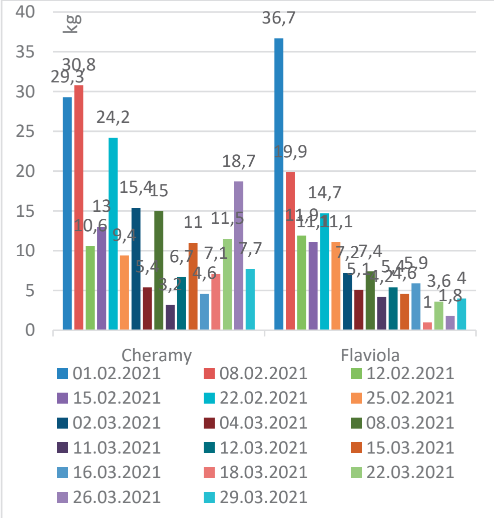


Figure 27. Influence of cumulative temperatures on production of 'Cheramy F1'

At the first harvest on 1.02.2021, 36.7 kg were obtained from the 'Flaviola' variety and 29.3 kg from the 'Cheramy' variety (Figure 28).



Figures 28. Harvests performed on the analysed cultivars

Figure 29. Aspect of culture - 'Flaviola'

The average production obtained per plant was in the case of the 'Flaviola' variety of 1.5406 kg/plant and in the case of the 'Cheramy' hybrid of 1.4519 kg/plant (Figures 29 and 30).

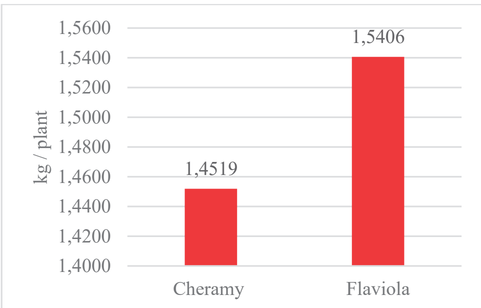


Figure 30. The average yield per plant of the 'Cheramy' and 'Flaviola' varieties

The productions of tomatoes were performed weekly and recorded. Thus, we found that they were higher in the 'Flaviola' variety compared to the 'Cheramy' hybrid. One conclusion would be that the 'Cheramy' hybrid is influenced by variations in the temperature and light factor (Figure 31).

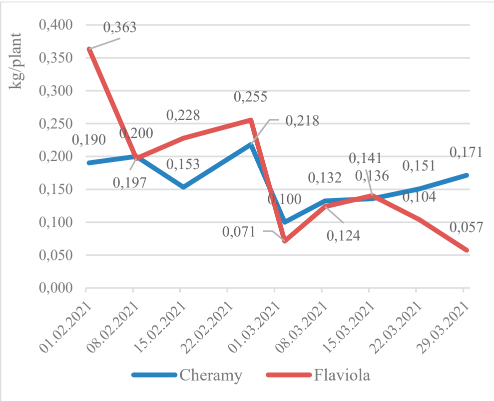


Figure 31. Average weekly harvest on plant

During the care works, we periodically carried out works to remove the leaves on the plant. The first deforestation was carried out after 20 days, and the rest of the deforestation of the vegetative mass of the leaves was made done as many times as needed. The figure shows the dates and the number of days after which the leaves were removed (Figure 32).

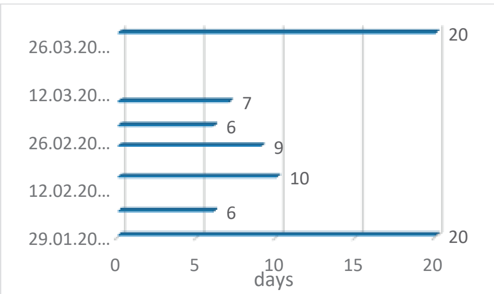


Figure 32. Number of days between leaf harvests

All vegetative mass was recorded. We found that, at the first and second harvest, the highest amount of vegetative mass was recorded for the 'Flaviola' variety of 11.7 kg at the first harvest and 10.6 kg at the second harvest. In this variety the vegetative mass harvested in the following stages was lower compared to the 'Cheramy' hybrid (Figure 33).

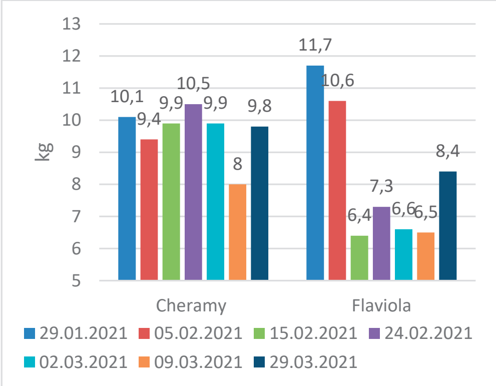


Figure 33. The total amount of leaves harvested in the greenhouse

In the case of the 'Cheramy' hybrid, a total of 67.6 kg was harvested per plant, with an average per plant of 0.439 kg, and for the 'Flaviola' variety, 57.5 kg, with an average per plant of 0.569 kg (Figure 34).

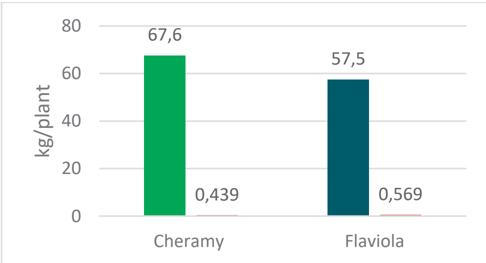


Figure 34. Total amount of leaves harvested at the analyzed cultivars and the average per plant

Analysing the relationship between the analysed varieties and the mass of harvested leaves in stages, we found that between varieties, the influence was small (R^2 = 0.0453), but the Pearson relationship shows that there was a weak positive relationship between the two varieties and leaf production (r = 0.213) (Figure 35 and Table 6).

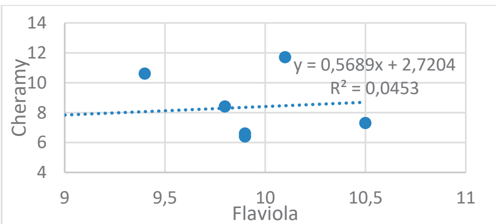


Figure 35. Influence of varieties on total leaf production

Table 6. The correlation between the two varieties on leaf production

Total mass Leaves	Cheramy	Flaviola
Cheramy	1	
Flaviola	0.213	1

Analysing the total accumulated vegetative mass, for each variety, we found that at the 'Cheramy' hybrid a quantity of 184.8 kg was obtained and for the 'Flaviola' variety 141.4 kg vegetative mass. On average, 1.2 kg/plant at 'Cheramy' hybrid and 1.4 kg/plant at 'Flaviola' variety were obtained (Table 7).

Table 7. Total vegetative mass harvested (leaves and stems)

Specification	UM	Cheramy	Flaviola
Total vegetative mass	kg	184.8	141.4
Total plants	No.	154	101
Average mass / plant	kg/plant	1.2	1.4

Table 8. Total vegetative mass and total fruit harvested on plant in the 'Cheramy' hybrid

Total vegetative mass and fruits	Vegetative mass	Total fruit mass
kg	kg	kg
408.4	184.8	223.6

In 'Cheramy F1', the percentage of harvested fruit represented 54.75% of the total accumulated vegetative mass, and the mass of leaves and stems 45.25% (Figure 36).

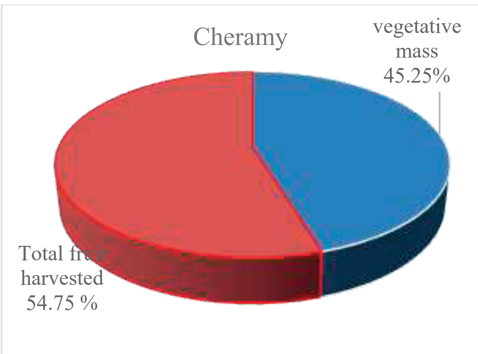


Figure 36 Percentage of vegetative mass and fruit yield in the 'Cheramy' hybrid

In the Flaviola variety, in the analyzed period, the percentage of fruits harvested on average per plant was 60.64%, and that of leaves and stems 39.36% (Figure 37).

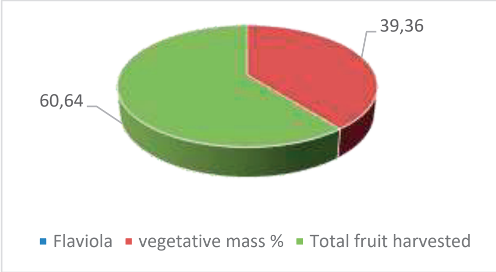


Figure 37. Percentage of vegetative mass and fruit yield in the 'Flaviola' hybrid

CONCLUSIONS

Analysing the temperatures recorded in the greenhouse during the period of vegetation and fruiting of tomatoes, we could see that its values were higher in February and March, which led to the acceleration of fruit ripening. In the 'Flaviola' variety, the temperature had a greater influence on the production compared to the 'Cheramy' variety. We noticed that each variety reacted differently both in terms of temperature and CO_2 values. The accumulated vegetative mass was higher in 'Cheramy F1' compared to 'Flaviola', this being a genetic characteristic of the cultivar. The percentage of fruits obtained per plant was 54.75% and only 45.25% vegetative mass in 'Cheramy F1'. For the 'Flaviola' variety, the percentage of fruits was 60.64% and 39.36% vegetative mass.

ACKNOWLEDGEMENTS

This research work was carried out with the research leading to these results has funding from the Norway Grants 2014, under Project contract no. 40/2021

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF FERTILIZER MEGAMIX ON VARIETY CHARACTERS INHERITANCE OF UTERINE ROOT BEETS BORDEAUX 237

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Abstract

The influence of liquid mineral fertilizer from the series of preparations "Megamix" ("Megamix-seeds" and "Megamix-profi") on the inheritance of varietal traits of uterine roots of table beet variety 'Bordeaux 237' was studied. contributed to the reduction of interphase periods and, in general, the growing season according to the variants of the experiment by 4-9 days. In the population of 'Bordeaux 237' variety, the rounded shape of the root crop prevailed. The maximum amount of such root crops was obtained by joint treatment of seeds "Megamix-seeds" and seedlings "Megamix-profi" - 84.0%, with 72% in the control. Root shape index was 0.9 - 1.1. The use of "Megamix" in the technology of growing table beets in vegetable crop rotation had a positive effect on the growth and development of plants. Biometric measurements showed that, on average, the weight of the root crop varied from 234.0 to 248.4 g. the formation of the economic part of the crop was higher than in the control by 0.01-0.04 g/cm^2. The maximum yield of table beet 28.4 t/ha was obtained by joint treatment of seeds and seedlings with Megamix-seeds and Megamix-profi, which is 6.2 t/ha higher than in the control. Evaluating the effect of the use of "Megamix" fertilizer, its influence on quality indicators, namely the marketability of 96-99%, was established.

Key words: *beet, cultivar, uterine roots, varietal characteristics, fertilizers.*

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is a widespread vegetable crop. It is rich in carbohydrates, mineral salts, organic acids and vitamins (C, B1, B2, B6, PP, P), biotin, folic and pantothenic acids and physiologically active substances - betaine and betanin. Beetroot is one of the early ripening, productive plants, its roots lie well during long-term storage (Burenin et al., 1998; Fedorova et al., 2017).

In the conditions of fierce competition in the market for selection achievements, high demands are currently made on the quality of new varieties and hybrids of vegetable crops. For dining beets, this is a high and stable yield, marketability, an attractive appearance of the root crop and, of course, resistance to diseases and pests. For mechanized cultivation, an important feature is an erect, compact leaf rosette, with thin petioles, which persists until the end of the growing season of plants, which makes it possible to use top-lifting combines during harvesting (Kozar, 2019).

To obtain high yields of table beet, vegetable growers use intensive cultivation technology that requires a large number of nutrients for plants. Some of them enter the plant from those available in the soil, the other from the fertilizers applied to the soil, and the third with foliar dressing. Each of these sources is only partially used. The amount of assimilated fertilizers is 30-60% of the applied fertilizers. The rest pass into hard-to-reach forms, being bound by the soil, washed out into groundwater or evaporated (in the form of ammonia), and the vegetable grower loses money (Romanyuk, 2000).

In this regard, it is possible to realize the high genetic productivity of plants without significant additional costs for their functioning, by optimizing the production process, by using new forms of micronutrient fertilizers in a chelated form in the cultivation technology. One of these forms is a complex highly effective liquid mineral fertilizer from a series of drugs "Megamix" (Kshnikatkina et al., 2010).

Presowing treatment with liquid mineral fertilizer from the Megamix series of preparations allows for strictly differentiated nutrition for each plant, enhance the starting acceleration in the development of seedlings and their resistance to unfavorable environmental factors, optimize mineral nutrition (http // megamix52.ru)

In recent years, the range of beets cultivated in the Middle Volga region has significantly expanded. Modern varieties of this culture differ in yield, quality, economically valuable traits, as well as in the ability to effectively use modification and genotypic variability in various environmental conditions. But the Bordeaux 237 variety is still unsurpassed (Gryazeva, 2007).

Beet varieties are complex population systems, that is, they consist of different biotypes. They are characterized by a complex splitting of characters in the offspring. Therefore, when growing uterine root crops, it is necessary to ensure the preservation of the initial level of economically valuable traits of the variety (Pivovarov, 1999).

In this regard, the optimization of the combination of methods for growing table beets, contributing to better growth and development of plants and ensuring high yields with good commercial and economic qualities of root crops in various environmental conditions, with the preservation of all varietal characteristics, determine not only the fundamental nature, but also the practical orientation , the relevance of research in this area.

Research goal is to study the effect of liquid mineral fertilizer from a series of drugs "Megamix" ("Megamix-seeds" and "Megamix-pro") on the inheritance of varietal and economically valuable traits, increasing the productivity of uterine beetroot varieties Bordeaux 237 in the Penza region.

MATERIALS AND METHODS

The research was carried out at the collection site of the Penza State Agrarian University in 2019-2020.

Experiment scheme: 1. Control - no treatment. 2. Treatment of seeds with Megamix-seeds. 3. Treatment of seedlings with Megamix-profi. 4. Treatment of seeds with Megamix-seeds and seedlings with Megamix-profi.

The counting area of the plot was 3 m^2, the repetition was six times, the arrangement of the plots was systematic, the sowing scheme was three-line, belt 20 + 20 + 50 cm. The seeding rate of seeds was 10 kg per 1 ha.

When laying the field experiments, we were guided by the main provisions of the Methodology for state variety testing of agricultural crops (potatoes, vegetables, melons) (1975).

Phenological observations and biometrics were carried out at the beginning of the emergence of shoots, the formation of 4-5 and 10-12 leaves, the mass setting of root crops, the onset of technical and commercial ripeness according to the Methodology of field experience in vegetable growing and melon growing (1992).

The assimilation surface of the leaves was determined by the gravimetric method.

Harvesting and accounting of the harvest was carried out manually, on a plot basis, taking into account standard root crops (GOST R 51811-72001, 2010) and non-standard root crops by fractions.

The description of morphological characters was carried out in accordance with the method of I.A. Prokhorov, S.P. Potapova (1975), Guidelines for the approbation of vegetable crops and fodder root crops (1982), Methodological guidelines for the study and maintenance of the world collection of root crops (1977).

The object of the research was the ‘Bordeaux 237’ beet variety, which is the most widespread in the vegetable growing practice of our country.

Variety ‘Bordeaux 237’ was bred at the Gribovskaya vegetable selection and experimental station - authors V.V. Ordynsky and S.P. Agapov - by individual and family selection from a hybrid population from free cross-pollination of a number of table varieties with the ‘Detroit’ variety. Root crops are round and oval-round with a small head, slight roughness and uneven surface. The pulp is intensely dark red, dense, with a slightly increased sugar content. The variety is quite fruitful, mid-season, few flowers. Keeping quality of root crops is good. The variety is suitable for long-term storage, fresh consumption and canning. Polyspermous (Agapov, 1975).

The research material was a complex highly effective liquid mineral fertilizer from a series of drugs "Megamix" ("Megamix-seeds" and "Megamix-profi"), which is based on a rich composition of micro- and macroelements. Most of the microelements are in a chelated form that is easily absorbed by plants. The drug has a significant effect on the life processes of the plant with a small application rate.

"Megamix-seeds" is a liquid mineral fertilizer for pre-sowing seed treatment based on micro- and macroelements (contains five macro- and 10 microelements), which ensures the best realization of the seedling development potential.

Purpose of "Megamix-seeds":

- full nutrition of seedlings in the initial phases of development, due to the content of macro- and microelements;
- the formation of a powerful root system, as the basis for the full development of culture;
- increasing the survival rate of culture, especially in the initial phases of development;
- an increase in the microbiological activity of the soil, and, as a result, an increase in immunity and the availability of nutrients;
- increased yield due to increased development of the root system and reduced risks in the initial, critical phases of plant development.

The consumption rate of the Megamix-seeds preparation is 2 l/t.

Megamix-profi is a liquid mineral fertilizer for foliar feeding with a rich content of microelements, aimed at complex stimulation of all plant processes. Synergy and antagonism of individual nutrients is also taken into account.

Appointment of "Megamix-pro":

- stimulation of root nutrition, activation of enzymes and replenishment of missing nutrients;
- elimination of the lack of trace elements in key phases and during the formation of the crop;
- increased productivity due to the stimulation of enzymatic processes and prolongation of the growing season;
- improving the quality of the crop, according to the indicators to which the variety is predisposed and to which the main fertilizers are oriented.

The consumption rate of the Megamix-profi preparation is 1 l/ha. Fork processing (http // megamix52.ru.)

RESULTS AND DISCUSSIONS

The accumulation of organic matter and the formation of the yield occurs during the growth of plants. The main criterion in the formation of a good harvest of beet root crops is the duration of the growing season.

Weather conditions have a significant impact on the transit times and the duration of interphase periods. The timing of the stages of development of table beet in different years is not the same.

Sowing of table beet was carried out on May 12 in 2019 and on May 18 in 2020. As a result of observations of the growth and development of plants, it was found that liquid mineral fertilizer from the Megamix series of preparations contributed to the faster growth and development of table beet plants, but this influence was ambiguous.

The duration of the "sowing-germination" period in the variants of treatment with liquid mineral fertilizer from the series of "Megamix" preparations ranged from 17 to 20 days, with 17 in the control (Table 1).

Table 1. Duration of interphase periods (average, 2019-2020)

Option	Sowing-seedlings	From germination to, days:			The length of the growing season
		phase forks	bunch ripeness	technical ripeness	
Control (no processing)	17	8	36	97	114
Seed treatment - "Megamix-seeds"	17	8	34	93	110
Treatment by seedlings - "Megamix-profi"	17	7	thirty	89	108
Seed treatment - "Megamix-seeds" + processing by seedlings - "Megamix-profi"	17	7	29	88	105

The longest period was 20 days in the treatment of seedlings with Megamix-Profi. The period of “sprouting, fork phase” was the shortest in the treatment options for seedlings and together of seeds and seedlings, which is one day shorter than in the control.

The duration of the period "shoots-bunch ripeness" ranged from 36 days in the control and up to 29-34 days in the treatment options. Small root crops with a diameter of 3-4 cm for beam ripeness are formed faster in the variant with joint treatment of seeds with Megamix-seeds and seedlings with Megamix-profi.

In general, the length of the growing season was shorter in the studied variants by 4-9 days compared to the control.

Thus, as a result of the research, it can be concluded that liquid mineral fertilizer from the Megamix series of preparations promotes the activation of seed germination processes and the reduction of interphase periods and, in general, the growing season of table beet.

The quality of canteen beetroot is determined by a set of features and properties. Modern varieties of table beet are mainly heterozygous populations subject to strong variability under the influence of environmental conditions (Burenin et al., 2016).

As a result of studying the forms of the root crop in the table beet variety ‘Bordeaux 237’, 3 biotypes were identified: round, round-flat and conical (Table 2).

In the population of ‘Bordeaux 237’ cultivar, the rounded shape of the root crop, characteristic of the cultivar, prevailed. The largest number of such root crops was obtained by joint treatment of seeds with Megamix-seeds and seedlings with Megamix-profi and amounted to 84.0%, with 72.0% in control.

An important feature that determines the technological qualities of varieties and hybrids of table beet is an erect, compact leaf rosette, which persists until the end of the growing season of plants and the shape of the root crop (Kozar, 2019).

Table 2. Influence of fertilizers from the Megamix series of preparations for varietal characteristics of uterine root crops of beet

Option	Root shape, %			Rosette of leaves, %			Color of the pulp
	rounded	rounded flat	conical	erect	sprawling	pressed	
The control	72.0	20.8	7.2	86.6	12.6	0.7	Maroon
Megamix seeds - seed treatment	79.6	14.2	5.8	89.3	10.6	-	Maroon
Megamix-pro treatment by seedlings	80.3	13,7	6.2	89.7	8.6	1.7	Maroon
Megamix-seeds - seed treatment + Megamix-pro treatment by seedlings	84.0	12.0	4.0	92.6	7.3	-	Maroon

The analysis of experimental data showed that in all variants the erect rosette of leaves prevailed, but in the variants of treatment with Megamix fertilizer, there were 2.7-6.0% more such plants than in the control.

In all variants, the roots had a maroon color of the pulp. Red-colored beet varieties have a higher content of vitamin C, betaine, betanin and ash elements, increased palatability and, often, more delicate pulp. Therefore, for the consumer and for canning, the intensity of the color of the pulp is an important feature, especially given the current market requirements (Burenin et al., 2016).

The shape of the root crop has a significant impact on the biometric indicators of beetroot (Table 3).

Table 3. Analysis of the morphological features of beetroot when processing Megamix (average 2019-2020)

Option	Root weight, cm	Root diameter, cm	Root length, cm	Leaves weight, g	Sheet length, cm	Number of leaves, pcs	Petiole length, cm	Root shape index
The control	234.0	8.6	8.4	90.0	15.0	13.0	13.2	0.9
Megamix-seeds - seed treatment	242.7	8.4	8.4	90.0	15.6	12.0	12.7	1.0
Megamix-pro - Processing by seedlings	246.0	8.0	8.5	88.1	16.0	14.0	12.7	1.1
Megamix-seeds - seed treatment + Megamix-pro treatment by seedlings	248.4	7.6	8.0	82.8	15.8	15.0	14.2	1.0

The combination of seed treatment and foliar treatments of beet plants in the forks phase had a significant impact on the growth and development of beet plants. "Megamix" stimulated the formation of leaves and the development of their assimilation surface, and also contributed to the thickening of root crops and an increase in their mass. Biometric measurements showed that, on average, the weight of the root crop varied from 234.0 to 248.4 g. In the variant, with the joint treatment of seeds and seedlings of beets, there was a tendency to decrease the weight of leaves by 7.2 g. The number of leaves varied from 12 to 15 pieces. on one plant.

Under the influence of Megamix, an increase in leaf length within the range of 15.8 - 16.0 cm was noted, with 15.0 cm in the control variant. The length of the petiole was in the range of 12.7 - 14.2 cm, in the control variant - 13.2 cm. These two indicators determine the plant height of the table beet, which is taken into account when mechanized harvesting of this crop.

Phenological and biometric studies have established that the most significant effect on the growth, development, dynamics of the formation of uterine root crops and the productivity of table beet was exerted by the complex treatment of seeds and vegetative plants in the fork phase with the fertilizer "Megamix".

Thus, the use of "Megamix" fertilizer in the technology of growing table beets in vegetable crop rotation had a positive effect on the growth and development of plants.

In our studies, we studied the relationship between the weight of the root crop and the weight of the leaves.

It has been established that the mass of the tops in the total mass of the plant ranges from 25.0 to 27.7%. The productivity of the work of the leaves - the number of grams of root crop mass created by the gram of the leaf ranged from 2.7 to 3 g in variants with the use of Megamix. The leaves worked most productively in the variant with joint treatment of seeds and seedlings with Megamix. In this variant, 3.0 grams of root crop mass was created with one gram of a leaf, with 2.6 grams in the control (Table 4).

Table 4. Relationship between root crops and beet leaf apparatus (average 2019-2020)

Option	Root mass		Leaf mass		The ratio of the mass of tops to the mass of the plant, %	Leaf productivity, g/g
	r	%	r	%		
The control	234.0	100.0	90.0	100.0	27,7	2.6
Megamix-seeds - seed treatment	242.7	103,7	90.0	100.0	27.0	2.7
Megamix-pro - seedling treatment	246.0	105.1	88.1	97.9	26.4	2.8
Megamix-seeds - seed treatment + Megamix-pro - treatment by seedlings	248.4	106.2	82.8	92.0	25.0	3.0

The degree of development of the leaf surface has a great influence on the productivity of agricultural plants. The leaf is not only a supplier of food and energy for the plant, it is the center of active and diverse regulation of the vital processes of the plant organism as a whole (Kshnikatkina et al., 2011).

It is believed that when applying new technologies, it is important to take into account the coefficient of economic efficiency (Khoz), which characterizes the level of use of assimilation products for the formation of the economic part of the crop (Timakova, 2011).

As a result of research, a close relationship has been established between the density of standing of plants and the coefficient of economic efficiency. So in the control it is equal to 0.35 g/cm^2 (Table 5). Treatment of seeds with "Megamix-seeds" and seedlings "Megamix-profi" contributed to an increase in the mass of the root crop and the area of the assimilation surface of leaves, and, accordingly, in these variants the Khoz was higher than in the control by 0.01-0.04 g/cm^2. The highest indicator of Khoz was in the variant with joint treatment of seeds and vegetative plants and amounted to 0.39 g/cm^2, which is 0.04 g/cm^2 higher than in the control. This indicates the formation of a more active photosynthetic apparatus in this variant.

Table 5. Coefficient of economic efficiency

Option	Density plants, thousand pcs/ha	Weight root vegetable, g	Square leaves, cm^2	Khoz., g/cm^2
The control	142.5	234.0	668.6	0.35
Megamix-seeds - seed treatment	151.9	242.7	674.2	0.36
Megamix-pro - Processing by seedlings	149.2	246.0	647.4	0.38
Megamix-seeds - seed treatment + Megamix-pro - treatment by seedlings	153.2	248.4	637.0	0.39

The main direction of increasing the coefficient of economic efficiency is the use of technologies that promote higher ratios of the mass of root crops and vegetative organs. One of these technologies is the use of liquid mineral fertilizer from the Megamix series of preparations.

The variety is able to realize its genetic potential for productivity when certain conditions are created by improving the elements of cultivation technology. Fertilizers play an important role among the factors influencing the yield of table root crops. According to I.I. Leunov, the share of the influence of fertilizers on the formation of the crop is from 30 to 45%, second only to the influence of meteorological conditions (Leunov, 1998).

The existence of any genotype is unthinkable outside a certain environment, therefore it is important to combine high productivity and ecological stability in one genotype under the influence of unfavorable environmental factors.

To determine the most optimal variant of the experiment that provides the maximum average yield in the entire set of environments, the selection criterion would be the value of OACi (general adaptive capacity). This is an indicator of the deviation of the average value of the characteristic for the options of experiencethe mean value of a trait in various environmental conditions (Kilchevsky et al.,1989).

Various years of cultivation were used as a set of media (Table 6).

The studies have shown that the yield of uterine beetroot crops significantly depended on the meteorological conditions of the growing season and on the treatment with preparations from the Megamix series.

So in the conditions of 2020, the yield was lower than in 2019, due to the unfavorable growing conditions of this year. The amount of precipitation during the period of root crop formation was 46% lower than the average long-term data. On average, for two years, the yield varied according to the variants of the experiment from 22.2 to 28.4 tons per hectare.

The maximum yield of table beet 28.4 t/ha was obtained by joint treatment of seeds and seedlings with Megamix-seeds and Megamix-profi, which is 6.2 t/ha higher than in the control.

Table 6. Productivity and quality of uterine root crops beetroot

Option	Productivity, t/ha		Average yield by options (Vi), t/ha	Deviation from the overall average (OACi), t/ha	Marketability, %
	2019	2020			
Control - seed treatment with water	23.8	20.6	22.2	- 3.4	90
Seed treatment with Megamix-seeds.	27.2	24.8	26.0	0,4	96
Seedling treatment with the drug Megamix-profi	26.0	25.8	25.8	0.3	95
Treatment of seeds with Megamix-seeds and seedlings with Megamix-profi	29.3	27.5	28.4	2.8	98
Average over the years	26.6	24.7	V Wed 25.6		

The positive value of the general adaptive ability was characteristic for all variants of the experiment with the Megamix preparation. Highest value OACi was in the variant with joint treatment of seeds and seedlings with Megamix-seeds and Megamix-profi preparations and amounted to 2.8 t/ha.

This allows us to conclude that there is a positive interaction between the studied options: the increase in yield from their combined action is much higher than when applied separately.

The quality of root crops as an economic feature is characterized by marketability, that is, the proportion of root crops suitable for sale and storage. The marketability of products is becoming increasingly important in connection with the increasing market requirements.

Evaluating the effect of the use of fertilizers from the Megamix series, one cannot fail to note their influence on quality indicators. Studies have shown that the studied drugs contribute to the improvement of the commodity structure of the crop. Marketability was in the range of 96-98%. When treating seeds and seedlings with Megamix, no root crops with ringing and lignification were observed in the harvest.

The marketability of beet roots is closely related to their shape. Varieties with a rounded shape of root crops have a higher marketable output than those with flat ones. They are characterized by good transportability and keeping quality during long-term storage. Root crops of this type are more convenient for processing in canneries. It is equally important that such root crops are better adapted when planted with special machines for growing seeds in the second year of life.

Thus, the research results show that the use of liquid mineral fertilizer from the Megamix series of preparations is effective, since it helps to increase the productivity and improve the marketability of beet root crops.

CONCLUSIONS

Presowing treatment of table beet seeds with Megamix-seeds contributed to the faster emergence of seedlings. The phase of mass emergence began 3 days earlier than in the control and in the variants where the seedlings were treated with Megamix-pro. In general, the growing season was shorter in the variants with the drug by 4-9 days compared to the control.

In the population of ‘Bordeaux 237’ cultivar, the rounded shape of the root crop, characteristic of the cultivar, prevailed. The largest number of such root crops was in the joint treatment of seeds with the drug Megamix-seeds and seedlings with the drug Megamix-profi and amounted to 84.0%, with 72.0% in the control.

In all variants, an erect rosette of leaves prevailed, but in the variants of treatment with Megamix fertilizer, there were 2.7-6.0% more such plants than in the control.

Average the weight of the root crop varied from 234.0 to 248.4 g. In the variant, with the joint treatment of seeds and seedlings of beets, a tendency was observed to decrease the weight of leaves by 7.2 g. The number of leaves varied from 12 to 15 pieces per plant. Under the influence of Megamix, an increase in leaf length within the range of 15.8-16.0 cm was noted, with 15.0 cm in the control variant. The length of the petiole was in the range of 12.7-14.2 cm, in the control variant - 13.2 cm.

The leaves worked most productively in the variant with joint treatment of seeds and seedlings with Megamix. In this variant, 3.0 grams of the root crop mass was created with one gram of the leaf, with 2.6 grams in the control.

As a result of research, a close relationship has been established between the density of standing of plants and the coefficient of economic efficiency. The highest indicator of Khoz was in the variant with joint treatment of seeds and vegetative plants and amounted to 0.39 g/cm^2, which is 0.04 g/cm^2 higher than in the control. This indicates the formation of a more active photosynthetic apparatus in this variant.

The maximum yield of table beet 28.4 t/ha was obtained by joint treatment of seeds and seedlings with Megamix-seeds and Megamix-profi, which is 6.2 t/ha higher than in the control. Highest value OACi was in the variant with joint treatment of seeds and seedlings with Megamix-seeds and Megamix-profi preparations and amounted to 2.8 t/ha.

The use of liquid mineral fertilizer from the Megamix series of preparations is effective, as it helps to increase productivity and improve the commercial quality of the crop. There is a positive interaction between the studied options: the increase in yield from their combined action is much higher than when applied separately.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF CULTIVAR AND PHYTOSANITARY TREATMENTS ON THE ATTACK OF SPECIFIC PATHOGENES AND TOMATO YIELD IN THE VIDRA AREA, ILFOV COUNTY

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Abstract

The aim of the research was to monitor the influence of the cultivar and the phytosanitary intervention on the attack of tomato pathogens. The 'Vipon' variety and the 'Perfect Peel F1' hybrid were studied. Variants with chemical and biological treatments were used. The best results were obtained in the variant of treatments with Copper Max 50 WP 0.2%, Bravo 500 SC 0.2%, Ortiva Top 0.1%, Melody Compact 49 WG 0.2% and Score 250 SC 0.05% the yield obtained was 6.55kg/sq.m (year 2016) and 7.85 kg/sq.m (year 2017) at 'Perfect Peel F1', and at the 'Vipon' variety the yield was 5.28 kg/sq.m (year 2016) and 6.65 kg/sq.m (year 2017). In the case of organic products, the best results were obtained for the Funres 0.3% treatment variant, the yield was 5.60 kg/sq.m (year 2016), 6.17 kg/sq.m (year 2017) at 'Perfect Peel F1', for the 'Vipon' variety the yield was 4.42 kg/sq.m (year 2016), 5.52 kg/sq.m (year 2017).

***Key words**: pathogens, degree of attack, efficacy.*

INTRODUCTION

Tomatoes are one of the most important horticultural crops in human nutrition and industry. In Romania, tomatoes are grown in field conditions and greenhouse, consumption increasing year by year. There are ongoing concerns in agricultural research for data on cultivated areas and the yield (Popescu et al., 2018; Toth and Cristea, 2018; Ichim et al., 2018).

In 2016 the area cultivated with tomatoes was 41.1 thousand hectares with a yield of 627.1 thousand tons, and in 2017 an area of 40.0 thousand hectares was cultivated with a yield of 679.8 thousand tons (FAO, 2018).

The pathogen *Pseudomonas syringae* pv. *tomato*, is manifested on the foliage of tomato plants by the appearance of brownish-black spots, surrounded by chlorotic zones. On the green fruits, black superficial points appear and on the ripe fruits the points are more prominent and are surrounded by a delayed ripening area (Preston, 2000).

This pathogen was reported in Taiwan and the U.S.A. in 1933, and in Romania was identified by Oprea and Rafailă in 1974 (Velichi, 2012). Timothy P. Denny (1988) studied the phenotypic diversity of the pathogen *Pseudomonas syringae* pv. *tomato*.

The pathogen *Xanthomonas campestris* pv. *vesicatoria* was reported in 1920 by Doidge, the bacterium being frequent especially in the years with abundant precipitation (Marinescu et al., 1986).

Alternariosis is a disease caused by the fungus *Alternaria solani*, which may manifest itself in all phases of plant development, on leaves, stems and fruits (Chaerani and Voorrips, 2006). Studies on the benefits of using fungicides for the control of this pathogen in tomatoes were conducted in New York and New Jersey in 1993 and 1994. Fungicides with the active substance chlorothalonil or mancozeb were applied at 7, 10 or 14 day intervals, to control pathogens *Colletotrichum coccodes* and *Alternaria solani*. Fungicide treatments reduced the severity of foliar diseases, and yield was significantly increased (Dillard et al., 1997). Flaherty et al. (2000), in the tomato crop, performed treatments with Infinito SC and Ridomil Gold MZ 68 WG for the attack produced by *Phytophthora infestans*, and following their application the severity of the disease was significantly reduced, compared to the untreated control.

Botrytis cinerea is a pathogen that causes significant losses to different species of vegetables. The pathogen infects the leaves, stems, flowers and fruits. The disease is manifested especially in tomato crops from protected areas, but also in the field. Outbreaks occur under moderate temperatures and atmospheric humidity >90%, which favours infections and may predispose the host to become susceptible. Important environmental factors that influence the occurrence and evolution of the disease are the high relative humidity in greenhouses and the free water on the foliage of plants. The application of chemical-based fungicides is a measure to slow and to stop the evolution of the attack. Another alternative method of control is the use of biological control products (Elad and Shtienberg, 1995).

Dillard and Cobb (1998), conducted two-year studies to evaluate the long-term survival ability of the pathogen *Colletotrichum coccodes* in the tissues of infected tomato plants and in the soil. In tomato fruits, studies have been carried out of infections and the development of lesions produced by the pathogen (Dillard, 1989).

Initially, the disease settles on the fruits by the appearance of light brown spots. As the disease progresses the lesions increase and become circular and deepened. The sporulation of the fungus are represented by blackish pustules, which form on the surface of the spots. In conditions of high atmospheric humidity they are covered with a mass of pink-orange spores (Dillard and Cobb, 1998).

MATERIALS AND METHODS

The experiments were carried out in 2016 and 2017, in field conditions at R.I.V.F.G Vidra, was organized a bifactorial experience, placed in randomized blocks, with 10 variants and 4 repetitions. Factor A was represented by the cultivar, with two graduations (A1-hybrid Perfect Peel F1 and A2-Vipon variety), and factor B with 5 graduations (B1, B2, B3, B4) representing the variants of phytosanitary treatments for control the pathogens and untreated control (B5).

Table 1. Cultivars and variants of applied treatments (Vidra - 2016; 2017)

No. crt.	Cultivar	Phytosanitary treatments	June	July	August
			Treatments 1.2	Treatments 3.4	Treatments 5.6
1.	A1 Perfect Peel F1	B1	1.Champ 77 WG 0.25% 2.Cabrio Top 0.2%	3.Ortiva Top 0.1% 4.Consento 450 SC 0.2% + Score 250 SC 0.05%	5.Ortiva Top 0.1% 6.Consento 450 SC 0.2%
2.		B2	1. Copper Max 50 WP 0.25% 2. Bravo 500 SC 0.2%	3. Ortiva Top 0.1% 4. Melody Compact 49 WG 0.2% + Score 250 SC 0.05%	5. Ortiva Top 0.1% 6. Melody Compact 49 WG 0.2%
3.		B3	1.2. Funres 0.3%	3.4.Funres 0.3%	5.6.Funres 0.3%
4.		B4	1.2. Citro Seed 0.2%	3.4.Citro Seed 0.2%	5.6.Citro Seed 0.2%
5.		B5	Untreated control		
6.	A2 Vipon	B1	1. Champ 77 WG 0.25% 2. Cabrio Top 0.2%	3.Ortiva Top 0.1% 4.Consento 450 SC 0.2% + Score 250 SC 0.05%	5.Ortiva Top 0.1% 6.Consento 450 SC 0.2%
7.		B2	1. Copper Max 50 WP 0.25% 2. Bravo 500 SC 0.2%	3. Ortiva Top 0.1% 4. Melody Compact 49 WG 0.2% + Score 250 SC 0.05%	5. Ortiva Top 0.1% 6. Melody Compact 49 WG 0.2%
8.		B3	1.2. Funres 0.3%	3.4.Funres 0.3%	5.6.Funres 0.3%
9.		B4	1.2. Citro Seed 0.2%	3.4.Citro Seed 0.2%	5.6.Citro Seed 0.2%
10.		B5	Untreated control		

Table 1 presents the variants of phytosanitary treatments that were used to control the pathogens present in the tomato crop. Were applied 6 treatments, at intervals of 7-14 days, correlated with the climatic factors.

Observations were made on the occurrence and evolution of the attack of pathogens (frequency F% and intensity of attack IA%). Based on the data obtained, the degree of attack (DA%) of the formula (F% xI%)/100 and the effectiveness (E%) of the formula (untreated GA% -treated GA%) x100/untreated GA were calculated.
The yields obtained on variants and repetitions were recorded. The yield data were processed with the ANOVA programm.

RESULTS AND DISCUSSIONS

During the vegetation period, the following pathogens were identified on the leaves and fruits of tomato plants: *Xanthomonas axonopodis* pv. *vesicatoria* (bacterial spot), *Pseudomonas syringae* pv. *tomato* (bacterial speck), *Alternaria solani* (early blight), *Botrytis cinerea* (gray mold), *Colletotrichum coccodes* (anthracnose), *Phytophthora parasitica* (root rot, stem and fruit rot) and *Phytophthora infestans* (late blight) in the two cultivars (Perfect Peel F1 and Vipon).
Analysing the data in the Table 2, it is found that in the case of the Perfect Peel F1 hybrid the degree of attack on the leaves was between 8.8% (*Xanthomonas axonopodis* pv. *vesicatoria*) and 11.7% (*Phytophtora infestans*) in the untreated control variant.
In the Vipon variety, the degree of attack on the leaves was 9.3% (*Xanthomonas axonopodis* pv. *vesicatoria*) and 12.3% (*Phytophtora infestans*) in the untreated control variant.
In the research carried out by Mândru et al. (2018), in tomato crops, under field conditions, the best results in terms of efficiency and yield, were obtained in the variants treated with copper hydroxide products of 50%, chlorothalonil 500 g/l, azoxystrobin 200 g/l + diphenoconazole 125 g/l, iprovalicarb 8.4% + 40% oxychloride, diphenoconazole 250 g/l, mancozeb 80% and mefenoxam 4% + macozeb 64%.

Table 2. Degree of attack (%) of the pathogens and efficacy (%) depending on cultivar and variant of treatments applied (Vidra, 2016)

Variant	*Pseudomonas syringae* pv. *tomato*		*Xanthomonas axonopodis* pv. *vesicatoria*		*Alternaria solani*		*Phytophtora infestans*	
	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)
A1B1	1.1	89.5	0.9	89.8	1.3	88.1	1.5	87.2
A1B2	0.7	93.3	0.6	93.2	1.0	90.8	1.2	89.7
A1B3	2.5	76.2	1.8	79.5	2.6	76.1	3.6	69.2
A1B4	3.0	71.4	2.3	73.9	3.3	61.5	3.0	74.3
A1B5(Ut.)	10.5	-	8.8	-	10.9	-	11.7	-
A2B1	1.4	87.7	1.4	84.9	1.7	86.1	1.9	84.5
A2B2	1.1	90.3	1.0	89.2	1.2	90.2	1.5	87.8
A2B3	2.8	75.4	2.5	73.1	3.0	75.4	4.1	66.7
A2B4	3.4	70.2	3.0	67.7	3.8	68.8	3.6	70.7
A2B5(Ut.)	11.4	-	9.3	-	12.2	-	12.3	-

Chemical control measures can be effective in controlling the pathogen *Phytophthora infestans*. During the research carried out, fungicides were used that may be applied before and after the disease has been installed. Fungicide treatments may be ineffective when the climatic conditions are highly favorable, phenylamide treatments have created a pathogen resistance (Nowicki et al., 2013).
The effectiveness of the treatments used is an important point of view in establishing phytosanitary intervention schemes for crops (Toth and Cristea, 2020; Jaloba et al., 2019; Alexandru et al., 2019; Buzatu et al., 2018; Cristea et al., 2017; Ichim et al., 2017).
Research on the pathogen *Phytophthora infestans* has also been carried out by Amin et al. (2013). They evaluated the efficacy of combating the pathogen with two fungicides Ridomil Gold and Victory 72 WP. Better results in reducing the late blight attack and increasing fruit production were obtained when applying Victory 72 WP fungicide compared to Ridomil Gold fungicide.

Gondal et al. (2012) studied, in five tomato cultivars, the effect of different doses of fungicide with the active substance mancozeb on alternariosis.
Steel (1996) studied the sensitivity of *Botrytis* pathogen to fungicide (iprodione and fludioxonil) treatments, and Bolton (1976) conducted studies on the resistance of the pathogen to fungicides.
The frequency of attack on the fruits (Figure 1) was higher in the case of the untreated control variant for both cultivars. It can be seen that the lowest values were recorded for variants A1B2 (Perfect Peel F1) and A2B2 (Vipon).

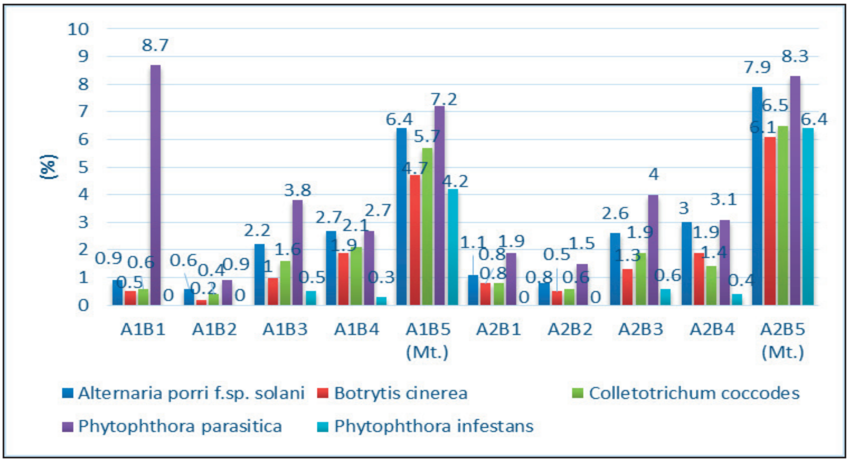

Figure 1. The attack frequency (%) on tomato fruits (Vidra, 2016)

From Table 3, it may be notice that in the B2 variant the highest yield was registered (5.92 kg/sq.m) followed by the B1 variant (5.80 kg/sq.m) for the treatments with chemical products. For the treatments with organic products variant B3 recorded the highest yield (5.01 kg/sq.m), followed by variant B4 (4.65 kg/sq.m), compared to the untreated control variant B5 (3.64 kg/sq.m). The yield differences, obtained in addition to the untreated control variant, are very significant in the case of variants B1 and B2, distinctly significant in the case of the B3 variant and significant in the case of the B4 variant.

Table 3. The influence of phytosanitary treatments on the yield (Vidra, 2016)

Factor B	Yield		The difference from the untreated control (kg/sq.m)	Signification
	kg/sq.m	%		
B1	5.8	159.2	2.16	***
B2	5.92	162.5	2.28	***
B3	5.01	137.6	1.37	**
B4	4.65	127.7	1.01	*
B5(Ut.)	3.64	100	0,00	Ut.

LSD 5%=0.994; LSD 1%=1.334; LSD 0,1%1.759

From the data presented in Table 4, it can be seen that the highest yield was obtained on the Perfect Peel F1 hybrid (5.63 kg/sq.m), compared to the Vipon variety (4.37 kg/sq.m), the difference of yield obtained in addition being significant (1.26 kg/sq.m).

Table 4. The influence of the cultivar on the yield (Vidra, 2016)

Factor A	Yield		The difference between cultivars (kg/sq.m)	Signification
	kg/sq.m	%		
A1	5.63	128.7	1.26	*
A2	4.37	100	-	-
LSD 5%=1.239; LSD 1%=1.657; LSD 0.1%=2.177				

Analysing the data presented in Table 5, we may notice that in the case of the Perfect Peel F1 hybrid, the highest yields were obtained in the variants A1B2 (6.55 kg/sq.m) and A1B1 (6.44 kg/sq.m) in the treatments with chemicals, being followed by the variants of treatments with organic products A1B3 (5.60 kg/sq.m) and A1B4 (5.21 kg/sq.m), compared to the untreated control variant (A1B5), where the yield was 4.35 kg/sq.m. In the Vipon variety, the highest yields were provided by the variants A2B2 (5.28 kg/sq.m) and A2B1 (5.15 kg/sq.m) for the treatments with chemical products, followed by the variants of treatments with organic products A2B3 products (4.42 kg/sq.m) and A2B4 (4.09 kg/sq.m) compared to the untreated control variant (A2B5) where the production was 2.93 kg/sq.m.

The yield differences obtained in addition to the untreated control variant are very significant.

Table 5. The influence of cultivar and phytosanitary treatments applied on yield (Vidra, 2016)

Variant	Yield		Difference from the untreated control (kg/sq.m)	Signification
	kg/sq.m	(%)		
A1B1	6.44	148.0	+2.09	***
A1B2	6.55	150.6	+2.20	***
A1B3	5.60	128.7	+1.25	***
A1B4	5.21	119.8	+0.86	***
A1B5(Ut.)	4.35	100.0	0.00	Ut.
A2B1	5.15	175.8	+2.22	***
A2B2	5.28	180.2	+2.35	***
A2B3	4.42	150.8	+1.49	***
A2B4	4.09	139.4	+1.16	***
A2B5(Ut.)	2.93	100.0	0.00	Ut.
LSD 5%=0.254; LSD 1%=0.342; LSD 0.1%=0.453				

For the second year of the study, in the case of the Perfect Peel F1 hybrid, the value of the degree of attack was 9.8% (*Xanthomonas axonopodis* pv. *vesicatoria*) and 13.3% (*Phytophtora infestans*) at the untreated control (Table 6).

At the same time, the value of the degree of attack, Vipon variety at the untreated control variant (A1B5), was 10.5% (*Xanthomonas axonopodis* pv. *vesicatoria*) and 14.1% (*Phytophtora infestans*). In the experiments performed by Mândru et al. (2017) the degree of leaf attack for *Alternaria solani* was between 1.6 and 2.2% in the treated variants, compared with 16.6% in the untreated control.

The lowest value of the attack degree was recorded in the variant that was treated with the following substances piraclostrobin + metiram, thiophanate methyl and chlorothalonil.

Table 6. Degree of attack (%) of the pathogens and efficacy (%) depending on cultivar and variant of treatments applied (Vidra, 2017)

Variant	*Pseudomonas syringae* pv. *tomato*		*Xanthomonas axonopodis* pv. *vesicatoria*		*Alternaria solani*		*Phytophtora infestans*	
	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)
A1B1	1.5	87.6	1.3	86.7	1.6	87.0	1.9	84.2
A1B2	1.1	90.9	0.9	90.8	1.3	89.5	1.5	88.7
A1B3	2.9	76.0	2.1	78.6	3.0	75.8	4.0	70.0
A1B4	3.5	71.1	2.7	72.4	3.7	70.2	3.5	73.7
A1B5(Ut.)	12.1	-	9.8	-	12.4	-	13.3	-
A2B1	1.8	86.7	1.7	83.8	2.1	84.0	2.3	83.7
A2B2	1.5	88.9	1.3	87.6	1.6	87.8	1.9	86.5
A2B3	3.3	75.6	2.9	72.3	3.5	73.3	4.6	67.4
A2B4	3.9	71.1	3.5	66.7	4.1	68.7	4.0	71.6
A2B5(Ut.)	13.5	-	10.5	-	13.1	-	14.1	-

Among the variants of treatments experimented with chemicals for the control of foliar pathogens *Pseudomonas syringae* pv. *tomato, Xanthomonas axonopodis* pv. *vesicatoria, Alternaria solani* and *Phytophthora infestans* stood out in the hybrid Perfect Peel F1, A1B2 and A1B1 and in the Vipon variety A2B2 and A2B1. And in the case of organic products, A1B3 was noted for the Perfect Peel F1 hybrid and A2B3 for the Vipon A2B2 variety.

The frequency of fruits attacked by *Phytophthora infestans* was reduced (1.5 - 2.1% in the untreated control), in the case of both cultivars. In the variants with treatments A1B1, A1B2, A2B1 and A2B2, the attack produced by *Phytophthora infestans* did not manifest itself even in the second year (Figure 2). Satisfactory protection of the fruits was also ensured by treatments with organic products (Funres and Citro Seed), the frequency of attacked fruits was between 0.3% and 0.8 for the Perfect Peel F1 hybrid and between 0.9% and 0.5% for the Vipon variety for *Phytophthora infestans* (A2B3 and A2B4).

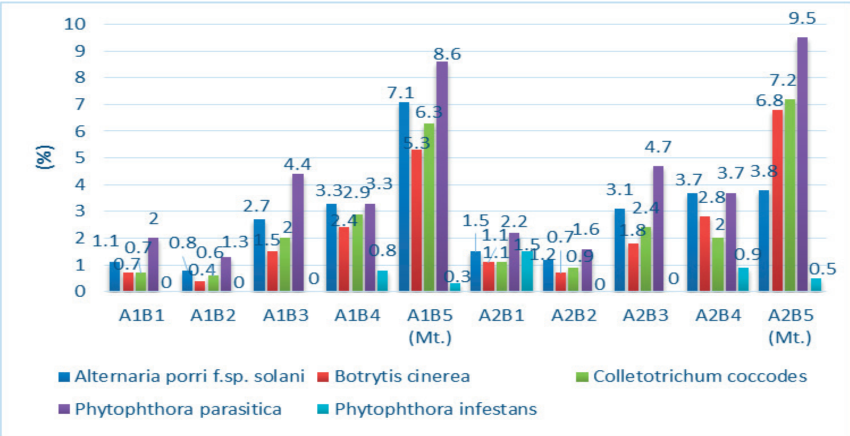

Figure 2. The attack frequency (%) on tomato fruits (Vidra, 2017)

Referring to the influence of the phytosanitary treatments with chemicals on the yield (Table 7) it can be seen that the highest yield (7.35 kg/sq.m) was obtained on the B2 variant followed by the B1 variant (7.20 kg/sq.m).

Table 7. The influence of phytosanitary treatments on production (Vidra, 2017)

Factor B	Yield		The difference from the untreated control (kg/sq.m)	Signification
	kg/sq.m	(%)		
B1	7.2	148.6	2.35	***
B2	7.35	151.7	2.5	***
B3	5.85	120.6	1	*
B4	5.57	114.9	0.72	-
B5(Ut.)	4.85	100	0	Ut.

LSD 5%=0.874; LSD 1%=1,173; LSD 0.1%=1.546

Treatments with biological products Funres (B3) and Citro Seed (B4) ensured a yield of 5.85 kg/sq.m and 5.57 kg/sq.m, respectively. At the same time, the yield obtained in the untreated control variant (B5) was 4.85 kg/sq.m. In the B1 and B2 variants, the yield differences obtained in addition to the untreated control variant are very significant, and in the B3 variant the difference is significant. Analyzing the data presented in Table 8, it is found that the highest yield was registered in the Perfect Peel F1 hybrid (A1=6.69 kg/sq.m). The difference of yield, obtained in addition to the Vipon variety, was insignificant.

Table 8. The influence of the cultivar on the production (Vidra, 2017)

Factor A	Yield		The difference between cultivars(kg/sq.m)	Signification
	kg/sq.m	%		
A1	6.69	118.8	1.06	-
A2	5.63	100	-	-
LSD 5%=1.450; LSD 0.1%=1.940; LSD 0.1%=2.548				

Analysing the data presented in Table 9, it may be notice that, in the case of the Perfect Peel F1 hybrid, the highest yield was obtained at variants A1B2 (7.83 kg/sq.m) and A1B1 (7.75 kg/sq.m) for chemical treatments, and for treatments with organic products variant A1B3 recorded the highest yield (6.17 kg/sq.m), followed by variant A1B4 (6.05 kg/sq.m) compared to the untreated control variant (A1B5) in which the yield was 5.66 kg/sq.m.

Table 9. The influence of cultivar and phytosanitary treatments applied on yield (Vidra, 2017)

Variant	Yield		Difference from the untreated control (kg/sq.m)	Signification
	kg/sq.m	(%)		
A1B1	7.75	136.9	+2.09	***
A1B2	7.83	138.3	+2.17	***
A1B3	6.17	109.0	+0.51	***
A1B4	6.05	106.9	+0.39	**
A1B5(Ut.)	5.66	100.0	0.00	Ut.
A2B1	6.65	165.0	+2.62	***
A2B2	6.87	170.5	+2.84	***
A2B3	5.52	137.0	+1.49	***
A2B4	5.09	126.3	+1.06	***
A2B5(Ut.)	4.03	100.0	0.00	Ut.

LSD 5%=0.224; LSD 1%=0.302; LSD 0.1%=0.400

A similar situation was registered in the Vipon variety, where, in the A2B2 variant, the yield was 6.87 kg/sq.m, followed by the A2B1 variant with 6.65 kg/sq.m.

For the biological products Funres (A2B3) and Citro Seed (A2B4) the registered yield was 5.52 kg/sq.m. and 5.09 kg/sq.m, respectively compared to 4.03 kg/sq.m. in the untreated control variant (A2B5).

The yield differences obtained in addition to these variants, compared to the untreated control variant, are very significant, only at A1B4 distinctly significant.

CONCLUSIONS

The pathogens detected in the tomato crop during the experiment period influenced the obtained yield.

The most effective for both cultivars were those with chemicals: B1: T1. Champ 77 WG 0.25%; T2.Cabrio Top 0.2%; T3. Ortive Top 0.1%; T4. Consento 450 SC 0.2% + Score 250 SC 0.05%; T5. Ortive Top 0.1%; T6. Consento 450 SC 0.2%; B2: T1. Copper Max 50 WP 0.25%; T2. Bravo 500 SC 0.2%; T3. Ortiva Top 0.1%; T4. Melody Compact 49 WG 0.2% + Score 250 SC 0.05%; T5. Ortiva Top 0.1%; T6. Melody Compact 49 WG 0.2%.

The biological products Funres variant B3 and Citro Seed variant B4 provided satisfactory protection against the attack of pathogens that attack the leaves and fruits of tomato plants for the both cultivars.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GENETIC-BREEDING VALUE OF THE TOMATO FORMS CARRYING THE *β* (CAROTENE) AND *R* (YELLOW FLESH) GENES

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Abstract

One of the main problems of modern vegetable growing is the creation of varieties with complex valuable traits, adapted to growing conditions and economic efficiency. The forms containing the β (carotene) genes and r (yellow flesh), which play an important role in the diet of allergenic people, especially children, are of particular importance for improving red tomatoes. To demonstrate the variability of agronomic characters and to elucidate the value of tomato genotypes carrying β (carotene), r (yellow pulp) genes, a comparative assessment of tomato genotypes was made taking into account a set of useful traits. The evaluation of genotypes was carried out based on the most valuable biological parameters (period of vegetation, number of fruits and productivity per plant, fruit mass, thickness of pericarp, heat resistance). Genotypes combining characters of early ripeness with high productivity were identified in the tomato collection. Analysis of tomato genotypes for heat resistance made it possible to reveal highly resistant genotypes that are of interest as an initial material for breeding.

Key words: *tomato, breeding, early ripeness, resistance, heat.*

INTRODUCTION

Drought and high temperatures in last decades of growing season became factors which significantly limited growth, reproduction and productivity of crop plants including tomatoes in the Republic of Moldova (Mihnea, 2016; Mihnea, 2018).

The optimum temperature for growing tomatoes is between 25-30^{0}C during the day and 20^{0}C at night (Camejo et al., 2005; Ribeiro et al., 2008; Carvalho et al., 2011). Temperatures above 35^{0}C affect seed germination, vegetative growth, flowering, fruit binding and ripening (Thomas & Prasad, 2003; Wahid et al., 2007). High temperatures can cause significant losses in fruit productivity and quality (Stevens & Rudich, 1978; Firon et al., 2006; Wahid et al., 2007; Pervez et al., 2009; Nahar & Ullah, 2012).

The yield and quality of tomato fruits depend both on the optimal conditions for plant growth and on the use of varieties with proper genetic characteristics. This indicator is a decisive factor for innovative progress in agriculture and ensures high quality and quantity of production including organoleptic properties (Seymour et al., 2002; Ercolano et al., 2008; Carli et al., 2011; Mihnea et al., 2016). The knowledge about the variability of characters which is determined not only by genotype but also by environmental factors has the crucial importance. The degree of variability of characters indicates the peculiarities of the reaction norm of the genotype under various environmental conditions (Haydar et al., 2007; Mohamed, Ali & Mohamed, 2012). The information about the variation of the character determined by the variability of the genotype indicates the possibility of changing the parameter in the necessary direction at this stage of selection. Establishing the specificity of variability and heritability of characters gives the breeder the opportunity to optimize the breeding program (Fasoylas, 1973).

For the efficient use of the tomato gene pool by both researchers and producers, it is necessary to create a special collection of tomatoes with identified genes. Within each collection, it is necessary to select genotypes with the most valuable characters for the selective improvement of the species genes.

Diversification of tomato germplasm, supplementation of new genotypes carrying *β* (carotene) and *r* (yellow flesh) genes in red tomatoes play an important role for the diet of persons with an allergy, especially children.

The aim of our study was to evaluate the collection of tomatoes carrying the *β* (carotene) and *r* (yellow pulp) genes according to a set of useful traits (early ripeness, yield, fruit size and quality, resistance to high temperatures) and select the most valuable forms for further breeding.

MATERIALS AND METHODS

As research material, 22 genotypes of tomatoes were used, 15 of all analyzed varieties (Rufina, Charovnita, Rosinca, Viking, Gold Nugget, Golden Jubilee, Timisorean landrace, MilOrang, Luci, Alex, Flacara, Chihlimbar, Hurma, Breeding Line, Mia) have the *β* (carotene) gene and 7 varieties (Dolgonosic, Buyan yellow, Oranjevie sosulki, Moldavian landrace, L 10B, Vrojainii, De-barao yellow) carry the *r* (yellow flesh) gene. The experiments were carried out in laboratory and field conditions, in the experimental field of the Institute of Genetics, Physiology and Plant Protection (Chisinau, Moldova). Resistance to high temperatures was studied under laboratory conditions. The analysis of the variability of the resistance was made based on the length of the embryonic root, stem and seedling.

The following regimes were used to analyze the influence of high temperatures on the seedling: A = 38^0C; B = 40^0C; C = 42^0C. The exposure was 6 hours. Thus, a differentiated background was created for the selection of forms resistant to high temperatures. Methodological recommendations were used to assess resistance of tomato genotypes to high temperatures (Ivachin, 1979) on the base of the capacity of embryonic roots to grow after maintaining them at high temperatures within 6 hours.

Cluster analysis was made by creating dendrograms on the base of agglomerative-iterative algorithm (Ward method) and the k-means method (Savary, 2010). Four clusters were programmed within the k-means method.

Tomatoes were grown by seedling cultivation in three repetitions according to the standard method (Ersova, 1978). Under field conditions, the morphological description was made according to the UPOV descriptor (2011). Seedlings were planted in the field in the third decade of May. The data obtained were statistically processed using the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

As a result of evaluating the forms of tomatoes by precocity, a rather high variability of the growing season and interphase periods was revealed depending on both the genotype and climatic conditions (Figure 1). Variability of the interphase period from mass appearance of seedlings to the beginning of flowering was within the ranges of 63-78 days. It was shown that climatic conditions significantly influenced the first interphase period. This is due to low temperatures and cold nights in the spring of 2020, which led to a later flowering of some varieties. The varieties Golden Jubilee, Timisoranean local population, Chihlimbar, Mia, De-barao yellow showed a later flowering. Ranges of the period from flowering to fruit ripening were within 39-64 days. A shorter period was registered in the varieties Dolgonosic, Mia, Gold Nugget, Luci.

The tested genotypes formed 4 groups of precocity: early - 106-110 days (5, 9, 10, 12 16), medium early - 111-115 days (1, 3, 4, 8, 13, 18, 19, 21, 22), late - 116-120 days (14, 15, 20), very late > 120 days (2, 6, 7, 11, 17) (Figure 1).

As a result of the evaluating the forms on the base of characteristics of the fruit, it was found a rather high variability (Table 1). In studied groups, the highest coefficient of variation has the fruit mass, the average being 31.2%, and the thickness of the pericarp - 21.0%. The data showed a wide range of variability in fruit length and width, mesocarp thickness and the number of locules. Average levels of parameters were of 12.8%, 14, 6%, 18.1% and 18,9 %. Thus, the medium variability of these analyzed characters in the studied groups was demonstrated.

When constructing dendograms, genotypes were divided into 2 clearly separated branches based on the classification of the following indicators: the mass of the fruit, the length and width of the fruit, the thickness of the pericarp, the thickness of the mesocarp, the number of locules. The highest similarity was recorded for varieties 3, 21, 11, 22, 19 and 1, 6, 8, 12, 13 (Figure 2).

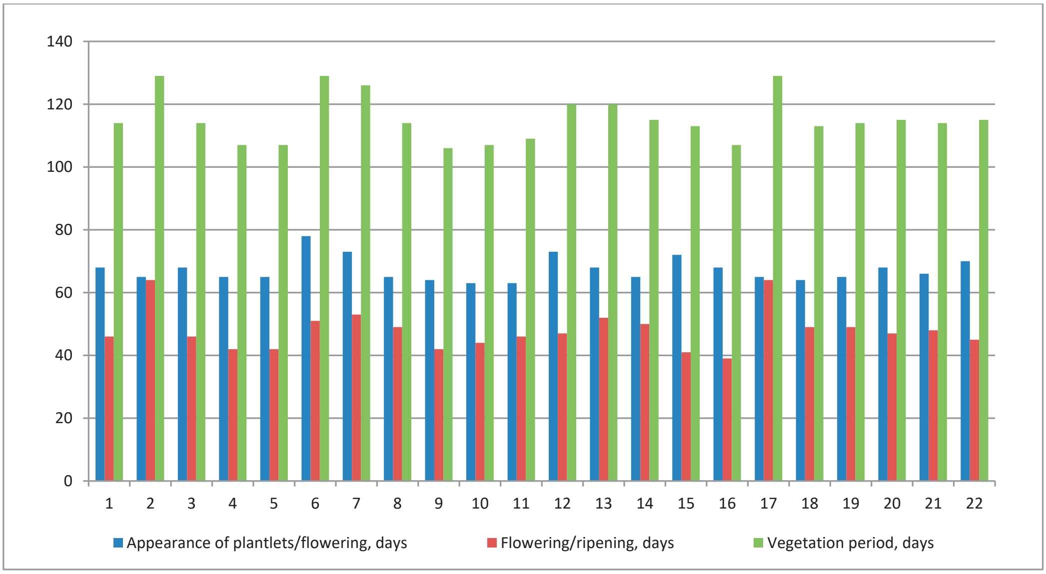


Figure 1. Phenotypic variability of interphase periods in tomato
1 – Rufina, 2 – Charovnita, 3 – Rosinca, 4 – Viking, 5 – Gold Nugget, 6 – Golden Jubilee, 7 – Timisorean landrace, 8 – MilOrang, 9 – Luci, 10 – Alex, 11 – Flacara, 12 – Chihlimbar, 13 – Hurma, 14 – Breeding Line, 15 – Mia, 16 – Dolgonosic, 17 – Buyan yellow, 18 – Oranjevie sosulki, 19 – Moldavian landrace, 20 – L 10B (Buzau), 21 – Vrojainii, 22 – De-barao yellow

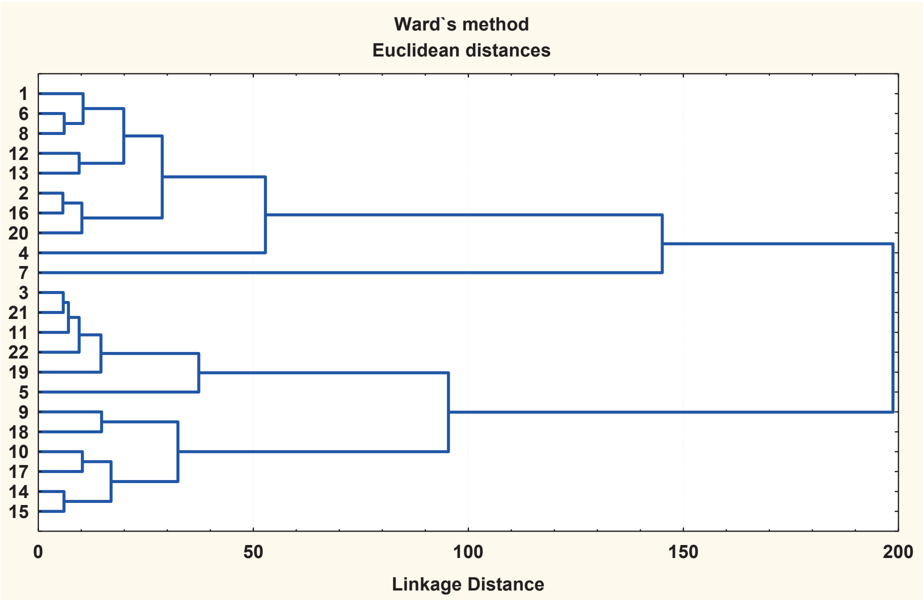


Figure 2. Cluster analysis of tomato varieties based on some fruit characteristics.
1 – Rufina, 2 – Charovnita, 3 – Rosinca, 4 – Viking, 5 – Gold Nugget, 6 – Golden Jubilee, 7 – Timisorean landrace, 8 – MilOrang, 9 – Luci, 10 – Alex, 11 – Flacara, 12 – Chihlimbar, 13 – Hurma, 14 – Breeding Line, 15 – Mia, 16 – Dolgonosic, 17 – Buyan yellow, 18 – Oranjevie sosulki, 19 – Moldavian landrace, 20 – L 10B (Buzau), 21 – Vrojainii, 22 – De-barao yellow

Table 1. Phenotypic variability of some fruit characteristics in tomato varieties

No.	Fruit mass, g		Fruit length, mm		Fruit diameter, mm		Pericarp thickness, mm		Mesocarp thickness, mm		Number of locules	
	$x \pm m_x$	V,%	$x \pm m_x$	V,%	$x \pm m_x$	V,%	$x \pm m_x$	V,%	$x \pm m_x$	V,%	$x \pm m_x$	V,%
1	65.2±3.37	23.2	43.8±0.95	9.8	50.8±1.28	11.3	6.6±0.31	21.2	36.4±1.20	14.8	3.9±0.22	25.6
2	54.6±3.28	26.9	48.3±2.95	27.3	45.3±1.47	14.6	5.0±0.15	12.8	30.0±1.30	19.4	2.9±0.13	20.7
3	36.0±2.29	28.3	35.2±0.86	10.9	39.2±0.86	8.5	3.6±0.20	25.0	26.4±0.65	11.1	3.1±0.15	22.6
4	87.4±8.0	39.9	41.8±0.88	9.1	60.3±2.29	16.6	3.5±0.19	24.0	46.1±1.85	17.6	6.6±0.21	13.6
5	14.4±0.94	29.2	29.1±0.62	9.6	27.8±0.64	10.4	3.2±0.15	21.9	15.2±0.77	22.7	2.2±0.09	18.7
6	56.3±10.39	52.2	37.8±0.94	7.1	48.4±4.43	26.0	3.9±0.30	21.3	35.5±2.74	22.0	5.3±0.25	13.2
7	146.5±7.18	17.7	50.3±2.87	20.6	63.5±3.93	22.4	6.4±0.30	16.1	49.5±3.10	22.7	5.5±0.40	27.3
8	58.7±3.71	28.2	42.9±1.14	11.9	49.0±1.75	16.0	4.7±0.15	15.0	35.1±0.71	9.11	3.6±0.11	13.9
9	40.4±1.81	22.3	66.7±1.36	9.1	33.6±0.61	8.1	5.4±0.21	17.4	19.4±0.70	16.3	3.0±0.09	13.3
10	48.2±2.05	19.1	49.5±0.97	8.7	42.9±0.91	9.6	3.8±0.19	21.9	24.1±1.92	35.7	2.9±0.14	22.1
11	33.7±2.28	30.3	39.3±0.93	10.7	38.1±0.90	10.5	4.7±0.19	19.1	23.2±0.92	17.8	2.7±0.11	18.5
12	55.1±4.52	29.6	49.8±3.20	23.1	51.6±3.23	22.7	5.2±0.34	23.6	41.3±2.32	20.3	4.4±0.18	15.9
13	58.1±6.84	52.5	45.4±2.03	4.6	49.0±1.88	17.1	3.9±0.20	23.3	48.3±1.75	15.8	6.6±0.41	18.2
14	38.9±3.42	39.3	52.3±1.65	14.1	35.8±1.18	14.8	7.2±0.44	27.8	21.8±0.82	16.5	2.3±0.11	21.7
15	35.9±1.17	14.8	56.1±1.87	15.0	33.5±0.55	7.5	5.7±0.24	19.3	19.7±0.69	15.8	2.4±0.11	20.8
16	57.4±2.23	17.4	53.2±1.00	8.5	44.8±0.51	5.1	5.6±0.21	16.8	29.7±1.34	20.2	2.5±0.11	20.4
17	46.2±6.23	48.7	54.1±3.37	22.4	34.5±3.02	31.6	3.7±0.36	35.4	23.5±1.19	11.5	4.8±0.23	17.3
18	51.5±4.87	42.3	74.3±2.56	15.4	36.5±1.61	19.8	6.0±0.31	23.3	24.5±1.06	18.8	2.0±0.00	0,00
19	26.4±2.00	34.1	34.3±0.84	11.1	36.5±0.96	11.8	6.1±0.42	31.1	22.1±0.86	17.6	2.5±0.11	20.4
20	52.8±4.60	39.0	42.5±1.46	15.4	44.1±1.57	15.9	5.4±0.28	22.8	28.7±1.27	19.9	3.2±0.18	25.3
21	41.2±2.33	25.2	37.4±0.67	8.0	41.5±1.16	12.5	5.8±0.14	10.9	29.5±1.01	15.3	2.7±0.12	21.4
22	33.0±1.88	25.2	38.6±0.85	9.8	37.2±0.77	9.2	5.0±0.13	12.0	24.3±0.89	16.5	2.4±0.13	25.0
Average, V, %		31.2±2.36		12.8±1.24		14.6±1.41		21.0±1.27		18.1±1.13		18.9± 1.26

Cluster analysis (k-means method) demonstrated that the interclusterian variance was much higher than the intraclusterian one for such characters as the fruit mass, fruit height and diameter and mesocarp thickness (Table 2). Thus, studied genotypes showed pronounced differences in this case. In contrary, the interclusterian variance was lower than the intraclusterian one for the pericarp thickness and the number of locules. However, the differences between genotypes based on these characters were insignificant.

Table 2. Analysis of the inter- and intraclusterian variance of some fruit characteristics

Character	Interclusterian variance	df	Intraclusterian variance	df	F	p
Fruit mass	12728.55	3	1524.626	18	50.09182	0.000000
Fruit length	1652.21	3	706.271	18	14.03611	0.000058
Fruit diameter	1290.79	3	365.569	18	21.18544	0.000004
Pericarp thickness	2.82	3	23.602	18	0.71613	0.555132
Mesocarp thickness	1465.69	3	542.697	18	16.20453	0.000024
Number of locules	17.26	3	24.160	18	4.28608	0.018961

Cluster analysis by the k-means method revealed that the groups of genotypes, separated into 4 clusters, differed according to the level and variability of the studied characters (Figure 3, Table 3).

By classifying the genotypes based on the 6 characters, it was found that cluster 1 included 9: Rufina, Charovnita, Viking, Golden Jubilee, MilOrang, Chihlimbar, Hurma, Dolgonosic, L 10B. Cluster 2 included the Timisorean landrace with the highest values of characters.

Cluster 3 included Rosinca, Gold Nugget, Flacara, Moldavian landrace, Vrojainii, De-barao yellow. Cluster 4 included Luci, Alex, Breeding Line, Mia, Buyan yellow, Oranjevie sosulki. Pericarp thickness and locules number were factors with lower discriminant capacity in classifying cluster genotypes.

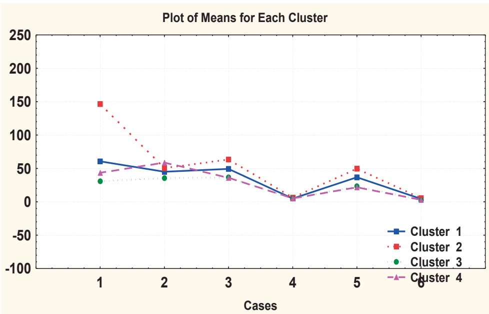


Figure 3. Ability to differentiate clusters (k-means method) using the characteristics of tomato fruit. Horizontal: 1. Fruit mass; 2. Fruit length; 3. Fruit diameter; 4. Pericarp thickness; 5. Mesocarp thickness. 6. Number of locules. Vertical: 1, 2, 3, 4 - clusters of tomato genotypes

Table 3. Descriptive analysis of clusters

Cluster	Character	x	Genotype
1	Fruit mass	60.61	1 – Rufina, 2 – Charovnita, 4 – Viking, 6 – Golden Jubilee, 8 – MilOrang, 12 – Chihlimbar, 13 – Hurma, 16 – Dolgonosic, 20 – L 10B.
	Fruit length	45.06	
	Fruit diameter	49.26	
	Pericarp thickness	4.87	
	Mesocarp thickness	36.79	
	Number of locules	4.33	
2	Fruit mass	146.50	7 – Timisorean landrace
	Fruit length	50.30	
	Fruit diameter	63.50	
	Pericarp thickness	6.40	
	Mesocarp thickness	49.50	
	Number of locules	5.50	
3	Fruit mass	30.78	3 – Rosinca, 5 - Gold Nugget, 11 – Flacara, 19 – Moldavian landrace, 21 – Vrojainîi, 22 – De-barao yellow
	Fruit length	35.65	
	Fruit diameter	36.73	
	Pericarp thickness	4.78	
	Mesocarp thickness	23.45	
	Number of locules	2.60	
Cluster	Character	x	Genotype
4	Fruit mass	43.52	9 – Luci, 10 – Alex, 14 – Breeding Line, 15 – Mia, 17 – Buyan yellow, 18 – Oranjevie sosulki
	Fruit length	58.83	
	Fruit diameter	36.13	
	Pericarp thickness	5.27	
	Mesocarp thickness	21.67	
	Number of locules	2.90	

The number of fruits per plant and the productivity per plant were evaluated in 15 varieties. The results are shown in Fig.4. The number of fruits per plant in the studied group varied within 8-28. More than 20 fruits were registered in the varieties Rufina, Dolgonisic, Local form (Moldova), Flacara (Figure 4A). The productivity per plant was: 0.186 kg to 0.91 kg. In Figure 4 it can be observed that the productivity of the studied varietie was rather low, because soil-climatic conditions of the year were quite harsh for the cultivation of tomatoes without irrigation.

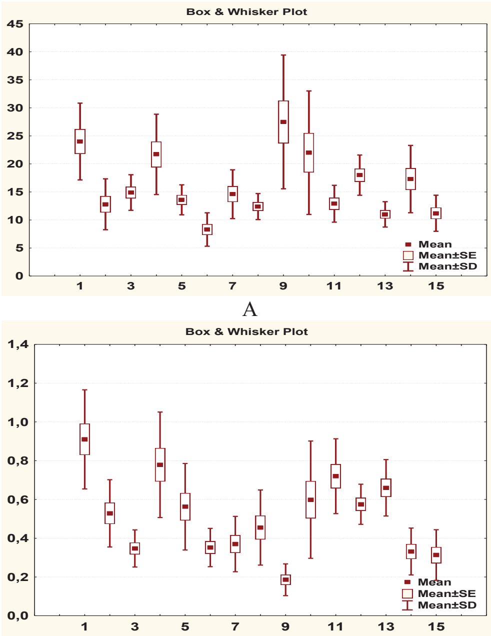
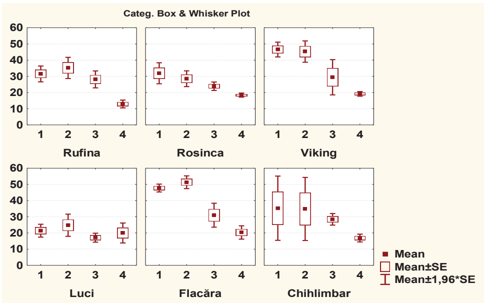


Figure 4. Variability of productivity traits (A - number of fruits per plant, B - productivity per plant, kg.) in tomatoes:
1 – Rufina, 2 – Charovnita, 3 – Dolgonisic, 4 – Rosinca, 5 – Viking, 6 – MilOrang, 7 – Luci, 8 – Alex, 9 – Moldavian landrace, 10 – Flacara, 11 – L 10B (Buzau), 12 – Vrojainii, 13 – Chihlimbar, 14 – De-barao yellow, 15 – Mia

The varieties manifesting a complex of economically valuable characters were tested at 4 temperatures: 25°C - optimal; 38^0C, 40^0C, and 42^0C - stressful. It was found that the root length varied within 26.2-44.8 mm under optimal conditions, while at 38^0C - 24.8-51.3 mm (Fig. 5A). The degree of growth inhibition in the varieties Rosinca, Viking, Amber was -0.7; -2.6; -11.4, respectively (compared to optimal conditions). Stimulation was registered in Rufina (11.7%), Luci (11.5%), and Flacara (7.3%). In the case of the temperature of 40^0C, the degree of inhibition of the embryonic root growth varied in the ranges of -10.8...-36.8%. There was no stimulation in any of the varieties. A relatively low inhibition was found in the varieties Rufina, Luci and Amber. This indicates the pronounced genetic determination of the root respons to stressful temperatures. The significant inhibition of the root growth was observed in all studied varieties under the temperature of 42^0C, with the exception of the variety Luci. In Luci this parameter decreased by 6.5%. The degree of growth inhibition compared to the control was: 59.0% (Rufina); 58.7% (Viking); 57.5% (Flame); 52.1% (Chihlimbar); 42.3% (Rosinca).
The length of the stem in the control variant varied in the ranges of 16.6-25.9 mm (Figure 5B). Under stressful temperatures, the genotypes showed a differentiated response and high variability of this trait, within 10.5-16.5 mm. The temperature of 40^0C significantly inhibited the growth of the stem in 5 genotypes compared to the optimal temperature. It is especially visible in the varieties Viking, Rufina and Flacara, in which the decrease of this parameter was -51.4%, -39.3%, and -39.0%, respectively. The stressful temperature of 42^0C inhibited the growth of stem in all studied varieties. Inhibition ranged from -12.3% to -58.6% compared to control. The greatest influence of temperature on the length of the stem was observed in the Viking (58.6%) and Rufina (-57.1%) varieties.
It was found that the temperature of 38^0C had a stimulating effect on seedling growth in the varieties Luci (+ 12.1%), Rufina (+ 7.6%) and Flacara (+ 3.8%) (Fig. 4C). In the other three varieties, inhibition of the character was insignificant: -2.7%, -5.4%, and -5.5%. The temperature of 40^0C determined a significant decrease in seedling length only in the Viking (-42.1%) and Flacara (-36.6%) varieties. The temperature of 42^0C decreased the length in the varieties Viking (-57.4%), Rufina (-56.6%), Flacara (-50.2%) and Rosinca (-39.3%). Therefore, maintaining germinated tomato seeds for 6 hours at 42^0C is the most effective treatment for differentiating genotypes by the level of resistance to thermal stress.
Based on the assessment of tomato genotypes by three test parameters, it can be concluded that the varieties Luci and Chihlimbar have shown complex resistance to thermal stress.

The statistical processing of the experimental data by the bifactorial analysis of the variance allowed the appreciation of the variability and the degree of influence of the temperature, genotype and their interaction on the variability of the evaluated characters (Table 4).
It was found that the contribution of genotype, temperature and genotype × temperature interaction to the growth of the embryonic root of tomato was 23.3%; 70.5%; 4.0%, to the stem growth 18.1%; 76.4%; 3.4%, and to the seedling growth 20.2%; 74,7%; 3.2%, respectively (Table 4). So, the most contributed factor in the growth of the root, stem and seedling is the temperature (70.5%, 76.4% and 74.7%).



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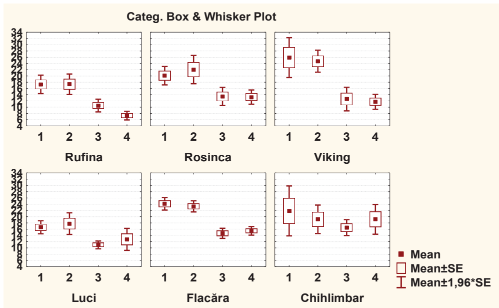

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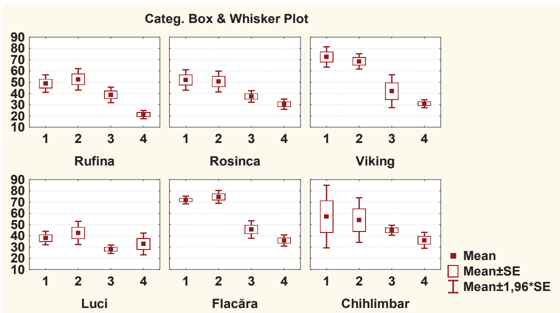

C

Figure 5. Influence of temperature on the length of the root (A), stem (B) and seedling (C) of tomatoes.
Horizontal: 1 = control (25°C); 2 = 38^{0}C; 3 = 40^{0}C; 4 = 42^{0}C

Table 4. Bifactorial analysis of tomato genotype x temperature relationships

Source of variation	Degree of freedom	Root length		Stem length		Seedling length	
		Mean sum of squares	Contribution in the source of variation, %	Mean sum of squares	Contribution in the source of variation, %	Mean sum of squares	Contribution in the source of variation, %
Tomato genotype	5	463.08*	23.3	83.20*	18.1	811.3*	20.2
Temperature	3	1399.40*	70.5	351.90*	76.4	3007.5*	74.7
Tomato genotype x temperature	15	79.26	4.0	15.90	3.4	127.6	3.2
Random effects	48	43.99	2.2	9.68	2.1	78.2	1.9

*- p<0.05.

CONCLUSIONS

The tomato varieties carrying the *β* (carotene) and *r* (yellow flesh) genes differ on the base of morpho-biological characters, precocity, fruit characteristics, productivity.
It was found using cluster analysis (Ward method) that studied varieties differ in

similarity of assessed characters such as fruit mass, fruit length, fruit height, pericarp thickness, mesocarp thickness, locule number. The highest similarity was recorded for the varieties Rosinca, Vrojainii, Flacara, De-barao yellow, Moldavian landrace

Cluster analysis (k-means method) of the varieties showed that the interclusterian variance was much higher than the intraclusterian one for the fruit mass, fruit height and diameter, and mesocarp thickness. It means that studied genotypes have clearly pronounced differences. The interclusterian variance was lower than the intraclusterian one for the characteristics of the pericarp thickness and the number of locules. However, in this case the difference between genotypes was insignificant.

It was found that the response of tomato plants (root, stem, and seedling growth) to stressful temperatures was different and depended on the growing organ, genotype and the temperature.

As a result of the bifactorial analysis, it was found that the contribution of the temperature in the variability of tomato growing organs is much higher than the contribution of the genotype.

The varieties Luci and Chihlimbar have manifested a low sensitivity to high temperatures and are therefore of interest in breeding as possible genetic sources of heat resistance.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOIL QUALITY ASSESSMENT BASED ON C:N RATIO IN AN ALLUVIAL SOIL TREATED WITH MICROBIAL INOCULANTS

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Abstract

Carbon and nitrogen are two of the most important elements found in soil structure and the relationship between them has a special relevance on soil characterization. This relationship is known as carbon-nitrogen ratio (C/N ratio) and indicates the rate of decomposition of organic matter. Degradation of organic matter will certainly modify the C/N ratio, and this modification is related to the existing microorganisms in soil. The present study follows the evolution of the C:N ratio in a calcaric alluvial soil from Buzau county used for organic tomato cultivation and subjected to a microbial treatment based on Beauveria bassiana inoculants. The experimental scheme includes three variants: (1) untreated soil, uncultivated (control); (2) untreated soil, cultivated with Florina 44 tomato variety; (3) soil treated with microbial inoculants, cultivated with Florina 44 tomato variety. Each variant was observed on two different lots and in two moments (autumn 2019 and autumn 2020). The soil samples were taken from topsoil, dried at room temperature, and analysed using the CHNS elemental analyser for C and N determinations. Regarding the C:N ratio, the results pointed out that there are some differences that correlate with the use of microbial inoculants in tested variants.

Key words: *C:N ratio, alluvial soils, microbial inoculants, CHNS elemental analysis, organic system*

INTRODUCTION

Lately, the idea of sustainability became the way forward for more and more farmers due to increasing demand for organic agricultural products. To deal with this challenge, the farmers must follow practices that help maintain a balance in natural systems. These practices cannot guarantee zero risk products but can minimize other problems such as air, water, and soil pollution (Klonsky, 2000).
The long-term soil organic management practices affect microorganisms and microbiological processes and change the quality and quantity of plant wastes, which are the main source of soil organic matter (Micuți et al., 2020). Between organic farming practices, the use of microbial inoculant for crop production gains more and more attention.
Although the first microbial inoculant was developed more than one hundred years ago, the true progress of this type of input was achieved in the last decades, regarding both production and the use of it (Santos et al., 2019). Microbial inoculants are natural products, and they are used for pest control, for quality improvement of soils or crops, and they can help reduce chemical fertilizer applications. Microbial inoculants could include bacteria, fungi, and algae (Alori & Babalola, 2018). There are a small number of studies that have investigated the use of microorganisms for enhancing the soil fertility. Application of entomopathogenic fungi was tested with many species as *Metarhizium anisopliae sensu lato, Beauveria bassiana, Isaria farinosa, Isaria fumosorosea, Aspergillus flavus and Lecanicillium lecanii* by Jaworska, in 1979 and 1981 and the studies about these fungal species were initiated continuously. Many studies focused on pest and disease management using fungias an alternative to chemical inputs in the organic agricultural systems (Sicuia et al., 2014; Canhilal, 2016). Also, the addition of

these fungal endophytes in composting materials could improve the decomposing process resulting in materials that have better properties such as high nutrient content, high population of beneficial microbes and low toxicity (Nchu, 2020). Elson et al. (1998) studied various effects of carbon concentrations, C:N ratios, and amino acids to *Helminthosporium solani* growth on different culture mediums. Similar work has been conducted by Gao et al. (2007), when the effects of several fungi were observed on carbon concentration and C:N ratio. The ratio of total organic carbon and total nitrogen indicates the rate of decomposition of organic matter. Recently, the amount of soil nitrogen and C:N ratio are used as indicators of soil carbon sequestration (Edu et al., 2012). Carbon is the main energyproducing element, while nitrogen helps building cell tissues. A C:N ratio of 20 indicates that there are 20 grams of C for each 1 gram of N. When organic matter decomposes, the carbon content decreases faster than nitrogen, reducing the C:N ratio (Miller, 2000). When the C: N ratio gets lower, nitrogen rapidly will be released into the soil for instantaneous crop use. When an organic substrate has a C:N ratio lower than 15, rapid mineralization takes place, with release of N, which is available for plant uptake. A C:N ratio bigger than 35 results in nitrogen immobilization. A ratio of 20-30 consists in equilibrium between mineralization and immobilization (Figure 1) (Brust, 2019).

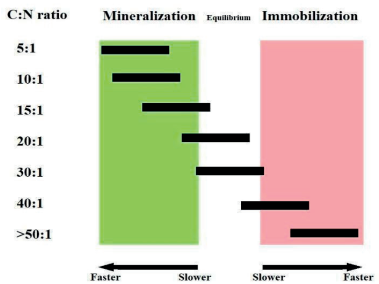

Figure 1. Nitrogen availability by C:N ratio variations. (After Brust, 2019)

Mineralization and immobilization are two soil processes related to nitrogen cycle; during mineralizationnitrogen is released for plant uptake while during immobilization the microbes utilize and tie up nitrogen.

During microbial degradation, the organic matter is used as a source of carbon by the soil microbial populations to fuel considerable population growth. These native microbial populations represent a natural antagonist to fungi, and they are one of the most active factors in determining the persistence of fungi in these environments (Jaronski, 2010). In an experiment about soil application of *Beauveria bassiana*, Swiergiel et al. (2016) noticed that the fungal density was maintained above the upper natural background level for about one season but came back to normal background levels within a year. A special attention must be paid to pesticide management, especially to fungicide use, because these treatments can influence or even stop the effects of *B. bassiana* soil inoculant (Soares et al., 2017).

This study follows the evolution of the C:N ratio of the soil when a microbial inoculant is used in a tomato crop. The results are compared with two control variants: the soil untreated with no crop and the soil untreated but cultivated with the same tomato variety.

MATERIALS AND METHODS

Experimental field

The experiment was conducted between 2019 and 2020 at the Vegetable Research and Development Station Buzău (S.C.D.L Buzău).

The study aims to determine the modification of soil C:N ratio after using a microbial inoculant based on *Beauveria bassiana* (‘Bbmi’) fungus, on a tomato crop. As biological material, tomato Florina 44 variety was used. The soil was characterized in 2018 as a calcaric alluvial soil, founded on fluvial deposits, on a meadow region, with the groundwater lower than 3 m (Musat et al., 2018). Experimental design consists in two different plots, each one holding three variants: V1- untreated soil, uncultivated (as control); V2 - untreated soil, tomato crop; V3 -‘Bbmi’ treatment, tomato crop. The experimental scheme is represented in Table 1.

The soil samples were taken in autumn 2019 and in autumn 2020, to find out if Bbmi treatment will keep his effects over time. The soil samples were collected from the surface layer of the experimental fields (20 cm), of the three variants (Figure 2).

Figure 2. Aspects from the experimental fields.
V1 - uncultivated lot; V2 - untreated soil, tomato crop lot; V3 - Bbmi treatment, tomato crop lot

Analysis of C and N content

The analyses were performed at the Laboratory of Agrochemistry, of the Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

All soil samples were air dried, ground with the laboratory soil grinder and sieved through a sieve of 250 microns. The samples were kept in a dry environment until analysed.

Table 1. Experimental scheme

Plot	Variant	Sample collection time
Plot A	V1 untreated soil, uncultivated	October 2019
	V2 untreated soil, tomato crop	October 2019
	V3 Bbmi* treatment, tomato crop	October 2019
	V1 untreated soil, uncultivated	September 2020
	V2 untreated soil, tomato crop	September 2020
	V3 Bbmi treatment, tomato crop	September 2020
Plot B	V1 untreated soil, uncultivated	September 2020
	V2 untreated soil, tomato crop	September 2020
	V3 Bbmi treatment, tomato crop	September 2020

*Bbmi - microbial inoculants based on *Beauveria bassiana*.

An amount of 5-10 mg of soil sample was used to determine the total nitrogen and carbon content. The analysis was performed using the CHNS elemental analyzer (EuroVector EA3100 Elemental Analyzer). Cystine was used as standard reference material. All determinations were performed in three repetitions.

Statistical analysis

The obtained values were processed using IBM SPSS (Version 27.0) statistical software. Duncan test (Alpha = 0.05) was used for multiple comparison between the three variants used in the experiment.

RESULTS AND DISCUSSIONS

Taking the plots each one separately, the effects of applied inoculants compared with the variants without microbial treatments differ from one year to another.

On plot A in 2019, the C:N ratio on untreated and uncultivated soil (V1) was 11.02. On cultivated soil, untreated (V2) and on cultivated and treated soil (V3) the C:N ratio was significantly higher, 11.83 and 12.00, respectively (Figure 3). This change may occur due to tomato crop which may influence the soil microbiology or due to nitrogen uptake by the plants which led to a decrease of nitrogen in soil. Even so, the modification of C:N ratio must be much higher to have a visible effect on the plants. At this value, the nitrogen content is adequate and there is no risk of immobilization of this element.

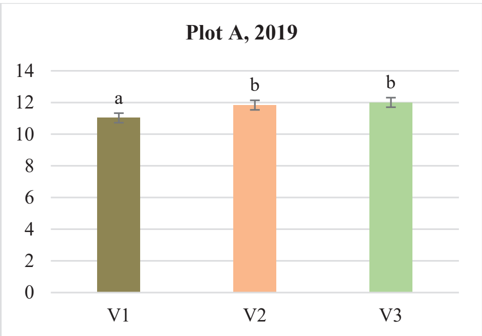

Figure 3. C:N ratio between V1, V2, V3 variants on Plot A in 2019

On the same plot (Plot A), in 2020, a significant increase of C:N ratio is observed on all variants, compared with the previous year. (Figure 4).

The modification of this indicator, even on the control variant may be explained by either the loss of nitrogen on this area or the increase of carbon content. It is also possible that the lower outside temperature at the first sample collection (October 2019) influenced the microbial activity compared to the second sample collection (September 2020), leading to this increase on C:N ratio. The influence of temperature on C:N ratio was studied by Kai et al. (1969), leading to the same response, that at lower temperatures, the nitrogen immobilisation is higher, resulting in a reduction of C:N ratio.

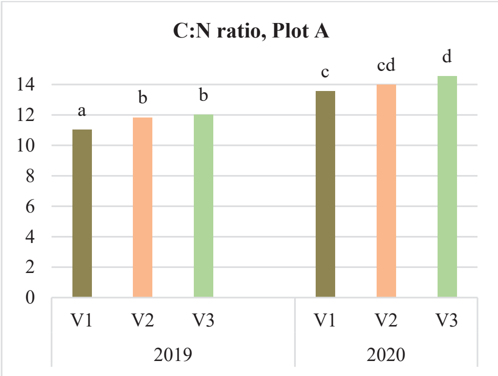

Figure 4. The evolution of C:N ratio over a year (2019 and 2020), on the same plot

Also, regarding the difference between variants on 2020 samples, it can be noticed that the MI treated variant (V3) had a significant increase of C:N ratio compared to untreated and uncultivated variant (V1) (Figure 5). This also may be due to soil treatment with microbial inoculation. The increase of this indicator, even far to the optimal level, can be beneficial for the crops on this treated soil.

Figure 5. C:N ratio between V1, V2, V3 variants on Plot A in 2020

Considering the plot B, no difference was observed on C:N ratio in any variant, for soil samples collected in the same year as the inoculation (Figure 5).

Comparing the two plots studied in 2020, the only significant difference was observed on

Bbmi treated variant on the plot A, where the treatment was 2 years old.

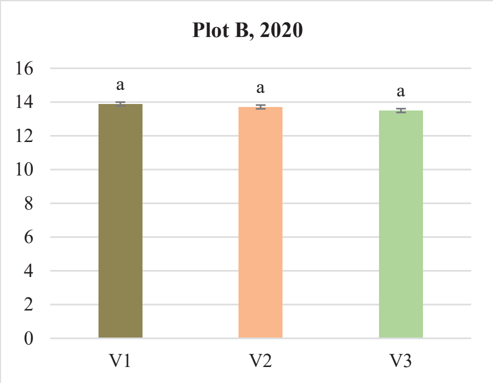

Figure 6. C:N ratio between V1, V2, V3 variants on Plot B in 2020

The rest of the variants did not relate any noticeable differences. To be mentioned that the samples from plot B, even though they were taken in the same year as the Bbmi treatment, have the C:N ratio closer to samples from plot A taken in 2020, two years after inoculation (Figure 7). This similarity may come from the fact that the samples were taken in the same month (September).

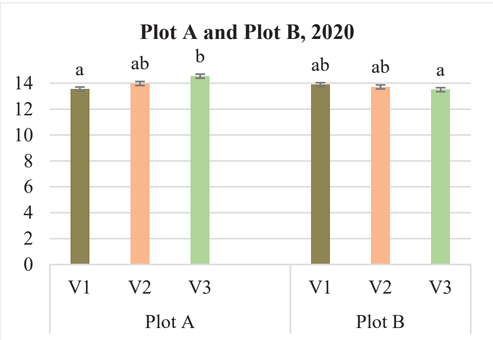

Figure 7. Difference between Plot A (treated in 2019) and Plot B (treated in 2020)

CONCLUSIONS

The treatment with microbial inoculants based on *Beauveria bassiana* fungus did not provide significant changes of C:N ratio in the same year of inoculations. As other study reported, this indicator has a rather limited variation over time and sometimes it takes years to notice a significant change (Cabral, 2012).

Anyway, the treatments may provide other beneficial effects, such as pest and disease reduction, but these aspects were not relevant for this paper, as no soil pests were impacting the tomato crop in the 2019-2020 period or in nutrient cycling, plant growth, nutrient uptake, aspects that will be considered in further studies, on medium term.

For the treatment performed in 2019, a significant increase of C:N ratio was observed in the treated variant compared with the untreated and uncultivated variant, one year after inoculation.

The modification of C:N ratio did not reach an optimal level in the studied period, but still the small increase over just one year indicates that on medium and long term the use of microbial inoculant on organic fields could improve the productive potential of agroecosystem.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0301/ 28PCCDI, within PNCDI III.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PHENOTYPIC EXPRESSIVENESS OF NEW *SIDERITIS SCARDICA* GENOTYPES OBTAINED AT VEGETABLE RESEARCH AND DEVELOPMENT STATION BUZĂU

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Abstract

Numerous species have been acclimatized and bred at VRDS Buzau, among which special results have recently been obtained for Sideritis scardica, also known as Mursalski chai. After completing the acclimatization stage, the valuable genotypes were subjected to intensive breeding works, obtaining six genotypes with distinct phenotypic expressiveness. During the vegetation period, biometric, phenological and biochemical observations were performed, applying the same crop technology for all studied genotypes. The results showed visible differences in terms of phenotypic expressiveness of the main characters. Regarding the weight of the inflorescence and the number of inflorescence/plant, G1 ranks first with an average value of floral mass of 790 g/plant, and G5 has the highest leaf mass value with an average value of 1198 g/plant, while G2 has the most intense fragrance on all vegetative aerial parts. All six genotypes showed genetic stability and uniformity in descent. In 2019, G1 genotype was patented and registered in the Official Catalog of Crop plant varieties from Romania under the name 'Domnesc' cultivar and used in this study as control variant.

Key words*: acclimatization, breeding, honey plants, inflorescence, Mursalski Chai.*

INTRODUCTION

Sideritis scardica is a medicinal and honey plant that belongs to the *Lamiaceae* family. Native of southeastern Europe, also called Mursalski Chai after Mount Mursalița in Bulgaria, it has been known since antiquity as Ironwort or Iron Grass, because it heales wounds caused by iron weapons. At the same time it was used for treating from simple colds to severe pulmonary, gastrointestinal, anemia disorders. In recent decades, *Sideritis scardica* has been extensively investigated for its content of phenolic compounds, terpenoids, hydrocarbons and related compounds, and essential oil composition (Kostandinova et al., 2007; Tadić et al., 2012). It has been suggested that phenolic and/or polyphenolic compounds are responsible for the antioxidant activity of the alcohol extracts, while terpene compounds from the essential oil and diterpenoids are responsible for the anti-inflammatory, analgesic, antiulcer, antibacterial, antifungal, cytotoxic, antitumor activities (Todorova and Trendafilova, 2014) and new studies made in rats suggested that it also fights against Alzheimer's disease (Hofrichter et al., 2016).

Sideritis is a species with a great diversity, its variability being expressed by many names, varieties, forms and names of varieties. It is a perennial, herbaceous, bluish-white plant with abundant hairs, which grows up to a height of 50 cm in the areas of origin, on mountain plateaus. The plant's root has a predominantly horizontal development. The opposite, obovate leaves are covered by small hairs with a double role: to protect the plant against extreme conditions in the alpine areas and to secrete a specific aroma, due to the volatile oils. Comparative studies of the volatile oils of *Sideritis scardica* Griseb from Macedonia, Albania and Greece, from dried and fresh plants, showed that the characteristic smell, for which Mursalita tea or Mountain Tea is so appreciated, is due to various components, the best known being: monoterpene, myrtenal, hexadecanoic acid, myristicin, menthol, limonene (Stefkov G. et al., 2014). *Sideritis* plants have four distinct stamens, similar to most species in the *Lamiaceae* family, and the clearest identifying feature is the characteristic smell of the leaves (Solomou A., 2019).

Perennial plant with a short lifespan (5-6 years on average), it reproduces by seeds. It is found in open, dry, grassy and stony meadows, on calcareous soil or eroded surfaces, but also in rocky areas, on slopes with southern exposure, at high temperatures and in dry conditions, in belts of subalpine and alpine vegetation, at altitudes of 1300-2100 m (Strid et al., 1991; Petrova et al., 2009, Bulgarian Academy of Sciences and the Ministry of Environment and Waters, 2011). There are no statistics available on the worldwide production of *Sideritis scardica*. In a working group study on medicinal and aromatic plants made in 2004, it was noticed that in Albania, Bulgaria, Greece, Macedonia, Serbia and Turkey, *S. scardica* is grown only on small areas in individual households, for personal use or for distribution in markets, health stores or drugstores. Due to its healing properties and the small quantities available, Mursalita is increasingly sought after, representing a business opportunity.

It has gained attention in recent years due to its medicinal properties. In order to maintain its healing properties, it is recommended that the cultivating area and the pedo-climatic conditions be similar to those of the natural habitat. The yield and lifespan of the culture largely depend on its care. The demand for *Sideritis* has increased because it is natural, ecological and recognized as a safe product, with high economic value (Aneva I., 2012). Therefore, for farmers in the areas of origin, it can provide added value, it can be grown in poorer areas or on degraded slopes in semi-arid areas, hence the need to expand studies to report additional information on the aromatic and medicinal importance of *Sideritis scardica*. Also, its cultivation can be used as a tool for biodiversity conservation, with beneficial effects for the environment. (Solomou A. et al., 2019). In Romania, the plant began to be studied in 2012 by the Genetics and Breeding Laboratory within V.R.D.S. Buzau, which showed a special interest in this species and studied varieties from *Sideritis syriaca*, *S. cyprea* and *S. congesta*. This is where it was introduced into the study a variety from the Bulgarian Mountains, considered authentic and highly valued for its many uses: food, medicinal, aromatic, ornamental, variety approved under the name of 'Domnesc' (Vînătoru C. et al., 2019).

MATERIALS AND METHODS

V.R.D.S. Buzau founded a Mursalski chai culture in 2015 and several genotypes with distinct phenotypes, valuable features and implicitly with high economic potential were noted in the culture. The Genetic, Breeding and Biodiversity Laboratory set out to study five of the new varieties. The Domnesc variety was a control variety and it was numbered G1, and the other five genotypes were numbered G2, G3, G4, G5 and G6.

The breeding method used was the negative mass selection for the 'Domnesc' variety, and for the other genotypes G2-G6, the repeated individual selection (an elite plant was used).

Compared to other vegetable plants, the growth rate in both young and mature plants is slightly slower.

The care works were classic: maintaining the soil humidity, destroying the weeds through mechanical and manual plows, loosening the soil and carefully avoiding bringing soil on the stem. In the case of irrigation, excess moisture has been avoided, which leads to wilting of the plants, as the very pubescent leaves easily adhere to the soil surface and can no longer come off, therefore rotting easily. During the vegetation period, phenological and biometric measurements and determinations were performed in the experimental field within the Biodiversity Improvement and The Genetic, Breeding and Biodiversity Laboratory, V.R.D.S. Buzau. Thus, the electronic balance Pioneer, Model PA413CM was used to determine the weight of the foliar and floral masses. Sensory and olfactory analyzes were also performed.

RESULTS AND DISCUSSIONS

Next, there will be presented the results of the study performed on the six genotypes of *Sideritis scardica*, obtained at VRDS Buzau: Genotype G1, which is also the control genotypes, or the 'Domnesc' variety, and the other five genotypes from G2 to G6, as follows: **Control genotype - G1**: perennial, herbaceous plant, with strongly developed root horizontally, strong appearance, rich foliage, and large number of stems with intense green to silver leaves. A characteristic of the variety is the high percentage (20%) of the branched floral stems compared to the other genotypes that have very small percentages of branched stems. The variety has large inflorescences with four edges, well outlined, compact, with light green vertices; the size of the bush is characteristic for this variety, with an average diameter of 91 cm and average height of 43cm, dimensions that give the bush a rich, strongly developed appearance, with large inflorescences that have an average length of 15 cm (Figure 1).

Figure 1. Control genotype G1 or 'Domnesc' variety

Genotype G2: it is also called 'Ossa', after the Greek mountain town it comes from; it is characterized by a very strong aroma with pleasant slightly mentholated accents, being the most fragrant genotype; the appearance is smaller both in diameter and height compared to G1 (71 cm and 32 cm, respectively - average values), with inflorescences with dark green, silvery, arranged stellate and spaced vertices, and the spike is not dense but sparse and airy; another noteworthy feature of the G2 genotype is that branched stems with 3 or more branches predominate (Figure 2).

Figure 2. Genotype G2 or 'Ossa' variety

Genotype G3: it is characterized by a stuffed appearance, with short inflorescences, with a small number of compact vertices rounded towards the top, which give the plant a blunt, unfinished look. The bush has medium dimensions, with weak foliage, rarely arranged leaves, being the genotype with the most spaced leaves; also to be noted is the total lack of branched stems, the plants having only solitary stems. The plant's fragrance is medium to intense, with early flowering. In the first week of June, the plants are fully flowered, the bushes are practically covered by bees (Figure 3).

Figure 3. Genotype G3

Genotype G4: distinguished by the large expanse of the bush, without excelling in height; thus, the average value of the diameter is 101 cm, with an average height of only 50cm; the solitary stems predominate and the branched ones are in small numbers, on average 16/bush. It has long stems with rich foliage, the largest number of leaves per stem (10); long, sharp leaves, large inflorescences (average value of 16 cm), with star-shaped, very sharp and light green vertices (Figure 4).

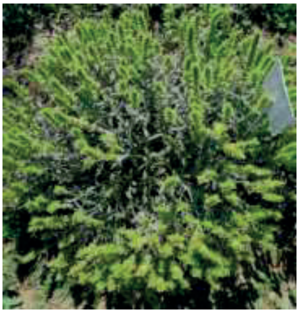

Figure 4. Genotype G4

Genotype G5: the plant has medium size and is characterized by: poor foliage, with small and narrow leaves, inflorescences colored in honey yellow; it has the largest inflorescences (average value of 13 cm) and very pubescent, spaced vertices. The spike is flexible, semi-erect, due to its very long length. The aroma is medium to weak (Figure 5).

Figure 5. Genotype G5

Genotype G6: it has a pleasant appearance, with spherical, globular bushes, dark green

Figure 6. Genotype G6

foliage, predominating solitary stems; vertices are intense green and the inflorescences are regular, truncated in size, compact, with a wider base and sharp towards the top, which gives the bush a spectacular appearance; this is the most beautiful of the 5 genotypes studied. It should be noted that the strong aroma is given by the yellow flowers, the bushes being covered almost entirely by bees during the flowering period, in mid-June (Figure 6).

Next, for an even more relevant presentation of the 5 genotypes and the control variety, in Table 1 below, are found the main quantitative characteristics of Mursalski bushes, namely the height and diameter of the bush, the length and width of the leaves, the distance between leaves/stem, number of leaves/stem, length of the stem and inflorescences, number of vertices/inflorescence and diameter of inflorescences.

Table 1. Calculation of average values, standard deviation and coefficient of variatio§n of quantitative traits

Variable	G1	G2	G3	G4	G5	G6	cv%
Plant height (cm)	43.2 ±9	32.20 ±9	40.60 ±6.06	50.30 ±8,5	53.10 ±7.7	39.5 ±4.40	17.25
Bush diameter (cm)	91.1 ±8.7	71.05 ±9.35	96.30 ±3.35	101.2 ±1.8	84.39 ±5.89	66.20 ±1.80	6.05
Leaf length (cm)	7.67 ±2.87	4.20 ±1.71	6.10 ±0.92	5.43 ±0.76	5.67 ±2.51	6.20 ±2.05	30.68
Leaf width (cm)	1.47 ±0.21	0.60 ±0.1	1.03 ±0.05	1.13 ±0.31	0.97 ±0.12	1.57 ±0.15	13.88
Distance between leaves/stem (cm)	7.13 ±1.00	5.00 ±0.50	10.00 ±0.93	7.33 ±1.53	7.37 ±1.15	7.6± 0.87	13.46
Number of leaves/stem	8±2	14.67 ±7.02	6.17 ±0.12	10.00 ±2	8±2	6±2	28.65
Stem length) (cm)	38 ±9.85	31.67 ±10.1	32.33 ±1.53	41.00 ±3	31.00 ±7.55	19.17 ±8.61	21.04
Inflorescence length (cm)	15±4	5.87 ±3.59	9±3.61	16.00 ±1	13.00 ±7.94	10.67 ±3.01	33.29
Vertices number/inflorescence	19.67 ±1.15	7.33 ±4.04	7.33 ±1.53	11.33 ±4.04	11.67 ±6.03	16.00 ±5.57	30.49
Inflorescence diameter (cm)	0.9 ± 0.1	1±0.1	1.8± 0.1	0.9 ± 0.1	1.7 ± 0.1	1.70 ±0.1	7.50

The measurements show significant differences between the six genotypes studied. The highest plant height was registered at genotype G5 - 53.1 cm and the lowest at genotype G2 - 32.2 cm. The largest diameter was measured at genotype G4 - 101.2 cm and the smallest at genotype G6 - 66.2 cm. Regarding the length and width of the leaf, the smallest values are found at genotype G2 - length 4.2 cm, width 0.6cm, and the highest values at genotype G1 - length 7.67 cm and genotype G6 - width 1.57 cm. Regarding the length of the stem and the inflorescence, genotype G1 presents the highest values - 38.9 cm (stem) and 15 cm (inflorescence) respectively, and the smallest values were registered at genotype G2 - 5.87 cm stem length and 5.87 cm inflorescence length. The smallest number of vertices in the inflorescences can be found at genotype G2 and G3, namely 7, and genotype G1 has the highest value - 19, while the smallest diameters of the inflorescences are found at the genotypes G1 and G4 - 0.9 cm; genotype G3 has the largest diameter - 1.9 cm. Regarding the coefficient of variability (cv%) it can be assessed that high values of the coefficient of variability were registered for the following quantitative characteristics: inflorescence length 33.29%, leaf length 30.68%, number of vertices in the inflorescence 30.49%. The lowest values of the coefficient of variability were obtained at the diameter of the bush - 6.05%, the diameter of the inflorescences - 7.50% and the distance between leaves/stem - 13.46%.

Next, several series of quantitative determinations were performed on the six distinct genotypes of *Sideritis scardica* to assess productivity, such as: number of solitary and branched stems, number of solitary and branched inflorescences, related percentage calculation.

As it can be seen in Figure 7, genotype G3 has only solitary stems, and the highest number of solitary inflorescences is 280 compared to the other genotypes.

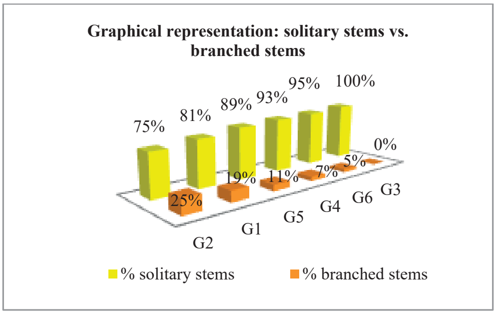

Figure 7. Graphic representation of solitary and branched stems

Genotype G2 has the lowest number of solitary stems - 143, with a percentage of 74.48%. Regarding the branched stems, we find the highest value in genotype G2, with 147 inflorescences, and the highest percentage 25.52%, while genotype G3, as we mentioned, does not have solitary stems. Next, the comparative analysis of the number of solitary and branched stems was transposed graphically.

The six distinct genotypes were subjected to quantitative measurements in order to determine the foliar and floral masses. These observations were performed both in the experimental field, to determine the number of stems / plant, and in the laboratory. Thus, based on the weight determinations of the stems (average value) and the number of stems / plant, the leaf mass/plant was calculated. More specifically, the highest leaf mass was found at genotype G5 - 1198.40 g/plant, and the lowest value was found at genotype G4 - only 505.55 g/plant. These values are represented in Figure 8.

Next, the quantitative measurements were performed in order to determine the average floral mass for each genotype. Therefore, genotype G1 is the most productive, with a weight of the floral mass of 790 g/plant, and genotype G2 is the least productive, with 278.4 g/plant.

For a better understanding of the quantitative characteristics, the values of the foliar and floral mass determined for each genotype are represented in Figure 8 below.

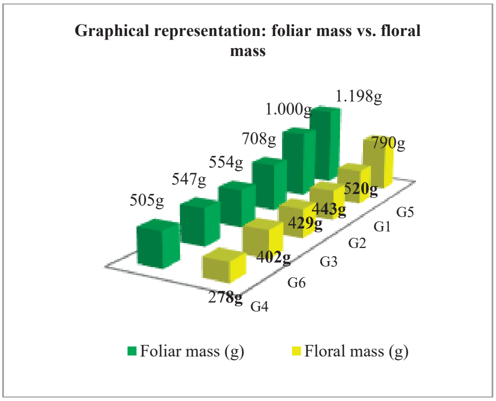

Figure 8. Graphical representation of foliar and floral mass for G1-G6

A relevant feature for *Sideritis scardica* is the fragrance released by leaves and flowers due to the presence of volatile oils. Therefore, the qualitative assessment was performed by carefully examining the fragrance of each genotype, the analysis was olfactory and was performed personally. At the same time, this evaluation was compared with the presence of bees on the bushes at the time of flowering (Figure 9). It was found that the most aromatic genotype with menthol and citrus accents is genotype G2, also called ‘Ossa’. This is, in fact, the genotype with the most pubescent foliage; on the leaves there are glandular brushes that secrete volatile oils. It is followed by genotypes G6 and G3 with intense flavors. Genotype G1 has medium intensity and geotypes G5 and G4 have weak flavors. 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 9. Bee on an inflorescence of *Sideritis scardica*

CONCLUSIONS

The first conclusion is that we managed at V.R.D.S. Buzau to successfully acclimatize the *Sideritis scardica* species approved under the name of ‘Domnesc’ variety, genotype G1 in this case. The researches were finalized achieving six different *Sideritis scardica* varieties, each one having distinct phenotypic expressions.

G1 - ‘Domnesc’ variety (control genotype), has a strong appearance of the bush, vigor, and the largest floral mass – 790 g/plant due to large inflorescences and the largest number of vertices - 19.

G2 - also called ‘Ossa’, is the genotype with the strongest fragrance and the most pronounced foliar hairiness, although it has the smallest floral mass - 278.40 g and the smallest inflorescences and leaves. It is also the genotype that stands out with the highest percentage of branched stems - 25%.

G3 - this genotype with medium floral and foliar masses has an intense fragrance and is the only genotype with 100% solitary stems.

G4 - genotype with a special appearance, the largest diameter of the bush - 101 cm, large inflorescences, and light fragrance.

G5 - this genotype has the largest leaf mass, 1198.4 g/plant and the largest inflorescences - 16 cm. The fragrance is light.

G6 - this genotype has a strong fragrance and a spectacular appearance of the bush, with dark green leaves and large truncated cone shaped inflorescences, deep yellow flowers and light green vertices.

Consequently, as a result of the valuable characteristics they present, all five genotypes studied will be the subject of intensive breeding works, some of which will be proposed for approval, as these genotypes can form new cultivars.

It is also necessary to emphasize the importance of honey plant features, due to the fact the flowering in stages for a long time offers bees an abundance of nectar and pollen, at a time when other honey plants are in decline.

Lately it is more and more sought after and can be a business opportunity, both in terms of selling seeds and selling tea, leaves and flowers. V.R.D.S. Buzau sells both seeds and seedlings of *Sideritis scardica*, the ‘Domnesc’ variety (genotype G1), thus making them available to farmers and amateur horticulturists, who set up crops or use them in arranging private gardens.

ACKNOWLEDGEMENTS

The work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE N, P AND K CONTENT IN PUMPKIN'S LEAVES IS INFLUENCED BY HERBICIDE STRESS AND BIOSTIMULANT APPLICATION

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Abstract

The aim of the study conducted from 2017 to 2019 is to evaluate the influence of the herbicide stress caused by the herbicide imazamox and biostimulatory treatment (preventive and curative) to the leaf N, P, and K content of pumpkins before the flowering stage. In the trial, the pumpkin variety 'Mathilda' F1 was grown. The experiment included 12 treatments. Number 1 was untreated weed-free control. Treatment 2 was Pulsar® 40 (40 g/l imazamox) applied at a rate of 1.00 l ha^{-1}. The other treatments (from 3 to 7) represented the application of the mentioned herbicide in tank mixture with biostimulant. The treatments from 8 to 12 showed the performance of therapeutic biostimulant application 7 days after the herbicide spraying. The herbicide was applied in BBCH 12-13 of pumpkins. The biostimulant products evaluated were: Shigeki®; Amino Expert® Impuls; Lactofol® O; Aminozol®; Terra-Sorb® Complex. The highest leaf N content for the untreated control was found. No influence of the treatments on the P content in the leaves was observed. The treatments that received the highest herbicide stress had increased leaf K levels.

Key words: *herbicide stress, biostimulants, pumpkins, NPK in leaves*

INTRODUCTION

Presence of weed infestation is one of the most limiting factors leading to rapid yield decrease. There is a large number of authors working on the weed control in the different crops (Yanev, 2020; Manilov and Zhalnov, 2018; Goranovska and Yanev, 2016; Kostadinova et al., 2016; Mitkov et al., 2016; Tityanov et al., 2016; Yanev, 2015; Yanev et al., 2014a; Týr and Vereš, 2012; Tonev et al., 2010a; Tonev et al., 2010b; Tonev et al., 2009a; Tonev et al., 2009b; Changsaluk et al., 2007; Masqood et al., 1999; Plew et al., 1994). In the fields of the late spring crops as pumpkins mainly late-spring weeds are developing.

The most distributed broadleaf weed species are common amaranth (*Amaranthus retroflehus* L.), wild mustard (*Sinapis arvensis* L.), fat-hen (*Chenopodium album* L.), wild hemp (*Cannabis ruderalis* L.), common cocklebur (*Xanthium strumarium* L.), creeping thistle (*Cirsium arvense* L.), etc.

The most distributed grass weed species are yellow foxtail (*Setaria* spp.), barnyard grass (*Echinochloa crus-galli* L.), johnson grass (*Sorghum halepense* L. (Pers.)) (Tonev et al., 2007).

The chemical control is one of the most commonly used weed control methods in crops. The proper herbicide choice is one of the most important and responsible parts of crop management. The proper herbicide must meet a number of requirements. It should be selective for the crop, highly effective against the weeds, its application rates should not lead to the accumulation of residues in plant production and in soil, it should not deteriorate the quality of production and it should be harmless to microorganisms in soil, as well as for the environment (Yanev et al., 2014b; Hristeva et al., 2014; Hristeva et al., 2015; Kalinova and Yanev, 2015; Semerdjieva et al., 2015; Yanev and Kalinova, 2020). Pumpkins are sensitive to herbicides and the chemical weed control is quite limited at that crop (Tonev et al., 2007).

When herbicide detoxification is not effective enough, various functional impairments may occur. A selective herbicide destroys or retards the growth of weeds, while causing little or no injury to crop species (Carvalho et al., 2009).

Herbicide phytotoxicity is most often chronic, but in some cases it can parish the crop. The extent of damage can be assessed visually (if visible) or by various physiological and biochemical indicators (Dayan et al., 2015;

Dayan and Zaccaro, 2012). Ability to recover the herbicide-damaged plants depends on the degree of the occurred structural-functional impairment. A number of studies have shown that chronic herbicide phytotoxicity can be overcome (to some extent or completely) by application of biostimulants, foliar fertilizers, growth regulators, herbicide antidotes, etc. (Jablonkai, 2013).

There are a great number of registered herbicides for grass weed control in pumpkins, but almost none for broadleaf weeds elimination.

The herbicide imazamox (that controls a wide spectrum of broadleaf as well as grass weeds) can be applied in pumpkins, but the application of the product Pulsar 40 (40 g/l imazamox) in rate of 1.00 l ha^{-1} caused visual phytotoxic symptoms to the pumpkins in the study. Also, the application of the herbicide imazamox with the biostimulant Amino Expert Impuls in tank mixture showed a protective effect to the plants (Neshev et al., 2020).

There is limited information for the effect of the herbicide stress on the plant's nutrient status. Zaidi et al. (2005) found that plant's N content lowered with increasing the herbicide dose.

The aim of the current research is to evaluate the influence of the herbicide stress caused by the herbicide imazamox and biostimulant treatment (preventive and medicative) to the leaf N, P, and K content of pumpkins before flowering stage.

MATERIALS AND METHODS

The experiment was situated in the experimental field of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 3 replications.

Studied products and evaluations:

Herbicide product: Pulsar® 40 (40 g/l imazamox). It is registered for Clearfield Technology at sunflower. This technology offers the farmers new and effective solution for weed control (Pfenning et al., 2008). Imazamox is applied as a spray when the sunflower plants are at 4-10 leaves stage (Kamburoglu et al., 2019).

Products with biostimulant mode of action:

- Shigeki® - Extract of algae (*Ascophyllum nodosum*) - 15%; Macronutrients: P_2O_5 - 7%; K_2O - 10%; Microelements: Fe-EDTA - 0.25%; Mn-EDTA - 0.17%; Zn-EDTA - 0.20%; Cu-EDTA - 0.10%; B - 0.20%; Mo - 0.04%.
- Amino Expert® Impuls - Amino acids - 5.00% (free amino acids - 4.43%); Macroelements: N - 2.53%; MgO - 0.50%; SO3 - 4.02%; Phytohormones - 0.0003%, Organic substances and natural adhesives: 73.96%; Microelements: B - 0.52; Cu - 0.39%; Fe - 0.38%; Mn - 0.38%; Mo - 0.08%; Zn - 0.78%.
- Lactofol® O (Macroelements: N - 21% = NO_3 - 7%, NH_4 - 4% and amide - 10%); P_2O_5 –-5%; K_2O - 10%; SO_3 - 0.6%; Microelements: B - 0.02%; Cu - 0.014%; Fe - 0.025%; Mn - 0.018%; Mo - 0.002%; Zn - 0.01%.
- Aminozol® - Macroelements: N - 9.4%; K_2O - 1.1; S - 0.25%; Na - 1.28 %; 66.3% organic substances obtained from animal sub products 3rd category EG (VO) 1069/2009; protein hydrolysates.
- Terra-Sorb® Complex - Macroelemets: N - 5.5%; MgO - 0.8%; Microelements: B - 1.5%; Fe - 1.0%; Mn - 0.1%; Zn - 0.1%: Mo - 0.001%; Free amino acids - 20%.

The study included the following treatments:

1. Untreated weed free control;
2. Pulsar® 40 1.00 l ha^{-1};
3. Pulsar® 40 1.00 l ha^{-1} + Shigeki® 3.00 l ha^{-1};
4. Pulsar® 40 1.00 l ha^{-1} + Amino Expert® Impuls 3.00 l ha^{-1};
5. Pulsar® 40 1.00 l ha^{-1} + Lactofol® O 6.00 l ha^{-1};
6. Pulsar® 40 1.00 l ha^{-1} + Aminozol® 3.00 l ha^{-1};
7. Pulsar® 40 100 ml da^{-1} + Terra-Sorb® Complex 3.00 l ha^{-1};
8. Pulsar® 40 1.00 l ha^{-1} + Shigeki® 3.00 l ha^{-1};
9. Pulsar® 40 1.00 l ha^{-1} + Amino Expert® Impuls 3.00 l ha^{-1};
10. Pulsar® 40 1.00 l ha^{-1} + Lactofol® O 6.00 l ha^{-1};
11. Pulsar® 40 1.00 l ha^{-1} + Aminozol® 3.00 l ha^{-1};
12. Pulsar® 40 1.00 l ha^{-1} + Terra-Sorb® Complex 3.00 l ha^{-1}.

Variant 2 was treated with imazamox only in BBCH 12-13 and did not receive preventive or curative biostimulant application.

At variants from 3 to 7 the treatment was accomplished in BBCH 12-13, but the plants

were sprayed with tank mixture of the herbicide and the different biostimulants – preventive approach.
At variants from 8 to 12, firstly the application of Pulsar 40 was done in BBCH 12-13, and 7 days later (in BBCH 19-21), treatment with the different biostimuants was performed – therapeutic approach. The size of the spraying solution was 500 l ha^{-1} for all treatments.
Predecessor of pumpkins in each year was maize. The performed tillage operations after predecessor's harvesting were deep ploughing and two times harrowing before planting. The crop was planted as preliminary grown seedlings with planting distance 1 x 1.5 m (6670 plants ha^{-1}).
For control of annual and perennial grass weeds 3-5 leaf stage of the annual weed species and 10-20 cm of height of Johnson grass (*Sorghum halepense* (L.) Pers.) application with Stratos® Ultra (100 g/l cycloxydim) in rate of 2.00 l ha^{-1} was done.
The whole experimental area was fertilized with 250 kg ha^{-1} NPK 15:15:15 after predecessor's harvest and tillage operations as well as 250 kg ha^{-1} NH_4NO_3 in spring before planting of the pumpkins.
To determine the content of nutrient elements in the leaves before flowering stage of the pumpkins fully developed leaves of the crop were collected. The plant samples were dried at 60°C, weighted and milled. They were mineralized with concentrated H_2SO_4 using H_2O_2 as a catalyst. The total nitrogen content was determined according to Kjeldahl method by distillation in Parnas - Wagner apparatus (Tomov et al., 2009). Phosphorus was determined colorimetrically on spectrophotometer Camspec E105 (Tomov et al., 2009) and potassium - photometrically by flame photometer PFP-7 (Ivanov and Krastev, 2005).
The visual herbicide phytotoxicity was determined by the 9-score scale of EWRS (European Weed Research Society) on the 7th day after the herbicide application as followed:
1. No damage/healthy plant;
2. Very slight symptoms, weak suppression;
3. Slight but clearly visible symptoms;
4. Severe symptoms (e.g. chlorosis) which do not lead to a negative effect on yield;
5. Thinning, severe chlorosis or suppression; yield reduction expected;
6. Severe damage up to complete destruction;
7. Severe damage up to complete destruction;
8. Severe damage up to complete destruction;
9. Severe damage up to complete destruction.

Statistical analysis of collected data was performed by using Duncan's multiple range test by the software SPSS 19. Statistical differences were considered proved at $p<0.05$.

RESULTS AND DISCUSSIONS

Despite the visual phytotoxic symptoms caused by the herbicide application for all treatments (with or without biostimulant application) the herbicide phytotoxicity on the 7th day after treatment varied from score 1 to score 3 in the different years of the research. The highest herbicide stress was found for variant 2 (Pulasr 40 alone), as well as for the treatments of Lactofol® O in tank mixture with imazamox or 7 days after the herbicide application – variants 5 and 10 respectively. The foliar fertilizer Lactofol® O contains high percent of nitrogen in its formulation (21%) that may be the cause of the high phytotoxic effect. In research findings it is reported that the co-application of fertilizers with different herbicides can cause injuries to the crop (Soltani et al, 2012).

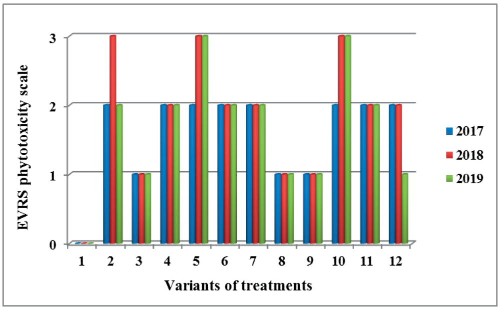

Figure 1. Visual phytotoxicity 7 days after the herbicide application (scores)

Chen et al. (1998) set optimum concentrations for nitrogen, phosphorus and potassium from fertilizer experiments conducted in Guangdong, with the leaves collected in autumn before flower initiation.
The leaf nitrogen (N) levels in the beginning of pumpkin's flowering are presented on Table 1.

Table 1. Nitrogen content in the pumpkin's leaves in the beginning of flowering stage (BBCH 61), %

Tr.	2017	2018	2019	Average
1	3,24 a	3,36 a	3,88 a	3,49 a
2	2,25 e	1,92 e	2,06 d	2,04 d
3	2,73 cd	2,63 cd	2,99 c	2,78 c
4	2,69 cd	2,55 d	2,90 c	2,71 c
5	2,32 e	2,16 e	2,00 e	2,16 d
6	2,58 d	2,62 cd	2,99 c	2,73 c
7	2,84 c	2,72 c	3,16 c	2,91 bc
8	2,66 d	2,51 d	2,97 c	2,71 c
9	2,77 c	2,64 cd	2,84 c	2,75 c
10	2,23 e	2,10 e	1,59 f	1,97 d
11	3,17 b	3,00 b	3,64 b	3,27 ab
12	2,70 cd	2,81 c	3,10 c	2,87 bc

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The optimal N content in the leaves of pumpkins vary from 3.00 to 6.00 % (Hochmuth et al., 2004). With the highest and optimal nitrogen content in the leaves according to these authors was variant 1 (Untreated weed free control) – 3.49% average for the period. The N content for this variant was with proved difference with the other treatments according to Duncan's multiple range test ($p < 0.05$). With 3.27% N in the leaves were the plants of treatment 11 (Pulsar® 40 1.00 l ha^{-1} in BBCH 12-13 + Aminozol® 3.00 l ha^{-1} in BBCH 19-21).

At all treatments, the foliar N concentration is below 3.00% and is under the optimal nutritional levels. Average for the period of the study, the lowest N content in the leaves of treatments 2, 5 and 10 was recorded – 2.04, 2.16 and 1.97% respectively. Most likely, herbicidal stress leads to disturbances in nitrogen metabolism in pumpkins and plants experience nitrogen deficiency, which later may have a negative impact on plant development.

The optimal phosphorus content in pumpkin leaves is from 0.30 to 0.50% (Silva and Uchida, 2000; Hochmuth et al., 2004).

The plants of variant 5 (Pulsar® 40 + Lactofol® O in a tank mixture) had the lowest phosphorus content - 0.52% on average for the experimental period.

The variants 2 (Pulsar® 40 1.00 l ha^{-1}) and 10 (Pulsar® 40 1.00 l ha^{-1} in BBCH 12-13 + Lactofol® O in BBCH 19-21) had a content of 0.54 and 0.56% phosphorus in the leaves before flowering of the plants, respectively.

Table 2. Phosphorus content in the pumpkin's leaves in the beginning of flowering stage (BBCH 61), %

Tr.	2017	2018	2019	Average
1	0,61 b	0,60 b	0,62 a	0,61 b
2	0,52 c	0,55 c	0,54 b	0,54 cd
3	0,60 b	0,63 ab	0,63 a	0,62 b
4	0,62 b	0,61 b	0,63 a	0,62 b
5	0,55 c	0,50 c	0,51 b	0,52 d
6	0,62 b	0,59 b	0,61 a	0,61 b
7	0,60 b	0,59 b	0,63 a	0,61 b
8	0,60 b	0,60 b	0,64 a	0,61 b
9	0,59 b	0,58 bc	0,52 b	0,56 c
10	0,53 c	0,55 c	0,59 ab	0,56 c
11	0,69 a	0,65 a	0,68 a	0,67 a
12	0,62 b	0,63 ab	0,66 a	0,64 ab

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The pumpkin plants from variant 11 (Pulsar® 40 1.00 l ha^{-1} in BBCH 12-13 + Aminozol® 3.00 l ha^{-1} in BBCH 19-21) had the highest phosphorus content (0.67%) on average for the experimental period.

The optimal potassium content in pumpkin leaves is from 2.30 to 4,00% according to Hochmuth et al. (2004) and from 3.00 to 5,00% according to Silva and Uchida (2000). The maintenance of optimum K nutrition status is very important for plant resistance to biotic and abiotic stresses and from other hand this nutrient element is helping the plants to overcome the stress conditions (Wang et al. (2013; Nikolova M., 2010). The results for the content of potassium in the leaves of pumpkin plants at the beginning of flowering are presented on Table 3.

Table 3. Potassium content in the pumpkin's leaves in the beginning of flowering stage (BBCH 61), %

Tr.	2017	2018	2019	Average
1	3,25 e	3,24 e	3,67 d	3,39 b
2	4,07 b	4,28 b	4,88 a	4,41 a
3	3,49 d	3,29 e	3,12 e	3,30 b
4	3,65 c	3,47 d	4,13 b	3,75 b
5	4,32 a	4,51 a	4,99 a	4,61 a
6	3,55 d	3,31 e	3,81 bc	3,56 b
7	3,29 e	3,44 d	3,94 b	3,56 b
8	3,59 c	3,66 c	3,80 bc	3,68 b
9	3,50 d	3,40 d	3,95 b	3,62 b
10	4,21 a	4,42 ab	4,80 a	4,48 a
11	3,46 d	3,65 c	4,01 b	3,71 b
12	3,14 e	3,33 e	3,54 d	3,34 b

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

On average for the study period, all plants had an optimal content of potassium in the leaves before flowering stage. In stressed plants from variants 2, 5 and 10 an increase in potassium levels in leaves was found - 4.41, 4.61 and 4.48%, respectively. Probably the plants of these variants absorb and accumulate higher quantities of this macronutrient in their leaves. This increased content is probably due to the high abiotic stress caused by the herbicide application and the inappropriate chose of foliar fertilizer for preventive and therapeutic treatment of the stressed plants, and potassium is the element helping the plants to overcome the conditions of stress (Nikolova M., 2010).

CONCLUSIONS

The application of the herbicide Pulsar 40 (40 g/l imazamox) in a rate of 1.00 l ha^{-1} caused temporary phytotoxic symptoms to the pumpkins on the 7th day after treatments. In time, the plants overcome the herbicide toxicity to some extent.

The highest N content in the leaves before flowering were recorded for the untreated control.

The highest P levels for the plants of the treatment with Pulsar® 40 1.00 l ha^{-1} in BBCH 12-13 + Aminozol® 3.00 l ha^{-1} in BBCH 19-21 were measured.

Increasing levels of K for the treatments with highest symptoms of phytotoxicity were recorded.

ACKNOWLEDGEMENTS

The research work was carried out with the support of Centre of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv, Bulgaria (Projects 08-17 and 17-12).

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECT OF DIFFERENT FERTILIZATION REGIMES AND RATES IN THE CARROT SEED PRODUCTION ON THEIR SOWING PARAMETERS AND CHEMICAL COMPOSITION OF THE SEEDS

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Abstract

The main aim of the present study was to establish the influence of different fertilization regimes and rates in carrot seed production on some sowing parameters and the chemical composition of the seeds. The experiments were carried out with carrot 'Tushon' variety. The seed plants were grown using the standard technology through stecklings. Three levels of NPK fertilization were tested, as follows N - 0, 50, 70, 90 kg.ha^{-1}, P - 0, 90, 140, 190 kg.ha^{-1} and K - 0, 100, 150, 200 kg.ha^{-1}, applied once and twice, respectively. The weight of 1000 seeds, germination energy and germination were studied. The content of dry matter, moisture, ash, raw protein, carbohydrates and total lipids in carrot seeds were evaluated. The highest germination energy and germination were counted at $N_{90}P_{90}K_{200}$ for once application and for twice one it was observed for $N_{90}P_{90}K_{200}$ and $N_{50}P_{190}K_{100}$. The highest changes were observed in the content of total lipids. Middle to strong positive correlation was found between the content of raw protein, lipids and carbohydrate and germination energy. Polynomial regressions between evenly increased fertilization rate and content of above-mentioned compounds with high determination coefficients were established.

Key words: *Daucus carota* L., germination, fertilization, protein, lipids, regression.

INTRODUCTION

The optimal nutrient regime has a strong influence both on the size and development of carrot seeds and also on their viability potential (Panayotov, 2005). Most of the cultivated plants are propagated by seeds therefore the quality of the seed is essential (Panchev, 2015; Ivanova and Panchev, 2016).

Chilingirov et al. (2018) and Haytova and Ivanova (2016) emphasized that the seed viability status play significant role for the normal development of the plants. In experiments conducted by Ilyas et al. (2013) found that by the increasing of fertilizer rates, especially phosphorus, a much higher effect is achieved in the development of seed plants and the quality of the obtained seeds. Hooda et al. (2014) expressed the opinion that with the increase of fertilizer norms the yield and quality of carrot seeds increases.

This is due to the better growth of the plants, as well as to the better development of the central umbels and the higher number of secondary umbels. A similar conclusion is expressed by Kushwaha (2009) that increasing the rate of nitrogen fertilizer to 100 kg.ha^{-1} improves the generative development and viability of seeds from this crop.

The main chemical ingredients in the seeds that determine the level of their vitality are proteins, fats and carbohydrates (Panayotov, 2015). According to Ozcan and Chalchat (2007), carrot seeds are rich in protein, fiber and ash. Significant is the content of essential oil, as the major constituent of seed essential oil is carotol. The authors add that the moisture, crude protein, ash, crude fiber and crude oil contents and fatty acid compositions of carrot seeds are affected mainly by the variety and growth conditions.

Yili et al. (2006) and Imamu et al. (2007) pointed out that essential oils are the main active compounds in carrot seeds. Foster and Duke (1990) and Lawless (1995) argue that the variation in the essential oils component compositions in carrot seeds is probably due to growing conditions. The main components of carrot-seed essential oils, depending on the region of growing are α-pinene, β-pinene, carotol and β-bisabolene and also asarone and cis-α-bergamoten.

The main aim of the present study was to establish the influence of different fertilization regimes and rates in carrot seed production on some sowing parameters and chemical composition of the seeds.

MATERIALS AND METHODS

The experiments were carried out in the period 2017-2019 in the experimental field of the Department of Horticulture at the Agricultural University-Plovdiv, Bulgaria with carrot variety Tucson. The standard, established and widespread in Bulgaria, technology of carrot seed production was applied, with preliminary production of stecklings (Minkov, 1984). The seeds for the production of stecklings were sown at the end of June, and their harvesting and storage in a pit took place in mid-November. The planting of the stecklings took place in mid-March, according to the scheme 80 x 30 cm. Each variant was grown in four repetitions with a plot size of 7 m^2 and harvested area of 6 m^2. The soil was prepared by deep plowing in autumn and in the spring the furrows were profiled.

Two regimes of fertilization were studied: Once fertilization - application of phosphorus and potassium fertilizers with autumn deep plowing and nitrogen fertilizer at planting; Twice fertilization - half of the phosphorus and potassium fertilizers were applied with the autumn deep plowing, the other half - in the spring before planting, and nitrogen fertilizer - half before planting, and the other half - at the beginning of flowering.

The following variants of fertilization in $kg.ha^{-1}$ were investigated: once fertilization: 1. $N_0P_0K_0$ - control; 2. $N_{70}P_{140}K_{150}$ (recommended); 3. $N_{50}P_{90}K_{100}$; 4. $N_{50}P_{90}K_{200}$; 5. $N_{50}P_{190}K_{100}$; 6. $N_{50}P_{190}K_{200}$; 7. $N_{90}P_{90}K_{100}$; 8.$N_{90}P_{90}K_{200}$; 9. $N_{90}P_{190}K_{100}$; 10. $N_{90}P_{190}K_{200}$ and once fertilization: 11. $N_{50}P_{90}K_{100}$; 12. $N_{50}P_{90}K_{200}$; 13. $N_{50}P_{190}K_{100}$; 14. $N_{50}P_{190}K_{200}$; 15. $N_{90}P_{90}K_{100}$; 16. $N_{90}P_{90}K_{200}$; 17. $N_{90}P_{190}K_{100}$; 18. $N_{90}P_{190}K_{200}$. Fertilizer rates were determined based on the recommended to this moment fertilization for carrot seed production in Bulgaria - $N_{70}P_{140}K_{150}$ (Madzharova, 1968; Kolev, 1977). Ammonium nitrate (N 34%), triple superphosphate (P_2O_5 46%) and potassium sulfate (K_2O 50%) were used. All necessary agro-technological practices were applied during the vegetation. The seed stalks were harvested when 60-70% of the seeds were in the stage of maturity, and the remaining ones were in waxy maturity, after that they were placed for ten days for post-harvest ripening and seed extraction was performed.

The germination energy and germination of the seeds were studied, according to the requirements of ISTA (2013), and the vigour - by the method of "Initial vegetative productivity of the seedlings" (Panayotov, 2013). The experiments were performed in four replications of 100 seeds each.

The content of absolute dry matter (by weight), moisture (by weight, AOAC 934.06, 2007a), carbohydrates by the the phenol-sulfuric acid method (Dubois, 1956; Nielsen, 2010), crude protein by Kjeldahl method (Bradstreet, 1965), total lipids according to the Soxhlet method (AOAC 920.39, 2012), ash (AOAC 940.26, 2007b) in the seeds were determinated.

Due to the uniformity in the trend of the results obtained during the study period, the presented values are three-year average data. The study data were subjected to analysis of variance and regression, with the least significant differences between the individual variants calculated by the Fisher test at p = 0.05. The methods of ANOVA, as well as regression analysis, are described by Fowel and Cohen (1992).

RESULTS AND DISCUSSIONS

The main elements that determine the main viability potential of seeds are germination energy and germination, which according to several authors depend on various factors, such as ecological and agrotechnological (Copeland and McDonald, 2001; Black et al., 2008 and Panayotov, 2015). In this regard, the tested regimes and levels of fertilization have a strong influence on the viability of the seeds (Table 1). Better germination energy, compared to the control, demonstrated all tested variants The highest values for once fertilization are reported for fertilizer combination $N_{90}P_{90}K_{200}$ - 78.88%, followed by fertilization with $N_{90}P_{190}K_{100}$ - 73.99%. In the other regime, the germination energy is the highest for variant $N_{50}P_{90}K200$ - 69.44% and also for $N_{50}P_{90}K_{200}$ - 68.88%. A slight decrease compared to the recommended fertilization rate was observed for $N_{90}P_{190}K_{200}$ in both regimes for $N_{50}P_{90}K_{100}$

as well as in once fertilization for $N_{90}P_{190}K_{200}$. The differences between most variants compared to the unfertilized control are mathematically proven, except for $N_{50}P_{90}K_{100}$ and $N_{90}P_{90}K_{100}$ (once fertilization) as well as for both regimes when using the highest fertilization rates.

Table 1. Sowing parameters of carrot seed after application of different nutrient regimes

Variants	Germination energy (%)	Germination (%)	Index of vigour
Once fertilization			
$N_0P_0K_0$	59.22	60.88	7.20
$N_{70}P_{140}K_{150}$	64.55	67.88	9.27
$N_{50}P_{90}K_{100}$	62.66	69.55	10.99
$N_{50}P_{90}K_{200}$	69.22	73.10	8.50
$N_{50}P_{190}K_{100}$	68.44	70.44	8.39
$N_{50}P_{190}K_{200}$	66.88	70.44	7.79
$N_{90}P_{90}K_{100}$	61.77	72.21	9.61
$N_{90}P_{90}K_{200}$	78.88	80.88	10.47
$N_{90}P_{190}K_{100}$	73.99	76.66	9.35
$N_{90}P_{190}K_{200}$	63.55	68.22	10.98
Twice fertilization			
$N_{50}P_{90}K_{100}$	69.32	74.55	9.84
$N_{50}P_{90}K_{200}$	69.44	76.22	9.25
$N_{50}P_{190}K_{100}$	67.10	79.77	8.97
$N_{50}P_{190}K_{200}$	65.10	69.10	8.48
$N_{90}P_{90}K_{100}$	66.44	70.44	10.59
$N_{90}P_{90}K_{200}$	68.88	75.66	10.14
$N_{90}P_{190}K_{100}$	66.11	71.10	9.66
$N_{90}P_{190}K_{200}$	62.11	65.44	9.04
LSD p=5.0%	7.24	7.34	2.04

The germination compared to the unfertilized and the recommended control, is higher for all studied levels and regimes of fertilization, except for $N_{90}P_{190}K_{200}$ (twice), where the reduction is insignificant. The highest values of this indicator - 80.88%, are reported after a once fertilization of plants with $N_{90}P_{90}K_{200}$. Next, with a very small difference was the twice application with $N_{50}P_{190}K_{100}$ - 79.77%. Relatively high germination was also developed by seeds from once regime with $N_{90}P_{190}K_{100}$ (76.66%) as well as twice - with $N_{50}P_{190}K_{200}$ (76.22%). Statistical significance of the differences compared to the control without fertilization was established, as such is missing only at $N_{90}P_{190}K_{200}$ (twice).

The high percentage of germinated seeds reported in the laboratory does not mean that the same seeds sown under field conditions will show the same germination and that the germinated seeds will develop typical seedlings and later normal plants. Therefore, the determination of the vigour is especially important for assessing the quality of seeds (Panayotov, 2013). No significant differences were found between the both periods of application of the fertilizer combinations. The vigour index reaches the highest levels of 10.99 and 10.98, with a once application of $N_{50}P_{90}K_{100}$ and $N_{90}P_{190}K_{200}$, respectively. In this regime, very good results are obtained also for variant $N_{90}P_{90}K_{200}$ (10.47). In the case of twice fertilization, high vigour is reported after fertilization with $N_{90}P_{90}K_{100}$ and $N_{90}P_{90}K_{200}$ - 10.59 and 10.14, respectively.

The viability behaviors of the carrot seeds were improved better after the application of the higher levels of phosphorus and especially potassium. Similar conclusions are also expressed by Rao and Maurya (1998), Hooda et al. (2014). The differences between the two fertilization regimes regarding to their viability status are relatively small.

The chemical composition of vegetable seeds is essential, as it must provide the seeds with the necessary nutrients and energy to be able to germinate, subsequently sprouting and develop the young plant (Copeland and McDonald, 1995; Panayotov, 2015). On Table 2 is presented the contents of the main substances that mainly affect the viability processes of the seeds.

Table 2. Chemical components of carrot seeds (%)

Variants	Moisture	Dry weight	Dust	Protein	Lipids	Carbohidrate
Once fertilization						
$N_0P_0K_0$	9.17	90.82	6.82	21.23	12.08	50.35
$N_{70}P_{140}K_{150}$	10.21	89.78	7.58	22.02	9.25	50.91
$N_{50}P_{90}K_{100}$	9.75	90.24	7.38	21.08	9.77	51.63
$N_{50}P_{90}K_{200}$	9.52	90.47	7.24	21.81	10.38	50.70
$N_{50}P_{190}K_{100}$	8.83	91.16	6.90	22.83	8.98	52.24
$N_{50}P_{190}K_{200}$	9.15	90.85	6.83	22.67	10.18	51.38
$N_{90}P_{90}K_{100}$	8.95	91.05	7.78	21.62	9.36	52.29
$N_{90}P_{90}K_{200}$	9.61	90.38	7.13	22.10	10.70	50.39
$N_{90}P_{190}K_{100}$	8.78	91.21	7.31	22.38	7.59	53.79
$N_{90}P_{190}K_{200}$	9.24	90.76	7.44	20.63	10.07	52.45
Twice fertilization						
$N_{50}P_{90}K_{100}$	8.85	91.14	6.68	23.14	7.55	53.42
$N_{50}P_{90}K_{200}$	8.99	91.01	6.83	22.72	10.46	50.86
$N_{50}P_{190}K_{100}$	12.30	87.69	7.70	21.44	8.71	49.47
$N_{50}P_{190}K_{200}$	9.31	90.68	7.26	21.91	9.93	51.35
$N_{90}P_{90}K_{100}$	9.14	90.85	7.07	21.39	10.30	51.37
$N_{90}P_{90}K_{200}$	9.90	90.09	8.26	21.71	8.17	51.34
$N_{90}P_{190}K_{100}$	8.70	91.29	7.49	21.34	7.70	53.65
$N_{90}P_{190}K_{200}$	9.00	90.99	7.72	21.74	9.22	51.18
LSD p=5.0%	2.19	2.18	0.95	2.10	4.22	4.75
r with germination				+0.74	+046	+0.41

According to the requirements of ISTA (2013), to be able to the vegetable seeds to be stored well and to ensure normal metabolism, the moisture content should not exceed 13%. In most of the tested variants, the average water content in the seeds is about 9%. Some increase was found for the fertilizer control - 10.21%, as well as for the combination $N_{50}P_{190}K_{100}$ applied twice - 12.30%. No seeds with higher moisture than the standard were observed.

Dry matter is a summary indicator that characterizes the overall condition of the endosperm and the content of nutrients in the seed. The percentage of dry matter in the seeds, which is average for the period ranged from 87.69% for $N_{50}P_{190}K_{100}$ (twice) to 91.29 % for $N_{90}P_{190}K_{100}$ (twice). With the highest ash content were characterized the seeds of the variant fertilized twice with $N_{90}P_{90}K_{200}$ - 8.26%. The amount of ash for most of the tested combinations was above 7%, and this percentage decreases more significantly in the combinations $N_{50}P_{190}K_{100}$, $N_{50}P_{190}K_{200}$ (once) and $N_{50}P_{90}K_{100}$, $N_{50}P_{90}K_{200}$ (twice).

ccording to Copeland and McDonald (2001), proteins are a major building block of the reserve nutrients used primarily by the embryo for germination. The values obtained for the amount of protein in carrot seeds were close and vary within narrow limits between the different variants. Some increase was observed in the variant fertilized twice with $N_{50}P_{90}K_{100}$ - 23.14%, in contrast to the others, where 21-22% were reported. There is a strong positive correlation between protein content and germination with a coefficient r =+0.74. The established regression dependence (Figure 1 and Figure 2) between the evenly increasing fertilizer rates and the protein content is polynomial with high determination coefficients R^2 = 0.64 and R^2 = 0.79, respectively, in once and twice fertilization, which means that between 60% up to 80% will have the indicated effect of fertilization on protein synthesis.

Lipids are the main source of energy and in metabolism during germination their hydrolysis begins first in the embryo. Carrot seeds are generally characterized by high lipid content (Panayotov, 2015). The data obtained for the lipid content ranged from 7.55% for $N_{50}P_{90}K_{100}$ (twice) to 12.08% for the unfertilized control on average for the reporting period. There was a large variation in their content depending on the fertilization, stronger with a once regime, especially with $N_{50}P_{90}K_{200}$, $N_{90}P_{90}K_{100}$ and $N_{90}P_{190}K_{200}$. The correlation with germination is average and positive with r =+0.46. Polynomial regressions of this indicator according to the evenly increasing fertilizer

rates are shown in Figures 3 and Figure 4. The determination coefficients for once and twice fertilization are R^2 = 0.98 and R^2 = 0.85, respectively, i.e. in approximately 90% these fertilization methods will cause the observed effect.
Carbohydrates are a very important source of energy and their content in vegetable seeds and their content in vegetable seeds is of the great significance. Lee et al. (1995) points out that the content of carbohydrates, and more specifically of the sugars released from the seeds, can serve to assess the level of their viability. The average content of carbohydrates was highest in the seeds of the once fertilizer with $N_{90}P_{190}K_{100}$ - 53.79%, which was almost 4% more than the two controls - fertilized and not fertilized. The same fertilizer rate provokes the highest percentage for the other term of application of mineral fertilizers - 53.65%, followed by $N_{50}P_{90}K_{100}$ - 53.42%. It can be pointed out that fertilization with $N_{90}P_{190}K_{100}$, both once and twice, as well as the combination of N_{90} with higher levels of P_2O_5 and K_2O contribute to an increase in the percentage of carbohydrates in the carrot seeds. The correlation with germination is average and positive with r =+0.41. The regressions of the evenly increased fertilization rates and the carbohydrate content (Figure 5 and Figure 6) are again polynomial, and the determination coefficients R^2 = 0.66 (once fertilization) and R^2 = 0.55 (twice fertilization) are relatively high and show that at approximately in 60% of this fertilization will have the identified impact.

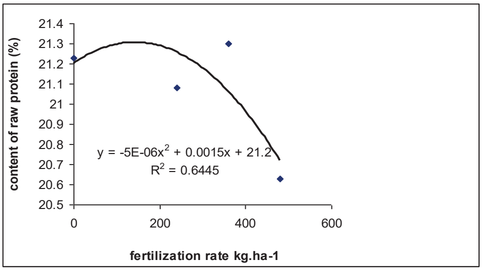

Figure 1. Regression dependence between evenly increasing levels in once fertilization and content of raw proteins
1.$N_0P_0K_0$, 2. $N_{50}P_{90}K_{100}$;
3. $N_{70}P_{140}K_{150}$; 4. $N_{90}P_{190}K_{200}$

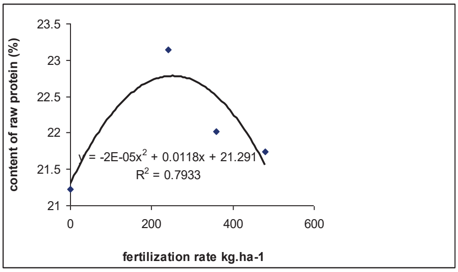

Figure 2. Regression dependence between evenly increasing levels in twice fertilization and content of raw proteins
1.$N_0P_0K_0$, 2. $N_{50}P_{90}K_{100}$;
3. $N_{70}P_{140}K_{150}$; 4. $N_{90}P_{190}K_{200}$

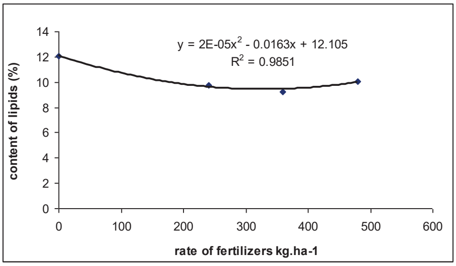

Figure 3. Regression dependence between evenly increasing levels in once fertilization and content of lipids
1.$N_0P_0K_0$, 2. $N_{50}P_{90}K_{100}$;
3. $N_{70}P_{140}K_{150}$; 4. $N_{90}P_{190}K_{200}$

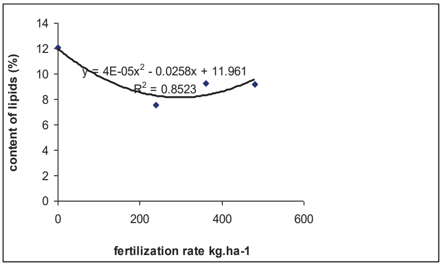

Figure 4. Regression dependence between evenly increasing levels in twice fertilization and content of lipids
1.$N_0P_0K_0$, 2. $N_{50}P_{90}K_{100}$;
3. $N_{70}P_{140}K_{150}$; 4. $N_{90}P_{190}K_{200}$

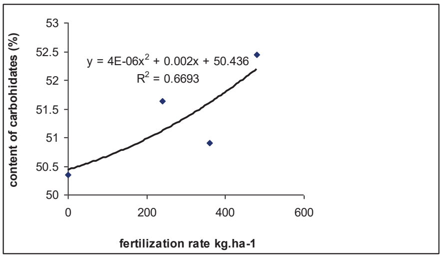

Figure 5. Regression dependence between evenly increasing levels in once fertilization and content of carbohydrates 1.$N_0P_0K_0$, 2. $N_{50}P_{90}K_{100}$; 3. $N_{70}P_{140}K_{150}$; 4. $N_{90}P_{190}K_{200}$

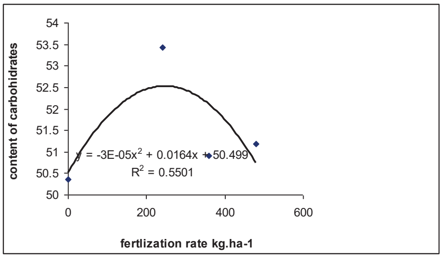

Figure 6. Regression dependence between evenly increasing levels in twice fertilization and content of carbohydrates 1.$N_0P_0K_0$, 2. $N_{50}P_{90}K_{100}$; 3. $N_{70}P_{140}K_{150}$; 4. $N_{90}P_{190}K_{200}$

CONCLUSIONS

The different fertilization regimes and rates significantly improved the viability of carrot seeds, and the effect is stronger when applying the higher levels of phosphorus and especially potassium.

Germination in once fertilization with $N_{90}P_{90}K_{200}$ and in twice fertilization with $N_{50}P_{190}K_{200}$ exceeds by approximately 20% that of the control variant.

The main chemical ingredients in carrot seeds are affected as a result of the application of mineral fertilizers. Higher changes were found in the content of crude lipids and carbohydrates.

Medium to strong positive correlations of the basic chemical components with germination has been established. The regressions of the evenly increase of the fertilizer rates and the content of proteins, fats and carbohydrates are polynomial with high determination coefficients.

ACKNOWLEDGEMENTS

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018"

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

POTASSIUM CONTENT IN TOMATO AND TOMATO PRODUCTS

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Abstract

Tomato (Lycoperscion esculentum) is an annual vegetable crop of the Solanaceae family. It can be consumed as fresh food or processed in the form of pelee, concentrate, juice or ketchup. Potassium activates enzymes and regulates permeability of living membranes. The goal of this research was to determine the content of potassium in fresh tomatoes and tomato products. Tomato sampling was conducted on 3 sales places in Zagreb, including fresh grappolo tomatoes, fresh cherry tomatoes, ketchup, double concentrate (28-30 % DW) and organic tomato pelée. In order to determine the content of potassium, the grinded samples were digested with concentrated HNO_3 and $HClO_4$ in a microwave oven, potassium is determined by flamephotometer. The content of dry weight ranged from 4.2 to 30.4%. Statistically the highest potassium content was found in fresh grappolo tomatoes (7.5% K dry weight and 1783 mg K/100 g in fresh weight). Slightly lower potassium content was determined in fresh cherry tomatoes and peeled tomatoes, while in ketchup was statistically the lowest (1.97% K dry weight and 65 mg K/100 g in fresh weight).

Key words*: food processing, Lycoperscion esculentum, macroelement, mineral.*

INTRODUCTION

Tomato (*Lycoperscion esculentum*) as one of the most widespread vegetable crops, has a wide use as a food. It can be consumed both, fresh or processed. It is especially widespread and highly valued in Mediterranean cuisine. Potassium, as a part of tomato mineral composition, is the most abundant, followed by phosphorus, magnesium, calcium, sodium and iron. Tomato is richer in iron than fish, chicken, and milk (Matotan, 2008).

Tomato cultivars differ in the type of plant growth, production purpose, fruit shape and colour, early-maturing, and other morphological and biological properties (Lešić et al., 2004). For commercial production, hybrids are used which, in relation to cultivars, give higher yields and better genetic resistance to diseases and pests (Matotan, 2004; Parađiković, 2009).

As a part of canning and processing, tomatoes have taken an important place due to their organoleptic and culinary properties as well as nutritional value as a raw material. The value of tomato as a raw material for processing is assessed on the basis of mechanical (e.g. fruit firmness) and chemical composition, suitability of the fruit for processing, as well as the intensity of changes in the chemical composition of the product in relation to the incoming raw material. Tomatoes are most often processed as puree, concentrate, peeled tomatoes (concentrated whole fruits), tomato juice (as a beverage), sauces, and dehydrated products (Lovrić & Piližota, 1994).

In modern agriculture, which point out high yields, there are also high needs for potassium. In order to meet the potassium need, its uptake is necessary by mineral fertilizers (Butorac, 1999). The physiological role of potassium, as an essential biogenic element, was determined quite late because it is not a part of organic compounds. The basic functions of potassium are enzyme activation and regulation of living membrane permeability. As an electrolyte, it affects the regulation of turgor, burst work and the regulation of water content in the plant (Parađiković, 2009). Potassium deficiency, due to its complexity and important function in metabolism, has an impact on the overall growth and development of the plant. In the absence of potassium, the edges of well-developed older leaves turn yellow (edge chlorosis), later irreversibly brown (necrosis) especially when the weather is hot (Petek, 2016).

As for plants, biogenic elements are also essential for normal human growth and development. Thus, after calcium and phosphorus, potassium is the most abundant mineral in the human body. There are about 160 to 300 g of potassium in the adult organism, of which 98% is found in cells, and only 2% outside them (Lovrić, 2004). Thus, potassium is an essential constituent of the human body which can be supplied by consuming foods rich in potassium, such as tomatoes.

The aim of this study was to determine the potassium content in different samples of fresh and processed tomatoes according to the place of purchase.

MATERIALS AND METHODS

For purpose of this investigation on 5 Dec 2017 in city of Zagreb (Croatia) samples of fresh tomatoes and tomato products were collected: organic peeled tomato (under the trade name Bio-Tomaten gewurfelt aus biologischer Landwirschaft), double concentrate (28-30% dry weight), ketchup, fresh cherry tomatoes and fresh grappolo tomatoes. All samples were collected in triplicate in the SPAR retail chain at the locations Jordanovac, Kvaternikov trg (Kvatrić), and Britanski trg (Britanac). Each sample was cleaned and washed in distilled water in the case of fresh tomatoes, and the products were removed from the packaging and each sample was chopped and ground. The homogenised and mixed average samples were dried at 105 °C, after which they were ground and homogenized. Potassium was determined by flame photometer (Jenway, PFP-7) after digestion with concentrated nitric acid (HNO_3) and perchloric ($HClO_4$) in a microwave oven (Milestone, Ethos One) (AOAC, 2015). Dry weight (DW) was determined gravimetrically by drying to constant mass.

The samples, collected in triplicate, were analysed individually and the results showed as average values. Statistical data analysis followed the variance analysis model (ANOVA). The SAS System for Win program was used. ver 9.1 (SAS Institute Inc.), and Tukey's significance threshold test (SAS, 2002-2003) was used to test the results.

RESULTS AND DISCUSSIONS

Figure 1 shows the percentage of dry weight (DW) in fresh tomatoes and tomato products. The average dry weight (DW) content in the samples, regardless of the place of purchase, ranged from 4.22 to 30.45%. The highest percentage of dry weight was found in ketchup, while the lowest was found in fresh grappolo tomatoes.

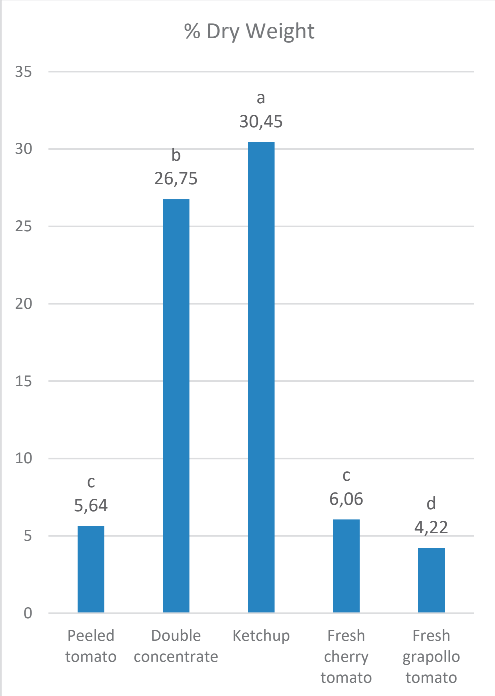

Figure 1. Average dry weight (DW) content (% DW) in fresh tomato and tomato products
Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different

Table 1 shows the percentage of dry weight in tomatoes and tomato products at different locations of the SPAR retail chain.

In fresh grappolo tomatoes was determined the lowest dry weight content, at the location SPAR Kvatrić 4.1% DM, followed by SPAR Britanac and SPAR Britanac (4.2 and 4.4% DW, respectively).

In fresh cherry tomato the dry weight content was 5.7% DW at the SPAR Jordanovac location, 6.0% DW at the SPAR Britanac

location, and 6.6% DW at the SPAR Kvatrić location.
Peeled tomatoes have similar dry weight content as fresh cherry tomatoes, at the location of SPAR Jordanovac and SPAR Britanac (5.3% DW). Finally, the highest percentage of dry weight in peeled tomatoes (6.3% DW) was determined at the SPAR Kvatrić location.
Double concentrate at the SPAR Jordanovac location obtained 25.6% DW, and at the SPAR Kvatrić and Britanac location it is very similar to the percentage of dry weight (27.5 and 27.2% DW, respectively).
Ketchup has the highest percentage of dry weight of all processed products, and the percentage of dry weight is almost the same in all samples: at the SPAR Britanac location 30.2% DW, at the SPAR Kvatrić location 30.4% DW and 30.7% DW SPAR Jordanovac.

Table 1. Dry weight (DW) content (% DW) in fresh tomato and tomato products according to sales locations

% dry weight	Sales locations		
	SPAR Jordanovac	SPAR Kvatrić	SPAR Britanac
Peeled tomato	5.3	6.3	5.3
Double concentrate	25.6	27.5	27.2
Ketchup	30.7	30.4	30.2
Fresh cherry tomato	5.7	6.6	6.0
Fresh grappolo tomato	4.4	4.1	4.2

The average tomatoes potassium content in dry weight, expressed as a percentage (% K DW), shows different values depending on whether the tomato is fresh or processed. Figure 2 shows the average values of potassium in dry weight (% K DW) ranging from 1.97 to 7.52% K DW. Quite similar average potassium content in dry weight was found in double concentrate, peeled tomatoes, fresh cherry tomatoes and fresh grappolo tomatoes ranging from 5.89 to 7.52% K DW, while the lowest potassium content was found in ketchup 1.97% K DW.

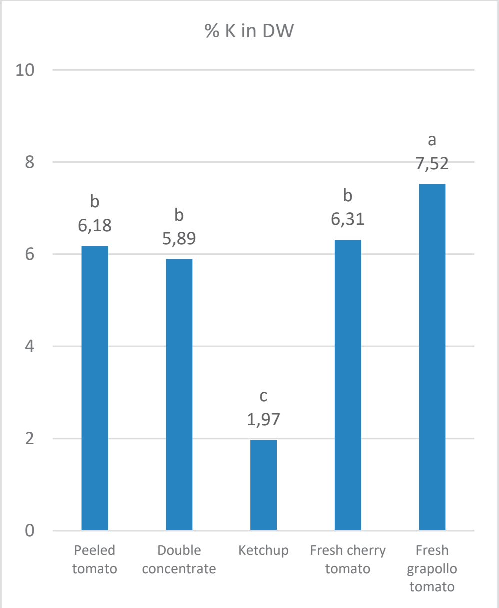

Figure 2. Average potassium content in dry weight (% K DW) in fresh tomato and tomato products Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

Table 2. shows the percentage of potassium in the dry weight in fresh tomato and tomato products samples at different locations of the SPAR retail chain.
The highest potassium content was determined in fresh grappolo tomatoes at the location of SPAR Jordanovac 7.99% K DW, followed by SPAR Britanac 7.50% K DW, and SPAR Kvatrić 7.08% K DW.
The fresh cherry tomato in the SPAR store at the Britanac, Jordanovac, and Kvatrić locations has the following percentage of potassium in the dry weight: 6.72, 6.49, and 5.73% K DW, respectively. In peeled tomato potassium content was determined as follows: 6.75, 5.91 and 5.88% K DW in SPAR stores at the locations Jordanovac, Britanac, and Kvatrić, respectively. In double concentrate in SPAR stores at the locations Jordanovac, Britanac, Kvatrić has the following percentage of potassium in the dry weight: 6.47, 5.76 and 5.44% K DW, respectively.
The lowest potassium content in the dry weight was found in ketchup at the SPAR Britanac location of 1.91% K DW, while at the Kvatrić and Britanac locations the percentage was

slightly higher at 1.93 and 2.06% K DW, respectively (Table 2).

Table 2. Potassium content in dry weight (% K DW) in fresh tomato and tomato products according to sales locations

% K DW	Sales locations		
	SPAR Jordanovac	SPAR Kvatrić	SPAR Britanac
Peeled tomato	6.75	5.88	5.91
Double concentrate	6.47	5.44	5.76
Ketchup	2.06	1.93	1.91
Fresh cherry tomato	6.49	5.73	6.72
Fresh grappolo tomato	7.99	7.08	7.50

Figure 3 shows the average potassium content in fresh weight (mg K/100 g FW). The highest average potassium content was recorded in fresh grappolo tomatoes 1783 mg K/100 g FW, while the lowest average potassium content was recorded in ketchup 64.65 mg K/100 g FW.

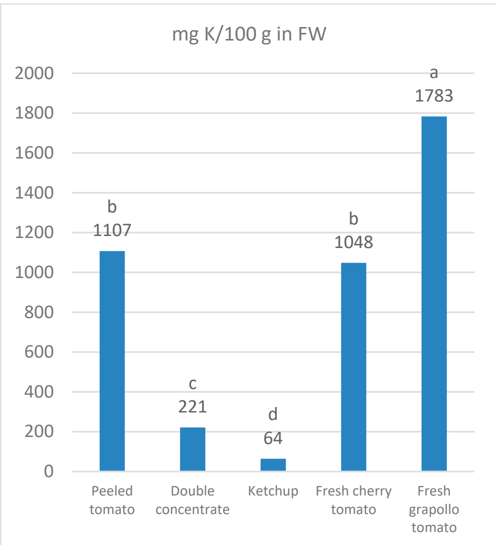

Figure 3. Average potassium content in fresh weight (mg K/100 g FW) in fresh tomato and tomato products Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Table 3 shows the amount of potassium in fresh weight (mg/K 100 g FW) in tomato samples and tomato products at different locations of the SPAR retail chain.

The following potassium content in fresh weight was found in fresh grappolo tomatoes in the SPAR retail chain at the Jordanovac, Britanac and Kvatrić locations: 1829, 1725 and 1797 mg K/100 g FW, respectively.

Fresh cherry tomatoes are rich in potassium in fresh weight. At the SPAR Kvatrić location, 873 mg K/100 g FW was determined, followed by 1128 mg K/100 g FW at the SPAR Britanac location and the highest value of potassium content in fresh weight at the SPAR Jordanovac location of 1145 mg K/100 g FW.

In peeled tomatoes, the highest potassium content was determined at the SPAR Jordanovac location 1269 mg K/100 g FW, followed by the SPAR Britanac location 1124 mg K/100 g FW and 928 mg K/100 g FW SPAR Kvatrić.

In double concentrate at the SPAR Jordanovac location, the highest value of potassium was found (253 mg K/100 g FW), followed by SPAR Britanac 212 mg K/100 g FW and in SPAR Kvatric with a minimum value of 198 mg K/100 g FW.

The lowest potassium content in fresh weight was found in ketchup, at all three locations the values are very similar. At the SPAR Britanac location 64 mg K/100 g FW, followed by SPAR Kvatrić with 63 mg K/100 g FW and finally SPAR Jordanovac with 67 mg K/100 g FW.

Table 3. Potassium content in fresh weight (mg K/100 g FW) in fresh tomato and tomato products according to sales locations

mg K/100 g FW	Sales locations		
	SPAR Jordanovac	SPAR Kvatrić	SPAR Britanac
Peeled tomato	1269	928	1124
Double concentrate	253	198	212
Ketchup	67	64	63
Fresh cherry tomato	1145	873	1128
Fresh grappolo tomato	1829	1725	1797

According to various literature sources, the percentage of dry weight in fresh tomatoes (% DW) is as follows: Matotan (2004) 4-6% DW, Parađiković (2009) 3-6% DW and Lešić et al. (2004) 5-7% DW. In this study, the % DW of fresh grappolo tomatoes in the SPAR retail chain is 4.22%, while the % DW of fresh cherry tomatoes is 6.06%. Both types of fresh tomatoes are in agreement with the values of dry weight according to the literature data.

The dominant mineral in the composition of tomatoes is potassium (Matotan, 2008). Different authors reported different K fresh tomato fruit content. So, Assuncao et al. (2019) reported only 1.7-2.1 and Barker et al. (2019) 3.27-5.33% K DW, while in this study determined K content was higher (5.73-7.99% K DW).

Tomato, according to Lešić et al. (2004), contains the potassium content between 92 to 375 mg K/100 g FW. In this study, the determined average potassium value in fresh grappolo tomatoes is 178 and in fresh cherry tomatoes 1048 mg K/100 g FW. The average values of fresh grappolo and fresh cherry tomatoes deviate significantly from the values according to Lešić et al. (2004). The reasons can be many: climatic conditions, cultivation method (open field production, greenhouse production, hydroponics), fertilization, harvest time, hybrid, cultivation care, etc.

Peeled tomatoes obtain an average percentage of dry weight in the fresh sample of 5.64%. This product bears the Demeter mark, and is grown in accordance with Council Regulation (EC) 834/2007 produced without GMOs, and without the use of mineral fertilizers. Organic fertilization of tomatoes is most often based on compost and manure (Lončarić et al., 2015). In an experimental study, Pribetić et al. (2000) fertilized tomatoes with mineral fertilizers, compost and a combination of mineral fertilizers and compost. The results of the research showed a higher percentage of dry weight of tomatoes that was fertilized only with compost (7.6% DW) compared to the treatment without compost (4.8% DW). Tomatoes, intended to be processed, should have a higher percentage of dry weight.

The total dry weight of the finalised ketchup product should be between 25 to 35% (Lovrić and Piližota, 1994), in this study the average value of dry weight in ketchup is 30.43%. According to Vargek (2019) the dry weight value in ketchup can be variable depending on the manufacturer. The double concentrate in this study has an average dry weight value of 26.77% DW.

Tomato products in this study: peeled tomatoes, double concentrate and ketchup have different values of potassium in both dry weight and fresh weight. The average value of potassium in peeled tomatoes was 6.18% K DW, in double concentrate 5.89% K DW and in ketchup 1.97% K DW. The average value of potassium in the fresh sample in peeled tomatoes was 1107 mg K/100 g FW, in double concentrate 220 mg K/100 g FW and in ketchup 64 mg K/100 g FW.

The reason why peeled tomatoes contain so much more potassium compared to double concentrate and ketchup, probably is the production technology. Peeled tomatoes are made of peeled pieces of tomato in an infusion of their own juice, with the addition of 0.2% salt. Double concentrate is a product obtained by evaporation of juice produced by mashing crushed and heat-treated tomato fruits, with the addition of 2-3% salt. The production of ketchup consists of the preparation of concentrates by the usual process or by diluting the concentrate with a larger amount of dry weight, a series of spices including salt 2-3%, and the addition of additives (Lovrić and Piližota, 1994). Significant amounts of potassium from food are lost through food processing, i.e. heat treatment and canning. During the preparation of tomato products, salt (NaCl) is added, which can disturb the ratio of potassium and sodium in humans. Processed foods, in which salt is added, contains an average potassium to sodium ratio of 7:1, compared to unprocessed foods (web 1). Richer K fertilization resulted with higher K content in tomato (Sontag et al., 2018; Daoud et al., 2020; Rahim et al., 2020; De Luca et al., 2021). So, in order to improve nutritional quality of tomato and tomato processed products fertilization should be applied.

CONCLUSIONS

In the study are determined potassium content and dry weight content in fresh tomatoes and tomato products. The dry weight content in the samples ranged from 4.22 to 30.45% DW. Determined potassium content in the dry weight was in range from 1.97 to 7.55% K DW and from 64 to 1783 mg K/100 g FW.

Statistically the highest potassium content in dry and fresh weight was found in fresh grappolo tomatoes. A slightly lower content was found in fresh cherry tomatoes and peeled tomatoes, while potassium content in double concentrate and ketchup was statistically the lowest.

Further research is needed with a precisely defined assortment, controlled cultivation that implies equal plantation care, equal fertilization, protection, irrigation, etc. in order to obtain more precise results.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOLUBLE SUGAR CONTENT AND pH IN CARROT'S ROOT JUICE

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Abstract

Carrot (Daucus carota L.*) is vegetable of Apiaceae family that has a very significant nutritional and health value in human nutrition. Furthermore, it is rich in elements that alkalize blood and keeps it at pH 7. Therefore, the aim of this research was to determine the content of soluble sugars and pH in carrot's juice and to compare the results regarding to the place of purchase. Carrot sampling was carried out in triplicate in city of Zagreb (Croatia) in 5 retail chains, 5 markets and 5 organic products stores. Soluble sugar content (% Brix) from the percolated juice was determined by refractometer, while the pH of the juice was determined by pH meter. The content of soluble sugar ranges between 6.47 and 10.13% Brix (average 7.43 in supermarkets, 7.95 in markets and 8.54 in organic shops). The pH of the carrot juice ranges from 6.17 to 7.02 (average 6.69 in supermarkets, 6.65 at markets and 6.55 in organic shops).*

Key words*: copper, Daucus carota* L.*, iron, manganese, zinc.*

INTRODUCTION

Carrot (*Daucus carota* L.) is a vegetable crop of the *Umbelliferae* family, grown primarily for its thickened and nutrient-rich root or hypocotyl. Due to the wider possibility of use in raw, fresh and processed form, as well as due to the nutritional values recognized in the food industry, it is one of the most important vegetable species and its potential is recognized not only in the food industry but also in other industries (Lešić et al., 2002).

A diet rich in processed foods acidifies the human body, which in optimal conditions should be within neutral pH. Carrot juice is recommended by many nutritionists as a valuable dietary supplement precisely because it is rich in alkaline elements that alkalize human blood and keep it at pH 7-8, and does not contribute to increased sugar blood level because it has a low glycemic index and low caloric value.

According to Schaller (1999) different varieties of carrots have different proportions of total sugars. In addition to genetic predispositions, sugar content is determined by environmental factors too. Sucrose, glucose and fructose make up 99% of the available carbohydrates in carrot. Sucrose is a storage carbohydrate of carrot root. It is synthesized in the process of photosynthesis in the leaf, after which it is transported to other plant organs, where is reduced to monosaccharides. Carrot absorbs a lot of potassium, so fertilizing with potassium-rich fertilizers, moderate-rich nitrogen fertilizers and sufficient exposure to sunlight will increase the intensity of sucrose synthesis as well as the storage of sugar in the root.

Bach et al. (2015) state that glucose, fructose, and sucrose were identified as individual sugars present in carrots. Sucrose is the most abundant sugar and makes up 57-69% of the total sugar content, while glucose and fructose make up 16-22% and 15-22% of the total content, respectively.

Schaller (1999) further states that in the vegetative phase of development, the sucrose content increases with maturity and growth time. In the post-harvest period, disaccharides are again reduced to monosaccharides and metabolized. The intensity of metabolism depends largely on the storage temperature. Research shows that carrot need a temperature of 0-2°C with high humidity for storage.

According to Bender et al. (2009) organically grown carrot contain more sugar than conventional ones, while differences in certain minerals are negligible.

The glycemic index (GI) is a scale for determining the rate of increase in a person's blood glucose level after consuming a particular food. The scale is ranked from 0 to 100. Foods that are rich in carbohydrates and easily digested cause a rapid rise in blood glucose levels. GI is particularly important given that the population is affected by chronic diseases such as diabetes and insulin resistance. The GI of fresh carrot is 30, while cooked 85. In addition, research by Gustafsson et al. (1995) studied the relationship between satiety and higher energy value of carrot-rich meals. It has been shown that the lower the reaction of glucose and insulin/C-peptide, the greater the feeling of satiety and that the minimum need is actually 200 g of carrot. But different preparations gave different results. Fresh carrot achieves greater satiety, but also a greater reaction of glucose and C-peptide in contrast to cooked carrot. Therefore, according to the recommendations of nutritionists, fresh carrot should be consumed to achieve a feeling of satiety, and sugars that are absorbed are consumed faster and are not stored in the form of fat.

Carrot, like all other types of fruits and vegetables, contain a large number of organic acids. According to the level of concentrations, malic and citric acids are distinguished with succinic, fumaric and tartaric acid, but they are found in low concentrations (Schaller, 1999).

However, high pH makes the juice more susceptible to spoilage and contamination by microorganisms, therefore for easier storage it is either mixed with juice of lower pH (apple, orange) or acids are added directly. Sinchaipanit and Kerr (2007) showed that the pulp size, diameter 332-283 μm, juice has no effect on pH and content of soluble dry matter, and that in pasteurized juice these values remain the same through two weeks of refrigeration. Homogenization of the samples had the greatest effect on viscosity, while other factors such as pH and soluble dry matter had no effect.

Schultz et al. (2014) state that reduced pH of the juice causes visually clear juice due to particle flocculation. Cloud stability decreases reciprocally with decreasing pH while particle size increases with decreasing pH. The effect of the added *Aframomum danielli* extract in carrot juice provided insight to into a new way of preserving said juice (Amanyunose et al., 2017). The high pH value (6.4) in the juice treated with 15% extract on the first day, resulted in low acidity thus characterizing the juice as a food with a low acid content. Thus, the addition of *A. danielli* extract increases the acidity of the juice at room temperature.

According to Reiter et al. (2003) acidification of juice causes coagulation of juice particles by the reaction of acid-sensitive proteins, which in their study takes place at pH 4.4. Nadulski et al. (2015) as part of their research also observed physical changes in freezing and thawing of carrot juice, where the established results showed that there were no significant changes in pH values (pH 6.35 was determined as the lowest value, while pH 6.53 was the highest). Türkmen and Takci (2018) investigated the influence of ultraviolet B and C rays on carrot juice, where one of the conclusions is that exposure of juice to ultraviolet rays does not cause significant changes in pH. The determined pH value of the control treatment is 6.34, for juices exposed to UV-C rays the reaction varied in the range between 6.31 and 6.39, while for juices exposed to UV-B rays between 6.28 and 6.54.

Da Silva et al. (2007) state in freshly squeezed juice a pH range of 6.01 to 6.14 while Demir et al. (2007) pH 6.2.

Therefore, the aim of this study was to determine the content of soluble sugars and pH in carrot juice and to compare the results according to the place of purchase.

MATERIALS AND METHODS

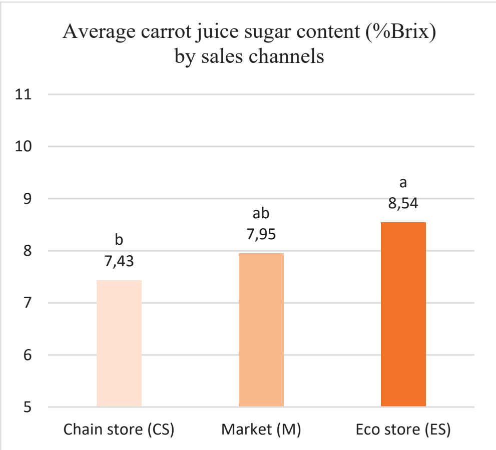
Samples of fresh carrots (orange root coloured) were collected in city of Zagreb (Croatia) on 4 Dec 2017. The sampling places represented different selling channels: chain stores, markets and organic products stores. Chain stores (CS) were represented by following selling points: CS1-Konzum, CS2-Lidl, CS3-Kaufland, CS4-Plodine, CS5-Spar. Markets (M) were represented by following selling points: M1-Britanski trg, M2-Branimirova, M3-Dubrava, M4-Dolac, M5-Kvatrić. Organic products stores (Eco stores; ES) were represented by following selling points: ES1-Priroda i društvo,

ES2-Garden, ES3-Grga čvarak, ES4-Biovega (bio&bio), ES5-Eko Sever.
It was randomly chosen 5 of each selling point, where the samples were sampled in triplicate.
Information on carrot cultivation was obtained at the point of sale (insight into the declaration or oral communication with sellers). As carrot samples from retail chains did not have the label that they are organic products, it is assumed that their cultivation is conventional, which presupposes the use of mineral fertilizers in carrot cultivation. According to sellers, cattle manure was used for growing carrots collected at the Britanski trg and Dolac markets, mineral fertilizer was used for growing carrots collected at the Dubrava and Kvatrić markets, and sheep manure was used for growing carrots collected at the Branimirova market. Carrots, collected in organic products stores, are from organic farming, because only products of certified organic origin are sold in these stores.
Collected carrot samples were peeled and cleaned of rotten parts, after which they were chopped and ground. The juice was squeezed with a press, in which a soluble dry matter (% Brix) was determined using the digital refractometer, and the pH value of carrot juice was determined using the pH meter.
The samples, collected in triplicate, were analysed individually and the results showed average values. Statistical data processing followed the variance analysis model (ANOVA). The SAS System for Win program was used. version 9.1 (SAS Institute Inc.), and Tukey's significance threshold test (SAS, 2002-2003) was used to test the results.

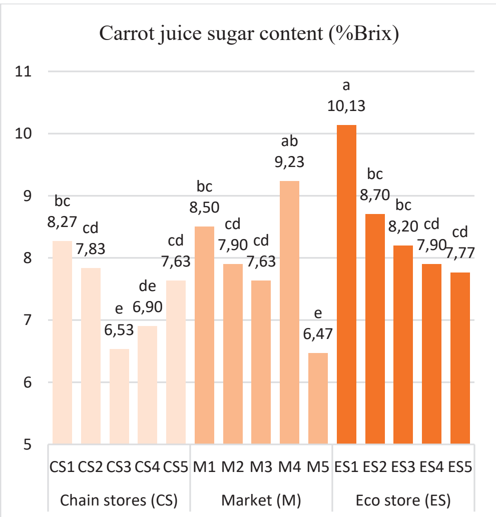
RESULTS AND DISCUSSIONS

Graph 1 shows the mean value of the soluble sugar (sugar) content in the analysed carrot juices according to sales channels, expressed in % Brix.
Statistically, the highest sugar content (8.54% Brix) was found in organic carrot juice, sampled in organic products stores, while statistically the lowest sugar content (7.43% Brix) was found in conventional carrot juice, sampled in chain stores.
Observing the Graph 2, it is determined the variation in the sugar content between 6.53 and 8.27% Brix in carrots from chain stores, 6.47 to 9.23% Brix in carrots from markets and 7.77 to 10.13% Brix in carrots from trade in organic products stores. The highest value was found in carrots sampled in the organic products store, Priroda i društvo (10.13% Brix), while the lowest value was found in carrots sampled at the Kvatrić market (6.47% Brix).
The obtained values match the literature citations looking at the mean value. The study by Gills et al. (1999) showed that depending on the carrot variety % Brix varies between 7.8 to 10.0, with the fact that the percentage sugar content is not directly related to the sweet taste of carrots. In one of the cultivars, which proved to be the sweetest in taste, a high % of Brix was found, but with the least sugar content. Significant differences existed in the perception of sweet taste as well as in the % Brix and the sugar content. However, the observed sweetness intensity was not related to % Brix or to the sugar content.
Da Silva et al. (2007) reported that % Brix, depending on the cultivars tested, ranged from 8.45 to 9.61. On the other hand, the research of Bender et al. (2009) showed that the difference in the total sugars content in conventional and organically grown carrots (6.5 and 6.0 % Brix) is not significant. Furthermore, Kaack et al. (2002) found 8.6 to 9.2% Brix in organically grown carrots and 5.0 to 9.1% Brix in carrots from conventional cultivation. The mean value in both cultivation methods was 8.9% Brix.
Graph 3 shows the mean pH of the juice of the analysed carrots according to sales channels. The mean pH of all juices is 6.63. Although the differences are not statistically significant, the relatively highest pH value is found in carrot juice from chain stores (pH 6.69), while the lowest value was found in carrot juices from organic products stores (pH 6.55).
Graph 4 shows all the obtained pH values where it can be seen that the pH of carrot juice sampled in chain stores varies between 6.49 and 7.01, sampled at markets 6.31 and 6.92 and sampled in organic products stores 6.17 and 7.02. The statistically highest pH value (7.02) was found in carrot juice purchased at Garden, an organic products store, while the statistically lowest pH value (6.17) was found in carrot samples purchased at the Grga Čvarak store, which is also an organic products store.

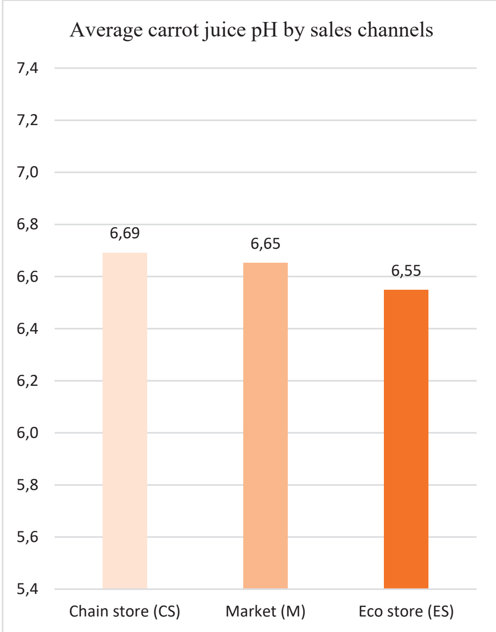
According to Gopalakrishnan (2007) due to low concentrations of organic acids, the reaction (pH) of carrots varies between 6 and 7. Da Silva et al. (2007) reported pH results ranging from 6.01 to 6.14, while in a study by Demir et al. (2007) pH was 6.2, and it is evident that the values determined from this study coincide with the literature data.



Graph 1. Average carrot juice soluble sugar content (%Brix) by sales channels
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different



Graph 2. Carrot juice soluble sugar content (%Brix) by selling points
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different



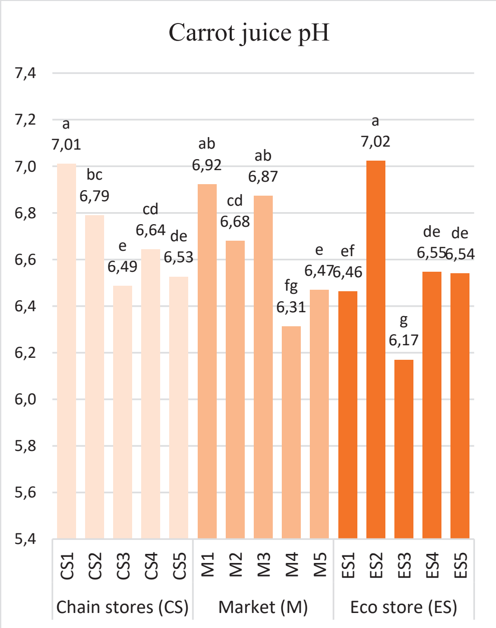
Graph 3. Average carrot juice pH by sales channels
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

CONCLUSIONS

The soluble sugar content (% Brix) in carrot juice ranged from 6.24 to 10.13% Brix (7.43 for carrots in chain stores, 7.95 for carrots at markets and 8.54 for carrots from organic products stores). According to the obtained results, it is visible that the juice from organic products stores has the largest soluble sugar content, while the lowest soluble sugar content was found in carrot juice from chain stores.

The reaction (pH) of carrot juices varied between 6.17 and 7.02 (6.69 in carrots from chain stores, 6.65 in carrots from markets and 6.55 in carrots from organic products stores). The obtained results show that the pH differences between the juices are not significant.

Regardless of the method of cultivation, the results showed that a slightly acidic to moderate juice reaction



Graph 4. Carrot juice pH by selling points
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.
(pH) is a property characteristic of carrots and that the soluble sugar content is a property that is related to the variety and method of cultivation

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INVESTIGATION OF THE SUBSTRATE WITH BIOCHAR ON THE DEVELOPMENT OF TOMATO SEEDLINGS

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Abstract

The purpose of the study was to determine the possibility of the application of Biochar as an additive substrate for seedlings growing. Three types of the substrate with woody Biochar were investigated. As a control, the peat and perlite (1:1) substrate was used. Some morphological signs (stem height, leaf number, leaf area, root volume, etc.) and physiological indicators of the seedling plants were determinate. The effect of biochar depends on both its quantity and the other components. The using of the substrate mixture with peat:perlite:biochar (1:1:1) provoke a positive effect on the development of the root system. The volume of the root system increased by 36.8% and the weight by 28.4% compared to the control. The physiological status of the plants has improved. The total chlorophyll content and the dry matter content in the plant organs increased.

Key words: *biochar, seedling mixture, tomato seedlings, morphological behavior of the plants.*

INTRODUCTION

The most widely used product in seedling production is a peat. The growing uses of peat in horticulture poses a serious danger of its rapid depletion, as a non-renewable resource. In connection with this, a global movement has been created to achieve sustainable use of peat and intelligent operation of wetlands. Efforts are being made to find and use sustainable peat substitutes that meet the specific requirements of plants, are easily available in sufficient quantities and, of course, are cost-effective (Fascella, 2015). A number of studies have been conducted related to the use of biochar in agriculture as a soil improver and as an alternative substitute for peat in seedling production. It is considered a potential soil substitute worldwide (Glaser et al., 2002; Lehmann et al., 2003). Biochar is a highly porous black material produced by pyrolysis of biomass under oxygen deficient conditions (Brewer & Brown, 2012). Pyrolysis is the thermal decomposition of hydrocarbons in organic biomass at a temperature in the range (450-500^{0}C) in the absence of oxygen. It is carried out by indirect heating of the biomass in an airless environment, or by its partial combustion with limited supply of air or pure oxygen. The most traditional product of pyrolysis is bio-oil, syngas, and charcoal (Manolova, 2014; Benev, 2008; Laird, 2008). Pyrolysis can be subjected to biomass from agricultural crops, forestry waste, sawdust (Chan et al., 2007).

In addition to playing a role in the uptake of carbon into the soil, biochar serves as a soil improver. Increases the pH of acidic soils because it has an alkaline pH (Yamato et al., 2006; Chan et al., 2007), increases cation exchange capacity in organically poor soils (Silber et al., 2010), increases nutrient retention in light soils (Glaser et al., 2002), improves their uptake by plants and helps to increase beneficial soil microorganisms (Kolb et al., 2009; Kolton et al., 2011). Biochar added to the soil increase the concentration of nitrogen and phosphorus by adsorption, helping to prevent their loss, leading to mineralization of organic N and P by activating microbial activity and promoting of root growth (Gul & Whalen, 2016; Gul et al., 2015,). This in turn leads to the degradation of P by the production of organic acids by the roots and rhizosphere microorganisms.

Weeds are a serious competitor of agricultural crops in terms of certain vegetation factors (Yanev, 2020; Yanev, 2015). Weeds losses are higher than other factors including animal pest, fungal and bacterial pathogens and viruses which caused 16%, 18% and 20% yield loss, respectively (Oerke, 2006). Carbonaceous materials have affect sorption, degradation, and bioavailability of pesticides to plants (Hilton and Yuen, 1963). According to Laird (2008) application of charcoal to soils is reduce leaching of pesticides and nutrients to surface and ground water.

Opinions on the application of biochar in seedling production are contradictory. According to Frenkel (2017) biochar have mainly positive or neutral effects on plant growth compared to peat media when they are in concentrations higher than 25% of volume of mixture. According to other authors, the biochar used as a substitute for peat and used in high concentrations may pose a latent risk as it may weaken plant protection or lead to the predisposition of roots to pathogenic attack (Jaiswal et al., 2015).

MATERIALS AND METHODS

The experimental work was done during the 2018-2019 in Agricultural University - Plovdiv, Bulgaria. Biochar of oak (*Quercus robur)* was added to a commercial potting substrate of peat and perlite to obtain three type of seedling mixture: peat:perlite:biochar in ratio 1:1:1; peat:biochar - 1:1; perlite:biochar - 1:1. The peat is from the company Durpeta from Lithuania and is factory enriched with N-250 mg/l, P2O5 - 270 mg/l, K2O-270 mg/l and Fe, Cu, Mn, B, Mo, Zn - 1.2 mg/l. The salt concentration of peat, measured in ms/cm, is 1.2, and the pH is 5.5-6.5.The seed of 'Aleno sarce' tomato variety were sown into 40 cells trays containing one of the investigated mixtures. For the control was used peat:perlite mixture (1:1). The seedlings were grown for 45 days. During the period of growing seedlings, care was applied according to the physiological condition and the degree of growth of seedlings. In both years, repeated feeding of seedlings with water-soluble fertilizers was applied.

On the end of the seedling period was measured some biometric indicators - stem height and thickness (cm), leaf number, leaf area (cm^2) by Konyaev (1970), shoot fresh and dry weight, root fresh and dry weight and root volume. It was determinate dry matter content (g) (Manuelyan, 1966). The total chlorophyll content was determined by Chlorophyll Meter SPAD-502. The mathematical processing of the data was done by standard program BIOSTAT.

Figure 1. Tomato plants grown in substrate with biochar

RESULTS AND DISCUSSIONS

Seedling growth parameters of tomato grown in growing media with biochar are better or worse compared to control. Shoots parameters of the plants in all investigated variants are smaller compared to the control. The stem height (Table 1) of the plants grown in a substrate containing biochar is from 13.6 cm to 20.6 cm, while in the control is 26.4cm. Biochar has a negative effect on the size of the leaf area. Plants grown in a substrate containing biochar have a leaf area of 1,54 to 4.84 times lower compared to the control. The plants grown in biochar and perlite (variant 4) are most affected - 66.89 cm^2. Comparable to the variant with the same percentage of biochar (var. 3), the leaf area is twice lower, despite the same number of leaves. The leaf area is greatest in plants grown in a substrate containing peat: perlite and biochar. A summary indicator of the vegetative growth of the seedlings is the shoot fresh weight. The value of this indicator, depending on the investigated mixture is from 4.63 (4) to 8.80 (2) g., compared to control - 11.51 g. The volume and weight of the root system are essential for the quality and vitality of seedlings. Despite the reported advantage of control on shoot parameters, the reported values for volume and weight of the root system are inferior to some of the variants with biochar. In variants with peat:perlite:biochar and peat:biochar, the volume and weight of the root system exceed the control by 23.3% and

36.8%, for the first indicator, and for the second the excess is by 10.2% and 28.4%
The higher values refer to variant 2 (peat:perlite:biochar), and the differences are statistically proven. The plants grown in perlite:biochar are distinguished by the least developed root system.

Figure 2. Tomato plants grown in substrate with biochar

Although the participation of biochar in the tested mixture in variant var.4 does not differ from the mixture in variant 3 (50% of the mixture), the root system of the plants develops much less. The volume of the root system is twice smaller and the weight is reduced by over 40%. Obviously, the effect of biochar on plant growth depends not only on its percentage application, but also on the type of other components in the mixture. Overall, the studies reported that application rates of biochar into the substrates under 25% by volume generally resulted in similar or higher plant growth compared to the referential commercial substrate (Huang L, 2019). According to Dumroese et al. (2011) the high content of biochar (over 50% relative to peat) can lead to undesirable changes in the structure of the substrate, increases the density, reduces the water holding capacity of the substrate, and the C: N ratio is much higher compared to a mixture containing 75 % peat and 25% biochar. The reason for the obtained results may be due to changes of this nature. The effect of biochar is changes when used in combination with perlite. All indicators concerning the morphological development of seedlings have the lowest values when using this type of mixture.

Table 1. Biometric indicators of plant, average for the period 2018/2019

Indications/Variant		Peat:perlite 1:1	Peat:perlite:biochar 1:1:1	Peat:biochar 1:1	Perlite:biochar 1:1	LSD 0,5
Stem height		26,4	20,6	17,7	13,6	2,58
Stem thickness (mm)		47,7	43,7	43,7	37,3	-
Leaf number		7,8	7,8	7,4	7,4	
Leaf area	cm^2	324,05	210,2	156,4	66,89	56,27
	%	100,0	64,9	48,3	20,6	-
Shoot fresh weight	g	11,51	8,80	7,30	4,63	1,63
	% to the control	100,0	76,5	63,5	40,2	-
Root volume	cm^3	2,81	3,85	3,47	1,69	1,02
	% to the control	100,0	136,8	123,3	60,1	-
Root fresh weight	g	2,75	3,53	3,03	1,78	0,54
	%	100,0	128,4	110,2	64,7	-
Shoot:root ratio		4,18	2,49	2,41	2,60	

The coefficient for all plants grown in a mixture containing biochar is lower than the control. The lower value of the coefficient indicates more harmonious combination in biological terms and is a prerequisite for better adaptation and recovery after planting in a permanent place. This means that the differences between the compared biometrics are smaller and the plants are more harmoniously developed. Changes in the morphological characteristics of seedlings are accompanied by changes in their physiological status (Table 2). The average results show that the shoots dry weight on the variants with biochar is from 0.515 to 1.255 g, in the case of the reported for the control plants - 1.475 g. The amount of shoot dry matter content is an important indicator that reflects the productive potential of young plants, and its higher content shows greater physiological potential of young plants and is a prerequisite for better adaptability to environmental factors in which they will fall after planting them in a permanent place.

Table 2. Dry weight and dry matter content of plants

Indicators / Variants	Period	Peat:perlite 1:1	Peat:perlite:biochar 1:1:1	Peat:biochar 1:1	Perlite:biochar 1:1
Shoot dry weight, g	2018	1,51	1,18	0,56	0,51
	2019	1,44	1,33	0,92	0,52
	average	1,475	1,255	0,74	0,515
Shoot dry matter content, %	(%)	12,81	14,26	10,14	11,12
	% to the control	100,0	111,3	79,1	86,8
Root dry weight, g	2018	0,21	0,31	0,23	0,15
	2019	0,31	0,55	0,41	0,21
	average	0,26	0,43	0,305	0,18
Root dry matter content, %	(%)	9,45	12,18	10,07	10,11
	% to the control	100,0	128,9	106,6	107

The higher shoot dry matter content can be used like a criterion for greater biological and physiological potential and a prerequisite for their faster recovery after planting (Shopova, 2014). The shoot dry matter is highest in variant 2 - 14.26%. The control plants also have an advantage in this respect. The root dry matter, in the variants containing biochar and peat (var. 2 and var. 3) as well as the amount of root dry matter content are higher than those reported in the control. The root dry matter content in variant 3 exceeded the control by 6.6%, and in variant 2 by 28.9%. In variant 4, a higher dry matter content was reported compared to the control by 7%, despite the significantly lower mass of the root system.

The results for the content of total chlorophyll in the leaves (Table 3) complement the characteristics of the seedlings. The data show that the total chlorophyll content for the experiment is between 33.8 to 42.7 SPAD units. The reported value in the control is 38,5 SPAD units. The content of total chlorophyll is highest in the plants of variant 2 , as the excess over the control is 10.9%. When using biochar in combination only with peat or only with perlite, the plants react differently in terms of chlorophyll content in the leaves. Despite the equal participation of biochar, in combination with peat the chlorophyll content increases, while in combination with perlite - decreases. In the second case, the decrease compared to the control is by 12.2%. Some authors (Akhtar et al., 2014) reported a significant reduction in chlorophyll content due to a reduction in nitrogen content in the leaves of plants grown in a substrate containing only biochar. In combination with peat, a favorable C: N ratio is observed in the substrate, which makes the available nitrogen readily available to plants (Scherer et al., 1996). Despite the presented results, other authors report that there is no difference in chlorophyll content in tomato plants when using a mixture containing pyrolysis residues in combination with compost and a mixture containing pure compost (Akhter et al., 2015).

Table 3. Content of total chlorophyll, average for the period 2018-2019

Variant	Content of total chlorophyll	
	SPAD	% to the control
peat:prelite 1:1		
1.	38,5	100,0
Peat:perlite:biochar 1:1:1		
2.	42,7	110,9
Peat:biochar 1:1		
3.	41,2	107,0
Perlite:biochar 1:1		
4.	33,8	87,8

CONCLUSIONS

Biochar, included in standard seedling mixture change the behaviour of the seedling plants. Its most effective when used as a component of a seedling mixture containing peat and perlite (1:1:1). The effect of biochar is most pronounced on the development of the root system. In combination with peat or with peat and perlite, the biochar leads to improvement of the physiological status of the plants. The content of total chlorophyll increases, as well as the amount of dry matter content in the plant organs. The use of biochar in combination with perlite has negative effect on the morphological and physiological development of tomato seedlings, growth is inhibited and the amount of chlorophyll is reduced.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Agricultural University - Plovdiv, Bulgaria, Department of Horticulture.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECT OF USING ORGANIC FERTILIZERS ON LETTUCE TYPE 'LOLLO ROSSO' UNDER OUTDOOR METEOROLOGICAL CONDITIONS

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Abstract

This paper presents the results of an experiment related to cultivation of red-leaf lettuce type 'Lollo rosso' variety 'Tuska' outdoors, in transition to organic production. The experiment has been conducted in field conditions on the premises of the Agricultural University in the region of Plovdiv. During the lettuce growth several variants were tested: 1. NPK (mineral fertilization); 2. Control (non-fertilization); 3. Italpollina; 4. Arkobaleno; 5. LC (Lumbricompost); and 6. Ekoprop NX. The effect of the different organic fertilizers has been studied through the main biometrical parameters. Statistically significant differences have been reported for the most important indicators - fresh mass weight g and number of leaves with highest values observed in variants 6. Ekoprop NX and 4. Arkobaleno. Organic fertilization successfully replaces the fertilization with a combined fertilizer (NPK) under the different hydrothermal conditions of the experimental period.

Key words*: Lettuce (Lactuca sativa), organic fertilization, climate change, hydrothermal coefficient, vegetative behaviour.*

INTRODUCTION

Organic farming helps to improve the health of the agro-ecosystem through its holistic approach, using agronomic, biological and mechanical methods on the farm and excluding all types of synthetic raw materials (Patle et al., 2020). It is defined as a unique production management system, which cares for the environment, including biodiversity, biological cycles and soil resources (Lester, 2006; Oberč et al., 2020; Duru et al., 2015; Dall'Asta et al., 2020). Its benefits for human health are undeniable (Shafi and Rennie, 2009) at a time when food is a major factor. In conditions of climate change and fluctuations, more and more often this direction in plant growing falls into the focus of modern scientific research with tendencies to increase the share of the fields for organic farming. The relationship between the environment and food production and the future development of agriculture is discussed (Margat and Vallée, 2000; Funk and Brown, 2009; Aggarwal, 2008) as well as its economic profitability over time. Serious attention is paid to the excessive use of chemical fertilizers, plant protection products and pesticides on the ecosystem (Sharma and Singhvi, 2017; Bourguet and Guillemaud, 2016; Lichtfouse, 2016). Emphasis is placed on the benefits of organically produced food over conventionally grown products (Forman et al., 2012; Tuomisto et al., 2012; Seufert et al., 2012; Meier et al., 2015). People nowadays are turning to sustainable farming methods with good yields and minimal impact on the environment (Horrigan et al., 2002; Jensen et al., 2020; Tuomisto et al., 2012). For products, intended only for fresh consumption and participating in many dietary programs, such as lettuce, purity of production and avoidance of fertilizers is of great importance. Lettuce is an economically important crop for small and medium-sized producers. When grown in adverse environmental conditions, lettuce is vulnerable to deterioration in yield and quality. Leaf salad variety Lollo Rosso is red in color, rich in carotene, with a slightly bitter taste and useful substances. Among the different varieties of lettuce, red coral lettuce shows the highest total antioxidants and antioxidant activity. It is most often used for decoration and as an addition to other types of leaf salads, as well as in combination with local types of food, which determines its small and standard sizes compared to other types of cold-resistant leaf and head salads. In Bulgarian weather conditions it is grown mainly in pre-winter time in unheated facilities, and in the open air in early spring, when the temperatures are still lower and sunshine less (short days). In recent years, increase in the air temperature and changes in the course and distribution of the precipitation in different regions of Bulgaria have been registered by a number of authors (Georgieva et al., 2017; Marinova et al., 2017; Alexandrov and Shopova, 2020). The observed climatic fluctuations in the study area over the last three decades have affected the conditions of growth and development of spring plants, and of the leaf lettuce in the open air, in particular. This study analyzes the influence of different fertilization technologies on the development of the Lollo Rosso leaf lettuce, grown outdoors under the hydro-thermal conditions of the experimental period 2018-2020 through its basic biometric indicators.

MATERIALS AND METHODS

The experiment was conducted on the experimental field of the Agricultural University of Plovdiv in 2019-2020 on alluvial meadow soil (Mollic fluvisol, FAO 2006). The red lettuce plants (type 'Lollo rosso') were planted on 12th of April in 4 rows according to the scheme 70+30+30+30/30 cm with a profile of the soil surface a high level bed (100+60cm). The experiment was based on the block method with four repetitions, using 28 plants per repetition, and a plot size of 3.36 m2. Organic seeds were provided for seedling production using container technology with 150-hole Styrofoam boards in the following combination - organic seeds - 80.0 %, Perlite - 20.0 %, Lumbricompost for bioproduction of seedlings (Kostadinov and Filipov, 2013). Several variants were tested: 1. Control (non-fertilization); 2. NPK (mineral fertilization); 3. Italpollina; 4. Arkobaleno; 5. LC (Lumbricompost); and 6. Ekoprop NX. The granular fertilizers were introduced as basic fertilization, with soil pre-transplantation at the following norms: N-12.5kg/da, P2O5-1.25 kg/da, + K2O-4.75 kg/da, Italpollina-25 kg/da, Arkobaleno - 100 kg/da, and Lumbricompost - 400 l/da. The liquid bio fertilizer Ekoprop NX was applied by double treatment in a dose of 100 g/da, before planting - in the 5th leaf seedling phase, and 10 days later on, after the adaptation to the soil. The remaining bio fertilizers are granulated and introduced into the soil before the last tillage and before planting the seedlings. The biometric measurements were taken three times at one-week intervals in stage-typical leaf mass reached. Meteorological observations from Plovdiv AU (Agricultural University) station (42 ° 14′N, 24 ° 75′E and 162 m above sea level) were used for the outdoor temperature analysis. The data were collected according to the recommendations of the WMO (World Meteorological Organization) and are comparable with the climatic norm for the region. The daily air temperature was recorded by means of a weather station Meteobot® Pro (https://meteobot.com/). For analysis of the hydro-thermal conditions, the hydrothermal coefficient of Selyaninov (1928), was calculated according to the formula: HTC = ΣR /0.1*ΣT, where ΣR is the sum of the rainfall in mm, and ΣT is the sum of the temperature for the months (IV-VI) with an average temperature above 10°C and classification: <0.4-extremely dry; 0.41-0.7 very dry; 0.71-1-0 dry; 1.01-1.3 quite dry; 1.31-1.6 optimum; 1.6-2.01 quite wet; 2.01-2.5 wet; 2.51-3 very wet. A statistical analysis was conducted using analysis of variance for the main effects; the means of the values were compared with the least significant difference (p = 0.05).

RESULTS AND DISCUSSIONS

According to some Bulgarian and foreign authors (Kartalov et al., 2007; Lorenz and Maynrad, 1988), the optimal temperature for lattuce development is 16.0-18.0°C. Well-rooted plants in phenophase 7-9 leaves can withstand temperatures down to minus 6.0°C (Divina, 2016; Cholakov, 2009). The range of development of the species is between 5.0°C and 25.0°C and below 5.0°C, the growth slows down and stops (Cholakov, 2009). Other authors (Kristensen et al., 1987; Morgan, 1999) consider the temperature of 4.0°C to be biological minimum. The degree to which lettuce can tolerate adverse temperatures varies considerably depending on the variety. Growing the lettuce outdoors during the three years of the experiment was carried out in different weather conditions. The moment of planting, according to the agro-technical terms, is in the middle of April and the plants adapt to the outdoor conditions for a week. In 2018, the planting and adaptation of the young plants was carried out in an extremely warm month, with an average temperature of 16.4°C and a positive deviation of 4.2°C (Table 1).

Table 1. Amount of precipitation, temperature and deviation compared to the period 1961-1990

Months/years	*av. t°C*	*δ t°C*	*max. t°C*	*date*	*min. t°C*	*date*	Σmm.	*Q/Qn*	*max.Σmm.*	*date*
April 2018	16,4	4,2	30,5	24	0,4	3	25	59	15	7
May 2018	19,2	2,1	30,2	5	10	3	58	93	17	27
June 2018	22	1,1	33,2	22	11,4	1	119	220	37	29
April 2019	12,6	0,4	27	26	-1,2	3	61	145	21	9
May 2019	18,4	1,3	32	28	2	9	22	34	6	31
June 2019	23,4	2,5	34,2	8	10,6	4	197	364	79	3
April 2020	11,5	-0,7	27,8	18	-2,5	8	90	215	37	5
May 2020	17,6	0,5	32	8	5,4	8	71	108	13	1
June 2020	21,5	0,6	34,7	30	11,3	4	54	101	14	16

In the second year, this period occurred at an average temperature close to the norm (12.6°C) and a positive deviation of 0.4°C, and in 2020 it took place in a colder April (11.5°C) with a negative deviation of (-0.7°C). Lettuce is a cold-resistant crop and the extremely warm April in 2018 was an unfavorable factor for its adaptation and initial development.

In general, during the three experimental years, extremely warm, close to the norm and cool conditions of development were observed. In April 2018, the amount of precipitation was 25 mm, less than usual, with the highest precipitation of 15 mm registered about 10 days before implantation - on 7.04. In the second year the amount was 61 mm, more than the norm, and there were conditions for appearance of an additional stress factor from over wetting. In April 2020 the rainfall was also extremely high - 90 mm, and a single amount of 37 mm fell down 5 days before planting the plants in the soil. During the months of active vegetation, of reaching economic maturity and harvesting in 2018, the temperature conditions were warmer than usual, May-19.2°C with a positive deviation of 2.1°C and June-22°C with a positive deviation of 1.1°C. In 2019, the development after adaptation in the soil and outdoor conditions took place at elevated temperatures with a deviation of 1.3°C for May and 2.5°C for April and May, insufficient rainfall in May and extremely wet June. During the last experimental year the vegetation took place at temperatures close to the norms: May - 17.6°C, June - 21.5°C; and excessive rainfall: May - 71 mm; June - 54 mm, which created conditions for deteriorating product quality, although to a lesser extent compared to 2019 (table 1). The lowest negative temperatures were registered on April 8, 2020 and April 3, 2019: -2.5°C and -1.2°C, respectively, before planting the seedlings. The maximum temperatures exceeded 33.0°C in 2018 and were slightly below 35.0°C in 2020.

The daily average air temperatures had the highest values in 2018 (Figures 1 and 2).

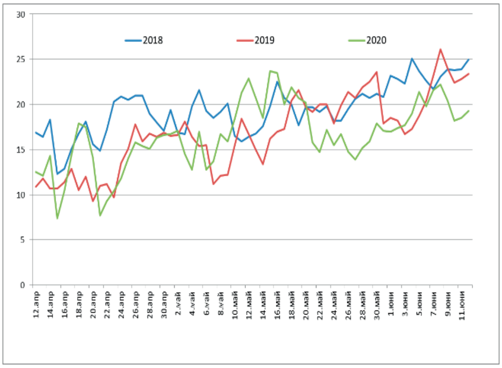

Figure 1. Daly air temperature (t°C), 2018-2020

Lettuce is a cold-resistant vegetable and the warmer weather conditions inhibit its development. The highest productivity and the best biometric indicators were observed for all variants in 2019 - the second year of the study.

During the vegetation until reaching economic maturity, the thermal conditions were closest to the requirements of the species and the warmest period was the one between the first and last harvest, which does not affect the growth of the lettuce, but can affect its taste and qualities. In the third year, the yield values were close, but slightly lower. High temperature amplitudes were observed during the period of planting and adaptation of the plants. In the period of growth and increase of the leaf mass the temperatures were the highest compared to the first and second year, being the lowest between the first and the last harvest.

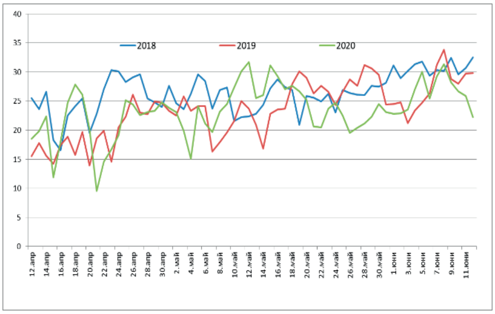

Figure 2. Daly max. temperature (t°C), 2018-2020

The Hydro-thermal Coefficient of Selyaninov, 1928 (HTC, Table 2) was calculated to characterize the humidification under natural conditions.

Table 2. Selyaninov's HTCoef., 2018-2020

Year	month	Selyaninov's HTCoef.	Classification
2018	April	0.51	very dry
2019	April	1.61	quite wet
2020	April	2.61	very wet
2018	May	0.97	Dry
2019	May	0.39	extremely dry
2020	May	1.30	optimum
2018	June	1.80	quite wet
2019	June	2.81	very wet
2020	June	0.84	dry

There were dry periods during adaptation and initial development in April 2018, during the period of accumulation of leaf formation in 2019 and when reaching economic maturity and harvest in 2020. Conditions for stress from overwetting were reported in April 2020, June 2019 and June 2020. In terms of climate, the area of the experiment has values <1.0, and during the experiment extreme manifestations of the weather with high frequency and intensity were observed, which is characteristic of the changes and fluctuations of the climate in the last decades.

Regarding the biometric measurements, the influence of both the combined synthetic fertilizer NPK and of the organic fertilizers on basic parameters such as the number of leaves, the weight of fresh mass g, the diameter of rosette cm, etc. was studied. Both statistically significant differences with respect to the control variants (Cheng-Wei Liu et al, 2014; Alisson Franco Torres da Silva et al, 2017) and higher but not significant ones (Reis et al., 2013; Reis et al., 2014) have been reported, according to the type and concentration of the used fertilizers.

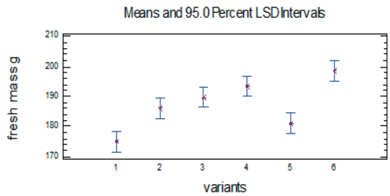

Figure 3. Statistical results - fresh mass weight (g) 29.05

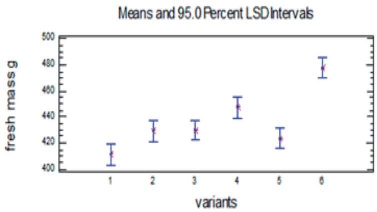

Figure 4. Statistical results - fresh mass weight (g) 12.06

In the recent years, research on the effect of fertilization has aimed at developing a strategy for production of healthy salads with low content of nitrates.

The results of previous studies show that providing lettuce with a combination of organic fertilizers and liquid fertilizers is superior in terms of soil quality, appearance, and the accumulation of nitrate (Cheng-Wei Liu et al., 2014).

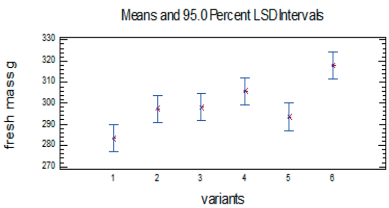

Figure 5. Statistical results at average values of the fresh mass weight

There are many varieties of lettuce in a huge range of colors, leaf shapes and sizes for almost any look. There are also burgundy or red leaved types like, and numerous types mottled or variegated in shades of green and red. Leaf shape varies with choices of smooth edged or deeply indented, such as oak leaf types, and flat or frilly. 'Lollo Rosso' type plants are characterized by a moderate growth rate and development and form a smaller vegetative mass, which can also be seen from the measurements of the 'Tuska' variety. In this three-year experiment, the influence of different fertilization technologies was verified by means of the basic biometric data on key indicators such as fresh mass weight and number of leaves formed outdoors in different weather conditions. The results show that the plants have an average weight of 299.43 g, varying from 171.16 g. at the first measurement up to 487.05 g. at the third. The data show the highest values for variant 6 Ekoprop NX, followed by variants 4 and 2. All organically fertilized variants are superior over the non-fertilized ones, with the exception of variant 5 and also variant 2 NPK. The reported differences are statistically significant for the first and third measurements, as well as for the average value of the fresh mass weight (Figures 3, 4 and 5).

Similar results were found regarding the influence of fertilization on the number of formed leaves. It varied from 22 at the first measurement to 38, and on average the plants had 29 leaves. The smallest number of leaves had the unfertilized variant - 28.51, and the others – as it follows: 2- 29.45 3-29.39; 4-29.67; 5-29.20756; 6- 30.35.

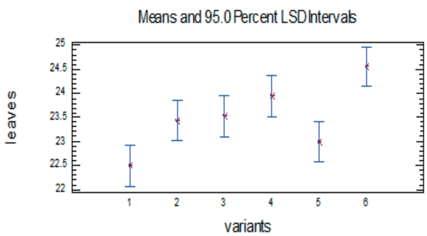

Figure 6. Statistical results - number of leaves 29.05

Variant 6 was with the largest number of leaves (with two more leaves), and all organic fertilization variants are superior both to the control one and to the synthetic fertilizer variant 2. The analysis shows statistically significant differences in this indicator as well (Figure 6). During the three years, the analysis of the biometric data showed a stable superiority of the biofertilized variants over the non-fertilized ones, variants 4 and 6 being superior over the NPK-fed variant as well.

CONCLUSIONS

Basic meteorological parameters such as air temperature and precipitation for the period 2018-2020 in the region of Plovdiv were measured and analyzed. The development of the plants in the considered variants took place in an extremely warm, a close to the norm and a slightly cooler spring. The hydrothermal conditions varied in a wide range, with HTC between extremely dry (0.34) and very wet (2.81) and there were prerequisites for deterioration due to over wetting. The three years of the experiment were characterized by the following values: HTC 2018 quite dry 1.05; 2019 wet 2.37; 2020 optimum 1.31. During the period extreme manifestations of the weather, characteristic of the climate changes in the last decades, were registered. This was also one of the reasons for studying outdoor organically grown lettuce as a method of adapting crop production to the current conditions. The biometric data on its fresh mass weight, rosette diameter and number of leaves were similar and stable over the three years of the experiment. Statistically significant differences in the weight of the fresh mass g and the number of leaves were reported, compared both to the control and to the NPK mineral fertilization variant, with the best indicators being with the variants fertilized with Arkobaleno and Ekoprop NX. The yields were highest in the second year, when the temperature conditions were closest to the norms, and lowest in the last experimental year. The organically grown plants consistently outperformed both the unfertilized plants and the synthetically fertilized variant, with the exception of the variant number 5 LC. The results show that widespread use of organic production of 'Lollo Rosso' lettuce in spring outdoor planting can be recommended, having in mind that the best indicators are reported for variants 4 and 6 - Arkobaleno and Ekoprop NX. It is possible to comment on the continuation of the research with higher concentrations of organic fertilizers, needless to say with consideration of nitrate content accumulation in the leaves of the 'Lollo Rosso' salad.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Centre of research, technology transfer and protection of intellectual property rights at the Agricultural University-Plovdv.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF THE MAIN QUANTITATIVE AND QUALITATIVE CHARACTERS FOR THE 'MAURA 2000' CLIMBING BEAN CULTIVAR

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Abstract

The 'Maura 2000' climbing bean cultivar was obtained at VRDS Buzau and patented in 2015. The cultivar was introduced into the culture and submitted to the conservative selection program in order to restrict the variability of the main characters, correlated with increasing production quality and productivity. 'Maura 2000' shows indefinite growth, a vigorous stem, with a linear appearance that has 4-6 main branches and a height between 2-2.6 m. In greenhouses, the height increases significantly. The stem and foliage are medium green, the flowers are white - yellow grouped 8-10 in raceme, linear, arranged in pairs. The pods are yellow, slightly pearly, have a length between 18-22 cm, width 1.8-2 cm and a thickness of 0.5-0.7 cm. They are straight, slightly arched towards the top. The pods forms threads late, after the optimal harvest period has passed. The seeds are medium-sized, single-colored, white, kidney-shaped. MMB = 355,654. The aim of the study was to present the phenotypic expressiveness of the main characters manifested by the cultivar in the fields of conservative selection.

Key words*: conservative selection, patented, Phaseolus vulgaris, VRDS Buzău.*

INTRODUCTION

Beans (*Phaseolus vulgaris*) are the member of the *Leguminosae*, family *Phaseoleae*, subfamily *Papilionoideae* and is the most important food legume for more than 300 milion people (Bressani, 1993; Graham and Ranalli, 1997). Common bean (*Phaseolus vulgaris* L.) have been recognized to be domesticated and originated in America on the basis of chronological, archeological, botanical and linguistic evidences (Gepts and Dpbouk, 1991; Papa and Gepts, 2003; Papa et al., 2005) and now consumed in every part of this globe especially by the people of low income group in the developing countries (Shimelis and Rakshit, 2005). Most often, beans are mainly used as dry seeds but there is possibility of their use as green pods as well as green shelled seeds (Lin et al., 2008). The important classes of dry beans include haricot beans (Shimelis and Rakshit, 2005), red kidney beans (Choung et al., 2003), black beans (Aparicio-Fernandez et al., 2006), Mexican beans (Hosfield et al., 2004), pinto beans, tirga beans (Amir et al., 2006), great northeren beans, navy beans and pink beans (Kelly et al., 2003; Luthria and Pastor-Corrales, 2006).

Nutritionally, beans are recognized as good source of proteins, which is 2-3 times that of cereal grains, (Siddiq et al., 2010). High dry matter content also contains high amounts of starch, dietary fibres, minerals and vitamins (Kutos et al., 2003; Costa et al., 2006). In addition to these, beans also contain rich variety of phytochemicals, antioxidant activity and an extensive array of flavonoids such as anthocyanins, flavonoids, proanthocyanidins, flavonols, phenolic acids and isoflavones (Beninger and Hosfield, 2003; Choung et al, 2003; Aparicio-Fernadez et al., 2005b; Lin et al., 2008; Granito et al., 2008). In beans, there are two varieties according to the type of stem growth: *nanus* convariety - having a determined growth, the stem is straight, branched and forms a dense bush, of small heights 40-60 cm (dwarf or oyster beans); conv. *vulgaris* - indeterminate growth, voluble stem, having lengths of 2-6 m (climbing beans). An essential feature of bean cultivation is the existence of a wide range of varieties that allows staggered production, but also the rhythmic supply over a long period of time.

In Romania, the area cultivated in 2019 with green beans is 5380 ha with a production of 26,690 tons.

Worldwide the largest cultivated area with green beans in 2019 is occupied by China, more precisely 743143 ha, with a production of 21761383 tons, followed by India 256271 ha with a production of 725998 tons (FAOSTAT).

VRDS Buzău has a number of approved bean of 6 approved bean varieties, of which: one variety is French Bean (*Phaseolus vulgaris* L.) - 'Doina'; two varieties are Climbing French Bean (*Phaseolus vulgaris* L. var *vulgaris* L.) - 'Maura' and 'Teodora'; four varieties Dwarf French Bean (*Phaseolus vulgaris* L. var. *nanus* L.) Aschers - 'Anisia', 'Clarisa', 'Ioana' and 'Menuet'.

MATERIALS AND METHODS

The Laboratory of Genetics, Breeding and Biodiversity from VRDS (Vegetable Research Development Station) Buzau has a valuable germplasm collection of *Phaseolus* sp. having 418 genotypes. Of these, 285 are stable and 133 are segregating. These were structured on use groups and stages of improvement as follows:

STGD-Stable genotypes with determined growth for pods

SEGD-Segregated genotypes with determined growth for pods

STGI-Stable genotypes with indefinite growth

SEGI-Segregated genotypes with indefinite growth

STGDSD-Stable genotypes with determined and semi-determined growth for dry grains

SEGDSD-Segregates genotypes with determined and semi-determined growth for dry grains (Figure 1).

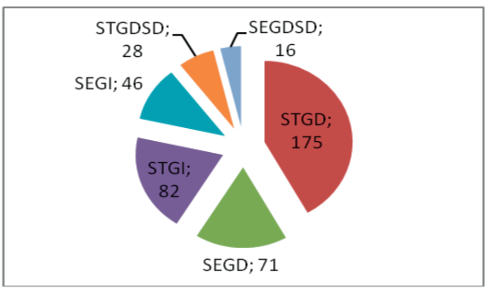

Figure 1. Germplasm collection from VRDS Buzău

The aim of this paper is to evaluate the main phenotypic and quantitative characteristics of the 'Maura 2000' climbing bean variety, in the fields of conservative selection.

Statistics indices used for each character were: the average ($\bar{x}$), standard deviation (SD), coefficient of variation (CV %), limits of variability ($\bar{x} \pm$ SD).

From the two varieties of climbing beans that SCDL approved, 'Maura 2000' was chosen for the present study.

Phenological, biometric and laboratory measurements were carried out during the vegetation period.

The experiment was conducted in the research site of VRDS Buzau in a sandy-loam soil.

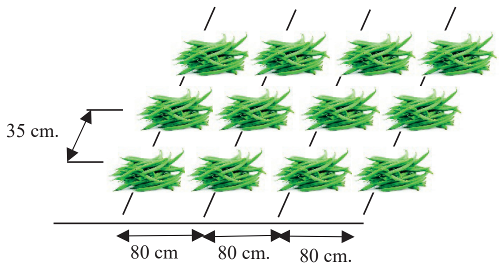

Figure 2. Planting scheme

The crop technology applied was the one specific for bean. The seed were sown at the beginning of May, the scheme used was 80 x 35cm (Figure 2).

The plants were grown in fenced system. Throughout the vegetation period a sets of 22 qualitative and 11 quantitative descriptors showing continuous variation were selected from the available literature on the crop, UPOV Guidelines.

Similar studies were made by us in our previous studies (Tănase et al., 2020; Gherase et al., 2020).

The quantitative characters analyzed were:, terminal leaflet (length of tip), flower (size of bracts), pod length, pod width, pod thickness, pod (length of beak), seed weight, seed (width in cross section), seed length, number of pods per plant, weight of pods per plant at maturity of consumption.

The qualitative descriptors targeted in the study were made on pod characteristics and plan (Tables 1 and 2).

Table 1. Qualitative traits plant

Descriptors	Polymorphism
Plant: anthocyanin coloration of hypocotyl(PACH)	1.absent 9.present
Plant: intensity of anthocyanin coloration of hypocotyls (PIACH)	3.weak 5.medium 7.strong
Plant: growth type (PGT)	1.dwarf 2.climbing
Plant: architecture(PA)	1.pyramidal 2.rectangular
Plant: start of climbing (80% of plants) PSC	3.early 5.medium 7.late
Plant: speed of climbing (PSpC)	3.slow 5.medium 7.fast
Leaf: intensity of green color (LIGC)	1.very light 3.light 5.medium 7.dark 9.very dark
Leaf: rugosity (LR)	1.absent or very weak 3.weak 5.medium 7.strong 9.very strong
Terminal leaflet: shape (TLS)	1.triangular 2. triangular to circular 3.circular 4.circular to rhombic 5.rhombic
Flower: color of standard (FCS)	1.white 2. pinkish white 3.pink 4. violet
Flower: color of wing (FCW)	1.white 2. pinkish white 3.pink 4. violet

Table 2. Qualitative traits pods

Descriptors	Polymorphism
Pod: shape in cross section (through seed) PSCS	1.eliptic 2.ovate 3.cordate 4.circular 5.eight-shape
Pod: ground color (PGC)	1.yellow 2.green 3.violet
Pod: intensity of ground color (PIGC)	3.light 5.medium 7.dark
Pod: presence of secondary color (PPSC)	1.absent 2.present
Pod: stringiness of ventral suture(PSVS)	1.absent 9.present
Pod: degree of curvature (PDC)	1. absent or very slight 3.weak 5.medium 7.strong 9.very strong
Pod: shape of curvature (PShC)	1.concave 2. s-shaped 3. convex
Pod: shape of distal part (excluding beak) PSDP	1.acute 2. acute to truncate 3.truncate
Pod: curvature of beak (PCB)	1. absent or very weak 3. weak 5.medium 7.strong 9. very strong
Pod: texture of surface (PTS)	1. smooth or slightly rough 2. moderately rough 3. very rough
Pod: constrictions (at dry stage) (PC)	1. absent or very weak 2. moderate 3. strong

RESULTS AND DISCUSSIONS

Throughout the vegetation period, the genotype studied was the subject to phenological and biometric measurements. Thus, the descriptive analysis of the qualitative and quantitative characteristics was made.

'Maura' is an early variety of beans with indeterminate growth (dryers), with a wide, long, yellow pod that does not show threads during maturation, intended for cultivation greenhouse and field in a fence system. The plant architecture is pyramidal, the start of climbing is medium and the speed of climbing is fast. The leaf are green, with medium rugosity. The terminal leaflet has a rhombic shape with a length varying between 10.6 cm and 13.6 cm. The color of wing (flower) and standard (flower) is white (Figure 3). The length of bracts has slight variations from 0.6-0.7 cm.

Figure 3. Flower of 'Maura 2000' garden bean variety

The pods are light yellow, shape in cross section is ovate. They present a texture of the surface smooth or slightly rough and a moderate constriction at dry stage. The stringiness of ventral suture of pods is present and the degree of curvature is weak (Figure 4 and Figure 6).

Shape of curvature is convex and the shape of distal part is acute. The beak has a length varied between 6.67 cm and 12.18 cm and a strong curvature.

The number of pods per plant varies between 80 and 100, with an average production of over

2.5 kg/plant in protected areas and over 1.5 kg in the field.

Figure 4. Crop detail 'Maura 2000' garden bean variety

Figure 5. Grains of 'Maura 2000'

Figure 6. Pods of 'Maura 2000'

Regarding the quantitative characteristics of the grain, it was found that it has an average length of 1.37 and a width of 0.76 cm. The weight of the grain varies from 0.37 g to 0.40 g.
The color of the grain is white, it is unicolor. The shape of the grain in longitudinal section is reniform, and in cross section is round. These have a medium degree of curvature (Figure 5).
Throughout years of study growth of the plant and the yield began to be relatively constant, being influenced only by environmental conditions. These data can be found in the Tables 3, 4 and 5.

Table 3. Variability in pod length

Year	SS	X+SD	X	SD	CV%
2016	SF	17.7-22.5	20.1	2.4	11.9
2017	PF	17.4-23.6	20.5	3.1	15.1
2018	PBF	18.5-21.7	20.1	1.6	7.8
2019	BF	18.2-21.8	20.0	1.8	9.0
2020	CF	18.0-22.0	20.0	2.0	10.0
Average	X	17.9-22.3	20.1	2.1	10.7

Over the years in which the 'Maura' bean cultivar was analyzed (2016-2020) the length of the pods varied from 17.4 cm to 23.6 cm, the highest value being recorded in 2017 in progeny field when the average was 20.5 cm .

Table 4. Variability in pod width

Year	SS	X+SD	X	SD	CV%
2016	SF	1.8-2.4	2,1	0.3	14.2
2017	PF	1.7-2.3	2.0	0.3	15
2018	PBF	2.0-2.2	2.1	0.1	4.7
2019	BF	1.9-2.1	2.0	0.1	5
2020	CF	1.8-2.2	2.0	0.2	10
Average	X	1.8-2.2	2.0	0.2	9.7

SF - selection field; PF - progeny field; PBF - prebase field; PB - base field; CF - commercial field

Regarding the variability of the width of the pods, no significant differences were identified. The recorded values ranged from 1.7 cm in the progeny field to 2.4 cm in the selection field. The average of these values was 2 cm.

Table 5. Variability in pod thickness

Year	SS	X+SD	X	SD	CV%
2016	SF	0.4-0.6	0.5	0.1	20.0
2017	PF	0.4-0.8	0.6	0.2	33.3
2018	PBF	0.5-0.7	0.6	0.1	16.6
2019	BF	0.6-0.8	0.7	0.1	14.2
2020	CF	0.5-0.7	0.6	0.1	16.6
Average	X	0.4-0.7	0.6	0.1	20.1

There were no major differences in the thickness of the pods, which attests to the fact that the 'Maura 2000' is a cultivar that has stability in the progeny field. The recorded values ranged from 0.4 cm to 0.8 cm. The coefficient of variation registered in the fields of conservative selection did not vary much between the length, width and thickness of the grain, which denotes a homogeneous population, but also that during the period of conservative selection a special attention was ensured on the quality characteristics of the cultivar.

CONCLUSIONS

Data presented showed that 'Maura 2000' variety is stable and distinct within the normal variability. The variety can be grown successfully in field and in greenhouse areas.
The Laboratory of Genetics, Breeding and Biodiversity from VRDS Buzau has a large variety of genotypes in *Phaseolus vulgaris* and the research will continue with assessment and enriching of the germplasm collection.

ACKNOWLEDGEMENTS

The work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI/2018, within PNCDI III.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE MAIN RESULTS IN ACCLIMATIZATION AND BREEDING OF *BENINCASA HISPIDA*

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Abstract

Benincasa hispida, known as winter melon, name inspired by the fruit shape similar to melon and also to the fact that fruits are kept very well after harvest, over winter time. The Breeding and Biodiversity Laboratory from VRDS 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 (G1, G2, G3) with distinct phenotypic expressiveness, especially in terms of fruit shape and size. G1 has elongated, cylindrical, rounded fruits at both ends, with an average fruit mass of 3.39 kg; G2 has round fruits, slightly ovoid, with an average fruit mass of 8.77 kg; G3 has large, round fruits with a pronounced depth on the top and with an average fruit mass of 14.42 kg. The G1 genotype has been proposed for patenting and from 2021 it will be registered in the Official Catalogue of Românian Crop Plants.

Key words*: Cucurbitaceae, phenotype, wax gourd, winter melon.*

INTRODUCTION

Benincasa hispida (Thunb.) belongs to *Cucurbitaceae* family and is also known as wax gourd, winter melon, Chinese watermelon, Kundur, tallow gourd, winter gourd and white pumpkin. The area of origin is not known exactly, but is probably native in Japan and Java and is widely cultivated or less throughout India and in warm countries from Asia. In Asian communities *B. hispida* is a popular vegetable crop both for nutritional and medical purposes (Nimbal et al., 2011; Zaini et al., 2011). The fruit may be used raw in salads, cooked with meat or different kind of vegetables (Stephens, 2012). In traditional medicine the plant was used in various complains such as respiratory disease, heart disease, diabetes mellitus, urinary diseases, gastrointestinal problems, menstrual disorders, fever, insanity, epilepsy, schizophrenia and other psychological disorders (Blatter et al., 1975; Joshi, 2000; Sharma and Medhyadi, 2005; Jayasree et al., 2011). Nowadays, pharmacological studies revealed that the plant exerted many pharmacological activities. Studies conducted by Huang et al. (2004) and Roy et al. (2007) on fruit of winter melon revealed that its juice and extract have antioxidant activity especially on human tissues like liver and brain. Roy et al. (2008) discovered that a dose of 400 mg/kg body weight, of *B. hispida* fruit seemed to have a protective effect on Alzheimer's disease. Other studies revealed anti-compulsive effect (Girdahar et al., 2010), anti-ulcer effects (Grover et al., 2001; Rachchh and Jain, 2009), anti-inflammatory effects (Cuzzocrea et al., 2001; Gill et al., 2010; Shetty et al, 2008), anti-obesity effects (Kumar and Vimalavathini, 2004; Zhang, 1996), anti-diarrheal agent (Mathad et al., 2005). The major constituents of *B. hispida* fruits are flavonoids, glycosides, sacchrides, proteins, carotenes, vitamins, minerals, volatile oils and uronic acid (Yoshizumi et al., 1998; Rana and Suttea, 2012; Chidan et al., 2012; Mandana et al., 2012, Busuioc et al., 2020). Zaini et al. (2011) recommends that due to high nutritional value and its growing demand, winter melon fruit should be used in different food commodities such as jams, juices, beverages, cakes and ice creams for value-addition.

Benincasa hispida is a particularly valuable species in term of its use as a rootstock. The species is compatible with most melon and cucumber cultivars. At the same time, the plant has a vigorous and well-developed root system which explores the deep layers of the soil and also has a genetic resistance to specific soil pests (Vînătoru et al., 2019).

In a survey made by Dobre and Toma in 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.

The aim of this work was to acclimatize the species in Romania and to obtain new genotypes with distinct phenotypic characteristics.

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 (G1, G2 and G3) with distinct phenotypic expressiveness, especially in terms of fruit shape and size. The breeding method used was repeated individual selection. The seedlings were produced in alveolar pallets with 70 cubes. After 60 days, the seedlings were planted in the greenhouse, in first decade of April.

The planting scheme used in the greenhouse was 70 cm between plants and 300 cm between rows (Figure 1).

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, especially by the bees. During the vegetation period, phenological, biometrical observations were performed.

In order to characterize the genotypes IPGRI descriptors were used and biometric determinations were made.

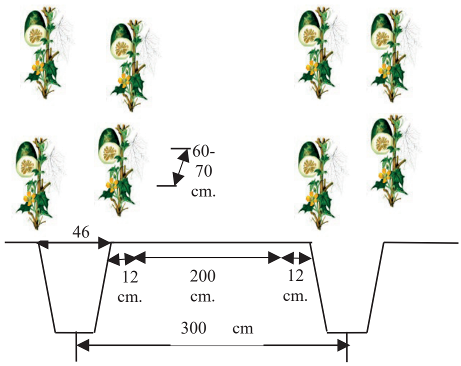

Figure 1. Planting scheme

RESULTS AND DISCUSSIONS

Winter melon is a monoecious vine, easier to grow than any other plants from Cucurbitaceae family. It can be grown spread on the ground/soil or trained to climb a support. The research has shown that the best results are obtained when the crop is vertically supported. A special attention should be paid to G2 and G3 genotypes because it has very large fruits that can detach easily from plant, reason why fruits needs support. The stem is hairless at the base, but as it grows it becomes pubescent and is covered with coarse hair. Foliage is simple, with large leaves of different length varying from genotype to genotype. The leaf width varies from 27.3 cm (G1) to 37.2 cm (G3). More details about phenotypic expressiveness of the leaves can be found in Tables 1-3.

The young leaves and flower buds can be steamed and eaten in different recipes. The flowers are solitary, yellow coloured and five parted. Male flowers appear first and female flowers bear miniature fruit.

The male flower diameter is large varying from 9.6 cm (G1) to 10.5 cm (G3), in return, the female flowers are smaller, with a diameter ranging from 8.1 cm (G1) to 8.7 cm (G3). Regarding petiole length there are also some differences, the male flower has a length varying from 22.9 cm (G1) to 25.7 cm (G3), while the female flowers registers values between 9.7 cm (G1) and 11.5 m (G3).

Table 1. Main characteristics of G1 leaf

Character	$\bar{x} \pm SD$	$\bar{x}$	SD	CV%
Leaf length (cm)	19-29	24	5	20.83
Leaf width (cm)	27.3-37.1	32	5	15.62
Petiole length (cm)	11-17	14	3	21.42
Petiole diameter (mm)	7.5-9.5	8.5	1	11.76

Table 2. Main characteristics of G2 leaf

Character	$\bar{x} \pm SD$	$\bar{x}$	SD	CV%
Leaf length (cm)	25.0-26.2	25.6	0.6	2.34
Leaf width (cm)	34.2-35.4	34.8	0.6	1.72
Petiole length (cm)	15.2-17.6	16.4	1.2	7.31
Petiole diameter (mm)	7.8-10.6	9.2	1.4	15.21

Table 3. Main characteristics of G3 leaf

Character	$\bar{x} \pm SD$	$\bar{x}$	SD	CV%
Leaf length (cm)	24.6-28.2	26.4	1.8	6.81
Leaf width (cm)	34.0-37.2	35.6	1.6	4.49
Petiole length (cm)	16.0-18.4	17.2	1.2	6.97
Petiole diameter (mm)	9.2-10.4	9.8	0.6	6.12

Mature fruits of *Benincasa hispida* are covered with a waxy coat which allows them long-term storage. The fruit shape may be elongated, ovoid or round (Figures 2 and 3).

Figure 2. Immature and mature fruit of G2

Figure 3. Mature fruit of G1 (left) and G3 (right)

G1 has elongated, cylindrical, rounded fruits at both ends; G2 has round fruits, slightly ovoid; G3 has large, round fruits with a pronounced depth on the top.

The first fruit reached technical maturity in the third decade of June and physiological maturity, in the last decade of September.

Fruit mass range from 2.390 kg (G1) to 16.840 kg (G3) and more details about the main characteristics of fruit genotypes can be found in Tables 4-6.

Table 4. Main characteristics of G1 fruit

Character	$\bar{x} \pm SD$	$\bar{x}$	SD	CV%
Fruit mass (kg)	2.390 -4.390	3.390	1.000	29.49
Fruit length (cm)	31-39	35	4.0	11.42
Median fruit diameter (cm)	11.5-.15.5	13.5	2.0	14.81
Pulp thickness (cm)	3.1-3.7	3.4	0.3	8.82
Pericarp thickness (cm)	2.5-3.5	3	0.5	16.66
Core thickness (cm)	5.5-6.6	6	0.5	8.33
Inner hollow median diameter	6.2-7.4	6.8	0.	8.82
Diameter of blossom end (mm)	8-12	10	2.0	2.0
Blossom end fruit dent (mm)	2-4	3	1.0	33.33
Stem end fruit dent (mm)	0-2	1	1.0	100.0

Table 5. Main characteristics of G2 fruit

Character	$\bar{x} \pm SD$	$\bar{x}$	SD	CV%
Fruit mass (kg)	8.210-9.330	8.770	560	6.38
Fruit length (cm)	28-40	34	6	17.64
Median fruit diameter (cm)	24-32	28	4	14.28
Pulp thickness (cm)	3.6-4.8	4.2	0.6	14.28
Pericarp thickness (cm)	1.5-2.5	2	0.5	25.0
Core thickness (cm)	6.8-7.6	7.2	1.6	22.22
Inner hollow median diameter	17-21	19	2	10.52
Diameter of blossom end (mm)	13-19	16	3	18.75
Blossom end fruit dent (mm)	8-12	10	2	20.0
Stem end fruit dent (mm)	12-16	14	2	14.28

The pericarp thickness is associated with shelf-life of fruit and the highest value was recorded by G1, with a thickness of 3 cm, followed by G2 and G3 with a thickness of 2 cm.

Table 6. Main characteristics of G3 fruit

Character	$\overline{x} \pm SD$	$\overline{x}$	SD	CV%
Fruit mass (kg)	12.000 - 16.840	14.420	2.420	16.78
Fruit length (cm)	31- 41	36	5	13.88
Median fruit diameter (cm)	28-38	33	5	15.15
Pulp thickness (cm)	5.1-5.7	5.4	0.3	5.55
Pericarp thickness (cm)	1,5-2,5	2	0.5	25.0
Core thickness (cm)	9.8-11.4	10.6	0.8	7.54
Inner hollow median diameter	21.5-23.5	22.5	1.0	4.44
Diameter of blossom end (mm)	19-25	22	3	13.63
Blossom end fruit dent (mm)	28-40	34	6	17.64
Stem end fruit dent (mm)	16-21	18	2	11.11

The flesh of mature fruit is white, spongy and juicy. The edible part of mature fruit is considered the core. The core thickness has the highest value registered by G3 with a mean of 10.6 cm, followed by G2 with a mean of 7.2 cm and G1 with a mean of 6.05 cm.

The number of fruits per plant depends when the harvest is made. If the fruits are harvested at technical maturity genotype G1 has 20-35 fruits/plant.

If the fruits are harvested at psychological maturity, the number of fruits per plant is lower, therefore 10-12 fruits. The G2 genotype has a yield potential of 15-20 fruits per plant when harvest at technical maturity and 8-10 fruits/plant when harvested at psychological maturity. The G3 genotype has larger fruits, and the number of fruits per plant is lower, it was noted that when fruits are harvested at technical maturity the plant can bear 10-15 fruits, but when the fruits are left to be harvested at physiological maturity, the number of fruits is lower, thus 6-8 per plant.

Longitudinal section of the fruit is presented in Figures 4-5.

Winter melon seeds contains high amount of fatty acids 24.3%, saturated fatty acids represents 75.38% and unsaturated fatty acid (Mandana et al., 2012). The seeds have different shapes (Figure 6), depending on the shape and type of fruit.

Figure 4. Longitudinal section of G2 (left) and G1 (right)

Figure 5. Longitudinal section of G3

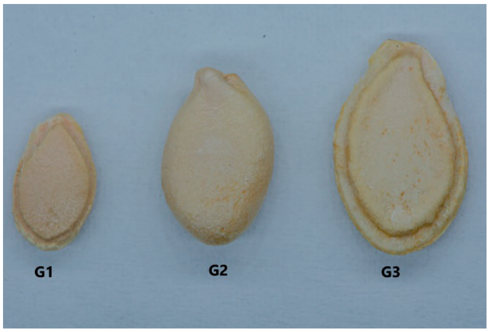

Figure 6. Seeds of *B. hispida*

The average seed length varies from 8.1 mm on G1, followed by G2 with 11.1 mm and G3 with 13.3 mm. Seed width also range from 4.2 mm (G1), to 6.3 mm (G2) and 8.4 mm (G3). Mass of thousands seeds weighs 23.34 g (G1), followed by 26.83 g (G2) and 33.04 g (G3). The seed mass in fruit differs depending on genotype as it follows, G1 weighs 23.83 g, G2 weighs 29.60 g and G3 weighs 126.26 g.

During vegetation period it was noted that no serious insects or disease problems were recorded and we recommend winter melon crop to be grown also in organic farming.

CONCLUSIONS

The research undertaken so far certifies that the species can be successfully grown throughout of Romania. During the research, three genotypes with distinct phenotypic expressiveness were obtained, of which, G1 will be registered in the Official Catalogue of Romanian Crop Plants.

Genotypes G1 and G3 are proposed for registration for year 2022. The Vegetable Research Development Station Buzau has promoted the species with its health benefits and the demand for seeds and seedlings has increased significantly from year to year among growers and also the consumers demand.

ACKNOWLEDGEMENTS

This work was supported by one founding source a grant of the Romanian Ministry of Research and Innovation, CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

TECHNOLOGICAL SOLUTIONS WITH BIOLOGICAL CULTIVATION OF GREEN BEANS AT FIELD CONDITIONS

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Abstract

The purpose of the study is to be determined the fertilization impact and the cultivation scheme on the biological manifestations and the productiveness of Bulgarian green beans varieties, which are cultivated as per the biological method in the region of the Thracian valley. The experiment is carried out during the period 2015-2017 in the test field of Institute as per vegetable crops Maritsa - Plovdiv, in strongly leached meadow-cinnamon soil after forerunner spinach (autumn planting). The spinach is cultivated in compliance with the principles of biological production. With three Bulgarian green beans varieties: Tangra, Pagane and Evros are tested two biological fertilizers: Emosan and Biosol, with two schemes of sowing (main with width of the row-crops 80 cm and additional one of 60 cm). Significant differences are determined in the productiveness of the studied green beans varieties, cultivated as per the biological method. The highest yield is obtained of variety Evros in comparison with the rest. With the main scheme of 80 cm under influence of the bioproducts brought in the yields vary from 14170.0 kg/ha (fertilized with Biosol) up to 15156.0 kg/ha (fertilization with Emosan), the increase is respectively by 17.8% and 26.0% in comparison with the control. Considerably higher yields for the three varieties are obtained with scheme of 60 cm. The effect is higher by the application of bioproducts with varieties Pagane and Evros - 23.3% and 20.3%, in comparison with the main scheme of 80 cm. The results are one-way for all the three varieties. The impact of the bioproducts brought in is expressed least with the variety Tangra - 14.6%, where the lowest yields are reported.

Key words: *biofertilizer, number of beans, organic production, Phaseolus vulgaris* L, *weight of beans, yield.*

INTRODUCTION

Increased demand for organic production in the EU, where the market is unlimited (Yordanova, 2003) imposes the extension of the production and expansion of the range of vegetable crops grown in accordance with the standards of the biological method in Bulgaria rapidly. In the leading countries of Europe, the USA, Canada and Australia legal, managerial and technological systems of farming and marketing of organic products are operating and constantly improving (Willer & Lernoud, 2017; 2019). Organic farming is a production system that combines best environmental practices with the aim of producing safe food, preserving natural resources and obtaining stable yields (Boteva & Cholakov, 2010; Vlahova, 2012; Vlahova, 2013). The use of biofertilizers as an alternative to chemical fertilizers increases vegetative growth, yield and fruit quality in vegetable crops (Aly, 2002; Aitbayev et al., 2018; Poudel et al., 2002). Breeding programs are directed to development of hybrid varieties that are more resistant and suitable for organic production (Antonova, 2012; Nacheva et al., 2013; Todorova, 2013).

Many experiments related to organic farming are being conducted around the world. The green beans participate in the most experimental schemes included in crop rotation or independently (Venturini et al., 2005). With the development of organic production, the importance of bean crops is growing up. In Bulgaria, beans are a traditional food for the population and the conditions for growing are favourable in more part of the country. The short vegetation period and production of organic N from the bacteria found in plant roots, defines green beans as economically important vegetable crops used as cover crops and green fertilizers (Nakhone & Tabatabai, 2008). The bean a very good pre-culture in vegetable production (Panayotov, 2000; Cholakov et al., 2015), suitable for incorporation in a system of organic production which enriches the soil with nitrogen, releasing vegetative masses in quantities equal to fertilizing with 30 t/ha. This crop is very appropriate for growing in organic vegetable farms (Karungi et al., 2006; Sharma & Chetani, 2017). The yields in organic farming of green beans are lower but the quality of the output is higher (Abubaker et al., 2007).

MATERIALS AND METHODS

The experiment was carried out during the period 2015-2017 in the field of Maritsa Vegetable Crops Research Institute - Plovdiv in strongly leached meadow-cinnamon soil after forerunner spinach (autumn planting). The spinach is cultivated in compliance with the principles of the biological production. Two schemes of sowing are tested and two biological fertilizers: Emosan and Biosol, they were brought in separately and in combination with three Bulgarian determinant varieties green beans: Tangra, Pagane and Evros.

Variants of the experiment

I. Main sowing scheme - with width of the row-crops 80 cm.

1. Control - unfertilized;
2. Mineral fertilization - N80P100K100;
3. Fertilization with 1000 kg/ha Biosol - with single bringing in before the sowing;
4. Fertilization with Emosan - 200 L/ha* - bringing in two times: before the sowing 150 L/ ha and phase beginning of blossoming 50 L/ ha
5. Combined fertilization with Biosol - 800 kg/ha + Emosan - 100 L/ha *.

II. Additional sowing scheme - with width of the row-crops 60 cm.

The variants are the same as with the main scheme.

The experiment is set as per the method of the long plots, in 4 repetitions, with reported area 4 m^2. The quantity of the mineral fertilizers is determined on the basis of agrochemical analysis of the soil. Ammonium nitrate, granulated fertilizer triple superphosphate and potassium sulphate are used. The phosphorous and potassium fertilizers and half of the nitrogen fertilizer norm are brought in with the main processing of the soil, the feeding with the rest nitrogen is carried out in phase beginning of blossoming.

The sowing is carried out in the period 30.05-06.06. The cares during the vegetation are: drip system irrigations, conformed with the development phase of the plants; against the weeds - manual earthing ups and weeding; feeding with fertilizers as per scheme; plant-protection practices - with bioinsecticides and biofungicides which are allowed for usage.

Materials

Variety Tangra – It is created at Institute as per vegetable crops Maritsa - Plovdiv. The plants are low-stemmed, shrubby, with height 47-57 cm, very well covered with leaves, with a strong, upright stem. The variety has a short vegetation period 44-46 days from the germination up to technological maturity and can be cultivated as it is sown per stages from April until the second half of July. It has high biological potential for yield and with double manual harvestings it exceeds the yield of the varieties, which are sensitive to *Pseudomonas savastanoi* and the bean common mosaic. The beans are flat, green, upright, 1.2-1.3 cm wide and 10-13 cm long. It has good resistance to the causes of the *Pseudomonas savastanoi*, sclerotic rot, as well as to the bean weevil. It is less attacked by bean common mosaic (BCMV) and cucumber mosaic (CMV).

Variety Pagane - An early and high-yield variety, determinant variety garden beans. The plants are high, very well covered with leaves, with a strong, upright stem and many nodes. The blossoms are white and typical for the common beans; the racemes are situated in the leaves. It is resistible to the most important warehouse pests of the beans – the bean weevil (*Acanthoscelides obtectus* Say). It is appropriate for direct consumption, as well as for processing.

Variety Evros - It has high productiveness, it is vital and very well adapted to the agroclimatic conditions of the country. The plants are with determinant type of growth with 50-55 cm height, with a strong and upright stem and average big frutex. It has cylindrical, long (13-15 cm); straight, green beans without fiber exceptionally appropriate for frozen vegetable mixes, whole frozen beans and canning. The beans reach technological maturity for 50 days and preserve their good taste qualities and appearance for a long period of time, because of their slow maturing dynamics.

Characteristic of bioproducts used in this experiment

Biosol (Sandoz GmbH) - A by-product of the production of penicillin, containing fumigant biomass (micelle). Contains: Dry matter – 95.60%; CaO -0.21%; Organic substance – 90.70%; MgO -0.05%; pH ($CaCl_2$) – 3.0; Cl – 0.04%; S – 1.80%; C: N = 5:1; B – 7.1 mg/kg; N (total) - 6-8%; Zn – 6.0 mg/kg; Phosphates (P_2O_5) – 0.5-1.5%; Fe – 10.1 mg/kg; Potassium (K_2O) – 0.5-1.5. Product is certified for organic production.

***Emosan* -** HemoZymNK (Arkobaleno, Italy) - Organic nitrogen fertilizer with long lasting effects on soil and plants. Contains total nitrogen (N) - 5%; organic nitrogen (N) - 5%; organic carbon (C) - 14%; protein - 34 p/p; humidity - 65 p/p; K - 0.4 p/p; P - 0.06 p/p, etc.; pH - 7.0-10.0. Product is certified for organic production

The experiment was set by the method of the long parcels in 4 replicates, with a reporting area of 4 m^2 and a scheme of sowing 80/30 cm.

Highly leached cinnamon soil has a heavy mechanical composition with mineral nitrogen content (NH4 - N + NO3 - N) – 2.01 mg/100 g soil (determined by distillation); 18.2 mg P_2O_5 and 17.5 mg K_2O per 100 g soil (determined by Egner River); soil response pH 6.8 in water (potentiometrically defined) with a humus content of 2.2% (by Thurin) (Tomov et al., 1999).

Study Indicators:

1. Biometric Measurements: Five plants were analyzed by replicates:

- ✓ Vegetative mass (leaves + stems) per plant (g);
- ✓ Weight and number of beans per plant (g);
- ✓ Weight of a bean (g).
- ✓ Number of beans

2. Morphology of beans: mass (g); length (cm) and width (cm) - an average of 10 peppers from repetition in technological maturity were analyzed.

3. Yield - Formed by standard beans of all harvests - kg/ha.

4. Statistical data processing - The treatment of the results obtained includes a Two-factor dispersion analysis and a Duncan's Multiple Range Test (Duncan, 1955; Lakin, 1990) comparative analysis performed with SPSS 12 for Windows.

RESULTS AND DISCUSSIONS

The results of the biometric measurements show that with scheme 80 cm row-crop, the plants of variety Evros form the biggest fresh over-ground biomass (average 272.24 g/plant). The plants, which are fertilized with mineral fertilizers (354.89 g) have the biggest mass. The impact of the used bioproducts on the formed vegetative mass of the plants is more strongly expressed with Emosan fertilization, followed by the variant with combined application of Biosol and Emosan, where the biggest mass of the beans is reported (85.32 g and 79.01 g) as well as number of pods per plant (23.08 pcs. and 22.25 pcs.). The separate fertilization with Biosol on the vegetative manifestations is less expressed.

With variety Pagane the difference in the formed total biomass /leaves + stems + pods/ among the variants, fertilized with Biosol and the combined application of Biosol + Emosan are statistically unproven. Regarding the mass of the formed beans per plant, the fertilization with Emosan significantly exceeds the rest variants.

The mass of the beans per plant with the variant Tangra is bigger with the combined fertilization with Biosol + Emosan. The general tendency is preserved as for the rest varieties, but the impact of the biofertilizers is less expressed (Table 2).

By decrease of the feeding area with scheme of 60 cm between the rows are decreased the values of the leaf-stem mass and the mass of the beans, respectively by 27,7%, 25,8% and 25,1 %. (Table 3). The biggest vegetation mass again has variety Evros (213.22 g/plant), followed by variety Pagane (196.29 g/plant), as the differences are small and statistically insignificant. The effect is the strongest on this indicator with fertilization with Emosan, followed by the combined application of Biosol + Emosan. The results are one-way for the three varieties. Significant differences are not determined with both schemes of cultivation on the indicator – number of the beans.

The biometric measurements (Tables 1 and 2) show that the differences among the variants, fertilized with Emosan and combined application of Emosan and Biosol are not big, as they are more significant among the varieties.

Table 1. Biometric measurements for green beans, with a scheme of 80 cm

Variant	Mass of plant, g	Mass of stems and leaves, g	Mass of peppers/ plant, g	Number of peppers	Length of plant, cm
Variety Evros					
Unfertilized	204,18 c	120,18	84,00 c	21,33 b	39,00
NPK	349,73 a	203,16	146,58 a	33,08 a	46,17
Biosol 100 kg/da	235,26 b	123,61	111,66 b	20,67 b	37,25
Emosan 20 L /da	325,94 a	177,21	148,74 ab	35,75 a	41,42
Biosol+ Emosan	286,55 ab	176,84	109,72 b	26,92 b	42,25
average	280,33	160,20	120,14	27,55	41,2
Variety Pagane					
Unfertilized	223,42 c	121,69	101,73 c	17,75 c	40,42
NPK	348,33 a	174,11	174,23 a	26,92 a	45,67
Biosol 100 kg/da	264,34 bc	142,54	121,80 b	19,42 b	45,29
Emosan 20 L /da	310,06 a	168,88	141,18 b	25,83 a	45,33
Biosol+ Emosan	283,48 ab	165,54	117,93 cb	19,75 b	41,83
average	285,93	154,55	131,37	21,93	43,71
Variety Tangra					
Unfertilized	172,78 b	122,01	50,77 b	16,08 b	45,42
NPK	239,40 a	161,31	78,09 a	19,83 ab	47,96
Biosol 100 kg/da	172,78 b	122,01	50,77 b	16,08 b	45,42
Emosan 20 L /da	231,17 a	164,23	66,94 ab	22,17 a	45,54
Biosol+ Emosan	206,36 ab	155,28	51,08 b	17,67 b	46,75
average	205,07	143,82	61,25	14,42	41,02

a, b, c - Duncan`s multiple range test ($p<0.05$)

Table 2. Biometric measurements in green beans, in a scheme of 60 cm

Variant	Mass of plant, кg	Mass of stems and leaves, кg	Mass of peppers/ plant, кg	Number of peppers	Length of plant, cm
Variety Evros					
Unfertilized	157,33 c	113,51	43,82 c	14,83 c	36,67
NPK	284,28 a	201,36	82,92 a	25,08 a	42,92
Biosol 100 kg/da	198,04 b	145,71	52,33 bc	15,92 c	39,67
Emosan 20 L /da	221,54 b	160,90	75,25 ab	17,75 b	37,71
Biosol+ Emosan	204,89 bc	121,24	62,40 b	21,08 ab	33,88
average	213,22	148,54	63,34	18,93	38,16
Variety Pagane					
Unfertilized	156,42 c	101,86	54,56 c	16,25 c	46,04
NPK	233,50 a	149,01	84,48 a	27,17 a	46,88
Biosol 100 kg/da	175,49 b	115,60	59,89 b	16,83 c	45,22
Emosan 20 L /da	228,92 a	147,79	81,14 a	21,00 ab	48,63
Biosol+ Emosan	187,14 b	124,59	62,55 b	19,08 b	46,04
average	196,29	127,77	68,52	20,06	46,56
Variety Tangra					
Unfertilized	124,00 c	94,60	29,41 c	9,25 c	42,17
NPK	195,83 a	152,48	43,35 b	15,08 ab	48,75
Biosol 100 kg/da	160,35 b	112,58	47,77 b	14,00 b	46,07
Emosan 20 L /da	186,51 ab	129,39	57,12 a	16,42 a	45,50
Biosol+ Emosan	152,87 b	98,81	54,06 ab	11,92 bc	38,13
average	163,91	117.57	46.34	13,33	44,12

a, b, c - Duncan`s multiple range test ($p<0.05$)

Higher values of the studied beans indicators are determined with variety Pagane out of the morphological measurements. The plants with the biggest beans mass are the plants, which are fertilized with Emosan (9.38 g). The results are analogical and for the rest varieties (Table 3).

Table 3. Morphological measurements in green beans, in a scheme of 80 cm

Variant	Mass of pepper, g	Length of pepper, cm	Width of pepper,cm
Variety Evros			
Unfertilized	7,43	12,37	12,72
NPK	8,52	12,97	12,36
Biosol 100 kg/da	7,03	12,43	13,04
Emosan 20 L /da	8,03	12,90	13,01
Biosol+ Emosan	7,87	12,63	12,92
average	7,82	12,70	12,81
Variety Pagane			
Unfertilized	8,08	13,23	13,11
NPK	9,59	13,07	14,20
Biosol 100 kg/da	8,39	12,83	13,45
Emosan 20 L /da	9,38	13,67	13,71
Biosol+ Emosan	8,59	13,30	13,70
average	8,81	13,22	13,63
Variety Tangra			
Unfertilized	5,69	11,05	8,32
NPK	7,77	12,93	9,97
Biosol 100 kg/da	5,90	12,11	8,83
Emosan 20 L /da	6,92	12,00	9,44
Biosol+ Emosan	6,03	12,60	8,92
average	6,46	12,46	9,10

a, b, c - Duncan`s multiple range test ($p<0.05$)

The impact of the sowing scheme on the parameters of the beans is well expressed after fertilization with Emosan. The results show that with the smaller row-crop the mass of the beans, their width and length decrease with the studied varieties. This, however, from technological point of view does not render impact on the final results of the production (Table 4).

Table 4. Morphological measurements in green beans, in a scheme of 60 cm

Variant	Mass of pepper, g	Length of pepper, cm	Width of pepper, cm
Variety Evros			
Unfertilized	7,02	12,09	12,58
NPK	8,14	12,47	13,25
Biosol 100 kg/da	7,34	12,73	12,65
Emosan 20 L/da	7,78	12,47	13,18
Biosol+ Emosan	7,62	13,00	13,10
average	7,58	12,55	12,95
Variety Pagane			
Unfertilized	7,29	12,73	12,76
NPK	8,89	13,33	13,70
Biosol 100 kg/da	7,66	12,05	12,94
Emosan 20 L /da	8,28	12,63	13,10
Biosol+ Emosan	8,09	12,65	13,01
average	8,04	12,68	13,14
Variety Tangra			
Unfertilized	5,55	11,37	9,41
NPK	5,83	11,33	8,78
Biosol 100 kg/da	5,92	11,47	8,52
Emosan 20 L /da	6,01	12,11	9,31
Biosol+ Emosan	5,27	11,37	8,00
average	5,72	11,53	8,80

Yields of the three varieties, registered with mineral fertilization, exceed the average of the bioproducts application as the increase is respectively 12.7% - for variety Evros; 10.8% - for variety Pagane and 8.2% - for variety Tangra.

The highest yields with bioproducts fertilization are reported with variety Evros in comparison with the rest varieties. The values among the variants with 80 cm row-crop vary from 14170.0 kg up to 15156.0 kg/ha. It is determined that the bioproducts increase the productiveness average by 21.5% in comparison with the unfertilized control. The obtained yields are with the highest values with fertilization with Emosan, followed by the variant with combined brining in of Biosol+Emosan as the increase in comparison with the control is respectively by 26.0% and 20.6%. The differences among the two variants are statistically proven (Table 5).

Significantly higher yields are reported with row-crop 60 cm, as the values vary from 16628.0 kg up to 18025.0 kg/ha. The increase of this indicator is average by 20.3%. The obtained results are not one-way with the main scheme of cultivation. The yields are the highest with the combined fertilization with Biosol and Emosan, followed by the separate bringing in of Emosan, but the differences among the two variants are small and unproven, respectively 22.1 and 24.2%. The effect of the brought in bioproducts with this scheme is by 24.2% higher than the main scheme.

The results are one-way with variety Pagane. With the main scheme of 80 cm under the impact of the bioproducts brought in the yields vary from 12130.0 kg/ha (fertilized with Biosol) up to 13317.0 kg/ha (fertilized with Emosan), the increase is respectively by 14.8 and 26.0 % in comparison with the control. Higher values for this indicator, average by 23.1%, are determined with the additional scheme of 60 cm.

The average effect of bioproducts included in the study is the highest with variety Pagane in comparison with the other varieties, expressed strongly with scheme of 60 cm row-crop - 23.3%, in comparison with the main scheme of 80 cm - 19.9%. In contrast to variety Evros, here the differences among the variants fertilized with Emosan and the combined bringing in of Biosol and Emosan are more considerable for both cultivation schemes.

The impact of the bioproducts brought in is less expressed with variety Tangra, where the lowest yields are reported. The tendency regarding the impact of the applied fertilization and the cultivation scheme is preserved, but the effect of the bioproducts in comparison with the unfertilized plants is less expressed - 14,5% (with scheme of 80 cm) and 14.6% (with scheme of 60 cm).

Table 5. Yields of green beans, kg/ha

Variant	Yield	% /K	Yield	% /K	% towards 60 cm
	80 cm between the lines		60 cm between the lines		
Variety Evros					
Unfertilized	12020,8 c	100	14510,5 c	100	120,7
NPK	15550,9 a	129,4	19560,8 a	134,8	127,8
Biosol 100 kg/da	14170,0 b	117,8	16620,8 bc	114,6	120,3
Emosan 20 L/da	15156,0 ab	126,0	17720,6 b	122,1	125,0
Biosol+ Emosan	14500,2 b	120,6	18025,0 b	124,2	127,3
effect		21,5		20,3	24,2
Variety Pagane					
Unfertilized	10560,9 c	100	12950,1	100	122,5
NPK	13990,5 a	132,4	16630,3 a	128,4	118,8
Biosol 100 kg/da	12130,0 b	114,8	15000,3 b	115,8	123,7
Emosan 20 L/da	13317,0 ab	126,0	16150,4 ab	124,7	121,3
Biosol+ Emosan	12560,8 b	118,9	16730,8 b	129,2	129,2
effect		19,9		23,3	23,1
Variety Tangra					
Unfertilized	9030,5 b	100	11670,7 c	100	120,2
NPK	10890,3 a	120,6	14080,5 a	120,6	123,3
Biosol 100 kg/da	9970,3 b	110,4	12830,0 b	109,9	118,6
Emosan 20 L/da	10370,5 ab	114,8	13380,4 ab	114,6	124,0
Biosol+ Emosan	10670,4 a	118,1	13930,8 a	119,4	126,6
effect		14,5		14,6	22,5

The yields level of the tested Bulgarian green beans varieties, in the conditions of biological production to the biggest degree depends on the variety peculiarities, and the technological elements - fertilization and cultivation scheme are with less impact (Table 6).

Table 6. Vatiation of the green beans yield under the influence of the fertilization and scheme of growing

Factors	**SS**	**df**	**F**	**P-value**	**F crit**
Variety	3615460	2	38,413	78,22***	3,885
Scheme	424151,2	1	9,013	9,18*	4,747
AxB	17827,7	2	0,189	0,39	3,885
Casual	564719,4	12		12,22	
Total	4622159	17			

CONCLUSIONS

The sowing scheme impact on the parameters of the beans is well expressed after fertilization with Emosan. The plants of variety Pagane have the biggest mass of the beans. The mass of the beans, their width and length decrease with the less row-crop with the studied varieties. This, however, does not render impact on the final results of the production from technological point of view.

Yields from the three varieties, registered with mineral fertilization, exceed the average ones of the bioproducts application, as the increase is respectively 12.7% - for variety Evros; 10.8% - for variety Pagane and 8.2% - for variety Tangra.

With variety Evros are reported the highest yields with bioproducts fertilization in comparison with the rest varieties. The values among the variants with 80 cm row-crop vary from 14170.0 kg/ha (fertilized with Biosol) up to 15156.0 kg/ha (combined fertilization with Biosol and Emosan), the increase is respectively by 17.8 and 26.0% in comparison with the control. Considerably higher yields for the three varieties are obtained with row-crop 60 cm.

The effect of the bioproducts is the highest with variety Pagane, more expressed with scheme of 60 cm - 23.3%, in comparison with the main scheme of 80 cm - 19.9%. The results are one-way for all the three varieties.

Appropriate for biological cultivation are variety Evros, followed by variety Pagane, which have realized high yields with both sowing schemes. Variety Tangra has relatively lower productiveness, but can also be used for the making of organic products.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF THE DIFFERENT TYPE OF TUNNELS COVERINGS APPLIED AT LETTUCE CULTIVATION

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Abstract

The aim of this study was to test different coverings used on low tunnels during the cultivation of lettuce for the winter-spring production, and to compare the results with those obtained in high tunnels. The experiment was conducted in the period 2019-2020 in the experimental field of the University of Forestry - Sofia (42°7' N, 23°43' East). There were selected 3 varieties (2 of Batavia and 1 of Romana) with different requirements for the terms and conditions of cultivation. For the purpose of the study, lettuces were planted in parallel in a high tunnel and in low tunnels with two different coverings - non-woven fabric (geotextile) and green polyethylene. Planting was done simultaneously during the second ten days of November (12.11.19) by the block method with four replications. Immediately after planting outdoors, low tunnels were placed over the bed. During the harvest (April) biometric measurements were made and reporting of quality indicators - dry matter content and nitrates. In low tunnels, the plants have more sugar and dry matter under the green polyethylene coating than the geotextile coating. Both varieties of lettuce grow better under a cover of green polyethylene, while lettuce has better performance when covered with geotextile.

Key words: *geotextile, green polyethylene, lettuce, low tunnels.*

INTRODUCTION

The greenhouse production of salads ensures their supply during the autumn-winter-spring period. They are one of the first vegetable crops grown in a polyethylene greenhouse to produce off-season production (Lamont, 2005).
The Lettuce (*Lactuca sativa* L.) is the most consumed leafy vegetable in world and has great economic importance. It is an herbaceous, delicate plant, with a short stem that holds leaves growing in a rosette pattern. Lettuce has a short growing cycle, large leaf area and shallow root system, requiring sandy-clayey soils rich in organic matter and with high concentrations of readily available nutrients (Silva et al., 2010).
High tunnels are large, framed structures covered with a single or double layer of greenhouse-grade plastic with no electrical or ventilation systems and are typically used to produce high-value specialty crops including lettuce and other leafy greens (Knewtson et al., 2010; Lamont, 2009). High tunnels are employed as crop growth enhancers, providing climate protection during severe weather conditions and enabling season extension (Lamont et al., 2002; Reeves and Drost, 2012).
During the winter months, high tunnels provide microclimates suitable for season extension allowing growers to plant lettuce earlier in the season, later in the season, or both. Optimizing the utilization of these microclimates can improve crop yield and quality (Zhao and Carey, 2009).
The product obtained in the high tunnels is of very good quality and the production is more economical than in the glazed greenhouses (Wells and Loy, 1993; Tüzel and Leonardi, 2009), the yields are higher and the harvests start more early, compared to growing lettuce in open areas. (Santos et al., 2009; Wallace et al., 2012; Golzar et al., 2018). For this reason, lettuce ranks as one of the main crops for growing in plastic tunnel greenhouses (Lamont, 2009; Favarato et al., 2020).
An alternative to growing lettuce in high tunnel greenhouses and obtaining earlier products from early field production is the use of low polyethylene tunnels. Low tunnels are temporary structures that are easy to assemble and disassemble with each crop. This mobility

offers an advantage over high tunnels because it allows for rotations with cover crops or other field crops to improve and maintain soil health and productivity. However, this requires an increase in labor costs to set up and remove the structures.

Low tunnels can be of various heights and covered mainly with three types of plastic materials: perforated or slit plastic film, spun-bonded fabrics, and insect nets, depending on the purpose of their use.

These structures are usually 50-80 cm high, arch-shaped and covered with plastic foil, so that they provide a suitable microclimate. (Scarascia-Mugnozza et al., 2011).

Accelerated plant development, earlier harvests, and higher yields lead to higher productivity during the autumn-winter season. (Arin and Ankara, 2001; Favarato et al., 2020).

In addition to the type of construction (high or low tunnels), the type of coverage also has an impact. The non-woven fabrics (agro-textiles) are one of the coatings used for low tunnels to improve the microclimate (Hamamoto, 1996; Hamouz et al., 2006; Buta and Apahidean, 2009; Olle and Bender, 2010).

Colorless polyethylene coatings are used, but colored coatings are also of interest. When comparing different color coatings, studies show that green colored ones stimulate vegetative development and slow down the onset of fruiting, while red colored ones improve fruit formation (Henschel et al., 2017). Quintero-Arias et al. (2021) found that it is necessary to select varieties of salads (green or red leaf type) with the type of plastic coating (UV-blocking or UV-transparent polyethylene) to improve the quality of production.

The aim of this study was to test different coverings used on low tunnels during the cultivation of lettuce for the winter-spring production, and to compare the results with those obtained in high tunnels.

MATERIALS AND METHODS

The field experiment was conducted during 2019–2020 at the experimental field (42°7′N, 23°43′E and 552 m above sea level) of the University of Forestry, Sofia, Bulgaria, on the fluvisol, which is slightly stony and slightly acidic (pH (H_2O)6.2).

For the aim of the experiment, a total number of 3 types of lettuces have been studied (2 of Batavia and 1 of Romana), as follows:

1. Gentilina: open type Batavia, cold-resistant and resistant to high temperatures variety (does not shoot). The recommended term for outdoor cultivation for Bulgaria is March-October.
2. Aquarel (Bejo Zaden): Batavia type salad suitable for planting spring-summer and summer-autumn outdoors.
3. Cherna Gyumyurdzhinska: Medium early winter variety of lettuce, intended for autumn planting. Characterized by good cold resistance. Grown by overwintering for earlier spring production.

Two different types of covering used on low tunnels were used and the results were compared with those obtained in high tunnels. The types of coverings are as follow:

Geotextile (GT) - for low tunnel: white non-woven polypropylene coating with UV protection. No chemicals - non-toxic and safe for plants. Helps warm the soil and protects plants from frost in early production. Allow of plants to breathe, be irrigated and treated with water-soluble substances. It does not absorb water and dries quickly after rain. The coating leads to an earlier harvest 1-2 weeks ahead.

Green polyethylene (GP) - for low tunnel: UV stabilized 120-micron polyethylene for covering greenhouses, with excellent transparency, high conductility and tensile strength. UV stabilizers protect the film from solar radiation and anti-oxidants by increasing the durability of polyethylene.

Reinforced and stabilized polyethylene (RSP) - for high tunnel: high-quality polyethylene coatings for greenhouses transmit, reflect, absorb and emit different parts of the spectrum of sunlight. Polyethylene film with reinforcement that makes it resistant to weathering and has a long service life. Polyester threads significantly improve the mechanical properties of polyethylene.

The cultivation technology is standard: by pre-produced seedlings. Transplanting in the polyethylene greenhouse and in the low tunnels took place on 12.11.19. One week later was carried out planting to replace the dead plants. The growing scheme used in the greenhouse is 50 + 30 + 30 + 30 x 30 cm, and outdoors is 30x30, in four rows.

For the purposes of the study were tracked: Biometric indicators: plant height (cm); plant diameter (cm); mass of one plant (g). Biometric indicators were measured at harvest.

Qualitative indicators: determination of the dry matter content in the production; determination of sugar content in the plant cell (Brix,%) with a refractometer (model - Digital refractometer 32145, manufactured by B & C Germany).

The collected data from these indicators were analysed by ANOVA and were expressed as mean ± standard deviations. Post hoc analyses were conducted using Fisher's protected LSD test.

RESULTS AND DISCUSSIONS

Climate may influence fresh market crop production, including lettuce yield and quality (Dufault, et al., 2006; Lamont, 2009). Early plant exposure to increasing irradiance, temperatures, and day lengths has been found to significantly reduce lettuce quality because of factors such as bolting (Dennis and Dulforce, 1974). Reduced lettuce yield and quality may also be associated with environmental stresses (high temperatures and winds) during later crop growth, or from damaging diseases and insect pests (Simonne et al., 2002; Zhao and Carey, 2009).

In terms of climate, the experimental field falls in the temperate continental climate area, in the mountain climate region. The climate is influenced by the general climate of the neighboring lowlands, which is why it bears the characteristic features of the temperate continental climate.

Long-term observations show that the coldest months of the year for this region are December, January and February, with average temperatures ranging between 0 and 1.8°C. Sometimes the daily temperatures drop below -20°C.

The warmest months of the year are July and August, typical of the entire continental subregion. During the experimental period (November-April), the air temperature is characterized by some peculiar dynamics.

The average daily temperature for November is 9.7°C (+4.7°C warmer for many years), which helped to take root the plants after transplanting (Figure 1).

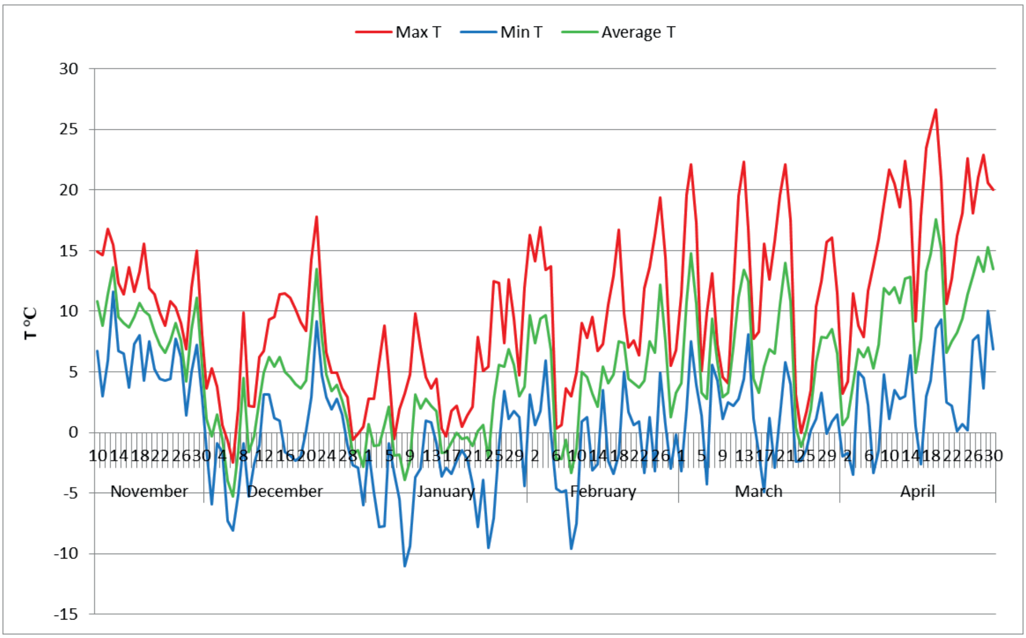

Figure 1. Atmospheric temperature for Sofia, during the experimental period (November 2019 - April 2020)

Over the next three winter months, the trend continues and despite the low temperatures that have been reported, they remain warmer than in the multi-year period (1981-2010), respectively +1.5°C for December, with +0.9°C for February and + 3.0°C for February (Figure 1). From the spring months, March is also warmer - by + 1°C, while April is cooler - the average monthly temperature is 9.7°C, which is -0.8°C lower than the average data from previous years (Figure 1).

During the lettuce growing the biometric indicators of the plants are influence more by the type of construction - high or low tunnels, as well as the various tested coatings.

In all three varieties the influence of the size of the facility on the diameter of the plants is significant for each variety: F (2.66) = 9.36, p <.05 - for the variety Gentilina, F (2.81) = 17.51, p <.05 - for the variety Aquarel and F (2.73) = 10.44, p <.05 - for the variety Cherna Gyumyurdzhinska. While the influence of different coatings is less.

In the Gentilina variety, the lettuces have a smaller diameter when grown under geotextile, in the Cherna Gyumyurjinska variety the plants are grown under green polyethylene. In Aquarel, the difference in the diameter of plants grown under low tunnels with different coatings is insignificant (Figure 2).

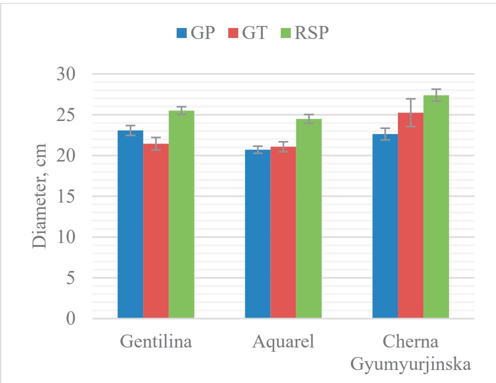

Figure 2. Diameter of plants grown under different coverings and tunnels

Significant is the influence of high tunnels on the height of plants - F (2.66) = 41.09, p <.05 - for the variety Gentilina, F (2,81) = 41,69, p <.05 - for the variety Aquarel and F (2.73) = 34.42, p <.05 - for the variety Cherna Gyumyurdzhinska. Again, the influence of the different coatings tested in the low tunnels is less, as the observed differences in the individual varieties appear as in the diameter of the plants In general, the Cherna Gyumyurdzhinska variety has a higher plant height, but this is a characteristic feature of the variety (Figure 3).

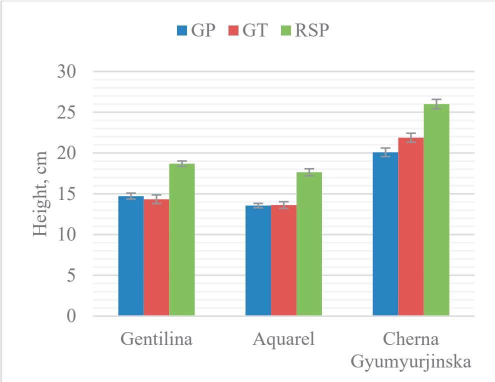

Figure 3. Height of plants grown under different coverings and tunnels

The average weight of one plant is also higher in lettuces grown in high tunnels, and the difference with those grown in low tunnels is significant: F (2.66) = 41.62, p <.05 - for the Gentilina variety, F (2.81) = 46.84, p <.05 - for the variety Aquarel and F (2.73) = 46.76, p <.05 - for the variety Cherna Gyumyurdzhinska (Figure 4).

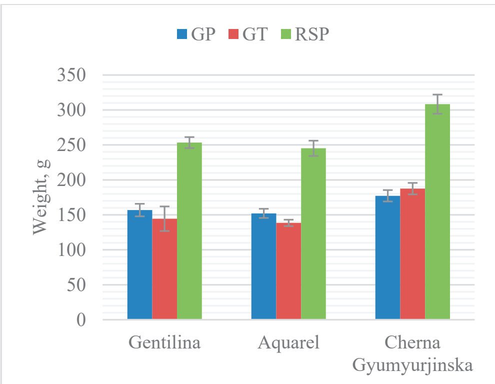

Figure 4. Mass of plants grown under different coverings and tunnels

In contrast to the diameter and height of the plants, in the case of Cherna Gyumyurjinska lettuce the mass of one plant is also influenced by the type of coverings tested in the low tunnels, as the plants grown under geotextiles have a higher mass than those grown, under green polyethylene $F(1.41) = 5.86$, $p < .05$. In addition to biometrics, analyzes of dry matter and sugar content were performed to determine whether and to what extent the coatings tested affected these parameters. The established differences are not unambiguous.

The Gentilina variety and the Cherna Gyumyurdzhinska variety have a higher dry matter content when grown under a low tunnel covered with geotextiles (Figure 5).

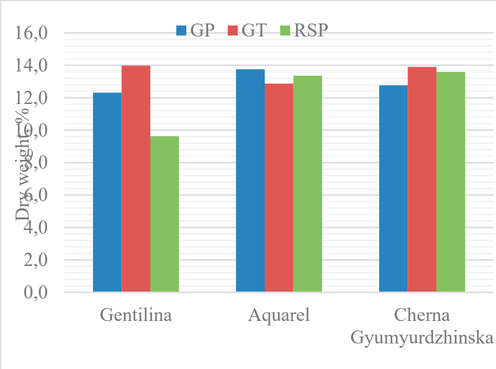

Figure 5. Dry matter content in% of plants grown under different coverings and tunnels

In the Aquarel variety, higher dry matter content was observed in plants grown under a low tunnel covered with green polyethylene.

In the Gentilina variety, a higher sugar content in the fresh mass was observed when it was grown under a low tunnel covered with green polyethylene (Figure 6).

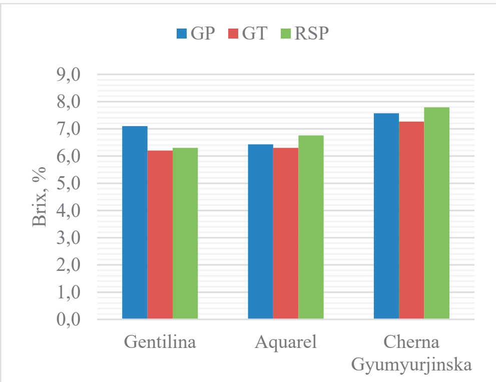

Figure 6. Total sugar content in % of plants grown under different coverings and tunnels

In general, lettuce has more sugars in the production than the two salads. When comparing the results obtained from plants grown under different coatings, it is seen that the differences between them are minimal. However, a higher sugar content is reported for both polyethylene coatings - a high tunnel with reinforced polyethylene and a low tunnel with a green polyethylene coating (Figure 6).

CONCLUSIONS

The present work was developed to evaluate the influence of different coatings on the development of salads.

Based on the results of biometric measurements, as well as some indicators responsible for the quality of production, the following conclusions can be made:

Of the two varieties of lettuce grown under a low tunnel, the Gentilina variety grows better when covered with green polyethylene - the plants are larger in diameter and height, with a larger mass, with a higher sugar content.

The Aquarel variety grows equally well under both types of low tunnel coverings.

Lettuce, Cherna Gyumyurdzhinska variety, is better developed when grown under a low tunnel covered with geotextile - the plants have a larger diameter, height and mass.

Compared to low tunnels, all three varieties grow better when grown under high tunnels.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF FERTILIZATION REGIME ON PLANTS GROWTH OF *PASSIFLORA CAERULEA*

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Abstract

Passiflora caerulea is a voluble ornamental plant appreciated in alternative medicine for its content in secondary metabolites. The aim of this research was to evaluate the influence of foliar fertilizers on the growth of biomass and ornamental characters in greenhouse-grown Passiflora, during 2019-2020. Thus, the Cropmax biostimulator and the mineral basic fertilizer Nutricomplex 20-20-20 were tested comparatively. Starting with the second week after transplanting the plant material into the soil, fertilizations were performed every 14 days, during the active growth and monthly during the winter. The plants were monitored for the main morpho-decorative characteristics, the results being interpreted statistically. The analysis of the recorded data shows that both treatments improved the growth of the tested plants compared to non-fertilized plants and the biostimulator Cropmax significantly influenced the growth of Passiflora caerulea plants compared to Nutricomplex 20-20-20.

Key words*: biostimulator, growth, mineral fertilizer, Passifora.*

INTRODUCTION

Passion flowers (*Passiflora* spp.) are tropical vines becoming more and more popular landscape plants. Climbing plants are important in the landscaping for their practical qualities because they can be used in purposes where trees and large shrubs are unsuitable (Berberich et al., 2006). The genus *Passiflora* includes approximately 550 species, of which more than 150 are native to Brazil which is one of the most important centers of passion flower diversity (Faleiro et al., 2005).

Passiflora caerulea (blue Passion flower) is the most gentle species having traditional use of its fruit as a sedative and anxiolytic. *Passiflora caerulea* is used medicinally in Uruguay. In West Indies, Mexico, Netherlands and South America, the root has been used as sedative and vermifuge. The aerial parts are used as antimicrobial agents in diseases like pneumonia (Patel et al., 2011).

Passiflora is one of the species with decorative potential in Romania for terraces and gardens due to exuberant flowers and the long period of decoration. Although due to the climatic conditions it needs protection over the winter in the conditions of this area, the decoration period is a long one, from the beginning of summer when the plant manages to reach a considerable size, until late autumn. *Passiflora* also behaves exceptionally in protected areas, decorating the interior terraces or spacious rooms of a home.

Passion flowers are fast growing plants and can make a sellable plant in approximately four months from sticking cuttings (Berberich et al., 2006).

Passion fruit is identify through a lot of uses of its different sections, such as pulp, seeds, bark, flowers, leaves, and branches (Nóbrega et al., 2017). These sections can be commercially valued in the aim of natural consumption, raw material for the food, juices, flowers for ornamentation condiment, cosmetic and pharmaceutical industries (Faleiro et al., 2015).

One of the impediments to expanding the production of *Passiflora* plants is the lack of informations or insufficient informations available to growers about growing practices necessary for the production of potted plants. Propagation by cuttings is successfully for *Passiflora caerulea*, offering the potential for economic profitability (Boboc et al., 2020). The growth and flowering of the passion flower is dependent on the fertilization regime

(Berberich et al., 2006; Borges et al., 2006 Menzel et al., 1991; Vieira Pacheco et al., 2018; Wanderley et al., 2020). Several factors influence the productivity of the passion fruit, among which can be highlight: the climate, the soil and the regime of fertilization and irrigation (Aular et al., 2014). Meeting nutritional requirements is essential to raise productivity and improve the quality of the fruits (Borges et al., 2006). Thus, the aim of the research was to establish an optimal fertilization regime for *Passiflora* grown in the greenhouse and to analyze the main morpho-decorative features to lead to the achievement of an optimal cultivation system for the conditions in Romania.

MATERIALS AND METHODS

The experience regarding the influence of fertilization regime on *Passiflora caerulea* plants growth was placed 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 (USAVM) Cluj-Napoca and was initiated on April 22, 2019. Plants were grown in a greenhouse with automatically controlled environmental conditions: 25/20°C day/night temperature, 60% relative humidity, and natural light. Plants were watered daily in the first 2 weeks after transplantation, and after that period twice a week. From the climatic point of view, the region in which the greenhouse is placed, according to the W. Köppen system is the climatic province Df, defined by the boreal climate with cold and humid winters and the lowest temperature recorded during winter below -30°C and the highest above 10°C (Bunescu et al., 2005).

Plant material consisted of young plants, 2 months old, obtained from cuttings of two knots harvested from mature plants at the end of February, 2019 and rooted in previous doctoral studies. After the plants root system was well developed, they were transplanted to soil.

For experimental purposes, two fertilizers were tested: Cropmax®, 100% organic biostimulator and Nutricomplex® 20-20-20 + M.E., mineral fertilizer. Cropmax® (Holland Farming, Groenekan, The Netherlands) is an organic growth biostimulant for all sorts of crops, contains amino acids, macro- and micro-elements, vitamins and polysaccharides. This fertiliser 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. 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 manufactured with high pure raw materials, enriched 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 recommended dosage is 250-400 g/hl.

Nutricomplex® 20-20-20 (n.d.)

The bifactorial experience was carried out during 2019-2020, in randomised block method with three repetitions (Ardelean et al., 2007) 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%

From the interaction of the two factors resulted 7 experimental variants.

The application of fertilizers (Cropmax and Nutricomplex) was done every 14 days by foliar spraying throughout the vegetation period. The treatments were applied after the first week after transplantation until the flowering. The results were compared to control, where *Passiflora caerulea* plants were treated with the same volume of water (without fertilizer). Tillage of plants was done using good agricultural practices.

Biometric variables have been used to assess the quality of *Passiflora caerulea* plant species. The morphological indices were represented by: average plant length (PL), average number of shoots per plant (ShNo), average number of internodes on the main stem (InNo), average length of internodes (InL) (cm), average number of leaves/plant (LeNo) and stem diameter at 50 cm from the ground (StD) (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 occured (FNFl), the average number of flowers per plant (FlNo), the flower diameter (FlD) and the average number of fruit per plant (FrNo). 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 = W2-W1/T,

Where: W1 = first measurement, W2 = second measurement, and T = the number of days between each.

The average values obtained in the period 2019-2020 were analyzed processed using the analysis of variance (ANOVA) followed by Duncan's multiple comparison test.

RESULTS AND DISCUSSIONS

Based on research conducted on the species *Passiflora caerulea*, the influence of fertilizer and dose applied on morpho-decorative features was analyzed.

Regarding the average growth of *Passiflora caerulea* plants in the first year after planting, the determinations were performed weekly, from transplanting date (April 22, 2019) until the end of the year (Figure 1). The plants fertilized with Cropmax recorded the highest average growth, with a value of 418.4 cm and the plants fertilized with Nutricomplex reaching an average height of 334.5 cm and unfertilized plants reached at 175.55 cm at the end of the year.

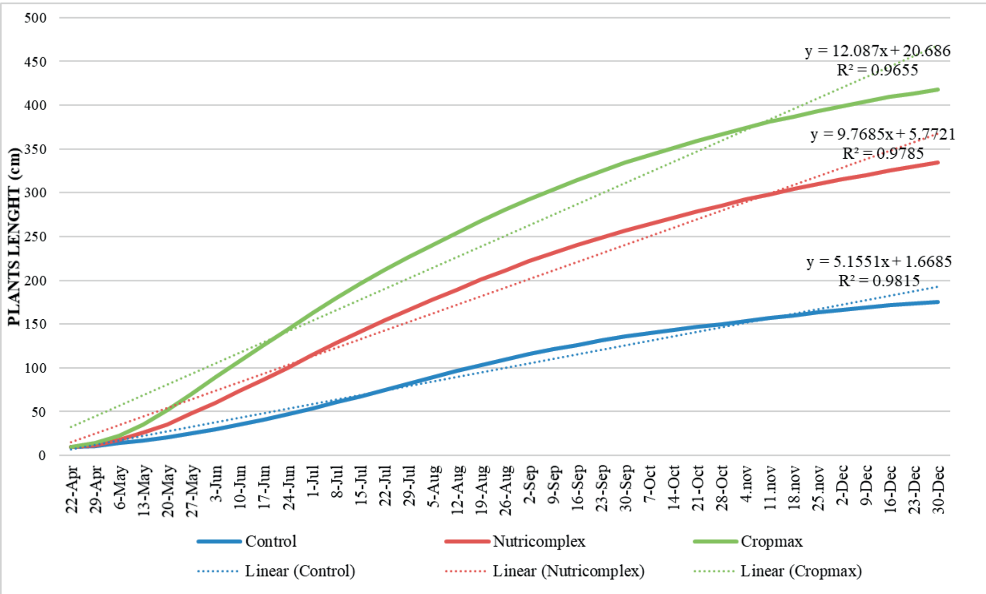

Figure 1. The evolution of average growths at *Passiflora caerulea* in 2019

Because a brief analysis of Figure 1 shows different growth rates, four time intervals were established for which the evolution of the relative growth rate was calculated. As shown in Figure 2, the highest RGR in all four time intervals (1.84, 2.44, 1.65, 0.92) and the highest average RGR/year (1.64) were recorded for Cropmax biofertilizer.

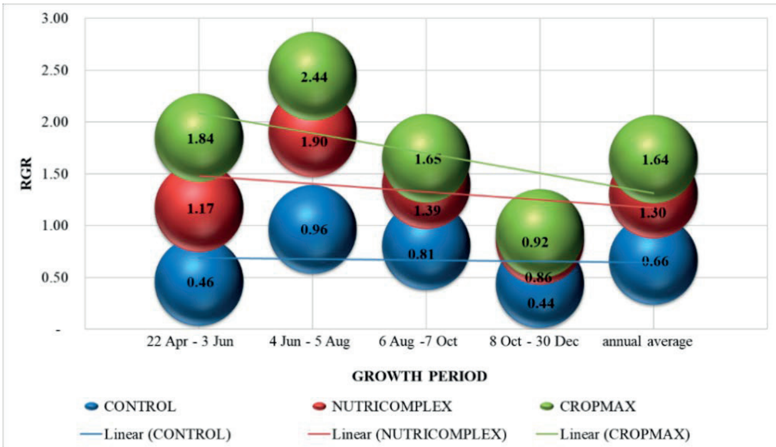

Figure 2. Relative growth rate (RGR) on growth intervals in 2019

Figure 3. *Passiflora caerulea* aspects of culture in the greenhouse didactic USAVM Cluj-Napoca: a, b - rooted cuttings; c - after 2 months from planting; d - specimen fertilized with Nutricomplex; e - specimen fertilized with Cropmax; f - fruiting, specimen fertilized with Cropmax; g, i - flowers of *P. caerulea*; i - fruit formation

According to Table 1, a bifactorial analysis of RGR was performed for the experimental years 2019 and 2020. On average, in 2019 the RGR (1.2) was higher than in 2020 (0.89). In the two years, Cropmax biofertilizer determines the highest RGR (1.64 and 1.35), followed by Nutricomplex (1.30 and 1.15) (Figure 3).
There were statistically assured differences between all the experimental variants

Table 1. Results on the influence of fertilizer on relative growth rate in 2019 and 2020 in *Passiflora caerulea*

Var. no.	Fertilizer	RGR 2019	RGR 2020
a_1	Nutricomplex	1.30 c	1.15 d
a_2	Cropmax	1.64 a	1.35 b
a_3	Unfertilized (C)	0.66 e	0.17 f
	DS 5%	0.03	

Note: The difference between any two values followed by at least one common letter is insignificant.

According to Table 2, the morphological indices showed increases in the application of all treatment variants compared to the control variant (V7).
Regarding the average length of the plants, there were statistically assured differences between each experimental variant (V1-V7), the average values ranged between 136.77 cm (V7) and 421.17 cm (V6).
The average number of shoots recorded values between 8.07 (V7) and 32.07 (V6). Cropmax-fertilized plants obtained significantly higher values, but there were no statistically assured differences (V4-V6) between the three applied doses.
The average number of internodes was shown to be directly proportional to the length of the plants. Thus, the average values varied between 25.27 (V7) and 63.33 (V6).
The average length of the internodes was between 4.85 cm (V7) and 6.65 cm (V7) but statistically assured differences were obtained only in the case of V2 (6.07 cm) and V7 (4.85 cm) variants.
The average number of leaves per plant ranged from 111.9 (V7) to 296.3 (V6). Each variant for which Cropmax was applied recorded statistically assured differences (V4, V5, V6), as well as for V1 fertilized with Nutricomplex and V7 (control).
The average diameter of the stem at 50 cm from the ground recorded values between 5.13 mm (V7) and 6.59 mm (V6), differences statistically ensured obtained in the case of variants V3, V5, V6 and V7.

Table 2. Results on the interaction of fertilizer and applied dose on morphological characters of *Passiflora caerulea*

Var. no.	Fertilizer	Doze	Morphological characters					
			Average plant length (cm)	Average number of shoots	Average number of internodes on the main stem	Average length of internodes (cm)	Average number of leaves / plant	Stem diameter at 50 cm from the ground (mm)
V_1	Nutricomplex	0.05%	325.17 e	24.81 b	56.10 d	5.79 c	217.67 e	6.18 c
V_2		0.1%	344.8 d	28.43 ab	56.83 cd	6.07 b	224.97 d	6.30 c
V_3		0.2%	385.83 c	26.83 ab	58.43 bcd	6.56 a	229.37 d	6.36 bc
V_4	Cropmax	0.05%	351.83 d	30.40 a	60.10 abc	5.82 c	273.6 c	6.28 c
V_5		0.1%	399.23 b	30.80 a	61.23 ab	6.53 a	282.00 b	6.54 ab
V_6		0.2%	421.17 a	32.07 a	63.33 a	6.65 a	296.73 a	6.59 a
V_7	Unfertilized (C)		136.77 f	8.07 c	25.27 e	4.85 d	111.9 f	5.13 d
	DS 5%		12.05-13.57	4.92-5.54	3.52-3.97	0.19-0.21	6.62-7.8	0.21-0.24

Note: The difference between any two values followed by at least one common letter is insignificant.

Regarding the unilateral influence of the fertilizer on the morphological indices (Table 3), there were significant value differences between the two fertilizers, the plants treated with Cropmax biofertilizer demonstrating values higher than those treated with Nutricomplex for each morphological index analyzed.

Table 3. Results on the influence of fertilizer on the morphological characteristics of *Passiflora caerulea*

Var. no.	Fertilizer	Morphological characters					
		Average plant length (cm)	Average number of shoots	Average number of internodes on the main stem	Average length of internodes (cm)	Average number of leaves / plant	Stem diameter at 50 cm from the ground (mm)
a_1	Nutricomplex	351.93 b	26.69 a	57.12 b	6.14 b	224.00 b	6.28 a
a_2	Cropmax	390.74 a	31.09 a	61.56 a	6.33 a	284.11 a	6.47 a
a_3	Unfertilized (C)	136.77 c	8.07 b	25.27 c	4.85 c	111.90 c	5.13 b
	DS 5%	17.72 - 18.08	4.78-4.87	3.35-3.42	0.08	25.04-25.55	0.30-0.31

Note: The difference between any two values followed by at least one common letter is insignificant.

Statistically assured differences between the two fertilizers were obtained only in terms of average plant length, average number of internodes on the main stem, average length of internodes, average number of leaves per plant. In the study conducted by Balint et al., (2018) fertilizations were performed on the commmon bean crop, and Cropmax significantly influenced the length and width of the bean pod. The comparative analysis of the unilateral influence of additional fertilization on ordinary pod beans shows a very significant positive difference (+0.04 cm) using Cropmax.

According to Table 4, the morphological indices regarding flowering and fruiting recorded average values higher than the control variant (V7). Thus, the first node at which a flower formed varied on average from 2.05 (V6) and 2.42 (V7), statistically assured differences were obtained only for V2 (2.23) and V6 (2.05). For this index, a lower average numerical value denotes a higher ornamental potential, with a higher number of flowers per shoot.

The average number of flowers per plant varies between 17.6 (V7) for non-fertilized plants and 193.6 (V6) for Cropmax fertilization 0.2%. Between each experimental variant, statistically assured differences were obtained, the number of flowers per plant being directly proportional to the applied fertilizer dose, thus V3 (173.66) and V6 represented significantly higher values compared to the rest of the variants.

The average diameter of the flower varies between 6.55 cm (V7) and 8.29 cm (V6), the differences being statistically ensured for the limit values.

The average number of fruits per plant ranges between 0.20 (V7) and 47.63 (V6). Plants fertilized with Cropmax produced a higher number of fruits compared to those treated with Nutricomplex, regardless of dose. Between each experimental variant, statistically assured differences were obtained.

Table 4. Results on the interaction of the fertilizer and the dose applied on the morphological-decorative characters in *Passiflora caerulea*

Var. no.	Fertilizer	Doze	Morphological characters			
			The first node at which the flower occurred	Average number of flowers / plant	Average flower diameter (cm)	Average number of fruits / plant
V_1	Nutricomplex	0.05%	2.34 a	111.35 f	7.67 c	14.67 e
V_2		0.1%	2.23 b	141.83 d	7.70 c	29.57 c
V_3		0.2%	2.14 bc	173.77 b	8.04 b	37.43 b
V_4	Cropmax	0.05%	2.34 a	126.33 e	8.05 b	21.30 d
V_5		0.1%	2.15 bc	154.6 c	8.14 ab	36.63 b
V_6		0.2%	2.05 c	193.6 a	8.29 a	47.63 a
V_7	Unfertilized (C)		2.42 a	17.6 g	6.55 d	0.20 f
DS 5%			0.10-0.11	9.49-10.69	0.15-0.17	4.20-4.74

Note: The difference between any two values followed by at least one common letter is insignificant.

Analyzing Table 5. on the influence of fertilizer on morphological and decorative characters in *Passiflora caerulea*, it is found that the application of a fertilizer does not statistically influence the first node at which the flower occurs on the shoot

Table 5. Results on the influence of fertilizer on morphological and decorative characters in *Passiflora caerulea*

Var. no.	Fertilizer	Morphological characters			
		The first node at which the flower occurred	Average number of flowers / plant	Average flower diameter (cm)	Average number of fruits / plant
a_1	Nutricomplex	2.18 a	142.32 b	7.80 a	27.22 b
a_2	Cropmax	2.24 a	158.18 a	8.16 a	35.19 a
a_3	Unfertilized (C)	2.18 a	17.6 c	6.55 b	0.20 c
	DS 5%	0.34-0.35	8.01-8.17	0.40	1.52-1.56

Note: The difference between any two values followed by at least one common letter is insignificant.

Regarding the average number of flowers and fruits per plant, between the two fertilizers there are statistically assured differences, in both cases Cropmax records higher values (on average 158.18 flowers and 35.19 fruits per plant) compared to Nutricomplex (which determined in average production of 142.32 flowers and 27.22 fruits per plant).
Regarding the average diameter of the flower, there are no statistically assured differences between the two tested fertilizers.
The results obtained from the correlation between each morphological index were presented in Figure 4. Using the Pearson correlation coefficient, indicates almost perfectly positive correlated direct links ($p < 0.001$) between PL ($r = 0.91$-0.99) and the rest of the analyzed characters except FNF1 together with which it achieves a strong negative correlation ($r = -0.89$). As can be seen, the FNF1 indicator makes direct connections negatively correlated with each indicator followed.

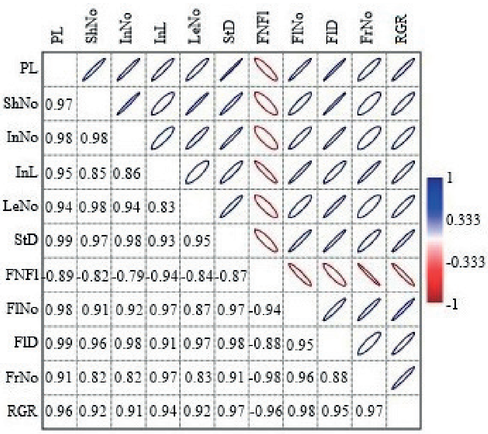

Figure 4. Correlation matrix with Pearson significance levels and graphical representation of the dispersion of each pair of variables in *Passsiflora caerulea*

In their research, Șesan et al. (2020) determined the effect of foliar treatments with a *Trichoderma* consortium on *Passiflora caerulea* on morphological, physiological and ultrastructural characteristics. Thus, the higher concentration of *Trichoderma* consortium was associated with larger leaves, increased number and size of chloroplasts, improved plant physiology characteristics, and an increased yield.
Pacheco et al. (2017) performed three types of fertilizers in the culture of *Passiflora edulis* for fruit: recommended mineral fertilizer, organic fertilizer represented by manure and the equivalence of recommended fertilization with potassium for the culture of *Passiflora edulis* by double dose of organic fertilizer. The single dose of organic fertilizer proved to be insufficient to maintain the quality of the fruit, but the fruit obtained from double-dose fertilized plants maintained their commercial quality better.
In another study on *Passiflora edulis*, Nascimento et al. (2016) evaluated the effects of bovine biofertilizer and mineral fertilization with NPK on the growth and production of fruit in passion flower plants irrigated with moderate salt water. Thus, the biofertilizer applied at the maximum dose combined with mineral fertilization with NPK led to a greater increase in stem diameter and increased plant productivity. Even in the treatments without mineral fertilizers, the biofertilizer increased the productivity to values close to 24 t ha^{-1} at the level of 60.65%.
In their research, Campos et al. (2015) demonstrated that organic fertilization had a positive effect on the *Passiflora incarnata* plant´s growth, improving the biomass production (dry matter). However, the synthesizing of bioactive compounds such as polyphenols, total flavonoids and the antioxidant capacity were not influenced by the effect of organic fertilization.
According to the study of Boechat et al. (2010), the application of NPK fertilizer is not enough to avoid adverse effects on plant growth or to correct the nutritional balance. Manure is the best option for the production of high quality seedlings under the conditions studied at *Passiflora edulis*.
In their research, Dinu et al. (2008) demonstrated that the treatments with 0.2% Cropmax and Vitaflora contribute for increase the starkness for the tomatoes plants, to increase plants resistance during the dryied time. The Cropmax biofertilizer can imporove the construct of tomatoes fruit.

CONCLUSIONS

Foliar fertilization influenced the culture of *Passiflora caerulea* in the first two years after cultivation. Regarding RGR, significant influences were found especially in the first year. The ecological foliar biofertilizer Cropmax has strongly influenced all the morphological and flowering characteristics of *Passiflora* plants. Regarding plant growth, differences between the two fertilizers can be seen from the first month of application, differences that have become more pronounced over time. This confirms the need for a fertilization regime and demonstrates its effectiveness. Thus, foliar fertilization is recommended for the cultivation of *Passiflora caerulea* from an ornamental point of view, or using organic fertilizers as a medicinal plant for its important therapeutic properties.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SEED GERMINATION RATE OF DIFFERENT TURFGRASS MIXTURES UNDER CONTROLLED CONDITIONS

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Abstract

Green areas are, in both domestic gardens and public places. In many cases, based on the landscape architecture design layout of a green space, lawns can occupy the largest surface. The lawn is used to gather all the elements of the designed landscape, thus finding the most suitable turfgrass seed mixtures is a priority in landscaping, but their success depends on the percentage of germination. The present research was undertaken to analyze the germination of eight different turf seed mixtures, available to Românian retail consumers in recent years, as follows: 'Landscaper Pro Finesse', 'Landscaper Pro Performance', 'Turfline DLF', 'Gebrauhsrasen Schattenrasen', 'Turfline DLF Sunshine', 'Landscaper Pro Rapid', 'DLF Ecolawn' and 'Landscaper Pro Sun&Shade'. Within the study, the seed mixtures were sown in a growing chamber with controlled temperature and lighting period for 14 days. The results indicated that the best germination percentage was obtained by the turf seed mixture named 'Landscaper Pro Rapid' (Festuca rubra 'Cathrine' 15%, Lolium perenne 'Vermino' 40%, Lolium perenne 'Groundforce' 35%, Poa pratensis 'Heatmaster' 10%).

Key words: *landscape, green area, grass seed, germination.*

INTRODUCTION

In many landscape architecture design layouts of a green space, the lawns occupy the largest area. Meanwhile, in the beginning, grass seeds were used more in an economical way, being a particularly important world crop from grasslands in the agriculture and animal production (Jones, 2013). Today green areas of grass have also an ornamental purpose, becoming more and more popular in landscaping (Dongmei, 2009; Haq, 2015). Lawns are a human-created and culture-shaped habitat in urban green areas (Yang et al., 2019), but etymologically defines a grass-covered surface and dates from the 15th century in America (Jenkins, 2015; Steinberg, 2006; Bormann et al., 2001; Teyssot, 1999). In medieval Europe, open expanses of low grasses became valued among the aristocracy because they allowed a better visibility on the surrounding space (Beard, 1972). The presence of the English lawn highlighted in the early 17th century during the Jacobean period (James VI of Scotland 1603-1625) and after, plays an important role in the setting of the English landscape garden style designed by William Kent and Lancelot Brown (Walpole, 1904). Named 'nature strip', the appearance of the lawn in Australia followed closely after its establishment in North America and parts of Europe (Hogan, 2003). Analyzing the Asian landscape design evolution, in Chinese cities for example, the lawn is still a new landscape feature and directly connected to the process of Westernization and globalization of urban environments, and the first record of a prototype of Chinese lawns 草坪 it was not until the 1960s, found in Fu on the Imperial Garden (Yang et al., 2019).

Studying the benefits of a green area covered with turfgrass in a landscape design proposal, evidence-based research indicates several environmental benefits (Figure 1) that improve our quality-of-life, like functional, recreational and aesthetic components summarize in the following diagram (Beard & Green, 1994). Another aspect of the lawns benefits is that absorbs noise, cool the air, reduce pollution (McKinley, 2005). Also, research result highlights that human exposure to green spaces influence emotional wellbeing, mental and physical health, by reducing stress, anxiety and depression and improves the cardiovascular and metabolic health, concentration and memory, energy level (Buru et al., 2021; Kwon et al., 2021; Cheng, 2020; Fesharaki et al., 2020; Hitter et al., 2019; Seresinhe et al., 2019; Li, 2018; Cooper Marcus and Sachs, 2014; Hartig et al., 2014; Van Herzele & de Vries, 2012; Gonzales et al., 2011; Sempik et al., 2010).

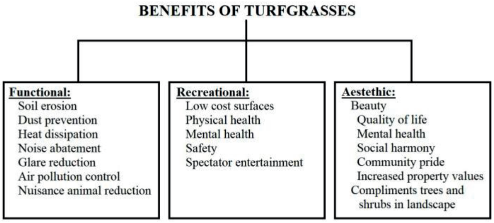

Figure 1. Diagrammatic summary of benefits derived from turfs (Beard & Green, 1994)

In the Romanian landscape architecture design, in accordance with the site dimension and the type of green spaces, technical instructions for design were recommended since 1973, to properly combine each part of the green space, depending on the destination of the landscape (Table 1). Following this recommended proportions, the lawn areas can be integrated in the hole landscape design concept, based on the different landscape units' types and requirements.

Table 1. Characteristic percentages related to the main component parts of the green space units (Negruțiu, 1980)

No.	Green space units	The green surfaces use					
		Vegetation					Other facilities (blue landscape, playground, pergolas) (%)
		Total (%)	Trees and shrubs (%)	Ornamental flowers (%)	Grass, laws (%)	Alley and accesses (%)	
1	Square	60-85	20-60	5-10	30-75	15-20	0-20
2	Allotment garden	40-70	30-60	4-8	32-66	10-20	20-40
3	Park	65-75	30-60	3-5	35-67	10-15	15-20
4	Planting strips	100	20-60	2-5	35-78	-	-
5	Residential green area	90-95	30-60	1-3	37-69	5-10	-
6	Kindergarten - Garden Unit	60-70	50-60	2-3	40-50	10-15	20-25
7	Educational institutions landscapes	45-60	60-70	2-3	30-40	10-15	30-40

In accordance with the green area use and purpose, different turfgrass seed mixtures is made and can be purchased in professional standard products. In many cases, producer's intent to use a mixture with minimum two or three different species having a better ability to adapt to various environmental conditions. To establish the lawn with a proper seed's mixture, several aspects must be considered, like environmental factors as soil type, soil moisture, soil slope, shading, temperature, or land use. Also, the lawn display aspect of the lawn is particularly important, like: color, texture, density. Based on research results, producers and economic agents supply the market with different types of turfgrass seed mixture, for different green areas and landscape types (Table 2).

Table 2. Turfgrass seed mixture (Dumitraș et al., 2008; Iliescu, 2008; Negruțiu, 1980)

Lawn for leisure			
Sandy soils	Heavy soils	Shaded areas	Sunny areas
40% *Lolium perenne*	45% *Lolium perenne*	35% *Lolium perenne*	40% *Lolium perenne*
50% *Festuca rubra*	40% *Festuca rubra*	35% *Festuca rubra*	30% *Festuca rubra*
10% *Festuca ovina*	15% *Poa pratensis*	15% *Poa nemoralis*	30% *Festuca ovina*
		15% *Poa trivialis*	
Sport field			
Sandy soils	Heavy soils	Shaded areas	
40% *Lolium perenne*	50% *Lolium perenne*	30% *Lolium perenne*	
20% *Festuca rubra*	25% *Festuca rubra*	40% *Festuca rubra*	
20% *Festuca arundinaceae*	15% *Festuca arundinaceae*	20% *Festuca arundinaceae*	
20% *Festuca ovina*	10% *Festuca ovina*	10% *Poa pratensis*	
English lawn			
Sandy soils	Heavy soils	Shaded areas	
20% *Lolium perenne*	20% *Lolium perenne*	60% *Festuca rubra*	
70% *Festuca rubra*	65% *Festuca rubra*	20% *Poa nemoralis*	
10% *Poa pratensis*	15% *Poa pratensis*	20% *Poa trivialis*	
Ornamental lawn			
Sandy soils		Heavy soils	
75% *Festuca rubra*		50% *Festuca rubra*	
25% *Agrostis tenuis*		30% *Agrostis tenuis*	
		20% *Festuca ovina*	
Lawn for road slopes, dams etc.			
Sandy soils	Heavy soils	Shaded areas	Sunny areas
50% *Festuca arundinacea*	50% *Festuca rubra*	50% *Lolium perenne*	60% *Poa pratensis*
25% *Bromus inermis*	40% *Festuca ovina*	30% *Bromus inermis*	35% *Festuca rubra*
25% *Dactylis glomerata*	10% *Poa pratensis*	20% *Poa pratensis*	5% *Agrostis tenuis*
Rustic lawn			
40% *Lolium perenne*			
50% *Festuca pratensis*			
5% *Poa pratensis*			
5% *Poa pratensis*			

MATERIALS AND METHODS

The present research study was carried out Institute of Advanced Horticultural Research of Transylvania (I.C.H.A.T) from the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, to test the germination of several turfgrass seed mixture available on the Romanian market in the spring of 2020. There were selected eight different turfgrass seed mixtures (M), considered experimental variants:

M1 - LANDSCAPER PRO FINESS: 40% *Lolium perenne*, 40% *Festuca rubra rubra*, 10% *Festuca rubra duriuscula*, 10% *Festuca rubra trichophylla*;

M2 - TURFLINE DLF: 80% *Lolium perenne*, 20% *Festuca rubra rubra*;

M3 - LANDSCAPER PRO PERFORMANCE: 20% *Lolium perenne*, 50% *Festuca rubra*, 20% *Festuca rubra trichophylla*, 10% *Poa pratensis*;

M4 - GEBRAUHSRASEN SCHATTENRASEN: 5% *Agrostis capillaris*, 20% *Festuca trachyphylla*, 45% *Festuca rubra*, 15% *Lolium perenne*, 10% *Poa pratensis*, 5% *Poa nemoralis*;

M5 - TURFLINE ECO LAWN: 35% *Lolium perenne*, 5% *Poa pratensis*, 12% *Festuca rubra trichophylla*, 45% *Festuca rubra rubra*, 3% *Trifolium repens*;

M6 - TURFLINE DLK SUNSHINE: 25% *Lolium perenne*, 45% *Festuca arundinacea*, 20% *Festuca rubra rubra*, 10% *Poa pratensis* ;

M7 - LANDSCAPER PRO RAPID: 75% *Lolium perenne*, 15% *Festuca rubra rubra*, 10% *Poa pratensis*;

M8 - LANDSCAPER PRO SUN&SHADE: 25% *Lolium perenne*, 30% *Festuca rubra rubra*, 15% *Festuca rubra commutate*, 30% *Festuca rubra trichophylla*.

The experiment was organized in randomizes blocks, with 3 repetition/each turfgrass seed mixture (100 seed/plastic containers), measured before sowing to determine the thousand seeds weight (TSW, g) and were sown after in plastic containers (195 x 125 x 75 mm) filled with rehydrated peat. The seeds were placed over the substrate, without being covered. Using a growing chamber - Ekochl 700, the controlled environment for the following 14 days was provided with 12 hours of photoperiod with day/night conditions of 15/10°C air-temperature and 55/75% relative humidity (Mollard & Naeth, 2014; Serpe et al., 2006). During the study, 7 days after the sowing the germinative energy (%) and after 14 days germinative faculty (%) were measured.

The obtained results from the present experience were statistically analysed using Duncan test and Fisher's Least Significance Difference to measure the differences between the eight turfgrass seed mixtures.

RESULTS AND DISCUSSIONS

Results regarding the seed germination of the eight turfgrass seed mixtures, to determine the best and fast germination percentage, in controlled conditions, using biometric observation and measurement were made on the important characteristics of the seedling, like thousand seeds weight, germinative energy and germinative faculty presented in the next tables (Tables 3, 4 and 5).

Table 3. Thousand seeds weight (TSW)

code	Mean (g)	±SD	±SE	Duncan	Diff.	Diff. %	LSD significance
M1	1.15	0.01	0.01	c	0.02	101.6	n.s.
M2	1.43	0.02	0.01	d	**0.30**	126.3	**
M3	1.00	0.16	0.09	bc	-0.14	88.0	n.s.
M4	0.77	0.08	0.05	a	**-0.36**	67.9	000
M5	0.93	0.11	0.06	ab	**-0.20**	82.2	0
M6	1.15	0.08	0.05	c	0.02	101.4	n.s.
M7	1.57	0.13	0.07	d	**0.44**	138.5	***
M8	1.07	0.08	0.05	bc	-0.07	94.1	n.s.
Average	**1.132**				0	100	Control

Note: ±SD – standard deviation, ±SE – standard error of mean
LSD significance p>0.05, (n.s.), p<0.05 (- ^{0}/ + *), p<0.01 (- ^{00}/+ **), p<0.001 (- ^{000}/ + ***)
Duncan test – differences between values followed by at least one common letter are n.s. at p<0.05

LSD (p 0.05) 0.18
LSD (p 0.01) 0.25
LSD (p 0.001) 0.35

Maximum thousand seeds weight value (Table 3) was registered by M7 - LANDSCAPER PRO RAPID (1.57 g.), and lowest by M4 - GEBRAUHSRASEN SCHATTENRASEN (0.77 g). The average thousand seeds weight for all eight variants was 1.13 g. Based on Duncan test, thousand seeds weight of M7 was significantly different superior to M1, M3, M4, M5, M6, M8. TSW of M4 was significantly different inferior to M1, M2, M3, M6, M7, M8 Thousand seeds weight of M1 was significantly different superior from M4, and significantly different inferior from M7, M2. There were no significant differences in thousand seeds weight between M3, M5 and M8. Also, thousand seeds weight was not significantly different between

M1, M6, M3 and M8. Based on Fisher's least significant different test was determined that thousand seeds weight of was M2 and M7 was significantly higher than the average for the eight variants. By comparison, the thousand seeds weight for M4 and M5 was significantly lower than the average for the eight variants.

Table 4. Germinative energy (%)

Experimental code	Mean (%)	±SD	±SE	Duncan	Diff.	Diff. %	LSD significance
M1	11.60	2.46	1.42	b	0.65	105.9	n.s.
M2	18.67	3.78	2.18	c	**7.71**	170.4	**
M3	4.83	3.12	1.80	a	**-6.12**	44.1	0
M4	1.50	0.82	0.47	a	**-9.45**	13.7	00
M5	6.80	1.54	0.89	ab	-4.15	62.1	n.s.
M6	4.30	0.20	0.12	a	**-6.65**	39.3	0
M7	28.00	4.54	2.62	d	**17.05**	255.6	***
M8	11.93	3.96	2.28	b	0.98	108.9	n.s.
Average	**10.954**				0	100	**Control**

Note: ±SD – standard deviation, ±SE – standard error of mean
LSD significance p>0.05, (n.s.), p<0.05 (- ^{0}/ + *), p<0.01 (- ^{00}/+ **), p<0.001 (- ^{000}/ + ***)
Duncan test – differences between values followed by at least one common letter are n.s. at p<0.05

LSD (p 0.05) 5.39
LSD (p 0.01) 7.47
LSD (p 0.001) 10.38

According to the germinative energy (Table 4), the highest percentage was registered by M7 - LANDSCAPER PRO RAPID (28.00%), and lowest by M4 - GEBRAUHSRASEN SCHATTENRASEN (1.50%) after 7 days from the sowing. During period of testing the germinative energy, the first seedlings could be observed at M5 (TURFLINE ECO LAWN) at day four (Figure 2).

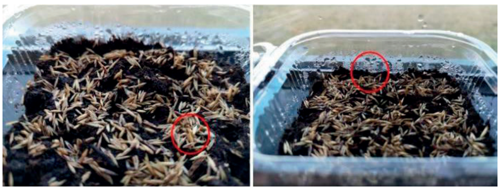

Figure 2. First seedlings registered in day four at M5 turfgrass mixtures, original

The average of the germinative energy for all eight variants was 10.954%. Analyzing the Duncan test results, it can be noticed that the germinative energy of M7 was significantly different superior to all other variants (M1, M2, M3, M4, M5, M6, M8). Germinative energy of the variants M3, M4 and M6 were significantly different inferior to V1, V2, V7, V8. There were no significant differences between germinative energy of variants M3, M4, M5, M6. Also, germinative energy was not significantly different between M1, M5 and M8. Considering the obtained date, Fisher's least significant different test was determined that germinative energy of was M2 and M7 was significantly higher than the average for the eight variants. Comparing the data, the germinative energy for M3, M4 and M6 was significantly lower than the average for the eight variants (Robins et al., 2020; Charif et al., 2019; Jones, 2013; Miller et al., 2013; Serpe et al., 2006; McKinley, 2005).

Table 5. Germinative faculty (%)

Experimental code	Mean (%)	±SD	±SE	Duncan	Diff.	Diff. %	LSD significance
M1	37.57	6.43	3.71	bc	-1.46	96.3	n.s.
M2	45.00	11.67	6.74	cd	5.97	115.3	n.s.
M3	28.50	16.15	9.32	bc	-10.53	73.0	n.s.
M4	6.43	3.03	1.75	a	**-32.59**	16.5	00
M5	43.80	10.04	5.80	cd	4.77	112.2	n.s.
M6	22.77	8.29	4.79	ab	-16.26	58.3	n.s.
M7	66.87	9.46	5.46	e	**27.84**	171.3	**
M8	61.27	10.46	6.04	de	**22.24**	157.0	*
Average	**39.025**				0	100	**Control**

Note: ±SD – standard deviation, ±SE – standard error of mean
LSD significance p>0.05, (n.s.), p<0.05 (- ^{0}/ + *), p<0.01 (- ^{00}/+ **), p<0.001 (- ^{000}/ + ***)
Duncan test – differences between values followed by at least one common letter are n.s. at p<0.05

LSD (p 5%) 18.05
LSD (p 1%) 25.02
LSD (p 0.1%) 34.76

Analyzing the germinative faculty (Table 5), the highest percentage was registered by M7 - LANDSCAPER PRO RAPID (66.87%), and lowest by M4 - GEBRAUHSRASEN SCHATTENRASEN (6.43%) after 14 days from the sowing. The average of the germinative faculty for all eight variants was 39.025%. Based on Duncan test, germinative faculty of M7 was significantly different superior to M1, M2, M3, M4, M5, M6 (Figure 3).

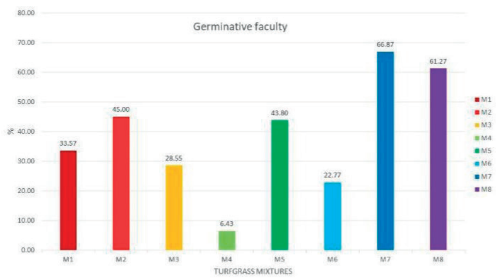

Figure 3. Compared result regarding the germinative faculty at eight different turfgrass mixtures, original

Germinative faculty of M4 was significantly different inferior to M1, M2, M3, M5, M7, M8. The germinative faculty was not significantly different between M1, M2, M3 and M5. Also, the germinative faculty was not significantly different between M4 and M6. Based on Fisher's least significant different test was determined that germinative faculty of M7 (Figure 4) and M8 was significantly higher than the average for the eight variants. By comparison, the germinative faculty for M4 was significantly lower than the average for the eight variants.

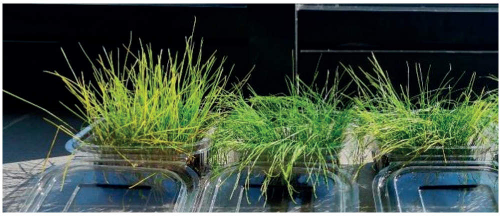

Figure 4. The highest germination was registered at M7 turfgrass mixtures, original

Turfgrass mixtures and blends are more resilient to environmental factors such as abiotic and biotic stress. The characteristics of a mixture and blend is given by the species and cultivars it contains and their proportion. A study on five turfgrass species showed these have different germination characteristics, with *Lolium perenne* displaying fast and high germination rate while *Poa pratens* presenting both slow germination at low rate. The species *Festuca arundinacea*, *Agrostis stolonifera* and *Pennisetum clandestinum* presented intermediate germination parameters compared with the two species mentioned above (Charif et al., 2019). A study conducted on eight turfgrass mixtures subject to low-input and two mowing conditions, has put in evidence a heterogenous response across variants, with tall fescue blend performing the best (Milller et al., 2013). Furthermore, another research has demonstrated that perennial wheatgrasses have a better performance in mixture with traditional turfgrass species (*Festuca brevipila, Poa pratensis*), resulting in a higher coverage and density than monoculture (Robins & Bushman, 2020).

CONCLUSIONS AND RECOMMENDATIONS

Based on the germination results can be observed that in the case of the seed mixtures of M2 (LANDSCAPER PRO PERFORMANCE) and M7 (LANDSCAPER PRO RAPID) a significantly higher thousand seeds weight was associated with significantly higher germinative energy. But only for M7 (LANDSCAPER PRO RAPID) the significantly higher thousand seeds weight was associated both with higher germinative energy and faculty, then this mixture can be recommended for grassed green areas in landscape constructions, based on the results of this study. This association between higher seed mass and higher germinative energy and faculty can be explained by the fact that larger seeds have larger foods reserves (more endosperm) for the growing embryo. The lowest performance regarding germinative energy and faculty was registered by M4 (GEBRAUHSRASEN SCHATTENRASEN), that also presented significantly lower thousands seeds weight compared to the average of all eight variants and this poor performance of M4 can be associated with smaller seeds with less nutrient reserves for the growing embryo. Thus, for landscape construction and maintenance services (or groundskeeping), green area and lawn can have an increase functional and aesthetic role by using professional seed mixture with a fast germination, but also with a high germinative faculty.

ACKNOWLEDGEMENTS

This work was supported by Advanced Horticultural Research Institute of Transylvania, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Authors are thankful to the students from Faculty of Horticulture UASVM Cluj-Napoca that were involved in this study.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IPOMOEA PLATENSIS – MULTIPLICATION OF A NEW ORNAMENTAL SPECIES IN ROMANIA

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Abstract

The Ipomoea platensis species belongs to the Convolvulaceae family. It grows as a wine and is widespread in tropical and subtropical areas. This morning glory is a caudiciform species, with tuberous roots, which also grow on the surface of the soil, thus having a good resistance to drought. In Romania's climatic conditions this species is flowering if fertilized but the capsule (bud) aborts usually before seed maturation, therefore the vegetative propagation is the only way to multiply it. In this research we investigated its optimum vegetative multiplication (ex vivo and in vitro). For ex vivo multiplication the best results were obtained using tap water without growth hormones. For in vitro multiplication we used nodal segments and leaf fragments. The nodal segments used as explant produced shoots, while the leaf fragments developed only callus. The explants were placed on MS medium containing cytokinines (BAP, KIN and TDZ) and auxins (IAA and IBA) in different concentrations. Optimal proliferation was observed when shoots were cultivated on MS medium supplemented with 0.5 mg/l IBA and 0.2 mg/l KIN. The concentration and type of cytokinine had an influence on the multiplication of I. platensis species, from nodal segments. Thus, Kinetin in 0.2 mg/l concentration induces the formation of a higher number of shoots compared to the cytokinines BAP in 0.3 mg/l and TDZ in 0.1 mg/l concentration. Consequently, the easiest way to multiply this species is ex vivo, but the highest ratio of multiplication is obtained in vitro, from nodal segments.

Key words*: Convolvulaceae, morning glory, micropropagation, ex vivo, in vitro.*

INTRODUCTION

Ipomoea platensis Ker-Gawl. is synonym with: *Ipomoea platense*, *Ipomoea digitata* var. *septempartita* Meisn.*; Ipomoea lineariloba* Peter; *Ipomoea platensis* var. *erecta* Hassl.; *Ipomoea platensis* var. *quinque-partita* Hassl.; *Ipomoea platensis* var. *subnovem-partita* Hassl.; *Ipomoea platensis* var. *subseptem-partita* Hassl. (http://www.catalogueoflife.org). This specie was first described by John Bellenden Ker Gawler in 1818. It belongs to the *Convolvulaceae* family which includes 50 genera and 1800 species (Orfila and D'Alfonso, 1995). According to some authors, the genus *Ipomoea* comprises 500 species (O`Donell, 1953; 1959), while other authors consider that it would comprise approximately 600-700 species (Meira et al., 2012). Given the large number of species of this genus, there is a great variability in their phenotype. Thus, we encounter annual and perennial species, in the form of shrubs and semi-shrubs, plants with voluble and climbing stems, with tuberous, caudiciform roots, etc. The species under study, as described by Orfila and D'Alfonso (1995), is native to Argentina, Paraguay and Uruguay, and is widespread in tropical and subtropical areas. It is a caudiciform species, with tuberous roots, which also grow on the surface of the soil, thus having a good resistance to drought. Prefers semi-shaded areas and not direct sunlight. The stem is voluble (4 - 5 m), the leaves are palmate, showing 5-9 lobes (Figure 1c), and the flowers are arranged in cymose inflorescences with 2 - 6 flowers in inflorescence. The caudex (tuberous root together with the stem) can reach up to 60 cm, and the voluble stems reach up to 4.0 m. The flowers are pink infundibuliform (Figure 1b, 1c). Propagation can be done by seeds, but unlike other species of the *Ipomoea* genus, it can be propagated very easily by stem cuttings or root separation. If it is multiplied by seeds, the flowering takes place after 3 years (in the conditions of our country and protected over the winter), and if it is multiplied vegetative, flowers appear already in the following year. This species is totally different from the well-known ornamental species of the *Ipomoea* genus, widespread in Romania. Due to the fact that the tuberous roots also grow on the soil surface, the species is also very suitable for pot cultivation (Figure 1a), providing a support system or can be grown even in the form of bonsai, because it behaves very well when pruning. This species of morning glory can be used to develop new ornamental genotypes of *Ipomoea* and for other utilizations (medicinal, agrotechnical etc.). It should be mentioned that this is the first detailed description of the species *I. platensis* in the literature of our country.

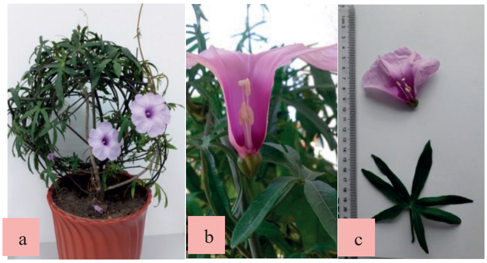

Figure 1. *Ipomoea platensis* in the pot (a); morphological flower characteristics (b and c) (original)

We decided to analyse *ex vivo* and *in vitro* multiplication because in the last three years it was impossible to obtain seeds from this plant. The objective of this study was to identify the proper way to multiply *Ipomoea platensis*.

MATERIALS AND METHODS

Ipomoea platensis is one of the species found in the germplasm collection of the Plant Breeding department of the Faculty of Horticulture Cluj-Napoca, Romania. Opposed to other common species of *Ipomoea* that multiply only by seeds, this one multiplies both by seeds and by stem cuttings. In the present research we tested the *ex vivo* and *in vitro* multiplication capacity of this species. For *ex vivo* propagation stem cuttings were used and for *in vitro* culture the initiation was performed from leaf fragments and nodal segments. The biological material was obtained from the potted mother plant.

Ex vivo multiplication of plants

For *ex vivo* multiplication, the stem cuttings were detached at 1-2 internodes and the foliar surface was reduced. The cuttings were placed in tap water, water with two concentrations of auxins and perlite substrate obtained four experimental variants: V1 - tap water; V2 -water +1.5 mg/l indolyl butyric acid (IBA); V3 - water + 3.0 mg/l indolyl butyric acid (IBA); V4 - perlite substrate moistened only with water. The cuttings were maintained at room temperature (22°C), periodically being carried out observation on the root system formation.

In vitro multiplication

The initiation of *in vitro* culture was made from leaf fragments and nodal segments. Disinfection of the explants was carried out by sterilization in ethyl alcohol 65% for one minute followed by immersion in solution of 3% dichloroiso-cyanuric acid Na_2 salt (commercial product Chlorom) to which were added a few drops of Tween 20. The sterilization time was 15 minutes after which the explants were washed three times with sterile distilled water.
Leaves were cut along the main rib to a size of 0.5-1.0 cm and placed with the abaxial surface down on the medium. The nodal segments were sectioned to a length of about 0.5 cm.
Explants were inoculated on basic medium MS (Murashige and Skoog, 1962) supplemented with cytochininis and auxines in two concentrations with the following variants: V1 MS + 1.0 mg/l IBA (Indole-3-butyric acid) + 0.5 mg/l BAP (6-Benzyl-Amino-Purine) and V2 MS + 0.2 mg/l IAA (Indole-3-acetic acid) + 0.1 mg/l TDZ (N-phenyl-N'-1,2,3-thiadiazol- 5-ylurea).
Sucrose was also added to the medium in a concentration of 3% and agar 0.65%. The pH was adjusted to 5.8 and finally, media were sterilized by autoclave at 121°C for 20 minutes.
For multiplication and elongation, the induced shoots were subcultivated on the MS culture medium with 0.5 mg/l IBA and 0.3 mg/l BAP and MS with 0.5mg/l IBA and 0.2mg/l Kin (Kinetine).
All cultures were incubated in growth chambers at a temperature of 23 ± 2°C, with a photoperiod of 16/8 hours and light intensity of 3000 lux provided by cool-white fluorescent light.
Determination of the number of shoots and measurements of their length were performed after six weeks of *in vitro* culture. All data were assessed using ANOVA and Tukey's HSD multiple-range test ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

Ex vivo multiplication of plants

Multiplication by stem cuttings is the most common method to propagate plants. The most important aspect in this context is the stage of stem maturity. In case of *I. platensis* the stem cuttings must be herbaceous. In our experiment the cuttings formed roots after 10 days in water and 15 days in perlite substrate. In the variants with two concentrations of IBA the stem cuttings were unrooted. According to these results, for statistical analysis we took in consideration only the variants with water and perlite. The results are presented in Figure 2. The highest rooting ratios were obtained in the water variant.

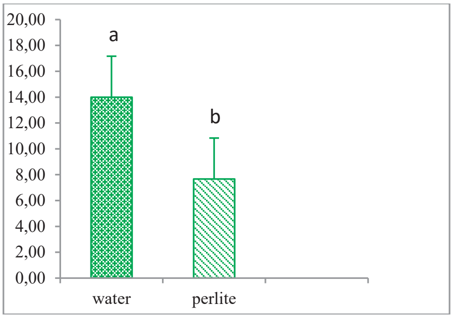

Figure 2. Number of rooted cuttings of *I. platensis*
(Values followed by the same letter are nonsignificantly different according to Tukey's test - $P \leq 0.05$)

The obtained cuttings formed 3-4 roots within 3-4 weeks, and after planting in a well-aerated soil they formed a thickened root which then turned into caudex, which assures better resistance to drought and also can be used as a decorative element (Figure 3).

Figure 3. The *ex vivo* rooting of *I. platensis* (original)

Results of in vitro multiplication

In our experiences, the culture was initiated from leaf fragments and nodal segments. The explants were inoculated on two media variants with two types of auxines and two of cytochinins.

Leaf fragments and nodal segments formed callus (Figure 4) on the V1 initiation medium (MS + 1.0 mg/l IBA + 0.5 mg/l BAP), although many plant species have the capacity to regenerate shoots from leaf fragments.

In the case of *Ipomoea batatas* species, Gosukonda et al. (1995) reported 25% regeneration of leaf fragments grown on medium with TDZ at a concentration of 0.1 and 0.2 mg/l.

Induction of caulogenesis, i.e. the formation of buds and stems, is stimulated by the presence of cytochinins in the culture medium. It is often necessary to associate cytochinins with auxins. An important role in the neoformation of buds is the nature and concentration of the growth regulators used.

Figure 4. Callus formation from leaf and nodal segments (original)

Since on the first variant of the medium plants have been callused, we tested a new variant that has been supplemented by 0.2 mg/l AIA + 0.1 mg/l TDZ.

In our experience on this culture medium, no multiple shoots have been obtained although TDZ is a cytokinin considered as one of the most active for inducing shoots in tissue cultures. Although no multiplication of shoots was observed, the explants had a vigorous growth reaching an average length of 3.4 cm (Figure 5).

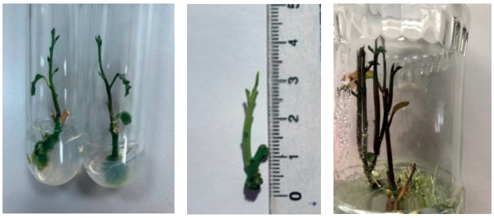

Figure 5. Development of shoots from nodal explant in *Ipomoea platensis* (original)

The BA's superiority over TDZ in the case of multiplication of axillary shoots in *I. batatas* cultivars, where no multiple shoots were formed on TDZ-supplemented media (Mohamed et al., 2007; Dewir et al., 2020).
Other studies reveal that TDZ induces the regeneration of shoots in many species (ornamental and woody) recalcitrant to *in vitro* propagation. The studies made by Pop et al. (2016) on the *Lisianthus* and *Vitis* species, led to the formation of a large number of shoots using a concentration of 1.0 mg/l TDZ and 0.5 mg/l AIA.
Morphogenesis induced by TDZ probably depends on the endogenous level of growth regulators being able to modulate also the endogenous level of auxines.
The TDZ medium obtained shoots were subcultivated for multiplication on two MS basic culture media supplemented with 0.5 mg/l IBA and cytockinins 0.3 mg/l BAP and 0.2 mg/l Kin.
With regard to the number of shoots/explant, the superiority of the medium supplemented by 0.5 mg/l IBA and 0.2 mg/l Kin is ascertained by an average number of 4.3 shoots obtained while on the medium supplemented by 0.3 mg/l BAP the number of shoots obtained was significantly lower (Figure 6).

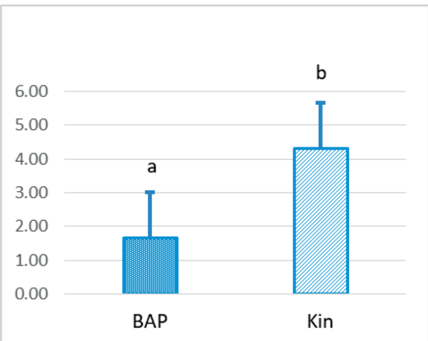

Figure 6. The influence of citokinine on shoots number

Mengs et al., (2018) reported that using cytokinine combinations in higher concentrations, namely BA 0.5 mg/l and Kin 2.0 mg/l, they obtained callus shoots in the *Ipomoea batatas*.
Our study demonstrates that the development of 3-4 shoots is influenced by the growth hormones used.
The cytokinins used also had an influence on the length of the shoots obtained. Thus on the culture medium supplemented with kin 0.2 mg/l the shoots had an average length of 5.6 cm with significant differences from the other two variants (TDZ and BAP) (Figure 7).

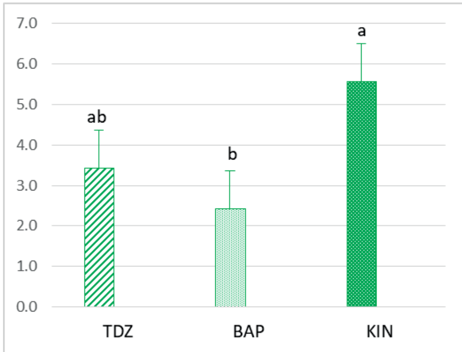

Figure 7. The influence of citokinine on length of shoots

The formation of a smaller and shorter number of shoots on the BAP medium at a concentration of 0.3 mg/l and 0.5 mg/l auxine IBA is due to callus formation at the base of the explants.

Some studies report the formation of a large number of shoots at 3.0 mg/L BA in combination with 0.5 mg/L NAA, with BA playing an important role in inducing and elongating shoots (Cheruvathur et al., 2015; Fira et al., 2016) and protocorm formation in *Phalenopsis* sp. (Cordea et al., 2019).

In our experiments, the concentration of BAP although very low, respectively 0.3 mg/l BAP, shoots have been callused. The callused tissue developed on the surface of the nutrient medium forming an amorphous mass of thin-walled parenchymal cells without a certain anatomical structure.

From some explants a mixed callus was formed, containing both regenerative and non-regenerative callus. During six weeks of cultivation the callus became green with a compact structure and meristemoid centers from which 1-2 shoots were differentiated (Figure 4).

In the case of *Ipomoea mauritiana*, massive callus proliferation was observed with the use of the additional MS basic medium with a higher concentration of BAP i.e. 1.0 mg/l in combination with 0.2 mg /l IAA (Islam and Bari, 2013).

It is well known that morphogenesis and *in vitro* growth are regulated by exogenously applied growth phytoregulators and the balance between endogenous hormones.

The response of explants to different growth phytoregulators also depends to a large extent on the genotype and species.

In our experiments, root formation was recorded in shoots grown on the basic MS medium supplemented by 0.5 mg/l IBA and 0.2mg/l Kin (Figure 5). The roots began to form after a three-week *in vitro* culture period. Similar data were also reported in studies by Islam and Bari (2013) in the *Ipomoea mauritiana* species, where root formation took place on the basic medium with 0.2 mg/l IBA, without other phytohormones.

CONCLUSIONS

In order to *ex vivo* multiplication of the *Ipomoea platensis* species the best rooting variant was obtained by keeping the cuttings in water without any auxines.

In the case of *in vitro* experiments, according to the results obtained, the MS culture medium supplemented by 0.5 mg/l IBA and 0.2 mg/l Kin was the best for multiplication and growth of shoots.

The BAP medium (0.3 mg/l) led to the proliferation of callus at the base of the explants and the formation of a very small number of shoots. This medium variant can be used to induce caulogenesis. Auxine IBA in combination with kinetine determines the formation of the root system by obtaining plants that can be acclimatized *ex vitro* and used in the perpetuation of the species *I. platensis*.

To our knowledge, this is the first report on multiplication and micropropagation of *Ipomoea plantensis* in Romania.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Advanced Horticulture Research of Transylvania (USAMVCN) project no. 946/SMIS 14064.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE USE OF WETLANDS AS SOCIO-EDUCATIONAL SPACES IN CHIAJNA COMMUNE, ILFOV COUNTY

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Abstract

This article discusses about the wetlands as areas of major importance for both biodiversity and socio-educational activities. Generally, wetlands located near densely populated urban areas, are oases of escape and educational spaces, relaxation areas where nature can be enjoyed and discovered. The site studied in this article is in Chiajna commune, in the immediate proximity of Bucharest. The commune dispose of natural spaces with a rich biological diversity, located in the outside urban area of Chiajna commune, where the Dâmbovița river creates a flood plain. This article proposes to identify and classify the flood plain ecosystem services by presenting them as inputs for valuing a sustainable manner in the area. Although undeveloped, this floodplain is a point of attraction for the inhabitants of the area, with a pronounced potential for recreation and leisure. Following research was developed as a planning solution which respects the natural character of the area by maintaining habitats and biological diversity. The floodplain is also designed to raise awareness and inform residents about its importance and fragility

Key words*: wetland, ecosystem services, planning solution, Chiajna commune, floodplain.*

INTRODUCTION

In the last 30 years, the process of urbanization has led to major environmental changes around the world, and these changes will continue because by 2050 most of the world's population will live in urban areas (Ahn & Schmidt, 2019). The figures show an increase in the urban population, this trend is still upward, so worldwide the urban population has increased from 31.3% in 1960 to 55% in 2019 (https: //data.worldbank.org/,2019). Urban pressure plays an important role in transforming and diminishing the most vulnerable areas. As a consequence of urban sprawl and human activities, wetlands near cities are declining dramatically (Jia, Ma, & Wei, 2011) simultaneously with the ecosystem services. Data show that wetlands are disappearing three times faster than forest ecosystems. Globally, in the last century, there has been an estimated reduction of wetlands between 64 and 70% (***, 2015). Loss and degradation of wetlands have a major impact on the economic and social environment through increased risk of floods, declining water quality with a direct impact on public health and affecting cultural values and living conditions(***, 2015).

Since they are fragile ecosystems, but at the same time represent large reservoirs of biodiversity and areas of recreational activities, subject to urban pressures and various threats, they become, in the current context, "research laboratories" we have to focus all our attention on.

Urban wetlands are seen as an integral part of the landscape and perceived as a complex ecosystem composed of ecological, social and economic factors (Jia, Ma, & Wei, 2011). In fact, when analysing these complex ecosystems, ecosystem services highlight features that emphasize the ability to regulate the microclimate, maintaining water quality, control and maintenance-prevention of floods, recreational areas and relaxation for those living in proximity (Jia, Ma, & Wei, 2011). Changes are also being made to the ecosystem services of these wetlands at the same time with the intervention and pressure on wetlands (Ahn & Schmidt, 2019).Moreover, these wetlands prevent and protect against floods, acting as natural sponges that absorb and then release water more slowly from precipitation, snow or floods, aided by nearby or indoor herbaceous and woody vegetation and contribute to improving the microclimate by providing

humidity and by decreasing the temperature (Jia, Ma, & Wei, 2011).
Therefore, the article aims to analyse urban wetlands as areas with major impact for biodiversity and socio-cultural activities in densely populated urban areas. They are perceived as escape oases and educational spaces, spaces for relaxation and leisure in the middle of nature or spaces for discovery and awareness of the natural environment.
The study area that we will investigate in this article is located in the administrative area of Chiajna commune, near Dudu and Roșu localities and is part of the hydrotechnical infrastructure of the Dâmbovița river. Located on the course of Dâmbovița, the studied site has undergone several changes over time. This fact was determined by changes in the configuration of the Dâmbovița river, caused by the major actions and projects of regularization and sewerage, started in 1868 and then continued at different periods of time until 1985-1987 when the Dâmbovița riverbed was dammed (***, 2008) (Figure 1).
Floods and other actions that could have endangered the neighbouring areas were prevented by building the Morii Lake, the largest body of water in Bucharest, creating lake areas (***, 2008) and wetlands of the type of hydrotechnical accumulation studied in Dudu. (Figure 2).

Figure 1. Dâmbovița River- drone photo, source: Atena Proca

Figure 2. Hydrotechnical accumulation on the Dâmbovița river at Dudu - drone photo, source: Atena Proca

The studied area has undergone several transformations over different historical periods, being presented as a swampy land subject to anthropogenic transformations. In the descriptions and specifications of eng. A. G. Vuzitas (Vuzitas, 1936) the area upstream of the Ciurel dam was a difficult area, with floodable portions, with many mills that used the waters of the Dâmbovița River changing the hydraulic regime of the river. The situation caused a series of undesirable events by generating "disturbances in the hydraulic regime of the natural and regularized course of Dâmbovița" (Vuzitas, 1936). Thus, the mills actively intervened by using the river water for their operation, the owners modifying and intervening in the river flow by restricting the water at will, causing quite a few problems downstream of the Ciurel dam, in the urban system of Bucharest (Vuzitas, 1936).
Along with the systematization and sewerage works, new projects started in 1942 and then continued after 1944 aimed at “the rectification and arrangement of the riverbed” (Stematiu & Teodorescu, 2012).These works took place between the Ciurel dam and the Roșu commune in order to regulate the course of the river and at the same time to rehabilitate the meadow, preventing the danger of floods for the Bucharest neighbourhoods and nearby localities (Stematiu & Teodorescu, 2012). An important moment in the configuration of the space was the construction of the permanent accumulation by building an earth dam upstream of the Ciurel road starting with 1985, when Lake Dâmbovița, best known as Lake Morii, was created on an area of 241.5 ha with a volume of 14 million m3 (***,2017).
The paper aims to present the wetland along the Dâmbovița River, near the densely populated urban areas of Chiajna commune, which are in the process of densification and continuous development. The article emphasizes the importance of this free space with natural value, with a mosaic of landscapes, used as a space for relaxation and leisure and wants to highlight the ecosystem services that accompany biodiversity. The research objectives focus on identifying and classifying vegetation, zoning green spaces with development potential, capitalizing on landscapes and developing proposals for activities that highlight the potential of the site.

MATERIALS AND METHODS

The studied wetland is the reserve of space that balances the urban context of the whole commune, because, according to statistics, almost the entire surface of the commune is integrated in the built-up area. With the massive migration of Bucharest residents to areas with territorial availability, Chiajna commune has tripled its number of inhabitants in the last 20 years (Table 1) (https://insse.ro/, 2017).The need to expand the built-up area (on almost the entire administrative area) in Chiajna arose as a result of the transformations after 1990, when agricultural lands became private property and more and more people from Bucharest acquired properties in Chiajna (Haneş, et al 2016). The real estate sector became very dynamic in the mid-2000s when more and more land was bought for housing construction (Haneş, et al 2016).

Table 1. The evolution of the number of inhabitants in Chiajna commune, Ilfov county
data source https://insse.ro/, 2017

Locality	Year		
	2000	2017	2018
Chiajna commune (Ilfov county)	7300	19800	22700

The works carried out upstream of Lake Morii are part of the flood protection infrastructure. Thus, the studied area is identified in the The General Urban Plan of Chiajna commune with several uses: a water surface area was established (UTR Exla), and green spaces with recreation and rest functions (UTR Vp) (***, 2013), adjoining with living areas and other activities. From the study of the urban documentations of Chiajna commune (***, 2013), but also of the integrated local Strategy for sustainable development of Sector 6 (***, 2017) it is easy to notice that the built-up area expanded (in Chiajna commune) due to a need for leisure spaces for both the inhabitants of Chiajna commune and for those of Sector 6 in Bucharest.
In this context, regular field visits were carried out between March and June 2020, simultaneously with the study of the bibliography to obtain detailed information about the site. According to the findings, there is a bibliography that gives details about the hydrotechnical characteristics of the area, but there are only a few studies and information on biodiversity and wetland landscapes. This highlights the marginal nature of the site, as it has never been researched and popularized for its natural value.
The accumulated information was interpreted, resulting in zoning of the site for a better representation of the studied areas; associations of vegetation in meadow areas were identified, as well as classifications of landscape typologies accompanied by inventory of activities encountered in the field. Photographs and field sheets were taken for a rigorous research.
The research was carried out following these steps (Figure 3).

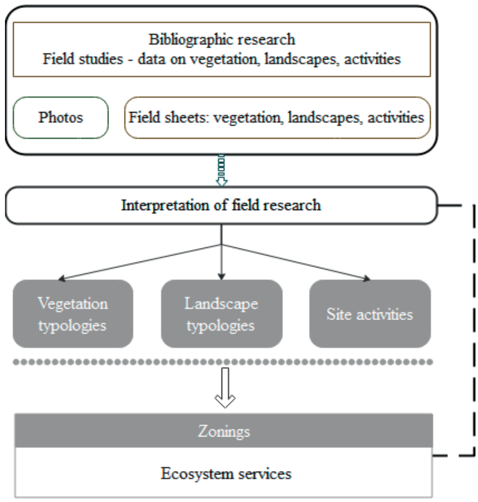

Figure 3. Conducting research

All the information was analysed and transposed in graphic form for a better clarity of the context and to be able to generate possible proposals regarding the future solutions for educational and leisure activities.

RESULTS AND DISCUSSIONS

The studied wetland is an important area for its ecological values, for its importance in flood prevention, but especially for ecosystem services: plant species adapted to the meadow environment, ecological diversity, habitats for wild bird species, aquatic ecosystems. The researched site was divided into three areas - A, B and C, corresponding to the three parts, which are separated by dams (Figure 4).

Figure 4. Zoning of the studied site, by Atena Proca

These three areas are characterized by various types of landscape, but also by vegetation with alluvial soils, swamps and ditches caused by stagnant water resulting from rains, floods and melting snow.

Being a floodable area, which is part of the flood protection infrastructure on the Dâmbovița River, the researched site has plant compositions that developed according to the activities and functions in the neighborhood or the interventions in infrastructure projects (Figure 5).

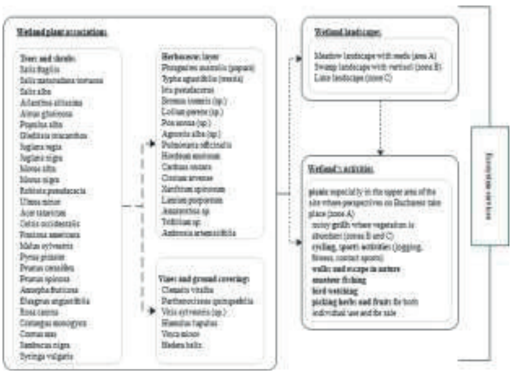
Figure 5. Plant associations-landscapes-activities

1. *Zone A*

Having the appearance of a floodplain, the landscape in zone A develops on two well-defined levels: the lower part determined by the lake basin, with a high degree of humidity, invaded by reeds and the upper part with abundant woody vegetation represented by large areas of trees and shrubs.

The Table 2 show us the representative species on vegetation floors, where predominant for this meadow landscape are the tall hygrophilous grasses represented by *Carex riparia, Typha latifolia and Phragmites communis.*

Area A (Figure 6) is accessible and subject to anthropization, and residential buildings have already been built around it.

The usual activities that take place in this area are walking, cycling, jogging, picnics, training and bird watching.

Figure 6. Zone A Meadow landscape with reeds, by Atena Proca

Figure 7. Swamp landscape with vertisol, by Atena Proca

Table 2. Vegetation typologies on zone A

Vegetation typologies		
Trees and shrubs	Vines and ground cover plants	Herbaceous layer
Salix matsoudana tortuosa Salix alba Ailanthus altissima Gleditsia triacanthos Juglans nigra Juglans regia Morus alba Morus nigra Robinia pseudacacia Ulmus minor Acer tataricum Celtis occidentalis Fraxinus americana Prunus cerasifera Eleagnus angustifolia Rosa canina Sambucus nigra Syringa vulgaris Rubus sp.	Clematis vitalba Parthenocissus quinquefolia Vitis sylvestris Humulus lupulus Vinca minor Hedera helix	Phragmites australis (papura) Typha agustifolia (trestia) Iris pseudacorus Bromus inemris Lolium perene Poa annua (sp.) Agrostis alba (sp.) Pulmonaria officinalis Hordeum murinum Carduus nutans Cirsium arvense Xanthium spinosum Lamium purpureum Amaranthus sp. Trifolium sp.

2. *Zone B*

Zone B has a special feature since it is an area with a dynamic swampy landscape that transforms completely when there are variations in humidity. The marshy area with patches of *Salix alba* and herbaceous species from the second part of the lake, dried especially in the central part, leaves room for the vertisol to take on various shapes, which arouse the curiosity of visitors. In this area the dominant species are represented by *Salix alba, Equisetum arvense, Achillea millefolium, Coronilla varia, Plantago sp., Berteroa incana, Ambrosia artemisiifolia, Hordeum murinum, Carduus nutans, Cirsium arvense and Xanthium spinosum,* with the swamp's landscape with vertisol (Figure 7).

The activities encountered in the area include the walking, educational exploration, cycling, jogging, and bird watching.

3. *Zone C*

The third area looks like a lake landscape, with high humidity that persists over time. It is an area with a great diversity of environments, a greater diversity of species, where the marshy vegetation is associated with the aquatic one (Figure 8)

Figure 8. Lake landscape, by Atena Proca

On this lake landscape the activities as walking, cycling, jogging, fitness, barbecues, fishing and bird watching are made almost all year round, but especially from spring to autumn when plant species cover this area. In Table 3 we are specified the vegetal typologies from woody to herbaceous species.

Table 3 Vegetation typologies on zone C

Vegetation typologies		
Trees and shrubs	Herbaceous layer	Emerging vegetation (on the banks)
Salix fragilis Salix matsoudana tortuosa Salix alba Alnus glutinosa Populus alba Populus tremula Gleditsia triacanthos Juglans nigra Morus alba Morus nigra Ulmus minor Celtis occidentalis Fraxinus americana Prunus cerasifera Prunus spinosa Amorpha fruticosa Eleagnus angustifolia Rosa canina Crataegus monogyna Sambucus nigra Syringa vulgaris	Holcus lanatus Achillea millefolium Festuca sp. Salvia sp. Polygonum sp. Carex sp. Lotus corniculatus Pulmonaria officinalis Euphorbia sp.	Phragmites australis Typha angustifolia Bolboschoenus sp. Lysimachia sp. Carex sp.

Having an area with increased biodiversity very close to inhabited areas, where people come in contact with nature, it is appropriate to develop socio-educational activities and conduct awareness campaigns to maintain and protect this wetland. Sustainable protection and management mean ecological, social, and cultural benefits that translate into ecosystem services. In this study the ecosystem services are identified with a great diversity of flora and fauna species, habitats of wild bird species, clean water resources, lake ecosystems, promenade spaces, where residents and visitors can meet nature, focusing on their well-being or practicing sports in nature (Walking, cycling, jogging, fitness). The cultural and aesthetic value of these spaces is an attractive factor that determines more and more visitors spend more time finding out more about the place. On the other hand, wetlands are appreciated for the role they play in relaxation and leisure activities, but also for scientific and cultural ones. (Jia, Ma, & Wei, 2011).

CONCLUSIONS

In the contemporary confrontation with urban development, the integration of wetlands in this dynamic can be one of the essential points that will make cities more resilient.

The development of these wetlands with park functions is a new option for the protection of habitats, the enhancement of landscapes, the development of ecological education activities and relaxation and leisure activities, as well as a means of raising awareness about to the environment. Urban wetlands have a great cultural significance by creating different types of landscapes, by capitalizing on the great diversity of environments, especially since the areas in the urban environment are limited and subjected to continuous pressure. When urban development affects wetlands, the associated ecosystem services may change. That is why the arrangement of wetlands can be perceived from the very beginning as an area that facilitates and develops the natural heritage, the diversity of landscapes, leisure and sports activities and last but not least process related to the research and monitoring of biodiversity.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

HIGH HETEROGENEITY OF SOILS IN THE RESIDENTIAL AREA OF IAȘI

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Abstract

The area of the residential spaces from Romania still has a tendency to expand. Residential spaces are part of the national economy and also plays a vital role in urban environment. One of the permanent concerns of the owners of the residential spaces is the creation of pleasant, comfortable and relaxing landscapes because he quality of environment directly influences physical, mental and spiritual life of people. For communities that pay special attention for relaxing landscapes on a site, it is usefully to focus on the characteristic of the soil at the site. Frequently, the urban soils from residential area are characterized by a strong spatial heterogeneity resulting from various input of exogenous materials and mixing of material from different soil horizons or even with lithological material on which the soil was formed. After carrying out field investigations we found different and varied soil limitations for plants even on small areas. The results obtained during the investigations made it possible to present some representative case studies of high heterogeneity of urban soils for the residential area in Iași.

Key words*: heterogeneity, urban soil, residential area.*

INTRODUCTION

According to Wikipedia, residential area is a land used in which housing predominates, as opposed to industrial and commercial areas. These include single-family housing, multi-family residential, or mobile homes.

The infrastructure required to support a residential development can be split into more categories such as transportation infrastructure (local road network, footpath, public transport, parking), waste management (waste collection and waste recycling), utilities (water supply, wastewater management, electricity network, gas network, telecommunications) renewable energy (solar energy, wind energy) community infrastructure, social infrastructure, digital infrastructure, green infrastructure (Bracknell Forest Council, 2012).

According to Stroganova et al. (1998), the soil surface can be covered (isolated from the impact of the atmosphere) by buildings, permeable road surfaces (pavement, gravel), impermeable road surfaces (asphalt, concrete).

The abundance of chemical elements in urban soils is a result of geogenic abundance and anthropogenic ones. Many times, anthropogenic influence is decisive for urban soils chemical, physical morphological and biological properties (Lăcătușu et al., 2008).

Dust pollution affects the atmosphere, vegetation and soils. The dust is generally toxic and hazardous and can pose a serious health threat to humans. The natural environment is also affected by dust emissions: soils in the surroundings of the plant are characterised by alkaline reaction and contain elevated levels of lead and zinc (Charzyński et al., 2013; Dabkowska et al., 1997).

The area's most heavily polluted with dust containing 7 to 9 heavy metals have been identified in Podu Roșu intersection, industrial area in Baza 3 (the Felicia Carrefour supermarket) and in the heavily trafficked Tudor Vladimirescu intersection. All the measures taken to limit the road dust (washing the streets; mechanized, sweeping of the streets; modernization of the roads etc.) are not enough to reduce the dust content below allowable limits (Oiste, 2013).

Soil from urban area are frequent contaminated with high highly chlorinated PCB. Studies conducted by Lăcătușu (2008) showed that most polluted areas in Bucharest are located in areas with intense automobile traffic like Rosetti Square, Sudului Square and Kisseleff Square, where the concentration of PCB exceed even the intervention threshold for sensitive use.

Housing constructions and another categories of infrastructures in residential area strong modifies soil characteristics and usually results in high soil compaction, low rainfall infiltration, and restricted aeration and low drainage porosity (Craul, 1985; Pouyat et al., 2007).
Soil modification can be intentional to strengthen soil for engineered loads, such as house foundations and roadways, or unintentional as a result of heavy equipment usage and site traffic.
It is obvious that following the construction of the infrastructure in the residential area, the floor covering also changes considerably.
One of the permanent concerns of the owners of the residential spaces is the creation of pleasant, comfortable and relaxing landscapes because he quality of environment directly influences physical, mental and spiritual life of people.
Achieving sustainable landscapes in individual gardens could be done only if the soil is kept loose, without high alkalinity or acidity, well supplied with nutrients and without pathogens contaminants.
Soil in individual gardens must also ensure organic matter recycling, rain or irrigation water retention for continuously supply of plants, vigorous root system development, water infiltration and, last but not least, plant protection against pathogens.
Our paper highlight the high heterogeneity of soils in urban areas in Iasi and to some negative influences on plants and human health.

MATERIALS AND METHODS

Most of the studies were conducted in urban area of Iasi, located in the central eastern part of Romania.
Iasi, the city of the seven hills, is situated in the North-East of Romania, at 47˚10’ northern latitude and 27˚35’ eastern longitude
The studied sites are located in the residential area of Iasi city (North-East of Romania).
We studied several locations in the urban area of Iasi. In each location we studied topography and realized several soil profiles.
The representative soil profile of the agricultural land o Iasi is Haplic Chernozems (Figure 1).

Figure 1. Haplic Chernozems

The studied soils have been diagnosed according to the Romanian System of Soil Taxonomy (Florea et al., 2012) and World Reference Base for Soil Resource (WRB, 2014).
Characterization of soil profiles was done following the instructions from guidelines for soil and land descriptions (Munteanu et al., 2009; Guidelines for soil description, FAO, 2006).
Soil samples were taken from each pedogenetic horizon in order to conduct laboratory analyses: according to the current methodology (Dumitru et al., 2009; Lăcătuşu et al., 2017).

RESULTS AND DISCUSSIONS

In residential spaces undergoes irreversible changes is the soil cover.
The extent and type of changes in the soil are influenced by many factors such as time and intensity of initial land use before the start of the residential area infrastructure, the physical, chemical and biological properties of the soils on which the new houses will be built and the related infrastructure, reclamation techniques etc.
Frequently, the largest share of urban soil constituents is owned by those of anthropogenic origin.
The natural constituents of the soils that are associated with those of anthropogenic origin

(artefacts), usually come from several pedogenetical horizons and from lithological layers which were excavated and stored on the surface of the land near the newly built houses.
Our studies carried out in urban areas of Iasi have shown a great heterogeneity of soils even in residential areas established on agricultural soils.
Part of the soils of the residential areas in Iași were removed by excavation during the construction of the designed buildings (Figure 2), another part is covered with permeable or impermeable roads surfaces.

Figure 2. The excavated soil is transported from the place prepared for the construction of the building in the residential area

During the execution of the construction, the upper part of the soil is compacted strongly and is often covered with different artefacts made of materials used in the construction of the building.
In order to preserve the initial state of loosening of the soil and after the construction of the building, we recommend that in the first phase only a part of the excavated soil be removed, and one part be used to cover the soil to prevent compaction (Figure 3).

Figure 3. Covering the soil surface with the soil resulting from excavation

After ending of the building, the ground cover can be removed and the soil can be used for creation of pleasant, comfortable and relaxing landscapes.
The removal of the soil used to cover the ground must be done separately from various resulting artefacts.
The soil covered by permeable roads (pavement, gravel), can be partially supplied locally with water both from lateral infiltrations and through the joints between concrete or brick pavers in the pavement.
The free spaces between the pavers can be infested with annual or perennial weeds such as little hogweed or couch grass (Figure 4).

Figure 4. Couch grass (*Cynodon dactylon*) and little hogweed (*Portulaca oleracea*) developed in the joints between the pavers

In some cases, paved alleys are infested with invasive and quarantine weeds (Figure 5) such as common ragweed, annual ragweed, and low ragweed (*Ambrosia artemisiifolia*).

Figure 5. Pavers pathway infested with quarantine weed *Ambrosia artemisiifolia*

This species harms the health of the population; many people are allergic to pollen. In

Switzerland borders several heavily ragweed colonised areas and up to 12% of the population suffers from allergies to ragweed pollen in these areas (Taramarcaz et al., 2005).
The soils covered with pavers in the studied areas are urbic ekranic Technosols or calcaric ekranic Technosols.
It is necessary to control the weeds in the paved alleys both to prevent their degradation and maintain a pleasant landscape and to prevent the disease of the population allergic to pollen of different species.
The soils of the residential areas covered with impermeable roads surfaces can be moistened with water from lateral infiltrations from adjacent lands.
In these cases, the ekranic Technosols allow the development of the roots of the plants that grow on adjacent lands (Filipov & Robu, 2013). Even if it seems impossible, the asphalt path can be traversed by perennial herbaceous plants such as *Convolvulus arvensis, Equisetum arvensis, Cynodon dactylon* (Figure 6).

Figure 6. Degradation ekranic Technosols covered by asphalt alleys after growth of couch rhizome

The developed cracks on the asphalt or small galleries formed as a result breakdown roots of couch grass allow growth of other plant species such as *Taraxacum officinale* (dandelion), *Polygonum aviculare* (birdweed, pigweed and lowgrass), *Hordeum murinum* (wall barley or false barley), *Lepidium ruderale* (narrow-leaf, pepperwort, roadside, pepperweed or peppergrass), *Poa pratensis* (Kentucky Bluegrass, Smooth Meadow-grass, or Common Meadow-grass), *Cardaria draba* (white top or hoary cress).

Preventing degradation of asphalt alleys by means of *Cynodon dactylon* can only be done by mechanical or chemical removing plant developed on both paths and those in areas in close proximity
Our investigation noticed that some plants species such as *Picea* sp. that grow in the vicinity of asphalted paths lead unevenness, cracking and perforation of asphalt path (Figure 7).

Figure 7. Degradation of asphalt path of ekranic Technosols by deformation cracks and perforation under influence of *Picea* sp. rooting

Soils from ornamental garden frequently contain large amount of artefacts such as bricks, pottery, glass, crushed or dressed stone, wooden boards, bitumen (Figure 8).
Such soils were also found in the residential area where we conducted these studies. Before establishing of the garden, the soil was levelled and then covered with fertile soil from the upper part of the Chernozems (Figure 8).

Figure 8. Mollic hyperatefactic Technosols

Soil has loamy clay texture and slight alkaline reaction.
The high content of artefacts reduces the volume of soil that can be used by plant roots.
The low capacity of the soil to retain useful water amplifies the negative effect of summer droughts on plants.
The resulting soil was diagnosed as Copertic urbic Tehnosol (after Romanian Soil Taxonomy System, 2012) or Urbic mollic Technosols.
Trees on these soils have a shallow root system (Figure 9), and can be knocked down by stronger winds.

Figure 9. Spruce roots developed in the fertile soil layer used to cover deposits with urban artefacts

We recommend avoid planting tall growing trees on these soils which are likely to knock down by strong winds.
In frequent cases, after finishing the buildings, the soil is levelled and covered with humic soil material. Input of good soils does not remove plant restrictions of soil covered with a fertile layer of soil. In the first stage the plants can develop in the imported soil, but later the growing stalling or even dried.
After finishing of construction, soil compaction processes can also occur under influences of some activities in new garden such as walking, sports, and even parking heavy vehicles on grassed areas.
After carrying out field investigations we found that the fertile soil material used to cover the land surface is not sufficient to ensure growth of all plants in individual gardens.
It is necessary to choose the ornamental species according to their requirements for the useful edaphic volume of the soil.

The required input data in order to identify area with where the deficient physical properties of soil for plant growing are uniformity of soil surface colour, soil crusting susceptibility, uniformity of the wetting strip after drip irrigation, the growing stagnation of plants, uniformity of root distribution (Filipov & Chelariu, 2020).
In the studied area we met soils with artefacts represented by crushed or dressed stone which was covered with soil from humic horizon (A mollic) of Chernozems (Figure 10).

Figure 10. Mollic urbic spollic Technosols

The distribution of roots in the soil layer used to cover the ground is uniform.
In lighter soil layers with a higher content of artefacts, the roots are very few and appear only locally.
The reaction of the upper part of the soil is weakly alkaline, the pH values being between 7.4 and 8.1.
The presence in individual gardens of soils with high amplitude of pH values requires the choice of suitable ornamental species.
The presence in individual gardens of soils with high amplitude of pH values requires the choice of suitable ornamental species. Some species prefer the acid reaction and do not tolerate weakly or moderately alkaline soils.
The different colour of *Hydrangea hortensis* flowers is a very good bio indicator of soils with high pH amplitude.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the research project 14644/2018 USAMV Iaşi.

CONCLUSIONS

In order to preserve the initial state of loosening of the soil and after the construction of the building, we recommend that in the first phase only a part of the excavated soil be removed. After ending of the building, the ground cover can be removed and the soil can be used for creation of pleasant, comfortable and relaxing landscapes.
The soil covered by permeable roads (pavement, gravel), can be partially supplied locally with water both from lateral infiltrations and through the joints between concrete or brick pavers.
In some cases, paved alleys are infested with invasive and quarantine weeds such as *Ambrosia artemisiifolia*.
It is necessary to control the weeds in the paved alleys both to prevent their degradation and maintain a pleasant landscape and to prevent the disease of the population allergic to pollen of different species.
After carrying out field investigations we found that the fertile soil material used to cover the land surface is not sufficient to ensure growth of all plants in individual gardens.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOME OBSERVATIONS ON THE ECOLOGY AND MORPHOLOGICAL FEATURES OF A *HUMULUS SCANDENS* (LOUR.) MERR. (*H. JAPONICUS* SIEBOLD & ZUCC.) POPULATIONS FOUND ON THE DÂMBOVIȚA RIVER BANKS

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Abstract

Included today in the list of invasive plants of community interest in Europe, Humulus scandens (Lour.) Merr., an annual vine from East Asia, was introduced as medicinal and ornamental species in the middle of nineteen century first in North America and then in Europe. In România this species was reported first as a subspontaneous one, of unknown origin, becoming then a naturalized species. The populations of H. scandens that we found were established on the Dâmbovița river banks in a nitrophilous plant community, alongside Sambucus ebulus and Rubus caesius individuals. The observations that we made showed the trichomes types and their distribution on the vegetative organs (stem, leaves and bracts) of the H. scandens plants.

Key words*: Conietum maculati, Humulus scandens, trichomes.*

INTRODUCTION

In 1952, in Flora RPR, *Humulus scandens* (*H. japonicus*) appears as an ornamental species which occasionally could be found in some areas as sub spontaneous (Grințescu, 1952). There are no mentions regarding its introduction in the country.

A paper from 1969 indicates the presence of the species in Orșova town, in flooded areas of the Danube's banks (Morariu et al., 1969). In 1970, *H. scandens* was found in a *Bidens vulgata* association in the Timis meadow, alongside *Galinsoga parviflora*, *Chenopodium ambrosioides*, *Ch. album*, *Bidens tripartita Impatiens roylei*, *Helianthus tuberosus*, *Setaria viridis*, *Rubus caesius*, *Solidago serotina*, *Rudbeckia laciniata*, *Salix alba*, *Calystegia sepium*, *Humulus lupulus*, *Echinocystis echinata*, *Sicyos angulatus* (Vicol, 1970). In 1974, Roman indicates the presence of this species in the area of the former Ada-Kaleh island and at Gura -Văii, in a *Calistegyon* type alliance.

Still, in 1979, *H. scandens* was presented as an ornamental plant, suitable for pergola, kiosks and grilles due to the abundant growth and elegance of the foliage, with the 'Variegatus' variety being recommended in this regard (Preda, 1979).

In two successive works of Ciocârlan (2000, 2009) it is specified that this species is rarely found in our country; in addition to the formerly indicated sites, the area around the irrigation canals of Chirnogi village, Giurgiu county is also mentioned.

H. scandens is also listed among other species in Alien Plants of Romania by Sârbu & Oprea (2011).

In a bibliography synthesis supplemented by their own observations Otves et al. (2014) preserved the indication of the *H. scandens* presence in Timiș meadow and in wetlands of the Banat area.

With the Inventory Guide and distribution map of invasive and potentially invasive allogeneic plant species from Romania, in 2019, *H. scandens* is placed on the list of alarming alien species for the European Union. The species presence is also indicated in many parts of Romania, such as Banat, Maramureș, Muntenia, Oltenia, Transilvania (Anastasiu et al., 2019).

Regarded today as a transformer invasive species in Hungary and considered an alien species with a negative impact on native European flora only in the last decade, *H. scandens* is a native plant from the Far East (Balogh & Dancza, 2008). It grows in China, Japan, Mongolia, North and South Korea, Taiwan, Vietnam, Russian Federation (EPPO, 2019).

In the late nineteenth century *H. scandens* was introduced in both North America and Western Europe to be used as an ornamental and medicinal plant (as tonic in Asian medicine) (EPPO, 2019). The first record of the establishment of the species in the wild in Europe is a voucher herbarium specimen collected in 1893 from the wastelands along the road Cours Journu-Auber (Bordeaux); in North America the escape from cultivation and the naturalization of *H. scandens* were observed in the 1900s in Delaware.

Today the sale and cultivation of *H. scandens* as an ornamental vine are not yet regulated in different countries of Europe and North America (EPPO, 2019). However, in an assessment worksheet of the Minnesota Department of Natural Resources (2016) the status of *H. scandens* in 2012 is stipulated as that of a noxious weed thus prohibiting its sale.

In their original habitat *H. scandens* is found in woodland areas at the borders of the forests, in meadows, on river banks, in ruderale habitats or in wastelands (Balogh & Dancza, 2008). It was also described as a common weed in orchards, wheat or reed fields in Northern China (Li & Cao, 1981).

In invaded habitats *H. scandens* prefers sunny or partially sunny areas located mainly in riparian zones but it can be found in grasslands, hayfields, on roadsides or in open disturbed areas. It installs quickly on bare surfaces following temporary floods of streams' sides (Balogh & Dancza, 2008; EPPO, 2019; Briscoe et al. 2019).

In relation to *H. scandens*' environmental requirements, it is mentioned that it can appear in plant communities installed in soil with high pH and moisture (Oh et al., 2008). On the other hand, Balog & Dancza (2008) have observed that the plants are indifferent to soil pH but prefer over-fertilized soil rich in nitrogen. A study on the ecological plasticity of *H. scandens* in 2013 emphasized that it can adapt to a moderate hydric stress by lowering its size but that brings a decrease in its competition capacity (Pinston, 2013).

It can be found in various plant community such as *Humulus japonicus - Artemisia princeps, Chenopodium album, Setaria viridis - Echinochloa crus-galli, Galium spurium - Stellaria aquatica, Equisetum arvense, Persicaria thunbergii, Echinochloa crus-galli - Digitaria ciliaris* (Oh et al., 2008). In their native range *H. scandens* plants are included in two main associations: *Bidentetea tripartiti* and *Commelinetalia communis*, but in Hungary they are classified in *Calystegio-Impatienti glanduliferae* and *Arction lappae (Conietum maculati)* syntaxomomical units (Balogh & Dancza, 2008).

In France *H. scandens* was reported as invasive in communities similar to those with native species such as *Galium aparine, Rumex crispus, Persicaria lapathifolia, Veronica anagalis-aquatica* and *Convolvulus sepium* and non-native species such as *Ambrosia artemisiifolia, Artemisia verlotiorum, Artemisia annuua, Bidens frondosa, Helianthus tuberosus* or *Xanthium orientale* subsp. *italicum* (EPPO, 2019). Its impact on the native communities in the invaded areas is significant, causing a decrease in vegetation diversity and an alteration of ecosystems functions. A paper from 2009 analysed the impact of *H. scandens* on *Miscanthus sacchariflorus* and *Phragmites australis* communities. The growth of *H. scandens* until it overtopped other species determined the collapse of those two species and their rapid decomposition (Kim & Kim, 2009).

H. scandens has an impact on human communities too: the pollen produced was reported to provoke allergenic rhinitis in East Asia increasing the risk of asthma development, the

pollen count being higher than that produced by Artemisia or Ambrosia species (Park et al., 1999; Jeong et al., 2018).
The morphology of *H. scandens* as an annual dioecious vine was studied in comparison with that of *H. lupulus*, perennial vine (Ehara, 1955; Balogh & Dancza, 2008). The stem of both species is twining clockwise and has opposite leaves. On the aerial organs of both species there are hairs - pubescent, glandular on *H. lupulus* and rigid, spinulose on *H. scandens*. The opposite leaves are dark green and 3-5 lobate to *H. lupulus* and light green, 5-7 (-9) lobate to *H. scandens*. There are no lupulin-glands in female inflorescences of *H. scandens* unlike those of *H. lupulus*.
With a less economic value than *H. lupulus* - the absence of lupulin glands does not allow the use of the species in beer flavouring, however various research has been conducted on the properties of *H. scandens* plants as an anti-aging and antioxidant agent (Sung et al., 2015), for their anti-mycobacterial effects (Hong et al., 2014) or to dyeing silk fabrics (Ha & Lee, 2015).
The paper emphasizes a new location in Romania of a *H. scandens* population and describes the micro-morphological aspect of the plant's aerial organs.

MATERIALS AND METHODS

Micro-morphological observations were carried on plants collected in the last decade of October from a *H. scandens* populations found on the Dâmbovița river's sides, near Budești (Călărasi County), at 40.2322 latitude and 26.4545 longitude; the township is located on the 4'th National Road, 40 Km South of Bucharest.
A stereomicroscope type S8APO, equipped with a video camera Leica DFC 295 and a SEM FEI Inspect S50 were used to reveal the specific morphological and micromorphological features of the stem, leaves and bracts.

RESULTS AND DISCUSSIONS

The population of *H. scandens* was identified on the banks of the Dâmbovița River, on either side of the bridge located at the entrance to Budești township (Figure 1).

Figure 1. View from the bridge to the southern bank of the river Dâmbovița occupied by a population of *H. scandens*

Plants were settled only on the left riverside with southern exposure. Household's waste and garbage are stored in the bridge proximity and a large amount of organic nitrogen, in the advanced stage of mineralization stimulated the occurrence of a community of nitrophilous plants from the association *Conietum maculati* I. Pop 1968, facies with *Sambucus nigra*, *Urtica dioica* and *Ballota nigra* Cristurean et Țeculescu 1970 (Sanda et al. 2008) (Figure 2).

Figure 2. General view of the *Conietum maculati* association, *Sambucus ebulus* facies

The *H. scandens* population was also expanded in areas with characteristic waterfront vegetation (*Phragmites australis* and *Agrostis stolonifera*) (Figure 3).

Figure 3. *H. scandens* on stalks of *Phragmites australis* plants

Plants colonized both vigorous herbaceous plants and woody specimens of the *Salix*, *Fraxinus* or *Populus* species (Figure 4).

Figure 4. *H. scandens* on *Salix alba* plants

Plants were reached the maturity phase and were in seed-spreading phenophases; tested, their achenes showed a good germination capacity.
On some leaves it could be seen a strong attack of hop's powdery mildew *Podosphaera macularis* (*Spaherotheca macularis*) (Wallr.) U. Braun & S.Takaram (Figure 5).

Figure 5. Leaves of *H. scandens* attacked by *Podosphaera macularis*

The surface of the plants is rough, the stems, leaves and bracts being covered with different types of hairs and glands.
According to Ehara (1955), on the young stem and petiole there are double and single hooked climbing hairs and many types of conic hairs. But if we use the terminology for plant's hairs from Payne (1978) we can describe the adult stem, leaves and bracts as bearing anvil, hooked (uncinate) hairs and short bristle (uncinate) with cystolith.
Anvil hairs are spread among a mass of uncinate hairs on the branches of the stem and on the petiole (Figure 6).

Figure 6. Anvil hairs on *H. scandens'* petiole

On the adaxial side of the leaf lamina, towards the edges can be observed uncinate hairs; between or on veins there are short, uncinate bristle with cystolith that make this surface very rough (Figure 7).

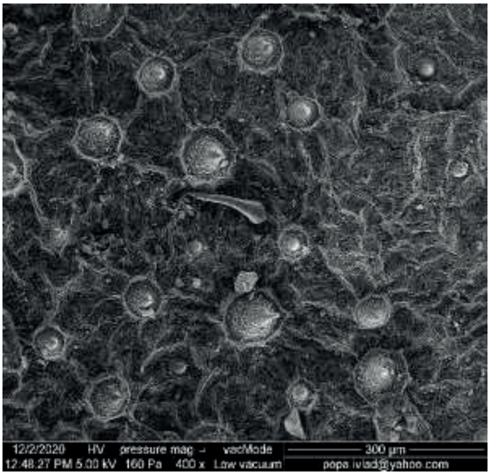

Figure 7. Short, uncinate bristle with cystolith on the adaxial side of lamina (SEM)

Uncinate hairs can be seen on the abaxial side of leaf lamina only on the main veins; also, there are glands scattered among the secondary veins (Figure 8).

Figure 8. Uncinate hairs and glands on the abaxial side of lamina

Bracts on the maturity are covered with uncinate hairs and it can be seen very rare glands on the abaxial side of them (Figure 9).

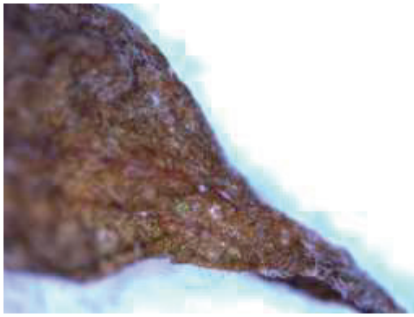

Figure 9. Uncinate hairs and glands on the adaxial side of bracts

CONCLUSIONS

The new location of a population of *Humulus scandens* (Lour.) Merr. in Romania confirms the occurrence of this species in association *Conietum maculati* I. Pop 1968, but in the facies with *Sambucus nigra*, *Urtica dioica* and *Ballota nigra* Cristurean et Țeculescu 1970.

Anvil, uncinate and short hairs with cystolith are spread on the aerial parts of the plant, and glands were observed on both leaves and bracts.

ACKNOWLEDGEMENTS

The paper is made under POIM/178/4/1/ 120008 project.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

TREE INVENTORY ANALYSIS IN VĂCĂREŞTI NATURAL PARK

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Abstract

The present study comprises a tree inventory analysis in Văcăreşti Natural Park from Bucharest. It aims to deepen the research regarding the vegetation habitats mapping conducted in Văcăreşti in 2019. The inventory has been realized by analyzing each tree over 4 m high. The analyzed aspects included: identification of the species and cultivars, (DBH) diameter at breast height, land use, tree height, health status etc. A database comprising those aspects was created through a specialized software, including also the spatial distribution of all the analyzed trees in the natural park. A total of 4676 trees have been inventoried, covering 29 different species from 18 genera. The results revealed: a high percentage of native species, a low presence of invasive species, the dominant species identified in each habitat and the relationship between the local pedoclimatic conditions and the spatial distribution of tree vegetation. This study helps us to analyse the evolution of vegetation in this natural urban area. Considering also the continuous extension of palustrine and tree vegetation, we can observe a tendency of the development of an urban forest in the next decades.

Key words*: tree inventory, vegetation mapping, urban wetland, Văcăreşti Natural Park Bucharest.*

INTRODUCTION

The study is exploring the identification and inventory of the mature trees of Văcărești Natural Park, declared the first urban natural area of Romania in 2016, by government decision (Guvernul României, 2016).
Urban green spaces offer many ecosystem services to the community: clean and fresh air, regulation of high temperatures in summer, balancing airflow, attenuation of precipitation, healthy recreation areas etc. Văcărești Natural Park is a good example of green space that, in addition to the ecosystem benefits offered, due to its natural character and biodiversity, also offers thematic spaces for education, information and awareness about the importance of nature in cities.
Thus, the evaluation of the ecosystem benefits offered by the tree vegetation, which also contributes to the well-being of the local community and the city, is an important future objective. Starting with November 2019, until March 2021, comprising a total of 16 months, data were collected on tree vegetation (Figures 1 and 2). This paper is an in-depth analysis of the structure of tree vegetation that completes the initial mapping study of plant habitats in the Văcărești Natural Park (Boc et al., 2020) (Figure 3).


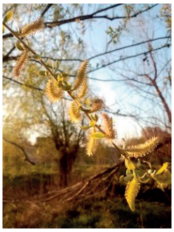

Figure 1. *Salix alba* in the natural park

Figure 2. *Juglans regia* in the natural park

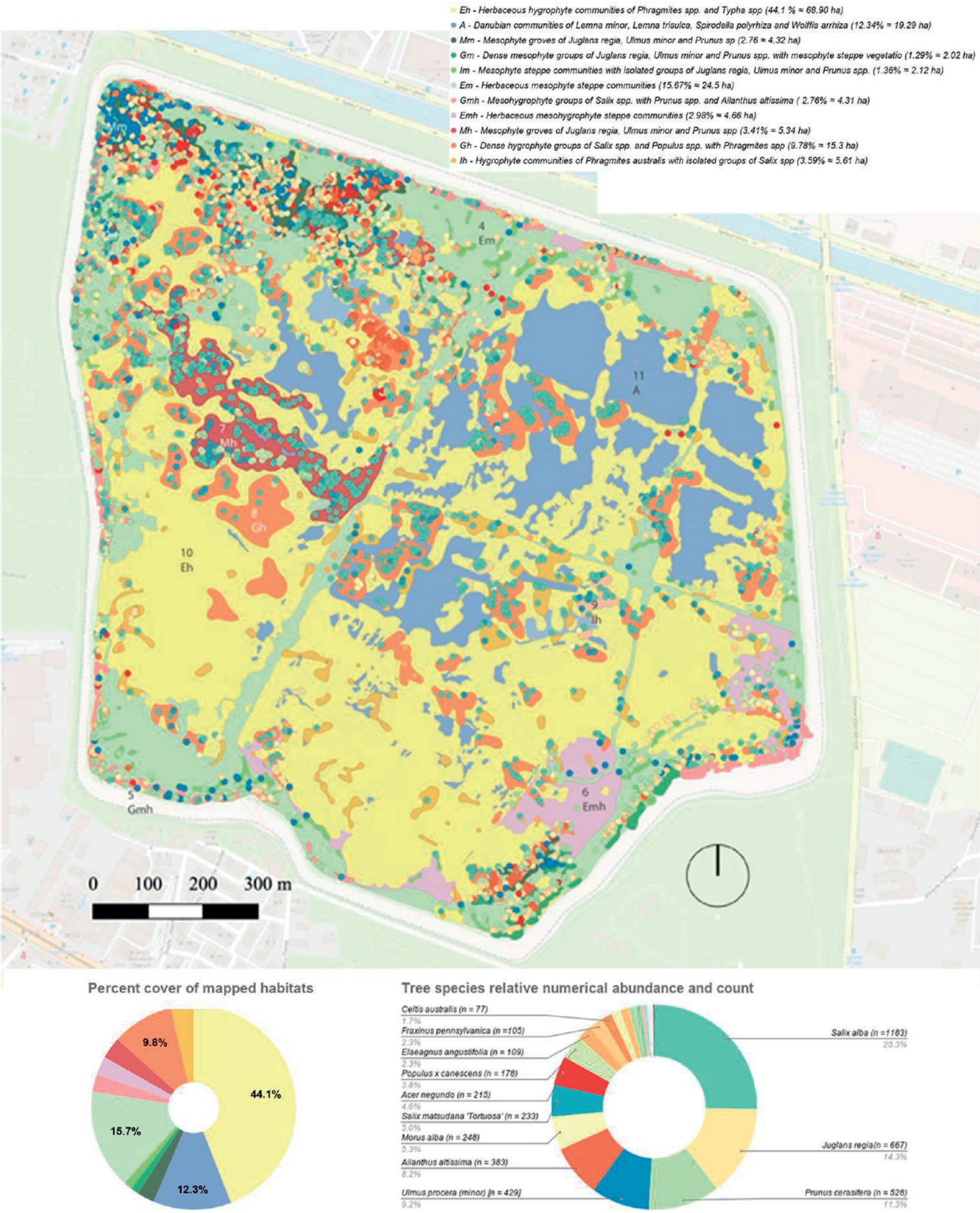

Figure 3. Tree vegetation overlapping the habitats

MATERIALS AND METHODS

The inventory of tree specimens and the ecosystem services offered by them was made with the help of the TreePlotter™ Inventory application (support.treeplotter.com), in the following 3 stages:

1. Species identification - the species and cultivars were identified using local scientific literature (Ciocîrlan, 1990; Iliescu, 2003; Pârvu, 2006) and previous studies conducted in Văcărești Natural Park (Anastasiu et al., 2017; Doniță et al., 2005; Stoican et al., 2014);

2. Field identification of the location of trees with a height of at least 4 m by GPS and registration of taxonomic and biometric data characteristic of each specimen with the help of the digital application TreePlotter™ Inventory. These data include: species and cultivars (if applicable), common name, height, trunk DBH (Diameter at Breast Height - about 130 cm), health. Health condition was determined upon visual inspection, classified according to the following four categories based on the observations list provided in the survey form of the TreePlotter app (support.treeplotter.com):

- excellent: no observations or only minor observations about the tree's condition;
- good: few observations that may include: cavity decay, crown dieback, frost cracks, pests, mechanical damage, and/or poor root system. This category should only have 1-2 observations which should affect < 10% of the tree;
- fair: same criteria applicable for "good" category, including also: poor structure; maximum of 1-4 observations which may affect 10-60% of the tree;
- poor: same criteria applicable to "fair" category, including also serious biological decline. There should be one or all of the observations reported which may affect >60% of the tree.

Where appropriate, additional observations which were not included in the original survey, such as the degree of burns or creeping vines, were made. In addition, recommendations were made for the future management of the specimens but the results are not included in this study.

Regarding DBH, since most of the trees were multi-stem trees (had more than 1 stem), their trunk DBH was calculated by taking the square root of the sum of each stem diameter squared. The specific tools used for measuring the DBH were tree calipers and measuring tapes. For height determination the Hagloff EC II electronic clinometer was used.

3. The last stage consisted in centralizing the data and interpreting the database resulting from the field inventory, by using specific software for statistical data. (R Core Team) and QGIS 3.4 for plotting thematic maps. At this stage, depending on the data collected in the field, the following results were identified regarding the structure of tree vegetation: species relative numerical abundance, percentage of native species, distribution of mesophytes, mesohygrophytes and hygrophytes species, tree condition, DBH range classes.

For the characterization of the tree community we calculated the relative abundace and derived the species domeninance (Preda, Rusti & Cogalniceanu, 2020). The species were sorted in descending order by relative abundances and dominance thresholds were chosen arbitrary by the researchers. If the relative abundance is above 10%, the species are considered frequent, while if it is beteween 1% and 10%, the species are classified as ocassional. If the relative abundance is below 1%, than the species are rare (Gomoiu & Skolka, 2001).

RESULTS AND DISCUSSIONS

As a consequence of the undertaken field inventory a **total of 29 tree species from 18 genera are identified** (Figure 4). Table 1 illustrates the number of individuals for each species and their relative abundance and their dominance. The total number of individuals is **4676**. The research was carried out on the surface of 156 ha within the concrete dam surrounding the park. The most frequent species identified are: *Salix alba* (25.30 %), *Juglans regia* (14.26 %), *Prunus cerasifera* (11.29 %) and *Ulmus procera* (9.17 %). These cover 60% of the park`s total tree population.

All the four species are native tree species that are expected to be present in an urban wetland habitat and have emerged in the last 20-25 years. This is the average age estimated for the oldest individuals. The dead individuals were not inventoried.

There are other 11 occasional species which cover an abundance interval between 8.19% and 1.20%. *Ailanthus altissima,* an invasive species of Community interest (*Handbook of Alien Species in Europe*, 2009) is the most abundant. Because of its fast vegetative reproduction through suckers it threatens the adjacent communities therefore management control is required.

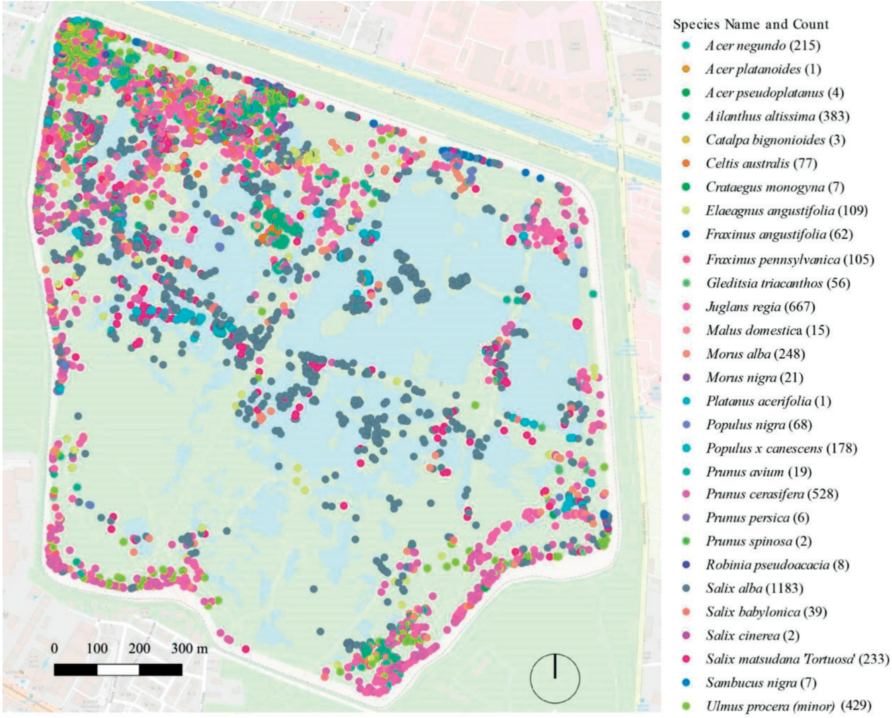

Figure 4. Tree species distribution

Table 1. Number of individuals and relative abundance of the tree species from the Văcăreşti Natural Park

Species name	n	Relative abundance (%)	Cumulative abundance (%)	Dominance
Salix alba	1183	25.30	25.30	Frequent
Juglans regia	667	14.26	39.56	Frequent
Prunus cerasifera	528	11.29	50.86	Frequent
Ulmus procera (minor)	429	9.17	60.03	Occasional
Ailanthus altissima	383	8.19	68.22	Occasional
Morus alba	248	5.30	73.52	Occasional
Salix matsudana 'Tortuosa'	233	4.98	78.51	Occasional
Acer negundo	215	4.60	83.11	Occasional
Populus x canescens	178	3.81	86.91	Occasional
Elaeagnus angustifolia	109	2.33	89.24	Occasional
Fraxinus pennsylvanica	105	2.25	91.49	Occasional
Celtis australis	77	1.65	93.14	Occasional
Populus nigra	68	1.45	94.59	Occasional
Fraxinus angustifolia	62	1.33	95.92	Occasional
Gleditsia triacanthos	56	1.20	97.11	Occasional
Salix babylonica	39	0.83	97.95	Rare
Morus nigra	21	0.45	98.40	Rare
Prunus avium	19	0.41	98.80	Rare
Malus domestica	15	0.32	99.12	Rare
Robinia pseudoacacia	8	0.17	99.29	Rare
Crataegus monogyna	7	0.15	99.44	Rare
Sambucus nigra	7	0.15	99.59	Rare
Prunus persica	6	0.13	99.72	Rare
Acer pseudoplatanus	4	0.09	99.81	Rare
Catalpa bignonioides	3	0.06	99.87	Rare
Prunus spinosa	2	0.04	99.91	Rare
Salix cinerea	2	0.04	99.96	Rare
Acer platanoides	1	0.02	99.98	Rare
Platanus acerifolia	1	0.02	100.00	Rare
Total	**4676**	**100**	**100**	-

Other 14 species are classified as rare, because their abundance in the park is less than 1%. *Platanus acerifolia* is a notable presence, with only one individual being identified. In addition, *Acer pseudoplatanus,* a species most common in the mountainous region has four individuals present in the park while *Acer platanoides* which is more common in the plains region only has one presence.

In Văcăreşti Natural Park, most of the specimens of trees inventoried are native species (69%) and less than a third (31%) are non-native species (Figure 5). Among the most common native species are *Salix alba* (Figure 1), *Ulmus procera*, *Prunus cerasifera* and *Juglans regia* (Figure 2). The most common alien species are *Ailanthus altissima*, *Morus alba*, *Salix matsudana 'Tortuosa'*, *Acer negundo*. Among the non-native species there are several groups of *Ailanthus altissima* and *Acer negundo* that have an invasive potential, and monitoring the evolution of those trees should be a priority for the management of the natural area.

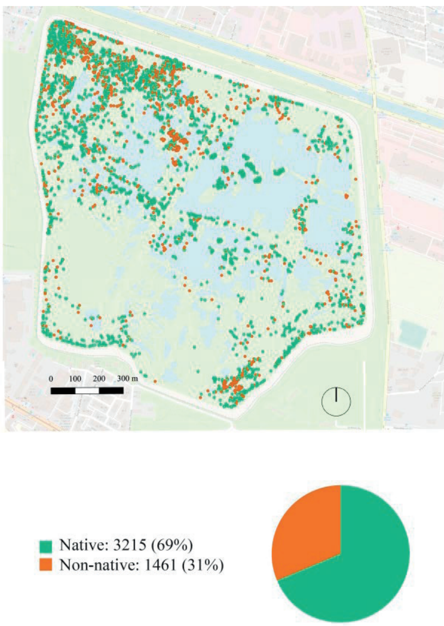

Figure 5. Tree classification by origin

Both the native and the non-native species frequently found in the park are representative for the spontaneous flora in the peripheral and ruderal areas of Bucharest.

From the point of view of ecological requirements, the species are divided into 3 categories: mesophytes, hygrophytes and mesohygrophytes. (Figure 6) Mesophyte specimens are generally located in the peripheral areas of the park, where the soil moisture is lower. These represent 63% of the total number of trees found in the park. The main mesophyte tree species encountered are: *Juglans regia*, *Prunus cerasifera*, *Ulmus procera*, *Ailanthus altissima* and *Morus alba*. Hygrophytes represent 31% of the total and are found in the wetland, on the banks of water mirrors and in floodplains covered with *Phragmites australis*. The predominant species in this type of habitat is *Salix alba*, along with *Salix matsudana 'Tortuosa'*. The third category - mesohygrophytes are adapted to both soil conditions with high humidity and soils with moderate humidity. They occupy 6% of the total individuals, and the two species that fall into this category are *Populus x canescens* and *Eleagnus angustifolia.*

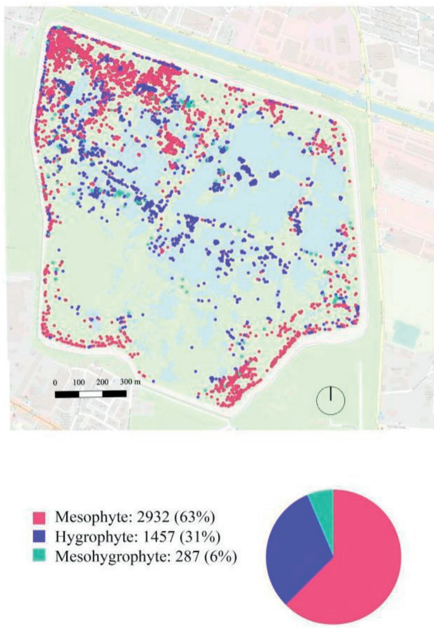

Figure 6. Tree classification by ecological requirements

Regarding DBH, it was found that mature trees with a diameter of over 30-45 cm are located mainly in the center of the park and close to the marsh areas. (Figure 7). In general, the most common specimens with high DBH, up to over

75 cm, are those of *Salix alba* (Figure 8) which are both the most mature trees, being the first to appear in the park about 20-25 years ago, but also characterized by a rapid growth rate when compared to other species found in peripheral areas, such as the genera *Juglans* or *Ulmus*. The analysis of the tree condition reveals a high percentage of trees in good (49.89%) and excellent (34.94%) condition (Figure 9) (the framing classes are described in materials and methods). Therefore, the young vegetation within the park may not be too affected yet by biological or anthropic factors.

The exceptions are the fair and poor tree individuals. The main reasons for these conditions are caused by observations of mostly mechanical damages, cavity decays or pests usually associated with each of the species.

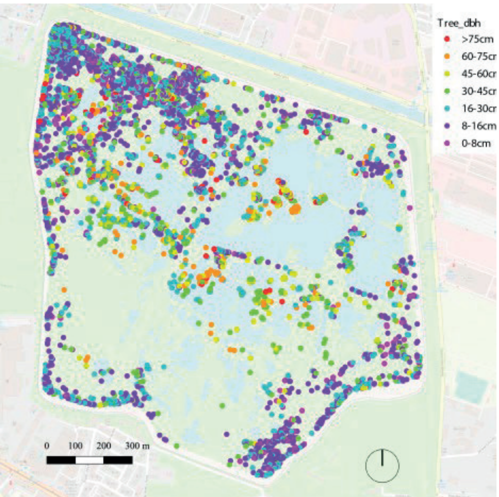

Figure 7. DBH range

Mechanical damages occur mostly due to natural phenomena, such as cracks from freezing rain, or fire damage.

There is also identification of mechanical damage such as tree cuttings observed especially in old *Salix alba*, or *Salix matsudana 'Tortuosa'* individuals, probably done mostly by the former unofficial inhabitants of the park.

Other recorded observations in the trees condition are poor root system or poor structure, due to the improper growing conditions of some of the trees.

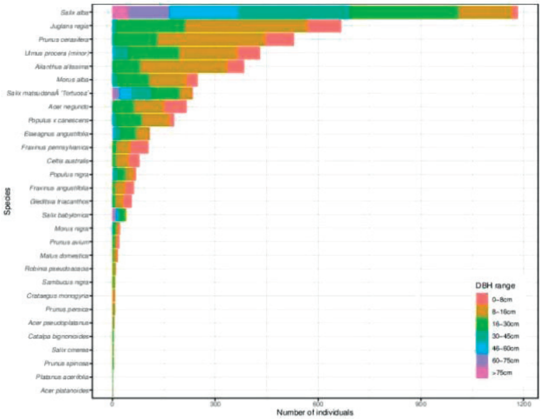

Figure 8. Species numbers by DBH

Figure 9. Proportion of tree condition

CONCLUSIONS

The diversity of tree species in the park is very high with a species richness of 29 tree species and 4676 individuals. The native species are dominant, but the non-native ones that were acclimatised here in the last century also occupy significant surfaces in the park.

Some of them have the tendency to be invasive: *Ailanthus altissima* - 8.19% (5th place) (Figure 12), *Acer negundo* - 4.6% (8th place) (Figure 10) and *Fraxinus pennsylvanica* - 2.25% (11th place).

The presence of a lot of young stems around the main trunks, because of the reproduction through suckers, is assuring a fast covering of the herbaceous areas and a limiting of the species depending on them (Figure 11). Thus, the necessity of maintenance works and control of the invasive species is required.

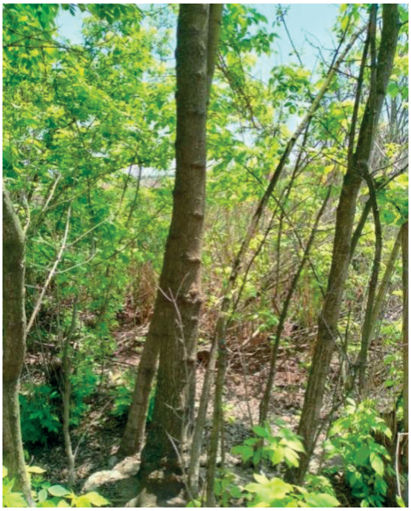

Figure 10. *Acer negundo* in the natural park

Figure 11. Future grove formed by suckers of *Fraxinus pennsylvanica* and *Acer negundo*. Medium height: 3-4 m

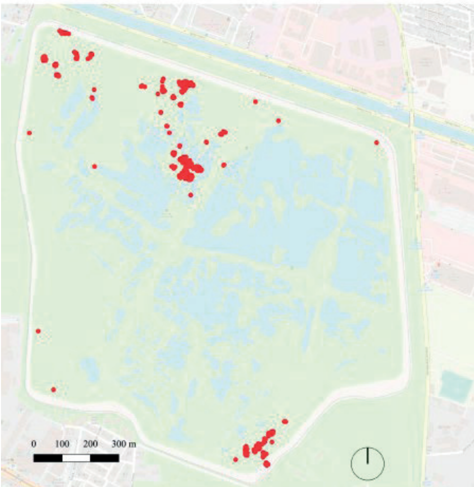

Figure 12. Invasive species (*Ailanthus altissima*) map

Future studies may look at the evolution of the different types of vegetation using different control plots. Some control plots can be maintained with this kind of vegetation, while in others it can be cut down.

An additional future study could investigate the succession of the vegetation and of its dynamic, through the repetition of the tree inventory every 5 years and comparing the results, to observe its evolution.

Another recommendation is that the inventory and mapping of the herbaceous species and areas in the park is done in order to find out their ecological benefits, but also for highlighting their relationship with the dendrological species. The extension of the study for the exterior zones of the park is also recommended.

A further study will be conducted in order to present the ecological benefits synthesized in the TreePlotter ™ Inventory application.

The species legend, visualisation of the satellite map and GPS coordinates of each tree can be found at: https://uk.pg-cloud.com/Văcăreşti/?utm_medium=email&utm_source=sharpspring&sslid=

ACKNOWLEDGEMENTS

This study was conducted with the help of Văcăreşti Natural Park Association - founder of the protected area and partners of the Romanian Ministry of Environment in the park's administration - alongside the following volunteers/employees of the park: Anita Aursulesei, Dan Barbulescu, Alice Dumitrescu, Nicoleta Marin, Eugenia Petrescu, Gabriela Poiana, Marius Predescu, Lia Stelea and the Camera Şepte Association. The study was part of a project in the RO SMART în Țara lui Andrei program, supported by Omv Petrom S.A.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ABUNDANCE OF INSECT SPECIES HARMFUL TO ORNAMENTAL PLANTS IN URBAN ECOSYSTEMS

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Abstract

Parks are the most important natural sources of fresh air and relaxation in a city like Timisoara. Therefore, in this paper we turned our attention to the factors that can disrupt the development of ornamental plants in the main parks. One of them is harmful insects that can affect in the medium or long term the species of plants. Thus, during 2020, between April and September, 125 observation points in 5 parks were analyzed to establish the level of abundance of insect populations. In this analysis, we considered 4 levels of evaluation: level I, extremely high (> 400 individuals), level II, high (250-400 individuals), level III, medium (100-250 individuals) and level IV, low (<100 individuals). Levels I and II included the species of Metcalfa pruinosa, Eriosoma lanigerum, Aphis rosae, Aphis gossypii, Trialeurodes vaporariorum, Corythucha ciliata and Halyomorpha halys, which had high and extremely high values in 2 parks (Botanical and Roses). Increased attention should be paid to these pests which in the absence of a control strategy can multiply excessively causing qualitative and quantitative losses of plant species.

***Key words**: urban ecosystem, insect population, harmful, abundance.*

INTRODUCTION

In the current context of modernization and technological and economic evolution, it is obvious that the human population is becoming more and more urban (Ritchie and Roser, 2018). That is why tools to support urban planning and management are needed to reconcile the needs for building development (UN, 2019), to ensure a high quality of life and to protect urban biodiversity (Pauna, 2007).
Increasing the impact of conservation measures of green space and parks through actions that promote correlating combinations between diversity and vegetation structure has become a target (Nielsen et al., 2013). In this sense, the conservation of spaces could offer enormous benefits for other urban ecological components but also for urban inhabitants (Lopucki & Kiersztyn, 2015). That is why we set out in this paper to address one of the destructive factors, such as harmful insects, which destabilize their proper functioning.
It is known that insects are important elements of urban ecological ecosystems (Cardoso et al., 2020) and are strongly affected by anthropogenic activities (EEA, 2020).
A comparative assessment of the frequency of insect species in different urban ecosystems providing decorative and relaxing services showed that there is a wide variety of insect species (Jaganmohan et al., 2013). Mata showed that herbivores and predators bugs show strong positive responses to vegetation volume in those urban ecosystems and that the plant diversity is distinctly species specific, or, otherwise said, high occupancy of bugs is obtained in green spaces with specific combinations of vegetation structure and diversity (Mata et al., 2017).
Numerous species of insects, native or non-native, have been identified in parks in Europe to date (Lesieur, 2018) and in parks in Romania (Grozea & Muntean, 2019). By the end of 2016, 1418 non-native insect species had been identified as being introduced and established in Europe. This acceleration (> 90%) was mainly due to accidental introductions related to human activities and, mainly, to the exponential growth of the world trade in ornamental plants (Villa et al., 2001), which leads to the transport of plant-associated species (Roques et al., 2010). These landscaped ecosystems are permanent sources of new

species. The rapid evolution of their expansion can also be measured in the number of years from the first reporting (Roques and Auger-Rozenberg, 2021); for example, the species *Cydalima perspectalis* and *Leptoglossus occidentalis* recorded an annual increase of over 150 km (EPPO, 2012).

Arthropods are key elements of urban ecological areas that can be influenced by anthropogenic activities. New methods are continuously developed for detection and quantification of insect species, that ultimately could lead to a reduction in population levels below the damage thresholds (NAPPO, 2014). Some insect species can be easily quantified, such as *Cameraria ohridella* in which adult flight monitoring is performed by using pheromones traps (Kalinova et al. 2003). Other are difficult to detect and control, as *Nezara viridula* because it has an accentuated polyphagism and is in continuous adaptation to new host plants (Kamminga et al., 2006).

To detect specimens of *Halyomorpha halys*, INRA launched a participatory detection program requested the assistance of the public (Streito, 2017).

One species for which monitoring involves diverse methods for adults and larval and nymph forms is *Metcalfa pruinosa*. Thus, adult specimens were quantified using traps and larvae and nymphs by cutting leaves and shoots directly from parks and gardens and placing them in plastic bags (Gogan, 2013; Vlad & Grozea, 2016).

In the following we will present the results of monitoring observations in order to highlight the species of problem insects, which due to the large number of individuals endanger the species of ornamental plants in public parks in the city of Timisoara.

MATERIALS AND METHODS

Observations for quantification and evaluation of the population level of insects were made during 2020, between April and September, in several parks in the city of Timisoara. Out of the 15 urban parks of the city, five were chosen mostly near the historic central area: Botanical Park, Central Park, Roses Park, Ion Creangă Park and Justice Park.

The above-mentioned parks were the main research areas. These areas were divided into 5 sectors each (S1-S5), and in each sector 5 observation points (OP) were established (Figure 1) depending on the presence of mixed plants, both woody plants (like trees and shrubs) and grasses (as periodic decorative plants).

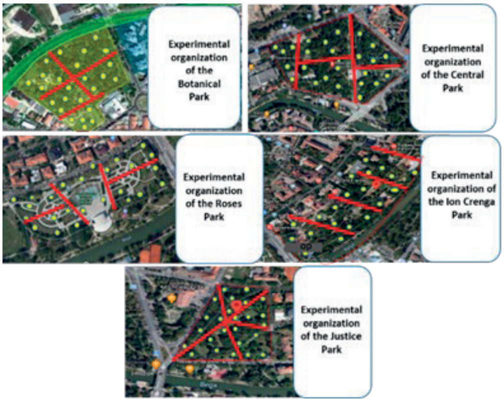

Figure 1. Geographical representation of the experimental organization by dividing the 5 parks in the city of Timisoara into observation points (OPs)

The direct readings (Figure 2) from the observation points included plants present on a radius of 4-6 m and where the free spaces without plants predominated, the observation surface was extended by 1-2 m. The observations were made on already existing plants. All plants in the observation points were analysed only at the aerial parts (stem, shoots, leaves, inflorescences, fruiting). The underground part (root) was not analyzed because we were not allowed to uproot the plants.

The categories of plants existing in parks and otherwise analysed were woody plants (shrubs and trees), shrubby woody plants, perennial woody plants (roses, ivy), herbaceous plants, annuals (flower layers) and perennial herbaceous plants (lawn). The monthly observations made in each OP/parks were focused on the following aspects: quantification of specimens of each insect species present, framing the population level at each observation and finally establishing the level and abundance curve (total number per monitored period).

We considered 4 levels of evaluation: level I, extremely high (> 400 individuals), level II, high (250-400 individuals), level III, medium (100-250 individuals) and level IV, low (<100 individuals).

Figure 2. Moments of observations to quantify the number of the insect species present in the five parks in Timisoara (a - Botanical Park; b - Ion Creangă Park; c- Roses Park; d - Central Park; e - Justice Park)

As working materials, in the pre-monitoring phase, in the Laboratory of Diagnosis and Phytosanitary Expertise within USAMVB "King Michael I of Romania" from Timisoara, the observation panels, collection containers, etc. were prepared. Post-monitoring, samples (insects, shoots with symptoms of attack, leaves and flowers with different forms of harmful insects) collected from parks were subjected to a more detailed analysis of determination and identification. Also, binocular magnifiers and other utensils like Petri dishes, tweezers, entomological needles, anaesthetic solutions, cutters and insect fixation substrate are used.

RESULTS AND DISCUSSIONS

The monitoring data obtained from the analysis of the five sectors (S1-S5) showed that in 2020, the following harmful insect species were present in the analyzed parks: *Metcalfa pruinosa, Nezara viridula, Cydalima perspectalis, Cameraria ochridella, Trialeurodes vaporariorum, Aphis gossypii, Eriosoma lanigerum, Leptoglossus occidentalis, Aphis rosae, Halyomorpha halys, Phylotreta sp., Cossus cossus, Lymantria dispar, Scudderia sp. and Corythucha ciliata.* These are part of the orders: Hemiptera, Orthoptera, Coleoptera and Lepidoptera. Each sector of the 5 analyzed had positive or negative results (+)/(-).

The periodic numerical evolution in Botanical Park showed that the highest size population of species and individuals (ind.) was registered in the summer and autumn period (July, August, and September), with average values (where x = 46.60, ind., x = 72.93 ind. and x = 42.93 ind.). Very high values (x > 50) were recorded only in August.

The abundance of species shows their inclusion in all 4 levels of population assessment. Figure 3 shows that in category IV, entered the species: *Cydalima perspectalis, Leptoglossus occidentalis, Lymantria dispar, Cossus cossus, Phylotreta* sp. and *Scudderia* sp. In the category middle level (III), the species *Cameraria ohridella* and *Nezara viridula* entered. High-level category II included *Aphis gossypii, Trialeurodes vaporariorum* and *Halyomorpha halis*, and category I, extremely dangerous species of *Eriosoma lanigerum, Aphis rosae, Metcalfa pruinosa* and *Corythucha ciliata* were identified.

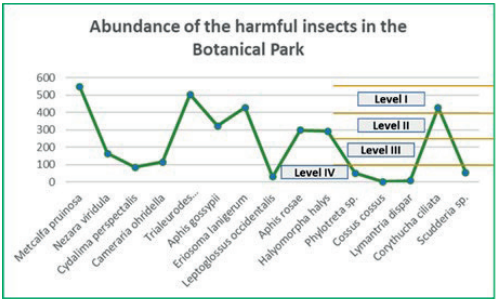

Figure 3. Abundance of harmful insect species present in the Botanical Park, in the city of Timisoara, in 2020 during April-September

For the Central Park, the sectors with positive values expressing the periodic numerical evolution highlighted the presence concentrated especially in the summer months (June, June, July and August), when the average values were between x = 12.87 and x = 13.87 individuals (x < 30).

Only 2 species *Metcalfa pruinosa* and *Aphis gossypii* entered in the category of medium-level III and all the other species with positive values entered in the category of level IV (low): *Nezara viridula, Aphis rosae, Trialeurodes vaporariorum, Halyomorpha halis, Cameraria ohridella, Cydalima perspectalis, Eriosoma lanigerum, Corythucha ciliata* and *Phylotreta* sp. (Figure 4). Unlike the Botanical Park, in the Central Park were identified fewer species but also fewer individuals.

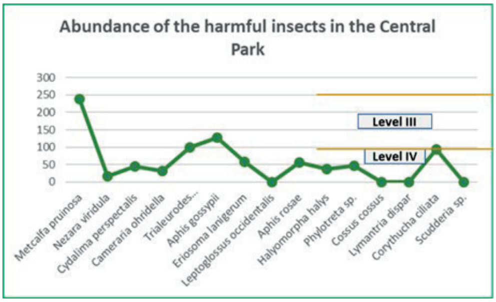

Figure 4. Abundance of harmful insect species present in the Central Park, in the city of Timisoara, in 2020 during April-September

In the Roses Park, several species identified in the Botanical Park (as a reference park) presented negative values, with the value x = 0/S1-S5; these were the species of *Leptoglosus occidentalis, Aphis gossypii, Phylotreta* sp., *Cameraria ohridella, Cossus cossus* and *Lymantria dispar*.

The periodic numerical evolution of species with positive values was concentrated in June, July and September (x = 44.40; x = 32.87; x = 30.13 individuals), where x > 30.

Related to the abundance of species, in Figure 5 it can be seen that in category of the level I, due to the large number of individuals present on the plants entered the species *Aphis rosae, Trialeurodes vaporariorum* and *Eriosoma lanigerum*. In category of the level II, the species *Metcalfa pruinosa* entered to a high level. Category of the level III (medium) included the species *Halyomorpha halys* and the category of the level IV (low) included the species *Cydalima perspectalis, Nezara viridula and Scudderia* sp. and *Corythucha ciliata.*

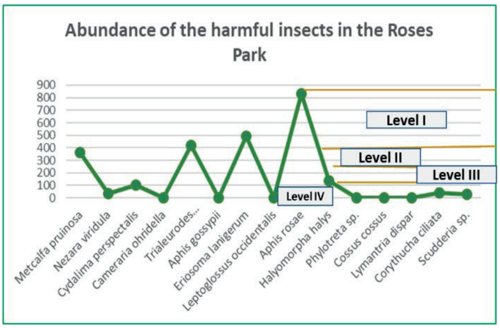

Figure 5. Abundance of harmful insect species present in the Roses Park, in the city of Timisoara, in 2020 during April-September

In Ion Creangă Park, in 2020 the species of *Lymantria dispar, Cossus cossus, Leptoglossus occidentalis* and *Scudderia* sp. were not present, their values in all the analyzed sectors being zero S (-) (x = 0). A concentration of species with positive values and individuals present in July was observed with an average value of 20.53 where x > 20.

In this park the abundance was expressed through 3 levels (level II, III and IV) (Figure 6). Only the species *Metcalfa pruinosa* entered in the level II. Level III included the species *Trialeurodes vaporariorum* and *Aphis gossypii*, and level IV included most other species with positive values: *Halyomorpha halys, Cameraria ochridella, Eriosoma lanigerum, Nezara viridula, Cydalima perspectalis* and *Corythucha ciliata.*

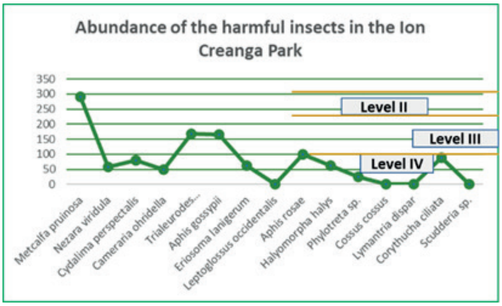

Figure 6. Abundance of harmful insect species present in the Ion Creanga Park, in the city of Timisoara, in 2020 during April-September

In the Justice Park, the observations highlighted the presence of a smaller number of species than in the other monitored parks. The absent species were: *Halyomorpha halys, Phylotreta sp., Cossus cossus, Lymantria dispar, Corythucha ciliata* and *Scudderia* sp. where (x = 0)/S (-). Their massive presence-was observed especially in June and July, with average values of x = 24.67 and x = 24.93 where x < 30.

The abundance of species represented in figure 7 shows that only one species (*Metcalfa pruinosa*) entered the high-level category II. In level III, medium, were included the species: *Cameraria ochridella, Trialeurodes vaporariorum, Aphis rosae* and in the category of level IV (low) entered *Nezara viridula, Eriosoma lanigerum, Aphis gossypii* and *Cydalima perspectalis*

The explanation for the high values registered in the Botanical Park compared to the other parks consists in the fact that it is a larger and more diversified park in Timisoara, being also known as the Botanical Garden (Ciupa et al, 2005). Another reason why the species of insects that feed on roses predominates in the Rose Park is the extremely varied range of rose species present in it.

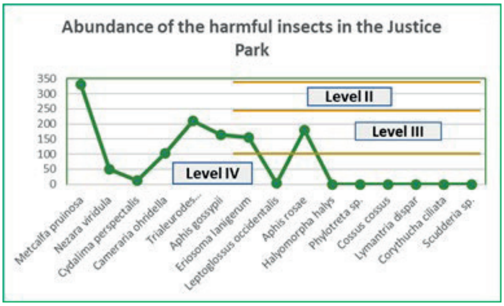

Figure 7. Abundance of harmful insect species present in the Justice Park, in the city of Timisoara, in 2020 during April-September

The species *Metcalfa pruinosa* and *Eriosoma lanigerum* species identification were easily identified and recognized, even remotely chosen after white secretions, fine whitish cloth left after attack for a long time on leaves, shoots, petiole and after glossy leaves due to transparent sticky liquid excrement deposited on them. Upon careful analysis, even adults, good jumpers, were observed on the stems or petiole of the leaves, placed in a longitudinal row (Figure 8).

Figure 8. Larval and adult colonies of *Metcalfa pruinosa* on park plants (a, b); Larval colonies of *Eriosoma lanigerum* on plants (c)

The other species were recognized either after direct observation (Figure 9) of adults (*Phyllotreta, Leptoglossus*) of larvae (*Cydalyma, Lymantria, Cameraria, Cossus*) and sometimes after direct observation of all active stages, ie larvae, nymphs and adults (*Nezara, Corhythuca, Halyomorpha*) and sometimes the damage caused helped to search and identify (such as *Scudderia*). In the case of aphids (*Aphis* sp.), they were permanently present on plants, in complex colonies (winged adults, non-winged adults, fundatrigens and virginogens, sexupares, eggs).

Figure 9. Virginogens colonies of *Aphis rosae* (a), young and larvae of *Cameraria ochridella* (b), adult form of *Nezara viridula* (c), larval attack of *Cydalima perspectalis* (d); mining by *Cameraria ochridella* (e)

CONCLUSIONS

In all monitored parks, the insects present were identified as various lepidopteran, hemipteran, coleopteran and orthopteran species, that varied both in terms of diversity and abundance.

The parks where the most diverse species were identified but also the most individuals were found were the Botanical Park and the Rose Park.

Among the most common species, the following are worth mentioning: *Metcalfa pruinosa, Aphis rosae, Trialeurodes vaporariorum, Eriosoma lanigerum, Aphis gossypii, Corythucha ciliata* and *Halyomorpha halys*. They recorded values between 250 and 400 individuals, but also values higher than 400 individuals.

Due to their presence in large populations, these species can cause considerable damage to ornamental plants in parks in Timisoara in the coming years. Therefore, it is recommended that the monitoring activity already started and presented through this paper, to continue in order to clearly establish their evolution. Also, a control strategy to keep problematic insect populations below the level of damage is necessary, and this must include environmentally friendly methods taking into account the presence of humans.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the people who manage the parks in the city of Timisoara because they allowed us to make observations on the plants. Part of this paper belongs to the doctoral thesis "Effective

management of harmful insects in the green spaces of Timisoara through monitoring and biological control strategies".

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARATIVE STUDY OF SPECIES OF THE GENUS *LUPINUS* ON THE SOWING QUALITIES OF SEEDS DURING THEIR TREATMENT WITH ULTRASOUND

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Abstract

The main purpose of the present study was to determine the effect of ultrasound treatment of seeds of different lupine species of the genus Lupinus on their sowing qualities. The experiments were carried out with seeds of two species of lupine (Lupinus polyphyllus Lindl.) and tarwi (Lupinus mutabilis Sweet.). The treatment of the seeds was performed with ultrasound, testing the following sound durations: 3, 6 and 9 minutes. Germination energy, germination, mean germination time and uniformity of germination, length of hypocotyls and embryo root, fresh and dry matter of seedlings were studied. Sonication with a duration of 6 minutes causes the highest increase of germination. As a result of treatment, the fresh weight of seedlings also increases. Polynomial regressions with high coefficients of determination about the effect of ultrasound on germination energy and germination were found. A higher effect was observed in the species Lupinus polyphyllus Lindl. It is recommended to improve the sowing qualities of lupine seeds to apply the sonication of 6 minutes.

Key words: *germination, seeds, ultrasound, dry matter, embryo root.*

INTRODUCTION

The genus *Lupinus* is one of the most widespread in natural conditions, characterized by a great diversity of species. It is found mainly in the Mediterranean, as well as in North and South America. Except for food, it is also widely used for decorative purposes (Wolko et al., 2011). According to Aniszewskiet al. (2001) did not find a significant difference in the seeds of ornamental species compared to the others. The authors emphasized that one of the widely used species in floriculture, such as *Lupinus polyphyllus* Lindl. is the result of mixing different species and botanical forms, which determines its great diversity in flowering and the shape and color of the seeds.

Other researchers report that these species are characterized by great adaptability, especially in the time of seed development, which explains the wide application of propagation by seed in ornamental horticulture (Sõber and Ramula, 2013). The great plasticity of the species of the genus *Lupinus*, as well as the diversity in their decorative manifestations, represents significant opportunities to expand the range of ornamental plants and to enrich the cultivated flora (Pantsyreva, 2019). Yves et al. (2020) observed uneven germination and sprouting of lupine seeds, especially *Lupinus polyphyllus* Lindl. and reported that their viability depends on both factors climatic conditions and the method of cultivation as well as their stage of development.

About the improvement of the germination processes of the seeds, various methods and ways of exposure with physical agents are applied, as one of the most widely used in the treatment with ultrasound (Panayotov, 2015). Sonication is an inexpensive and easy-to-apply way to treat seeds and thus the water uptake is accelerating, the seed porosity increases and the metabolic processes are improved (Hielscher Ultrasound Technology, 2015). When applying temperature treatment, as one of the physical methods, Carl et al. (2011) found a species response to improvement of the germination in different members of the genus *Lupinus*. Guo and Pan (2016) found that after exposure to ultrasound on lupine seeds, their viability, germination and vigour are improved and at the same time the soluble protein content is increased. Aguilar-Acosta et al. (2020) point out that the effect of ultrasound application depends on the lupine species. In *L. mutabilis*

L. the improvement in the seed status is significant and the increase in the protein content is higher, while in *L. mutabilis* L. there are almost no differences.

The main purpose of the present study was to determine the effect of ultrasound treatment of seeds of different lupine species of the genus *Lupinus* on their sowing qualities.

MATERIALS AND METHODS

The experiments were carried out in the Scientific laboratory of the Department of Horticulture at the Agriculture University-Plovdiv- Bulgaria. Seeds from two species of lupines (*Lupinus polyphyllus* Lindl.) and tarwi (*Lupinus mutabilis* Sweet.) were subjected to ultrasonic treatment. The different periods from 3 minutes, 6 minutes and 9 minutes of sonic were applied, as control was not treatment seeds (0 minutes). The apparatus Ultrasonic Water Bath "Nahita" model 620-1 with the capacity of 0.6 L, frequency of 50 Hz and total power (220-240 V) was used.

After treatment, the seeds were placed to analyze their sowing parameters. The germination energy (first cont) and germination (final count) were determined according to ISTA Rules (ISTA, 2013) in four replicates each from 100 seeds. The mean germination time (MGT) by the method of Battle and Whittington and uniformity of germination by the method of Strona, both described in Panayotov (2015) were calculated. The length of embryo root and length of hypocotyls on 15 seedlings of each replicate as well as fresh and dry weight of each developed seedlings was measured on the day when the germination was established. The dry weight was determined by the method described in detail by Georgiev et al. (1980).

The dispersion analysis of data (ANOVA) and regression analysis were processed (Foel and Cohen, 1992).

RESULTS AND DISCUSSIONS

Germination energy (Table 1) is one of the main signs that describe the possibility for earlier and faster germination of better seeds with the highest viability (Black et al., 2008). Strong differences in this behavior between both investigated lupine species were registered. The germination energy was higher in the seeds of *Lupinus mutabilis* Sweet., two to three times more, in comparison with other species. Application of ultrasonic wave also provokes higher effect in this species and maximum was in variant 3 minutes -35.0% after that decrease. In *Lupinus polyphyllus* Lindl. however, with an increase in the time of treatment, the values gradually increase and reached 30.0 % in variant 9 minutes. In this variant, but in other species, a considerable reduction towards the control with 8.33% was observed. There is a statistical significance of differences between the variants. Polynomial regression for the influence of studied ultrasound treatment was established (Figures 1 and 2) The determination coefficients are high $R^2 = 0.98$ and $R^2 = 0.99$ in *Lupinus polyphyllus* Lindl. and *Lupinus mutabilis* Sweet., respectively. This indicates that around 99% of the treatment of the above-mentioned tendency will be obtained.

Data for germination provide essential information on the seed status and this identifies this indicator as one of the most important elements for the qualification of the sowing material (Copeland and McDonalds, 2001). The germination in *Lupinus mutabilis* Sweet., regardless of the higher germination energy, was much lower than in the other lupine. In both species it increases in the application of the ultrasonic treatment with 6 minutes and the highest values are 78.33 and 35.00 for *Lupinus polyphyllus* Lindl. and other lupines, respectively. The differences between separate variants are small while the variations with control are higher. For the first species (*L. polyphyllus*) they are between 45% (9 minutes) to 46.66% (6 minutes). Each sonication with ultrasound helped for higher germination except for 9 minutes in *L. mutabilis*, when a very small decrease to the control was observed. In this variant for other lupines, the decrease towards the previous variant also has been registered. The recommended duration of sonication for lupine can be pointed out which is 6 minutes. Similar results about the higher effect of 6 minutes sonication s reported also Farahani et al. (2015). The positive effect of ultrasound seeds treatment established also Chilingirov et al. (2018) and Dakova et al.

(2018). The differences between variants and control in *L. polyphyllus* are with statistical significance. The effect of ultrasound treatment on germination also described by polynomial regression (Figures 3 and 4) with a high determination coefficient R^2 = 0.94 and R^2 = 0.99. This suggests that more than 90% of the application of ultrasound the effect discussed above trend is expected to be obtained.

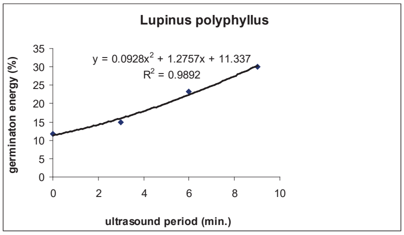

Figure 1. Regression dependence of sonication to germination energy *of L. polyphyllus* seeds

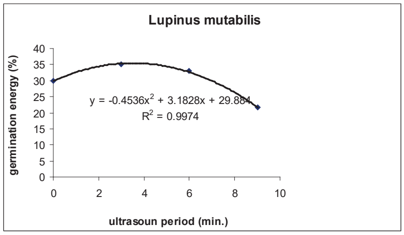

Figure 2. Regression dependence of sonication to germination energy of *L. mutabilis* seeds

Table 1. Viability characteristics of lupinus seed after treatment with ultrasound

Variants	Germination energy (%)		Germination (%)	
	Lupinus polyphyllus	*Lupinus mutabilis*	*Lupinus polyphyllus*	*Lupinus mutabilis*
Control	11.67	30.00	31.67	32.67
3 min	15.00	35.00	76.77	35.00
6 min	23.33	33.33	78.33	35.00
9 min	30.00	21.67	76.67	31.67
LSD p=5.0%	3.2	2.8	6.3	5.2

About a more complete assessment of seed potential Panayotov (2015) recommended evaluating the additional behaviors such as needed days for germination of one seed (MGT) and their uniformity. Table 2 is shown the results for these two parameters. The mean germination time in both investigated species after sonication improves, especially for variant 6 minutes with 1.17 and 0.36 days. The next increase of ultrasound wave decreased this characteristic in comparison with the previous variant, but the data are higher than the control. Better results are obtained for *L. mutabilis*.

A similar trend was observed for uniformity of germination. The highest results also were established for treatment with 6 minutes 10.78% and 14.33% for *L. polyphyllus* and *L. mutabilis*, respectively and after that, the percentage of germinated seeds per day decreased. A small decrease also indicated the seed of *L. mutabilis* in 3 minutes. Most of the differences between the variants and control are statistically significant. López-Ribera and Vicient (2017) also emphasized that the ultrasound improves the sowing parameters of the seeds.

The fresh weight of the seedlings very often use as an indicator for seed vigour index (Panayotov, 2013). The data are presented in Table 3. The higher period for sonication provokes an increase of this index and it reached in 9 minutes to 144.74 mg (*L. polyphyllus*|) and 102.18 mg (*L. mutabilis*), which is more towards the control with 56.58 mg and 34.67 mg, respectively.

The dry weights of the seedlings are characterized by small differences between species as well as investigated variants. It is in diapason 8.95% (control) to 10.92% (6 minutes) and 8.82% (9 minutes) to 9.86% (6 minutes, for first and second species, respectively. The data are mathematical proved.

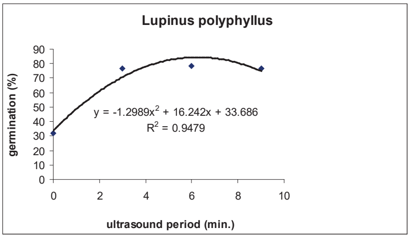

Figure 3. Regression dependence of sonication to germination of *L. polyphyllus* seeds

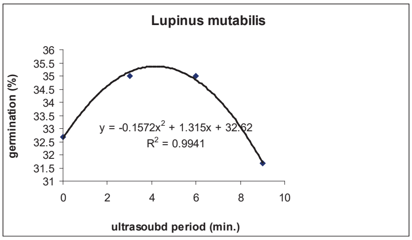

Figure 4. Regression dependence of sonication to germination of *L. mutabilis* seeds

Table 2. Sowing behaviors of lupinus seed after treatment with ultrasound

Variants	Mean germination time (day)		Uniformity of germination (%)	
	Lupinus polyphyllus	*Lupinus mutabilis*	*Lupinus polyphyllus*	*Lupinus mutabilis*
Control	6.87	3.69	5.45	12.08
3 min	8.58	3.77	10.23	10.33
6 min	8.56	4.05	9.31	14.33
9 min	7.16	5.42	10.79	9.72
LSD p=5.0%	1.4	1.9	4.0	3.6

Seedling morphology is also influenced by sonication (Table 4). Species differences are recorded about the length of the embryo root. The effect is higher in *L. polyphyllus*. In this species, the higher period of treatment provokes a decrease while the contrariwise was established for *L. mutabilis*. The highest values are measured in the first species in 3 minutes - 7.30 cm or with 86.70% above the control while for other species it is in 9 minutes - 3.46 cm that is 45.99% more than non-treated seeds.

Table 3. Behaviors of lupinus seedlings after treatment with ultrasound

Variants	Fresh weight (mg)		Dry weight (%)	
	Lupinus polyphyllus	*Lupinus mutabilis*	*Lupinus polyphyllus*	*Lupinus mutabilis*
Control	88.16	67.51	8.95	9.10
3 min	136.78	69.79	9.88	9.18
6 min	142.75	100.33	10.62	9.86
9 min	144.74	102.18	9.99	8.82
LSD p=5.0%	12.1	10.8	1.6	1.2

The lengths of hypocotyl were stimulated highly from treatment with ultrasound. The highest for *L. polyphyllus* was registered in variant 6 minutes - 3.66 cm and for other species - in 3 minutes - 1.81 cm, this is with 50% more than the control seedlings. The strong effect of sonication on the development of seedlings root also reported Machikow et al. (2013). The effect of ultrasound treatment is due to better absorption of nutrients from the endosperm, as well as increased activity of enzymes and other reactions in the seed (Miano et al., 2015).

Table 4. Morphological characteristics of lupinus seedlings after treatment with ultrasound

Variants	Length of embryo root (cm)		Length of hypocotyl (cm)	
	Lupinus polyphyllus	*Lupinus mutabilis*	*Lupinus polyphyllus*	*Lupinus mutabilis*
Control	3.91	2.37	2.34	1.29
3 min	7.30	2.59	3.66	1.81
6 min	5.57	3.42	3.95	1.44
9 min	5.91	3.46	3.48	1.53
LSD p = 5.0%	3.30	1.24	1.6	0.92

CONCLUSIONS

Ultrasound treatments highly improve the viability status and the sowing parameters of lupine seeds and the effect depends on species responses.

The germination energy and germination increase after sonication and the highest values were found in the period of treatment of 6 minutes. This duration of the ultrasound wave is recommended to apply for augmentation of sowing quality of lupine seeds. The seedling morphological behaviors are also with better characteristics.

Effect of sonication on the germination energy and germination of lupine seeds described by polynomial regression with high determination coefficients.

ACKNOWLEDGEMENTS

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018"

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

REHABILITATION OF GREEN SPACES WITH ARCHEOLOGICAL VESTIGES FROM DÂMBOVIȚA COUNTY

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Abstract

The present work involves the rehabilitation of the Metropolitan Park in Târgoviște, located between Liberty Boulevard and Mircea the Old Boulevard, identified with Cadastral Number 83026, registered in the Land Book at no. 83026, site where the "Old Metropolitan Church of Wallachia" is located, respectively the Church of the Ascension, declared a historical monument, built between 1508-1537, demolished in 1889, and rebuilt between 1890–1923. Nearby are the ruins of dated buildings from the 16th century.
To enhance and protect them it was proposed, according to the Urbanism Certificate, the consolidation and protection of the stone walls, the marking of the basements of the metropolitan palace on the east side and the rehabilitation of the green spaces related to them, the surface of which amounts to 17629.28 square meters.
The proposal for the redevelopment of the Metropolitan Park and its implementation, according to the design theme, will be done in such a way as to preserve the initial architectural-landscape style and conception, but with the improvements and rehabilitations necessary for better functioning and maintenance, and at the same time the wishes of the citizens of Târgoviște are taken into account. Trees and shrubs of decorative value at the site will be preserved, and degraded ones have been proposed for replacement. at the same time the vegetation of the park will be completed with young specimens that will create valuable landscape compositions.

Key words*: rehabilitation, landscaping, archaeological vestiges, public park.*

INTRODUCTION

This paper presents a case study in terms of environmental and landscape design, architecture and archaeology of the Metropolitan Park - Târgoviște Archaeological vestiges present in this park are part of the historical monument inscribed in the List of Historical Monuments and Archaeological Sites of Dâmbovița County, position 598, code LMI DB-II-a-A-17283, dated XVIth century.
The main goal of the present project is exploring the potential of archaeological sites in the images of landscape and habitat, and namely that ruins are ideally suited to match the vegetation, lines and colours of landscape.
The myth of vestiges associated to nature is described in Hypnerotomachia Poliphili (Stanley-Price, 1997), in the dreamlike encounter of Poliphilo, the main character, with Polia, his lover, in the midst of ancient ruins lost in the wilderness.
Rehabilitation and the new landscape design of the public park satisfies the need for peace and relaxation and supplies the inhabitants of the area with conditions for outdoor movement, contributing to the embellishment of the urban aspect of the city and the preservation of the health of the inhabitants (Burgess Harrison, 1988).
The green space will be planted with trees and shrubs species. For the remaining areas there will be used grassing.

MATERIALS AND METHODS

Methodological approach to any restoration or rehabilitation project is based on such principles as minimum intrusion, reversibility, authenticity-recognisability, which applies to all sectors and disciplines, including archaeology, architecture, landscape and arts (Hartig, 2003).
The proposal for the rehabilitation of the Metropolitan Park and its implementation, according to the design theme, will be done in such a way as to preserve the initial

architectural-landscape style and conception, but with the improvements and rehabilitations necessary for better functioning and maintenance, and at the same time the wishes of the citizens of Târgoviște are taken into account. Trees and shrubs of decorative value at the site will be preserved, and degraded ones have been proposed for replacement. at the same time the vegetation of the park will be completed with young specimens that will create valuable landscape compositions.

To help preserve nature in the city, the shrubby and arboreal vegetation has been carefully placed at the site for maintenance and possible correction work where appropriate, so as not to pose a danger to residents (Glaeser, 2010).

Rehabilitating this space, the green area is restored to its functionality and vitality, in order to restore the connection between man, nature and history (Pálsdóttir et al., 2015).

The Metropolitan Park in Târgoviste, located between Liberty Boulevard and Mircea the Old Boulevard (Figure 1), identified with Cadastral Number 83026, registered in the Land Book at no. 83026, site where the "Old Metropolitan Church of Wallachia" is located, respectively the Church of the Ascension, declared a historical monument, built between 1508-1537, demolished in 1889, and rebuilt between 1890-1923. Nearby are the ruins of dated buildings from the 16th century.

Figure 1. Area layout plan

We have included in this project (Table 1) species that are also suitable for planting in early spring. However, much better results can be obtained by planting trees in autumn between October and November, maybe even later, but not less than 15-20 days before the frost.

Table 1. List of proposed tree and shrub species and quantity

Nr. crt.	Specie name	Container type	Size (cm)	Quantity.
1	*Berberis thunbergii* 'Atropurpurea Nana'	pot	30-50	20
2	*Berberis thunbergii* 'Maria'	pot	30-50	20
3	*Betula utilis* 'Jacquemontii'	pot	250-300	15
4	*Betula utilis* 'Jacquemontii'	pot	300-350	15
5	*Buxus sempervirens*	ballot	80-100	20
6	*Buxus sempervirens* 'Angustifolia'	ballot	40-60	20
7	*Ligustrum ovalifolium* 'Aureum'	pot	30-50	30
8	*Ilex aquifolium* 'Crispa'	pot	80-100	20
9	*Spiraea japonica* 'Crispa'	pot	40-60	10
10	*Spiraea x vanhouttei*	pot	150-175	10
11	*Syringa vulgaris* 'Katherine Havemeyer'	pot	150-175	10
12	*Viburnum opulus* 'Roseum'	pot	40-60	10
13	*Juniperus horizontalis* 'Blue Forest'	pot	40-60	15
14	*Pinus mugo* 'Hesse'	pot	30-40	20
15	*Thuja occidentalis* 'Smaragd'	ballot	150-175	15
16	*Lavandula angustifolia* 'Rosea'	pot	15-20+	60
17	*Pinus ponderosa*	ballot	300-400	10
18	*Magnolia tripetala*	ballot	300-400	12
19	*Platanus orientalis*	pot	600-650	6
20	*Quercus robur* 'Fastigiate Koster'	ballot	300-350	10
21	*Tilia cordata* 'Greenspire'	pot	200-300	40
22	*Picea abies* 'Inversa'	pot	175-200	6
23	*Picea pungens* 'Hoopsii'	ballot	200-250	4
24	*Prunus cerasifera* 'Nigra'	pot	T 220	30

Mainly there will be uses ancient topiary species as boxwood, pine, thuja, oak and juniper (Paraskevopoulou et al., 2010).

Landscaping will involve deforestation of existing vegetation; land modeling; planting of trees, shrubs, flowers, etc. with complete coverage of areas and care of existing vegetation. The arrangement of green spaces refers to those with unlimited access, in this case the arrangement of the Metropolitan Park, the transformation of this public space into a space for recreation, socialization, culturalization (Derek, 2002).

RESULTS AND DISCUSSIONS

The landscape objective of the investment is to arrange and transform the land into leisure areas for the community while ensuring the improvement of environmental factors and living conditions in urban areas and beautifying the urban aspect of the municipality.

All the solutions aim to lead to the symbiosis of greenery and archaeology that offer the chance to provide a romantic, or otherwise architecttural and evocative, setting for the ruins. By greenery we mean not only the plants, but the overall arrangement and decoration contributing to turning an archaeological site into an enjoyable park.

Currently, the land is used as a park for leisure, but is in a fairly advanced state of degradation (Figure 2). The project provides for the arrangement of green areas consisting of park and garden for unlimited public access. On the site and within the limit of 10% of the surface of the green space registered in the Register of Green Spaces, constructions and arrangements will be placed consisting of: pedestrian alleys, modern, sustainable and good quality urban furniture, recreational areas and artesian wells.

To help preserve nature in the city, the shrubby and arboreal vegetation has been carefully placed at the site for maintenance and possibly correction work where appropriate, so as not to pose a danger to residents. In this space, by rehabilitating the green area, the place is restored its functionality and vitality, in order to restore the connection between man and nature. Thus, in this space by respecting the design principles, the beneficiary will not be aware of the specific forms in the plan, but will be delighted by the countless pleasant relationships produced by the designed environment (Nordh et al., 2009).

Particular interest will be given to the regeneration and rehabilitation of the extrapolated area throughout the city, which can provide the basis for highly appreciated and publicized examples. In order to confirm the need for good practices in sustainable design, the present study considered the regeneration and rehabilitation of the site in a different way, but the result is as expected.

All these choices are representative of the basics of an archaeological park, the union of nature and archaeology. Vegetation, terrain modelling and natural materials furniture can be designed purposefully for archaeological sites.

Thus, in order to highlight the ruins of the Metropolitan Park and protect them, stone masonry will be provided at the top, consolidating or marking the ruins at a higher level of the walls of the old cells, marking the cellars of the metropolitan palace on the west side, including their protection at the top with stone masonry, the restoration of the main access tower to the precinct, the restoration of the walls of the old chapel of the Metropolis, the marking of the fountain of the old Metropolis and the arrangement of access alleys (Soromenho, 1994).

Given the analysis of the existing situation, the following main interventions in vegetation are proposed:

- deforestation of the incomplete lawn area that does not meet the requirements from the landscape point of view;
- cleaning the entire surface of plant debris;
- installation of an automatic irrigation system;
- planting trees and shrubs;
- land preparation works in order to install the lawn by sowing;
- in order to create the entertainment areas, it was decided to remove three trees that had an unbalanced crown and were in a period of decline.

The development project (Figure 3) of the Metropolitan Park in Târgoviște, Dâmbovița County, aims to achieve a landscaping that supports both the street alignment and completes it with the proposed volumes of vegetation and green species related to the park, and aimed the following aspects:

- the realization of a delimitation on the street esplanade of the road and in the afferent course of this street for the realization of a microclimate inside the site;
- planting trees, shrubs and flowering species in this area will achieve a special atmosphere and decor, throughout all decorative seasons;
- making alignments of medium shrubs to mark the street esplanade.

Figure 2. The existing situation at the site level

Figure 3. Systematization of the Metropolitan Park, located in Dâmbovița County, Târgoviște

In the proposed landscaping solution (Figure 3), the natural landscape of the area was taken into account, and finally the Park must be integrated into the landscape, the final effect also being as natural as possible. The central alignment of this arrangement, we can say that it is perceived as the point of interest, because all perspectives converge towards the central area. The species used for this purpose (*Prunus cerasifera* 'Nigra', *Platanus orientalis*, *Tilia cordata* 'Greenspire'), due to their size, arrangement and canopy, ensure openness and accessibility.

It is important that the periphery of the arrangement will be less highlighted than the center, to frame and fill the sides and to avoid the scattering effect (Csemez & Jombach, 2011). The general design of the park depends primarily on the position of the compositional units (groups) but also on the position of each plant, related to each other, also on the structure of the place, rather than on the characteristics of the plant. Therefore, the design proposes the beautification and rehabilitation of the area, so that it has a natural, landscape aspect (Figure 4).

The plants used in the arrangement of this park, have different ways of growth, forming a unique mass and volume, intervening changes as the plant matures. These shapes of the species used, whether pyramidal, spherical, weeping, columnar or spread, compartmentalize and define space. Some shapes are much more dynamic than others and have been used to attract attention The groups of trees and shrubs were placed so that in addition to the decorative purpose, they serve other purposes such as sanitary, protection against the prevailing wind, masking certain areas, visual and acoustic insulation of the pedestrian surface from the road surface and not lastly for highlighting the compositional units and for making the connection between the pedestrian area and the park (Papafotiou et al., 2017).

Figure 4. Proposal for landscaping of the Metropolitan Park, located in Dâmbovița County, Târgoviște

In the production of mixed groups of deciduous trees (*Platanus orientalis*, *Betula utilis* 'Jacquemontii') and conifers (*Picea abies* 'Inversa', *Picea pungens* 'Hoopsii', *Pinus ponderosa*), a special effect was obtained by placing different specimens of deciduous trees in the planes close to the viewer and using softwoods in the planes, to create a dark background, which highlights the first (leaf species). Another special effect was obtained by combining in the same group specimens of different heights and ages, not indicating the homogeneity of ages and heights in a group. The association of ornamental shrub species and their grouping also took into account the psychic influence of the crown shape on passers-by (Irvine et al., 2009).
Thus, it was desired that the compact groups of trees and shrubs print order, solemnity and determination, those formed by species with pyramidal or conical bearing give the impression of stability and height, the groups formed by specimens with spherical, tubular or umbrella crown to inspire feelings of calm, protection and tranquility, being located in areas intended for passive rest, where many banks are present.
It was proposed to use an odd number of copies and arrange them according to different irregular geometric shapes such as triangle, quadrilateral or pentagon, positioning the highest specimen or specimens in the area of interest and the smallest to the periphery of that geometric shape (Lindal & Hartig, 2013). Thus, the visibility of the group from all directions is ensured, the decorative effect being complete.
The urban grid is even more emphasized with the use of shrubby native plants (Papafotiou et al., 2017).
For turf surfaces, a lawn roller and a 20 cm layer of topsoil were provided, which should

ensure a good development and maintenance of the lawn over time. All surfaces that will be provided with turf will have the level of the land before mounting the rollers 2 cm lower than the upper level of the curbs inside the arrangement, to avoid migration of soil outside the green space thus avoiding soiling of paved or asphalted surfaces (Gatersleben & Andrwes, 2013).

The large lawn area (6000 sqm) functions as a common denominator, connecting the compositional units, ensuring the balance of the arrangement (Girardet, 2001). Also, the groups of shrubs that provide the colour palette of the arrangement are much better highlighted, in contrast to the green background of the lawn (Figure 4).

There were designed pathways naturally created that connect its main interest spots composing an organized system. Additionally, the ornamental vegetation that was chosen alongside would add aesthetic and functional value to the site.

CONCLUSIONS

In the proposed solution, the symbiosis of plants, landscape and archaeology offers the chance to provide a romantic atmosthere, a symbiosis of ruins and greenery, with an architectural approach respectful of the principles of conservation, authenticity, reversibility. The solution considers the regeneration and rehabilitation of the site in order to provide recreational, educational and aesthetic qualities in modern growing cities.

The plant species (boxwood, pine, thuja, and juniper) were chosen based on historical information on their use in ancient times, designating the local character of the site, but also can provide elegant formal structure in harmony with green spaces with archeological vestiges.

Making use of an odd number of copies and arrange them according to different irregular geometric shapes such as triangle, quadrilateral or pentagon and positioning the highest specimen or specimens in the area of interest and the smallest to the periphery of that geometric shape ensure the visibility of the group from all directions is ensured, the decorative effect being complete.

The arrangement of green area and nature-based rehabilitation of the Metropolitan Park, transform this public space into a space for recreation, socialization and culturalization.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

METHODS OF REHABILITATION OF A DEGRADED AREA IN ORĂŞTIE CITY

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Abstract

In any city we find spaces that, either because of unsustainable exploitation or because of the wrong approach, fail to reach their full potential. As a result, there is a need to pay particular attention to these types of sites for their introduction into the landscape circuit. Thus, the paper intends to come up with a solution that transforms a space without identity of 35,125 m^2, with a great need for completion, innovation, within a reasonable limit, in a space-volumetric environment that is more accessible to both the inhabitants of Orăștie city, as well as for the municipal administration. The architecture of the present arrangement proposes a different architectural concept, in which the landscape elements that come into its composition define the mixed style. The scenario, is proposed to be constructed from regular shapes that combine with free forms and creates a harmonious, balanced rhythm and thus strengthens the feeling of spatiality. The interior path of the proposed green spaces within the layout, offers a gradual transition, between the different functional areas.
By designing the rehabilitation solution within this project, we aspired to increase the value of the subconscious and the consciousness of the inhabitants of Orăștie city, adopting a timely energetic attitude to heal a land of no value.

Key words: *urban environment, landscaping, rehabilitation.*

INTRODUCTION

Sustainable development movement underline the role of green area and in order to achieve this goal, green space is considered the central character for improving urban sustainable development. Green elements connect the spaces and create green networks. Those networks can be planned and managed for multidimensional purposes such as ecological, recreational, cultural, recognizable beauty, body and mental health and many other reasons, together crating the concept of sustainable city (Karade, R.M., Kuchi, V.S., Kabir, J., 2017).
Our research draws the attention to the role of green space for sustainable landscape development. The design of the arrangement proposes a different architectural concept, the landscape elements that are part of it defining the mixed style. The scenario, 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.
The vegetation proposed in this arrangement is a fundamental element of the natural environment and is the main component of green spaces in Romania, being composed of rustic species with good resistance to weather conditions at the site. Through it, the objective of improving the environment and harmonizing the modified or landscaped landscapes with the natural ones can be achieved, so as to create environmental conditions appropriate to the development of social activities (Ward Thompson, C., 2011).

MATERIALS AND METHODS

The rehabilitation project of the site considered by this paper has two components, which are proposed to be carried out in Orăștie Municipality, on Târgului Street, in locations located as follows:

Objective 1: Landscaping component (park and garden). The land is in the public domain of Orăștie Municipality according to the Government Decision no. 1352/2001. The land proposed for rehabilitation has an area of 35,125 m2, tabulated according to C.F. no. 65603 U.A.T. Drastic.

Objective 2: Modernization of Târgului Street component. The land is in the public domain of Orăștie Municipality according to the Government decision no. 1352/2001, land that has a surface area of 9,629 m2, tabulated according to C.F. no. 65610 U.A.T. Orăștie.

Based on the regulations of the urban planning documentation no. 11/2009, PUG phase, approved by HCL Orăștie no. 18/2014, the location is in M2 - Proposed mixed subzone (LFCm2 + ISP2), LFCm2 - Subzone of low-rise housing and complementary functions proposed on flat land (P-P + 2), ISP2 - Subzone institutions and services proposed public interest, max. Gf + 4 (sports, administrative, financial-banking, culture, trade, tourism, education, cults, health, social assistance). Category of use: unproductive - plot with cadastral number 65603; road - parcel with cadastral number 65610.

Currently, the land is unused being degraded and abandoned. The project provides for the arrangement of a green area consisting of a park and a garden for unlimited public access. Within the arrangement of the green space, pedestrian alleys will be built made of self-locking concrete 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 alleys is variable between 2.00 and 5.00 m, they also serve for the occasional access of the means of intervention. On the green space, within the limit of 10% of its surface, placed constructions and endowments will be, subjects of this proposal.

From a climatic point of view, the location is in the area with a temperate-continental climate with strong Baltic influences, which gives a rich rainfall regime both in winter and summer and temperatures 1-2^0 lower compared to other regions of the Transylvanian depression. From the multiannual meteorological observations, it is found that from a thermal point of view, the analyzed area is characterized by average annual temperatures of 8-9ºC. 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).

According to normative P100/1-2013, the location falls into the following characteristics:

- horizontal acceleration of the terrain - ag = 0.10 g;
- corner period - Tc = 0.7 sec.

The region is classified in the 6th degree of seismic zoning according to the Msk scale.

The surface of the green spaces that are proposed for development is estimated to be around 31,633.73 m^2. Their arrangement is suggested to be done by carrying out the following categories of works:

- Landscaping (deforestation of existing vegetation; land modeling; planting perennials / lawning of surfaces, including planting of trees and shrubs). The arrangement of green spaces refers to those with unlimited access, in this case park, square and garden.
- Creation of pedestrian alleys, creation of facilities for recreation on the landscaped grounds (sports area, skate park area, outdoor events stage, playgrounds for children and fitness area for adults).
- Equipped with urban furniture (benches, trash cans, ecological toilets, urban billboards, drinking fountains).

- Installation of Wi-Fi in public spaces, installation of video surveillance systems of the spaces arranged by the project.

- Construction of an irrigation system and an outdoor lighting system for the spaces.
- Construction of a new road structure on Târgului Street, with asphalt pavement.
- Extension of the water supply and sewerage network to the site area.
- The water supply of the irrigation system for these green spaces (Figure 1) is proposed by this project to be achieved through several options:
- Water supply from the drainage groundwater storage tank. For this solution, considering the high level of groundwater, it is recommended to design a drainage system, according to the execution details,

which will store the infiltration water in an underground tank.
- Water supply directly from the public network in case there is no water accumulated in the storage basin and in case the necessary connection pressure is ensured.

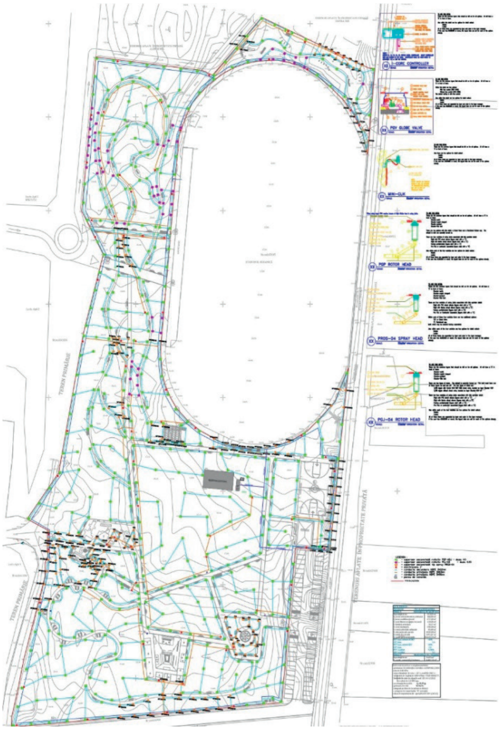

Figure 1. Irrigation system plan

The power supply of the site is proposed to be made from the National Energy System located in the area of the site through a three-phase electrical connection.

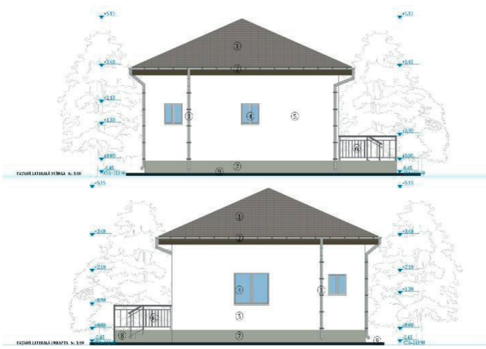

Figure 2. Administrative annex plan

The supply of electricity to the consumers of the objective (of the multifunctional field, of the event scene, of the administrative annex, of the video surveillance equipment, of the installations related to the wireless internet networks and of the automatic irrigation system) is proposed to be made from the general electrical panel (hereinafter referred to as TEG) located on the ground floor in the outbuilding in the porter's lodge (Figure 2).

For the lighting of the site, the project proposes a lighting system for pedestrian traffic that uses “green energy” and that has the role of ensuring a sufficient level of lighting for pedestrian traffic (Figure 3).

Figure 3. Electrical networks coordination plan

We suggest the lighting of pedestrian traffic to be done using LED lighting fixtures, installed on metal poles with a height of 3.50 m equipped with photovoltaic kits with autonomous operation. The designed photovoltaic lighting system consists of 100 lighting poles placed in the green space, according to the plan of electrical installations, in insulated foundations made of simple concrete in which the fixing bolts of the pole are embedded. The arrangement of the pillars will be done equidistantly at an interval of approximately 10-15 m.

RESULTS AND DISCUSSIONS

The design of the arrangement puts forward a different architectural concept, the landscape elements that are part of it defining the mixed style. The scenario, built of regular shapes that combine with free shapes, creates a harmonious, balanced rhythm and strengthens the feeling of space (Brookes, 2009). The interior path of the proposed green spaces provides a gradual transition between the different functional areas, as can be seen in Figure 4.

Figure 4. Systematization of public green spaces with unlimited access in the degraded urban area "Stadion Mecanica" in Orăștie municipality, Hunedoara county

Landscaping will follow the application of the principles of harmony, unity in diversity and aesthetics, so that the final composition will ensure a pleasant, pleasant, sustainable architectural silhouette (McPherson, E.G. and Peper, P.J. (2012).
As a result, the arrangement of green spaces was done in the first phase by dividing the site into 7 areas. Thus, it is proposed in Zone 1 (Figure 5) to arrange a space for the promenade with pets, given that they need to be properly trained in an appropriate sector, regular outings to reduce boredom and deplete energy and for this reason the area in Zone 1 was created to give dogs different activities, along with other dogs, for example: throwing a ball, chasing a frisbee or a relaxing walk together with the master (Figure 6), aspects that contribute significantly to maintaining the health of both parties.

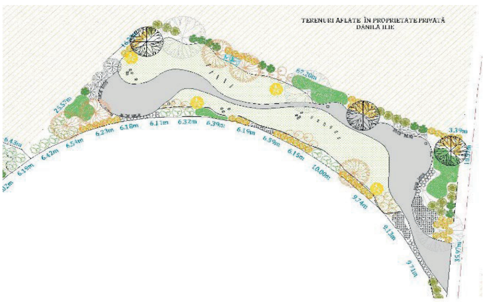

Figure 5. Zone 1 - Dog park

In each of these alveoli presented in Figure 6, it was wanted to satisfy the needs of the owners of quadrupeds in the city, who until now did not have a specially arranged place where they could walk their pet in peace. As can be seen, this space is a place where they can train and play without a leash in an area under the supervision of their owners.
This space for quadrupeds was introduced in the landscape of the site not only to facilitate traffic in the park but also as a way to concentrate the different areas of interest according to the selection criteria of the inhabitants of this city.
In an age where people are often reluctant to each other or are afraid to socialize, a leash-free and exercise area for quadrupeds also contributes to people's openness, creating a greater sense of community.

Figure 6. Systematization of the playground for pets

The most obvious reason dogs need an open public space is because of their popularity. For this reason, access to a park close to home or even at a reasonable distance is the safest and most efficient way to ensure a minimum of comfort for pet owners. Also, for the comfort of pet owners, siting places (Figure 7), ramps, jumping hoops and tires were introduced in the arrangement.

Figure 7. Main area

In organizing the space, all necessary precautions were taken into consideration, taking into account certain rules, namely, to avoid the appearance of potholes in the lawn but also to create a special chromatic effect, a space of over 1000 sqm of lawn with net inside the canine area, for the moments when the

animals are left without a leash, considering first of all the protection of the lawn.
The protective net of the lawn prevents the formation of ditches when passing frequently through that space and ensures an excellent anchoring of the roots, improving the resistance of the grass and avoiding the situation in which the lawn would become muddy. In addition to all these advantages, the lawn protection net is a product made of high-density polyethylene (HDPE) with rhomboidal mesh, for strengthening the lawn, offers a natural look and can be mounted immediately after planting the lawn rollers, being easy to mounted on their surface.
Studies have shown that people are able to talk to each other more easily by focusing on the common center of interest, which is why in Zone 2 of this arrangement, shown in Figure 8, was created as a forest-park space, thus demolishing all barriers. constraint on socialization in which people regularly perceive themselves as strangers (Irvine, KN, Warber, SL, Devine-Wright, P. and Gaston, KJ, 2013).

Figure 8. Zone 2 - Park forest

The functional urban furniture and the beautifully arranged green spaces that can be seen in Figure 9, will create a pleasant and civilized environment in the city. In this space, the points of interest will be represented by the diversity of plants and woody vegetation.

Figure 9. Location of resting places in zone 2

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 (Hanus-Fajerska, E., Karczewska, I. and Ciarkowska, K., 2010).
The vegetation proposed in this arrangement is a fundamental element of the natural environment and is the main component of green spaces in Romania, being composed of rustic species with good resistance to weather conditions at the site. Through it, the objective of improving the environment and harmonizing the modified landscapes with the natural ones can be achieved, so as to create environmental conditions corresponding to the development of social activities.

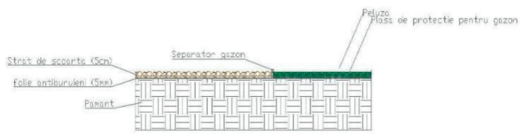

Figure 10. Section through the lawn with mesh

Taking into account the need of the population to get rid of daily stress, in the newly arranged space, it was necessary to take measures to amplify the atmosphere created by the forest (Jorgensen, A., Hitchmough, J. and Calvert, T., 2002) and because of these considerations in this area built alleys were not introduced, but only rustic lawn trails on which a protective net was mounted, which would create a special circuit at the site and also prevent the easy formation of mud in wet seasons. In order to avoid the monotony and to respect the mixed style of the arrangement, alveoli were created on the edge of the alleys where the benches were placed. In the alveoli, the use of built elements has also been avoided and, therefore, for a better sustainability of the arrangement and to facilitate the maintenance process, the surfaces under the benches we propose to be covered with protective net for lawn that provides a nice look to the site.

Man is closely connected with nature and will always feel the need to return to its midst. For this reason, serious measures have been taken to preserve the existing green areas at the site and to capitalize on them as constructively as possible (Honold, J., Lakes, T., Beyer, R. and van der Meer, E., 2015).

To help preserve nature within the city, the shrubby and arboreal vegetation has been carefully placed at the site (Figure 11) for maintenance and possible correction work where appropriate, so as not to represent a danger to the inhabitants.

Figure 11. Systematization of vegetation in zone 3

Taking into account the sustainability of plant compositions, in the area with ornamental shrubs it was decided to cover the soil with an anti-weed foil that allows water to enter the soil, but does not allow weeds to spoil the appearance of plant compositions, over which it is suggested to place a layer of at least 5 cm of tree bark (Figure 12).

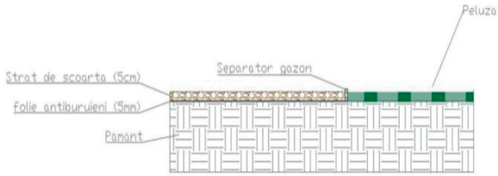

Figure 12. Section through the bark area

As a result, it is considered that green spaces represent that functional category within the localities or areas connected to them, whose specificity is determined, first of all by vegetation and secondly by the built environment, including endowments and equipment intended for cultural and educational activities, sports, recreation of the population (Oguz, D. and Çakcı, I., 2010).

This concept of urban planning combines two aspects of maximum relevance, namely, an aspect related to the reduction of extreme climatic manifestations that lately manifests itself as a clear reaction to the ongoing abuses on forests and the environment in general and an aspect related to the dimension of the human psyche through which it is desired to determine those who live in this environment to be aware of the importance of returning to nature, by bringing to the fore a landscape close to the human soul (Morales, D., 1980).

Utilizing this type of land contributes to the sanitary function precisely through the ability of vegetation to retain, fix and sediment particles in the atmosphere, in suspension, smoke or fine dust. For these reasons, a buffer zone has been created around the children's area, which aims to protect this space but also to create the much sought-after shade from the hot season (Szarek-Łukaszewska, G. and Grodzińska, K., 2007).

An important note that was taken into account when the systematization of the plants at the site level was also related to the physical characteristics of the land in sight, namely:

location, size, surface and shape of the land, topography, parceling, 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 space for children determines the exponential increase in the value of the site. Also, a sense of unity is created throughout the arrangement by introducing the species of *Betula pendula* and the areas where the *Ajuga* specimens were placed.
Because the appearance of order and cleanliness has been taken into account, numerous dining areas have been placed at the site, as can be seen in Figure 13, and in order to avoid crowding in the hot season, shaded areas have been introduced. in the landscape numerous trees to provide shade in the summer season.

Figure 13. Recreation zones in the park forest area

Given the inappropriate initial operation of the site, due to a wrong approach that failed to reach the maximum potential of the space, this project proposes a different approach to cover all the needs of the inhabitants of this city as can be seen in Figure 14.
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 (Mansfield, C., Pattanayak, SK, McDow, W., McDonald, R. and Halpin, P., 2005).

Figure 14. Detail from area 6

Thus, in this space, by respecting the design principles, the beneficiary will not be aware of the specific forms in the plan, but will be delighted by the countless pleasant relationships produced by the designed environment.
By proposing this concept of development within the site, it was considered to send an ecological message in the context of climate change, but also saw the possibility of bringing to the fore a good opportunity to get closer to nature and socialize the inhabitants of this city.
In this space, by rehabilitating the green areas, the place is given functionality and vitality, in order to restore the connection between man and nature (Figure 15).
The vegetal compositions that make the transition between the parking space and the arranged area create volume in a special way and at the same time lead the eyes to the area with water which is highlighted by creating a brightly colored background behind it.
Since the artesian fountain does not have a central position, it is proposed to create a large plant composition (Figure 16), consisting of both deciduous and coniferous species, a

composition created in an organic form that "dissolves" even more the geometric center of rounding while providing a background to the proposed plant compositions to the edges of the round.

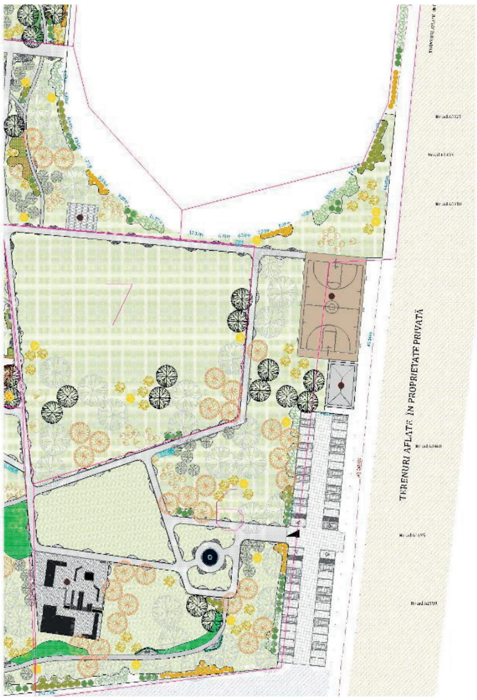

Figure 15. Detail from area 6 and 7

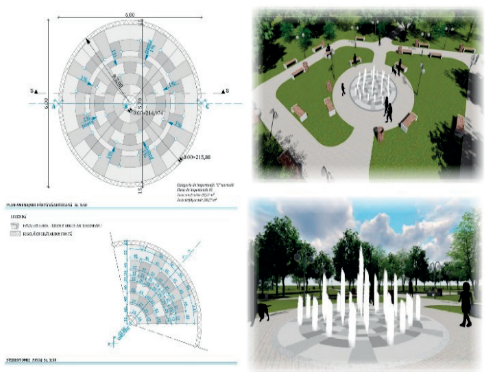

Figure 16. Artisan fountain arrangement plan

In order to achieve the sustainability of this arrangement, plant species were chosen due to their resistance to pollution and poor quality of the substrate, but also for their decorative properties (color and texture of foliage, flowers, fruits, vines, rhytidome, etc.). 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 colors in terms of aesthetic orientation, can interrupt the daily delimitations of the rehabilitated landscape.

CONCLUSIONS

Considering the importance of green spaces, especially in urban areas, the concept of Urban Orchard is meant to create a space, not only with an aesthetic role, but also with an important sanogenic, economic and social role. This desire can be achieved by introducing an oasis of relaxation, as close as possible to nature for site visitors.

The advantage of using this type of arrangement of fruit species, initially induces visitors the feeling of modernism, but in reality, the landscape created is a rustic, natural and local, being easy to maintain.

The site has been designed so that each space has a well-defined role, both as an orchard, but also as a space for relaxation, trying to please all types of visitors.

The planting of trees and shrubs species will take into account the optimal conditions for these works to ensure the highest possible planting success.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MORPHOMETRIC AND MOLECULAR CHARACTERIZATION OF *PARATYLENCHUS NANUS* COBB, 1923 (TYLENCHIDA: PARATYLENCHIDAE) ASSOCIATED WITH SOIL FROM *GLADIOLUS* PLANTS

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Abstract

Nematodes belonging to the genus Paratylenchus are widespread throughout the world and are associated with many plant species. Morphological identification of Paratylenchus species is a difficult task because it relies on many characters with a wide range of intraspecific variation. In this study we provided morphological and molecular characterisation of the species Paratylenchus nanus. In 2019, 5 soil samples were taken from Argeș county from different flowering plants to detect and identify phytoparasitic nematodes. Soil samples were taken from the following species of flowering plants: Lilium, Chrysanthemum, Gladiolus and Lisianthus. Based on laboratory tests, the nematode Paratylenchus nanus was detected in a soil of Gladiolus plants. Species were identified by morphobiometric analysis and molecular biology. To confirm the species, sequencing and phylogeny analyses were performed. Thus, following this study, we report for the first time, in Romania, the presence of the nematode Paratylenchus nanus, on another host plant, than the one previously reported.

Key words: *DNA sequencing, identification, morphometric, Romania, phylogeny, new host.*

INTRODUCTION

The pin nematode in the *Paratylenchus* genus Micoletzky, 1922 (Nematoda: Tylenchulidae) was first described based on a single female, belonging to species *P. bukowinensis* Micoletzky, 1922 coming from a flooded and sandy soil on the riverbank of Prut, once part of Romania (Ghaderi et al., 2014). The nematodes of *Paratylenchus* genus Micoletzky, 1922 feed on many species of plants and are spread throughout the world (Van den Berg et. al., 2014). As host plants, they have varied horticultural and agricultural crops, ornamental and forest plants (Raski, 1991). *Paratylenchus* genus contains over 120 species (Siddiqi, 2000). Depending on the stylet size, the pin nematode species are classified into two genera, i.e., *Gracilacus* and *Paratylenchus* (Raski, 1962). The short-stylet species are epidermal feeders whereas the long-stylet ones will go deeper into the root cortex, as they are ectoparasit-feeding nematodes (Siddiqi, 2000). *P. hamatus* is a parasite for wheat, pea (Riga et al., 2008) and celery (Lawnsbery et al., 1952); P. *neoamblycephalus* acts as a parasite for the plum saplings (Braun and Lawnsbery, 1975) and apricot saplings (Fisher, 1967); *P. projectus* is a parasite for the tobacco (Coursen and Jenkins, 1958), while *P. nanus* for the rye (Bell, 1999). *Paratylenchus nanus* has been so far reported in Europe, Australia, North America, Asia, Africa, and Antarctica (Upadhaya et al., 2019). *P. nanus* is associated with the following crops - grasses, fruit-bearing trees, vegetables, and grains (Upadhaya et al., 2019). In Romania, species *P. curvitatus* van der Linde 1938, was reported by Popovici in 1974, as having been identified on an arable land in the vicinity of Cluj-Napoca. *P. microdorus* Andrassy, 1959 was collected by Popovici and Ciobanu in 1977 and 1978 from grasslands around the Southern and Eastern Carpathians. *P. neoamblycephalus* Geraert, 1965 was reported by Popovici in 1993, as found in the grasslands with spruce-fir forests, located in Retezat Mountains and *P. (Gracilacus) aciculus* Brown, 1959 was collected by Popovici in 1974 from grasslands and arable soil nearby the city of Cluj-Napoca and, more recently, in 1998, from meadow areas in Retezat Mountains, Cernei Mountains and Mehedinti Mountains. Ciobanu et. al, 2003, gives a short desciption of the *Paratylenchus* species present in Romania, namely *P. nanus* Cobb, 1923, *P. microdorus* Andrassy, 1959, *P. projectus* Jenkis, 1956 and *P. (Gracilacus) straeleni*. Pin nematodes are hard to be identified, due to their small size and variable features (Raski, 1962). Nevertheless, in order to be able to distinguish between the species of the pin nematode, we can use the following characteristics for the morphological identification - length of stylet, number of lateral lines, presence or absence of the vulval flap (Ghaderi et al., 2014). For a more accurate taxonomy within the species, of the nematodes in *Paratylenchus* genus, there are currently used molecular biology techniques that include contrastive analyses of the ribosomal RNA gene sequences (rARN) and phylogenetic analysis based on rARN sequences. Subotin et al., 2005, Chen et al., 2008, 2009 and van Megen et al., 2009, have provided a molecular description of the species contained in this genus, by using the D2-D3 expansion genes of the gene sequences 28S rARN, ITS rARN and gene sequences 18S rARN.

The main objectives in this study are represented by the morphological identification and the molecular charaterisation of the species *Paratylenchus nanus*, collected from Romania by making use of the D2-D3 expansion sequences of the gene sequences 28S rARN and ITS rARN. Phylogenetic trees have been built, based on both fragments of the genes and relations examined within the *Paratylenchus* species.

MATERIALS AND METHODS

In the summer of 2019, 5 soil samples were collected from a private person in Pitesti locality, Arges county, Romania and from diverse flowering plants, such as *Lilium, Chrysanthemum, Gladiolus* and *Lisianthus*. Besides the *Gladiolus* crop, which was cultivated in the open field on an acreage of 600 sqm, the other flower species were planted in protected spaces (greenhouses). Out of the 5 samples analyzed, only one was positive and phytoparasitic nematodes were detected, from *Paratylenchus* genus. The geographic coordinates (GPS) of the sampling site are 44°52'28.0"N 24°52'51.6"E (Figure 1).

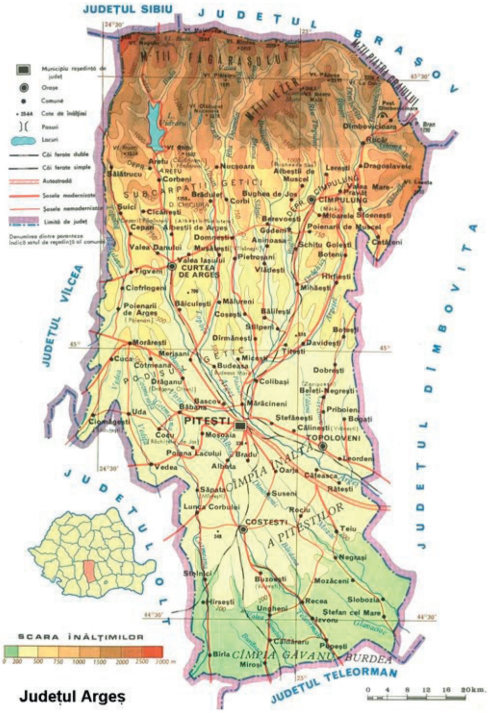

Figure 1. Geographic location of Pitesti locality, Arges county, Romania

The extraction of the nematodes from the soil was conducted by using Cobb method, 1918, with around 200 ml of soil was used for each extraction. The nematodes derived from the soil extraction procedure were collected and preserved in formaldehyde 4% for at least 10 days, in order to achieve the permanent slides (Yoder M. et al., 2006). The species *Paratylenchus nanus* was noticed and identified in a soil sample collected at the level of the radicular system, with host plants as flowering - plants in the *Gladiolus* spp. genus, mentioned above. The measurements of nematodes were done with a microscope Zeiss Axio Image 2 equipped with a digital camera Zeiss Axiocam 506 and an incorporated soft Zen 2.6 (Blue edition).

MOLECULAR ANALYSES

DNA was extracted for molecular analysis. Individual nematodes were transferred in 8 µL worm-lysis buffer (50 mM KCl, 10 mM Tris-Cl pH 8.3, 1.5 mM $MgCl_2$, 1 mM DTT, 0.45 % Tween-20) and 1 µL of proteinase K (1.2 mg ml^{-1}). The mixture was incubated for 1 h at 65°C and 10 min at 95°C followed by a centrifugation step for 1 min at 16000 g. For the amplifying of the rDNA-ITS region we used the primers TW81b (5'-GTAGGTGAACCTGCAGCTG-3', adapted TW81 primer on ILVO, not published) and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3', Joyce et al. 1994). A 50 µL PCR reaction volume contained 22.4 µL distilled water, 25 µL MyFi Mix (Bioline, Germany), 0.3 µL of each forward and reverse primer (50 µM) and 2 µL DNA. For the amplification of a part of the 28S rRNA gene the primers D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3', De Ley et al. 1999) and D3B (5'-TCGGAAGGAACCAGCTACTA-3', De Ley et al. 1999) were used. A 50 µL PCR reaction volume contained 21.4 µL distilled water, 2 µL $MgCl_2$, 25 µL 2X BIO-X-ACT (Bioline, Germany), 0.3 µL of each forward and reverse primer (50 µM) and 1 µL DNA. The thermal cycling profile was as follows: initial denaturation step at 96°C for 3 min; 35 cycles of 96°C for 30 s, X°C for 30 s, 72°C for 1 min with X being the annealing temperature of 49°C for the rDNA-ITS region and 55°C for the 28S rRNA gene; final extension step at 72°C for 10 min. The PCR products were visualized after electrophoresis (100 V, 30 min) on agarose gels (1.5 %) with Midori Green Advance stain (Nippon Genetics) using a UV_transilumminator. Purification was done following the protocol accompanying the Wizard SV Gel and PCR Clean – Up System purification kit (Promega, Belgium). The amplified fragments were sent for sequencing to GeneWiz (Germany). All fragments were sequenced in forward and reverse directions. The overlapping parts of the forward and reverse sequences of three *Paratylenchus* nematodes were removed using FinchTv version 1.4 and the contigs were compared with all DNA-sequences available in GenBank using the BLASTn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

PHYLOGENETIC ANALYSES

Sequenced fragments were edited and assembled using DNA Dynamo software and deposited into the GenBank database. Subsequently the obtained sequences were aligned with the sequences of the D2-D3 expansion segments of the 28S rDNA or sequences of ITS rDNA, respectively, of other *Paratylenchus* species published in the GenBank using the nucleotide BLAST program in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).
Multiple alignments of sequences were done using MUSCLE integrated in MEGA software (Kumar et al., 2008). The evolutionary history was inferred by using the Maximum Likelihood method and the robustness of the ML trees was inferred using 1000 bootstrap replicates (Felsenstein J., 1985). The initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model (Tamura ,1992), for D2-D3 expansion segments of the 28S rRNA sequences, and Kimura 2-parameter model (Kimura, 1980) for ITS rRNA sequences respectively, and then selecting the topology with superior log likelihood value, a discrete Gamma distribution being used to model evolutionary rate differences among sites. Evolutionary analyses were conducted in MEGA X (Kumar , 2018).

RESULTS AND DISCUSSIONS

***Paratylenchus nanus* Cobb, 1923**
(Figure 2)

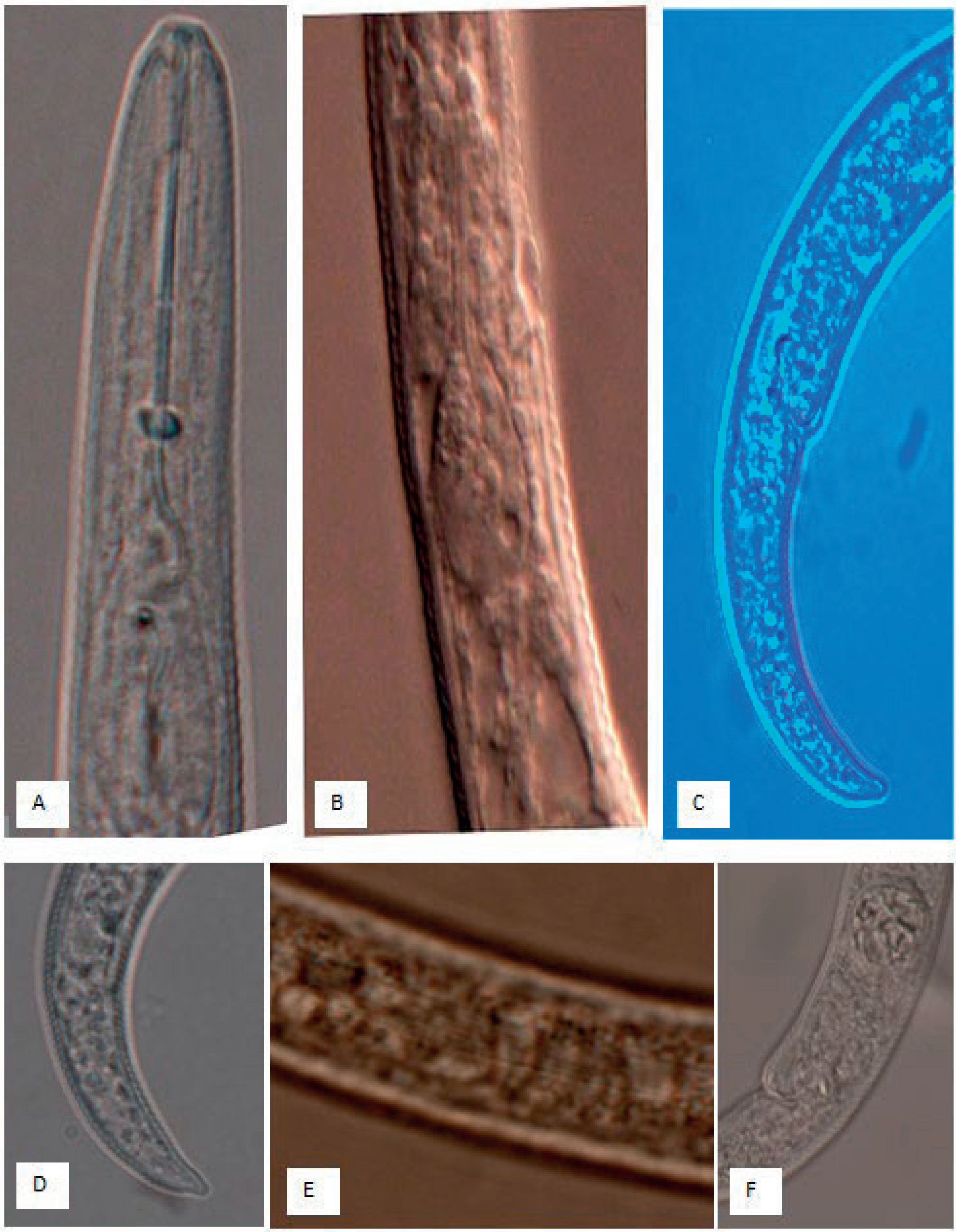

Figure 2. *Paratylenchus nanus*, Romanian population, (A-F) Female. A: Anterior region; B: Excretory por; CD: Posterior region; E: Lateral field at mid-body; F: Vulvar area.

MEASUREMENTS (See Table 1)

Table 1. Morphometric data for *Paratylenchus nanus* Cobb, 1923, female, from Romania

Species:	*P. nanus*	
Site location: Characters / ratios	After Brzeski, 1998	Pitești - Argeș Plant host: *Gladiolus* spp.
n		16 ♀
L (µm)	340 (270-460)	370 ± 19.56 (325-397)
a	22 (17-28)	20 ± 1.49 (17-23)
b	3.8 (3.2-4.4)	4 ± 0.18 (3.6-4.3)
c	13.8 (9.6-18.9)	14.3 ± 1 (13-17)
c'	2.6 (1.9-4.4)	2.4 ± 0.18 (2–2.7)
V%	83 (79-86)	83 ± 1.01 (82 - 85)
Stilet length	28.5 (23-34)	27 ± 1.41 (25 – 30)
Pharynx	91 (83-104)	92.69 ± 3.77 (86 – 101)
Anterior end to excretory pore	77 (65-103)	76.8 ± 3.13 (72 -82)
Head – vulva	-	308 ± 15.57 (276-334)
Tail length	26 (18-36)	26 ± 2.42 (22-31)
Max. body diam.	-	18 ± 0.83 (16.5-19)
Anal body diam.	-	10.7 ± 0.70 (10-12)

Note: All measurement are in µm and in the form of mean ± s.d. (range).

DESCRIPTION

Female

When relaxed, the female and the juveniles are arcuate ventrally and their cuticle has distinct annulations.

The lateral lines are four in number, taking up approx. 1/5 of the body width. The head is round, in a continuous shape, with the lip or labial plate not protruding.

The stylet has an average length of 27 µm (25-30µm), with the stylet cone holding 63-70% of its length, knobs with the width of 4-5 µm, while DEGO is posteriorly located compared to the knobs, with the length ranging from 4.5 to 5 µm.

Spermatecha is full, round in shape, with the advulval flaps distinct, in a round form. In comparison to the head, the excretory pore is located about 76.8 µm length, with a average between 72 and 82 µm (Table 1).

The median bulb is unexpectedly well developed, occupying half of the oesophagus length. Vulva has a massive transversal slit, with strong lateral membranes.

The tail is variable, in most cases slightly indented on the dorsal side close to the top, annulated towards the tail end, with a rounded terminus (Figure 2).

Male not found.

Note: In 1965, Geraert made a comment concerning the species in the group of *P. curvitatus,* more exactly that they are difficult to be distinguished from one another. Among the species in this group, we can enumerate *P. nanus, P. neoamblycephalus, P. dianthus, P. hamatus, P. amblycephalus, P. nainianus, P. projectus.* In 1965, Fisher considered the species *P. nanus* synonymous with *P. projectus.* Five years earlier, Tarjan was upholding the idea that species *P.nanus* and *P. neoamblycephalus* are similar, whereas species *P. projectus* is close to species *P. nanus* and *P. curvitatus.* Other researchers did not accept these synonyms later.

MOLECULAR PROFILES AND PHYLOGENETIC STATUS

The species was molecularly characterized using partial gene D2-D3 of 28S and ITS field of rDNA gene and deposited in the GenBank. The genus contains over 120 species but only a dozen species have been molecularly characterized. *Paratylenchus nanus* was reconstructed and is shown in (Figures 3-4). Ghaderi et al., 2014 has used the most stable characters to identify the species in *Paratylenchus* genus, namely the length of stylet, number of lateral lines and the presence or absence of the advulval flaps. Based on these three characters, the species of nematodes in *Paratylenchus* genus were classified into 11 groups.

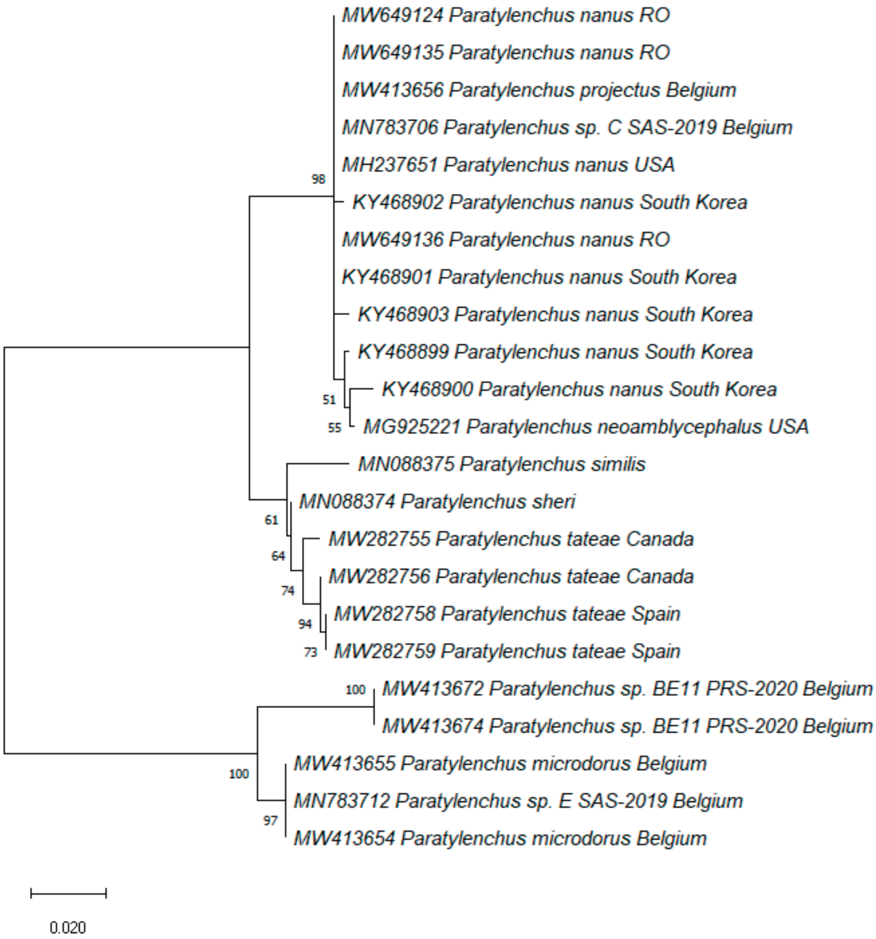

Figure 3. Phylogenetic relationships of *Paratylenchus nanus* isolate collected from Romania and isolates from other geographical regions based on the sequence alignment of the D2-D3 expansion segments of the 28S rDNA. The phylogenetic tree was inferred by using Maximum Likelihood method and Tamura 3-parameter model with 1000 bootstrap replication. The tree with the highest log likelihood (-1628.43) is shown. Bootstrap values are indicated at the nodes. The analysis involved 23 nucleotide sequences and there was a total of 686 positions in the final dataset. The scale bar indicates 0.020 nucleotide substitutions per nucleotide position

The obtained sequences of the ITS rDNA and the D2 - D3 of the 28S rDNA regions of each of the six nucleotides were 709 - 714 - 727 bp and 735 - 774 - 787 bp long, respectively.

Based on the 28S gene (Figure 3), the three nucleotide sequences in Romania, two are grouped *P. nanus* (MW649124; MW649135), and the third (MW649136) is at a distance of four nucleotides from the first because it is shorter but it is in the same clade.

P. nanus (MW649124; MW649135) are nearly identical with *P. projectus* (MW413656) and *Paratylenchus* sp. (MN783706) from Belgium, *P. nanus* (MH237651) from USA and *P. nanus* (KY468902) from South Korea.

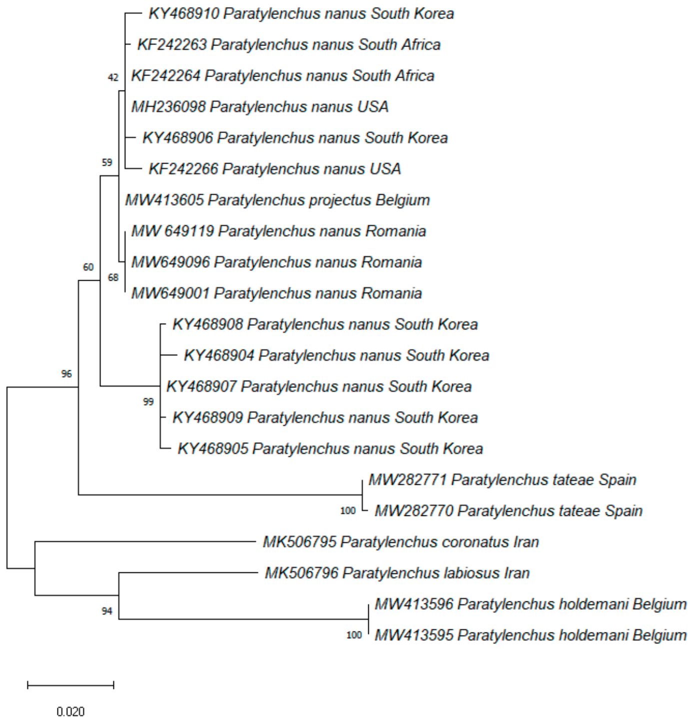

Figure 4. Phylogenetic relationships of *Paratylenchus nanus* isolate collected from Romania and isolates from other geographical regions based on the sequence alignment of the ITS rDNA. The phylogenetic tree was inferred by using Maximum Likelihood method and Kimura 2-parameter model with 1000 bootstrap replication. The tree with the highest log likelihood (-2096.95) is shown. Bootstrap values are indicated at the nodes. The analysis involved 21 nucleotide sequences and there was a total of 749 positions in the final dataset. The scale bar indicates 0.020 nucleotide substitutions per nucleotide position

The other nucleotide sequence in Romania *P. nanus* (MW649136) is almost identical to *P. nanus* in South Korea (KY468902; KY468901; KY468903) and *P. nanus* (MH237651) from USA, *Paratylenchus* sp., (MN783706) *P. projectus* (MW413656) from Belgium and *P. nanus* (MW649124; MW649135) from Romania. The analysis involved 23 nucleotide sequences and there was a total of 686 positions.

Based on the ITS gene (Figure 4), the three sequences belonging to the *P. nanus* species from Romania (MW649119; MW649096; MW649001) are clearly differentiated from those of other species.

The three sequences belonging to the species *P. nanus* from Romania are very close to the species *P. projectus* (MW413605) from Belgium.

They are sisters to *P. nanus* (KF242266; MH236098) from the USA, *P. nanus* (KY468906; KY468910) from South Korea and *P. nanus* (KF242263; KF242264) from South Africa.

Paratylenchus nanus from Romania is sister to the five species of *P. nanus* (KY468908; KY468904; KY468907; KY468909; KY468905) from South Korea and *P. tateae* (MW282771; MW282770) from Spain.

The analysis involved 21 nucleotide sequences and there was a total of 749 positions.

All species that are part of the phylogenetic tree for both 28S and ITS genes are part of G3 according to Ghaderi et al., 2014.

CONCLUSIONS

The species is present in Romania and it was described and morphobiometrically identified by Ciobanu et al., 2003, in Fagaras Mountains, at an altitude of circa 740-900 m, in mixed deciduous forests (Carpino-Fagetum).

Besides the description and the morphobiometrical identification, a molecular characterization has been completed.
The species *Paratylenchus nanus* was found on a new host plant (*Gladiolus* sp.), in a different area.

ACKNOWLEDGEMENTS

The author would like to extend her warmest thanks to the team of the nematology laboratory at the Institute for Agricultural and Fisheries Research (ILVO) - Belgium for their support in molecular biology analysis and sequencing of this species, Mrs. Nicole Damme, head of laboratory and her colleagues. Similarly, grateful recognition to Prof. Dr. Reza Ghaderi at the Plant Protection Department, Islamic Republic of Iran, for her support in the morphological identification of the species.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDIES AND RESEARCH ON THE SPECIES AND VARIETIES OF DAHLIA IN CULTIVATION

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Abstract

The Asteraceae family (Compositae) contains a multitude of species, over 23,000, spreading all over the globe. The genus Dahlia includes about 42 species, falling into the category of ornamental plants, because the flowers are characterized by a multitude of shapes, sizes and colours. The floral qualities of the species of the Dahlia genus make these plants indispensable in gardens and the decoration of green spaces. In addition to their ornamental qualities, these plants are highly valued for their medicinal qualities and nutritional properties. Although Dahlia tubers have a low caloric value, it is still notable for its high fiber content, especially soluble fiber, such as inulin, but also considerable amounts of minerals (potassium, magnesium, phosphorus, calcium, iron). The paper presents studies and research conducted in recent years on the morphological, physiological, biochemical and nutritional properties of species from Dahlia genus.

Key words: *biochemical, flowers, morphology, nutritional properties, ornamental.*

INTRODUCTION

The genus *Dahlia* belongs to the family Asteraceae (Lord, 2003) or Compositae (Șelaru & Mucescu, 1976) and includes about 15 perennial species (Toma, 2009). It is known, especially, as a plant for ornamenting flower gardens characterized by the variety and brightness of the colour, their composition and size, the duration of flowering of the plant and high production capacity (Șelaru, 1998).
The colour of flowers and calatidia, their special shape and their long shelf life, make dahlia one of the most appreciated species used as cut flowers for summer bouquets (Cantor et al., 2007). Sho Ohno et al. (2011) state that in recent years, more than 50,000 varieties of Dahlia with different colours, shapes and sizes of inflorescences are grown in gardens (Figure 1).
Their easy cultivation technique and also the prolonged flowering, make dahlias among the most popular decorative flowering plants (Kiselev, 1956). Dahlia is widespread everywhere, but shining in the hilly and submontane areas (Șelaru, 2007).

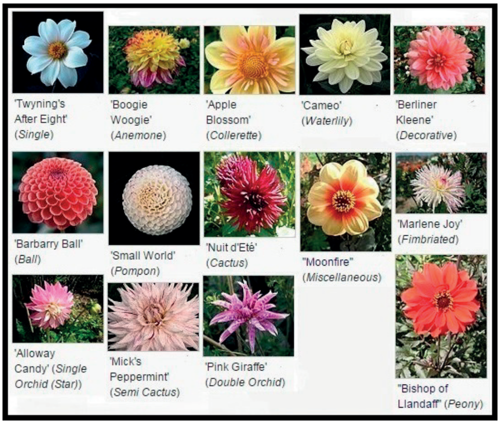

Figure 1. Types and varieties of *Dahlia hybrida*
Source: http://justfunfacts.com/interesting-facts-about-dahlias/

The sketches with Dahlia flowers drawn by the Spanish doctor Francisco Hernandez (1570), but also the notes of the Frenchman Nicolas Thierry de Menonville (1787) signaled the existence of these species on the territory of Mexico (Șelaru & Mucescu, 1976). In the wild, dahlias grow in South America in short day conditions and cold nights, which explains their abundant flowering in August-September when the day shortens and the nights become colder (Kiselev, 1956). The use of Dahlia plants has been varied since Aztec times; the same is true today: dahlias are used in landscaping, as a cut flower in pots, in the cosmetics industry, in the food industry, as a raw material for obtaining dyes, in the diet of diabetics, in the treatment of gastrointestinal problems, obesity and skin infections (www.healthbenefitstimes.com/dahlia).

Currently, Dahlia is widely used for economic purposes: in landscaping, in the flower shop as a cut flower, for the pharmaceutical, cosmetics, food industry and as a raw material for the extraction of dyes (Moldovan I., 2017). In the country of origin, dahlia flowers are used to dye natural fiber materials (Cretu, 2007). The first species of Dahlia appeared in Mexico and were inexhaustible sources for the emergence of new and new varieties, each interesting by the way the flowers are composed, their size or colour (Șelaru & Mucescu, 1976). Dahlias grew and still grow like weeds in the mountainous regions of Mexico and Central America and the Aztecs used them for food (Santana et al., 2016) and medicine, motifs decorated the helmets of the Aztec warriors; the petals were used in ceremonies, including human sacrifice to their sun god (https://www.mexinsurance.com/dahlia/).

When Hernan Cortez and the Spanish conquistadors entered the Aztec city Huaxtepec in 1519, they were the first Europeans to view the most impressive of Emperor Montezuma's botanical gardens. Undoubtedly, one of the most curious sights in the garden at Huaxtepec would have been a specimen of what we know as *Dahlia imperialis* (Figure 2), the tree dahlia that the Aztecs called acocotli, which means water pipe in their language (https://www.mexinsurance.com/dahlia/).

Hernan Cortez wrote of the flowers known in the Nahuatl language as acocotli, and even sketched them. Sadly this and other Spanish works are all that remains of the Aztec records detailing how dahlias were used in garden and as a medicinal (https://moplants.com/aztec-dahlia-flowers-of-mexico/). The first seeds belonging to the species *Dahlia rosea* and *Dahlia pinnata* appeared in Europe in 1789, more precisely in Spain (Șelaru & Mucescu, 1976).

Figure 2. *Dahlia imperialis*
Source: https://www.pacifichorticulture.org/articles/the-tree-dahlia/

STUDIES AND RESEARCH ON THE IMPACT OF FERTILIZATION ON FLOWER PRODUCTION, BIOMETRIC AND PHYSIOLOGICAL PARAMETERS IN DAHLIA (ASTERACEAE)

Research by Mahgoub et al. (2011) to *Dahlia pinnata* plants sprayed foliar with putrescine and thiamine (vitamin B1) in different concentrations (50, 100, 150 ppm/30 and 60 days) from transplanting and plants sprayed with tap water it was found that the results obtained indicate higher values when using 150 ppm putrescine and thiamine at 100 ppm. Also, spraying plants with putrescine and thiamine significantly increased plant height, number of leaves, number of branches, fresh and dry weight of leaves, stem diameter and fresh and dry weight of stem. The chlorophyll content was higher in foliar sprayed plants compared to untreated ones. Following the results obtained by Abd-Elkader et al. (2020) on certain vegetative parameters and chlorophyll content in plants treated with Tryptophan at 100 ppm , Arginine at 100 ppm, Glycine at 100 ppm, Tryptophan + Arginine + Glycine at 100 ppm per each compared to untreated plants indicated that treatment with mixture of amino acids tryptophan + arginine + glycine gave the maximum chlorophyll a, b and total chlorophyll values, followed by arginine treatment then the tryptophan treatment, whereas untreated plants (control plants) gave a minimum values of chlorophyll a, b and total in both seasons, respectively. It is evident from the presents data that the plants sprayed with the amino acids mixture (tryptophan + arginine + glycine at 100 ppm) produced the superior values of tallest plants, the maximum branches number /plant, the largest leaves number/plant, and the largest stem diameter.

Kashif et al. (2014) used foliar fertilizers to observe the impact on Dahlia plant growth. Four treatments were applied that were: T0, control (no foliar application of nutrients); T1, NPK (17:17:17); T2, NPK (15:32:7) + micro power); T3, NPK (15:32:7) + chelated mix micro-nutrients. Micro power contain N, 1%; K2O, 1%; Zn, 2.5%; B, 1%; Fe, 1%; Mn, 1% and Cu, 2% nutrients and chelated mix micro-nutrients (CMM) also contained Zn, 1.5%; B, 2%; Fe, 2%; Mn, 2% and Cu, 1% nutrients. The results endorsed the benefits of foliar fertilization by witnessing the improved growth traits of the plant.

In the study conducted by Younis et al. (2014), was used source of macro and micro nutrients like Foliber a macro nutrient product containing ingredients with concentration g/L: nitrogen (N) 80, P O 80 and K O 60 and Unipower solution a micro nutrient product containing ingredients with concentration in mg/100 ml: Zinc (Zn) 5000 ± 200, Boron (B) 4000 ± 200, Iron (Fe), 5000 ± 200, Manganese (Mn) 1000 ± 200, Copper (Cu) 1000 ± 100, Chloride (Cl) 1000 ± 100, Molybdenum (Mo) 50 ± 10 and pH of the solution 2.5 ± 0.5 were used. There were 16 treatment combinations comprising T = Control, T = 2.5 Unipower ml/L), T = 5.00 Unipower (ml/L), T = 7.5 Unipower (ml/L), T = 2.00 Foliber (ml/L), T = 2.00 Foliber (ml/L)+ 2.5 Unipower (ml/L), T6= 2.00 Foliber (ml/L) +5.00 Unipower (ml/L), T7= 2.00 Foliber (ml/L) +7.5 Unipower (ml/L), T8= 4.00 Foliber (ml/L), T9= 4.00 Foliber (ml/L)+2.5 Unipower (ml/L), T10= 4.00 Foliber (ml/L)+5.00 Unipower (ml/L), T11= 4.00 Foliber (ml/L) +7.5 Unipower (ml/L), T12= 6.00Foliber (ml/L), T13= 6.00 Foliber (ml/L) +2.5 Unipower (ml/L), T14= 6.00 Foliber (ml/L) +5.00 Unipower (ml/L) and T15= 6.00 Foliber (ml/L) +7.5 Unipower (ml/L). Plants were allowed to grow and data regarding following growth and flowering was collected using standard procedures. The parameters studied were: plant height (cm), number of lateral shoots, length of lateral shoots, number of leaves /plant, leaf area (cm^2), days to first flower emergence, root length, blooming period (days), size of flowers (cm), number of flowers/ plant and number of tubers/ plant. In this study, the results associated with plant growth parameters indicated that the maximum values for plant height, number of leaves, number of side shoots, leaf area, number of tubers plant, flower diameter and least days toflower emergence were observed in combined application of macro and micronutrients containing 6 ml/L Foliber and 7.5 ml/L Unipower. This combination has sufficient amounts of the nutrients essential for plant growth so this best combination of macro and micronutrients having 6 ml/L Foliber and 7.5 ml/L Unipower for good vegetative and flower growth in Dahlia is recommended.

Vlad et al. (2013) used foliar fertilizers to observe the quality production of *Dahlia variabilis* “Golden Wonder” cultivar (fam. Asteraceae) flowers. The experiments on the influence of phase fertilization on quality production of Dahlia flowers were conducted during 2010-2012 in a nursery from the locality Leş, situated in Bihor County, North-Western Romania. Propagation was performed by means of root cuttings forced in a warmed greenhouse solarium (16-22°C), using a substrate composed of peat and sand in equal parts. The obtained cuttings were stimulated to root in a substrate of peat and sand in equal proportions. The rooting took place within 30-35 days. The rooted cuttings were planted in the nursery soil with the aim of producing plants devised for cut flowers. During the vegetation period, foliar fertilizations were performed using Wuchsal (N:P:K=1:2:1). Apart from nitrogen, phosphorus and potassium, Wuchsal contains microelements (Fe, Cu, Zn, B, S, Co). Flower yield was enhanced in blocks were the foliar fertilization was applied weekly using a concentration of 0.2 and 0.25% (V4 and V6).Blocks where fertilization was applied every two weeks resulted in enhanced yields as compared to the blank (V3, V5, V7 and V8) - Tabel 1. Excepting the blank, in all blocks the proportion of first quality flowers raised over 80%.The best results with regard to yield and the number of quality flowers were obtained in block 6 (fertilized with Wuchsal, concentration 0.25% weekly).

Tabel 1: The influence of foliar fertilizers upon the quality of *Dahlia variabilis* flowers (Surce: Vlad, 2013)

Blocks	Flower yield in *Dahlia variabilis*		
	Totals of stalks/m^2	Of which, first quality	
		Absolute stalks/m^2	Relative %
V1 – blank (unfertilized)	68	49	72
V2 – fertilized with Wuchsal, concentration 0.1% weekly	100	85	85
V3– fertilized with Wuchsal, concentration 0.1% every two weeks	90	72	80
V4- fertilized with Wuchsal, concentration 0.2% weekly	120	102	85
V5- fertilized with Wuchsal, concentration 0.2% every two weeks	96	78	81
V6 - fertilized with Wuchsal, concentration 0.25% weekly	128	104	81
V7 - fertilized with Wuchsal, concentration 0.25% every two weeks	108	86	80
V8 - fertilized with Wuchsal, concentration 0.3% every two weeks	115	92	81

The results of the research conducted and presented highlighted the benefits of fertilization on growth and flower production.

NUTRITIONAL AND MEDICINAL PROPERTIES IN DAHLIA

Studies and research conducted by students and researchers of Chapingo Autonomus University showed that Dahlia tubers have a low caloric value, between 180-193 kcal/100 g of dry matter; provides a low calorie intake, which means that a large amount of this food needs to be consumed. The total dry matter of Dahlia tubers contains between 4.8 - 11.1% fiber, which means a rich source of fiber (https://www.ddfgg.de/dahlien/pdf/DAHLIA-FLOWERS-AND-TUBERS.pdf).

Studies indicate that in the prehispanic era the tuberous roots of this plant were consumed as a source of carbohydrates, that is, as an equivalent of potatoes (*Solanum tuberosum* L.) (Santana et al., 2016).

After Lara-Cortés et al. (2014) in Mexico, Dahlia flowers are commonly consumed in different type of dishes; however, there are no reports on characteristics as a functional food. Compared to other foods, it is a very high percentage of fiber. According to the World Health Organization (2003) fiber intake in children aged 5-8 years is 8 g/day, in children between 9-10 years 22 g/day, for adults between 18-59 years 25-30 g/day. According to the Academy of Nutrition and Dietetics (DNA), an intake of 30 g/day is required for men after the age of 60 (Mahan and Raymond, 2017) and 21 g for women (Palafox and Ledesma, 2015). Nsabimana and Jiang (2011) highlight a large number of substances in the tuberous roots of Dahlia: minerals (Fe, Zn, Na, K, Cu, Mg, Ca, Co, Cr, P), fats, proteins, fiber, ash, vitamins fat-soluble (A, E), water-soluble vitamins (B1, B2, B3, B6, B7, C). Dahlia tubers exhibited varying concentrations of minerals, among which potassium, calcium, magnesium, phosphorus, zinc and chromium were predominant (Nsabimana & Jiang, 2011).

Dahlia is a plant with a high content of soluble fiber (40-80%), such as inulin a carbohydrate storage that has a wide nutritional and pharmaceutical importance (Melanie et al., 2015).

Inulin (Figures 3 and 4) is a carbohydrate with the following properties: lowers lipid metabolism, reduces the risk of colon cancer, lowers body mass, lowers blood sugar, stimulates the immune system and increases intestinal flora (https://laverdadnoticias.com/estiloyvida/Las-dalias-un-gran-aliado-para-las-personas-con-diabetes-tipo-2-20200117-0250.html).

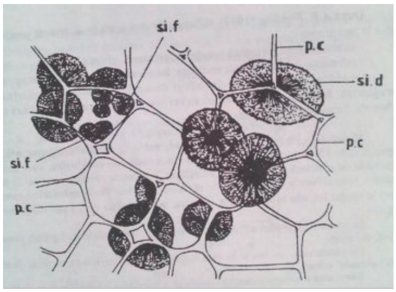

Figure 3. Spherocrystals of inulin in the tuberous root of the Dahlia: p.c - cell wall; d - spheric crystal of inulin developed in contact with cell walls; f - spheric crystal of inulin in training (Source: Burescu, 2002)

It also does not contain other significant soluble carbohydrates, which means that it does not provide energy in this way. A low protein content was found in Dahlia, between 6.5-15.1% (https://www.ddfgg.de/dahlien/pdf/DAHLIA-FLOWERS-AND-TUBERS.pdf).

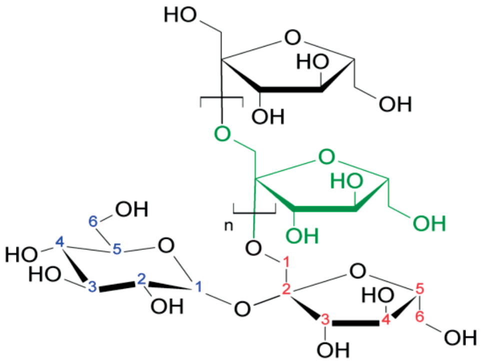

Figure 4. The chemical formula of inulin
Source: https://ro.wikipedia.org/wiki/Inulin%C4%8

In Dahlia tubers are found lipids in small amounts, about 1%. Dahlia tubers contain significant amounts of potassium, magnesium, phosphorus, calcium, iron, and in the presence of vitamin B are an important source of minerals for bones, especially in women, helping to absorb calcium; Potassium also helps the proper functioning of the kidneys and heart, muscle contraction and the transmission of nerve influx. The Aztecs used Dahlia as a treatment for epilepsy (Mareș, 2004). Both the tuberous roots and the flowers of this ornamental and medicinal plant are used for therapeutic purposes (Moldovan I., 2017). For the past 20 years, Chapingo Autonomous University in Mexico has conducted many studies on the growth, reproduction and use of Dahlia species. The results are publicized to encourage as many people as possible to grow dahlias for both food and their medicinal benefits for people with diabetes, gastrointestinal problems, high cholesterol and blood triglycerides and obesity. Flowers are also promoted in the diet, with a recipe book based on Dahlia flowers and tubers (Martinez-Montes et al., 2008). The flower petals are consumed by adding to salads, the sweet extract from the tubers is combined with cold, hot water, milk or sprinkled on ice cream. (https://www.healthbenefitstimes.com/dahlia).

Lara-Cortés et al. (2014) studied the composition, minerals, vitamin C, phenolic compounds, total anthocyanins, carotenoids and antioxidant activity of Dahlia flowers. In general, the highest values of phenolic compounds, anthocyanins and antioxidant capacity were found in purple Dahlia (127.5 mg AG.g^{-1}, 257.5 mg pelargonidine, 100 g^{-1} and 24% of inhibition). The type and concentration of phenolic compounds varied depending on the colour of the flower. The highest value of the phenolic compound was for hesperidin (398.9 mg.g^{-1}), while the most detected phenolic compounds in flowers were gallic and caffeic acids. Based on these results, we can recommend the consumption of Dahlia flowers as a functional food, because they provide phenolic compounds (especially dark Dahlia flowers, because they have the highest phenolic composition and antioxidant activity). Dahlia tuber fibers function as a prebiotic, meaning the food of the intestinal microbiota that ensures better digestion and better intestinal transit helping to maintain gastrointestinal health and prevent health problems such as colon cancer (Nsabimana and Jiang, 2011). According to the recommendations of the American Diabetes Association (ADA), people with diabetes should consume between 10-13 g of fiber per 1000 kcal to maintain the health of the body. In order to maintain good health, it is necessary for the continuous fiber intake to vary between 20-35 g/day, depending on the age and clinical condition of the person (according to American Diabetes Association). The Dietary Guidelines for Americans recommend a minimum of 14 grams of fiber per 1,000 calories. Foods that are naturally high in fiber and contain at least 2.5 grams are often labeled as a "good source," and foods labeled as "excellent source" contain more than 5 grams of fiber per serving (https://www.diabetes.org/healthy-living/recipes-nutrition/understanding-carbs/get-to-know-carbs). Abscisic acid is an important plant stress-induced phytohormone. Gouveia et al. (2020) also found in tuberous dahlia roots. Recent results of Italian researchers confirm that the health benefits observed in people with diabetes are related to the plant hormone, abscisic acid, present in large quantities in latent plants and it plays an important role in managing glucose homeostasis in humans (Zocchi et al., 2017).

CONCLUSIONS

The special floricultural qualities of the species of the genus *Dahlia* make these plants suitable for cultivation in gardens and green spaces. The genus *Dahlia* is distinguished by a multitude of species and varieties as different as possible. They are easy to grow, the decoration period is quite long, and the flowers are numerous and with an impressive colour. The nutritional and medicinal properties of these plants have been known since Aztec times, and all these uses have led to the study of *Dahlia* species and varieties by researchers around the world. Thus, numerous morpho-anatomical, physiological, nutritional and medicinal researches with a great scientific value have been carried out, which confirms that these plants bring benefits to humans.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCHES REGARDING THE GERMINATION CONDITIONS FOR THE SEEDS OF SPECIES USED IN THE LAWN MIXTURES

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Abstract

In the arrangement of the residential spaces, one of the most important stages is to identify the best mix of species that are suited to be used for the establishment or rapid correction of turf surfaces by modern techniques, which involve the use of pre-germinated seeds under the specifical condition of each site. In this research was tested the seeds of the species most often used in lawn mixtures, namely Poa pratensis, Festuca arundinacea and Lolium perenne. The research was consisting of a bifactorial experiment where the A factor was the germination temperature (in a range from 5 to 20°C), and the B factor was the seeds treatment. The analyzed parameters were the germination indicators. The statistical analysis for the differences between germination means was done using Student's t-test and Wilcoxon test for paired samples (done with the Jasp 0.14.3. program). Using the seed treatment is not determine an increase of germination characteristic in all de the cases by comparing with untreated variant for all the tested species of seeds. The increase in the temperature level was followed by the increase in the speed and in the level of germination.

***Key words**: germination, lawn mixtures, seeds.*

INTRODUCTION

By definition, a grassy surface should be flat, uniform, without spots or damaged areas, strong green in color, with an optimal density and a relatively soft texture (Steinberg, 2007; Slater, 2007). There are few species of grasses that fully meet these requirements and although the perfect lawn is said to be a classic example of monoculture (Stewart et al., 2009; Ignatieva and Stewart, 2009), in current practice mixtures of 2-3 species are used for the realization of turf surfaces.

These seed mixtures are based on grass species from humid climates in northwestern Europe, where they have been used since 1,000 years ago (Fort, 2000; Smith and Fellowes, 2014). With the economic and scientific development of society, innovative technologies in all fields of horticulture (including ornamental and landscape horticulture) could be widely accessed, so that the lawn becomes an important part of the urban landscape (Macinnis, 2009).

Lawn seeds used in landscaping in Romania are mostly imported (Netherlands, Denmark, France), not always meeting the specific pedo-climatic local. Imported from other countries and do not always meet the requirements of climatic conditions in our country, which can lead to lower results, with increased costs (establishment, correction or maintenance).

It is therefore imperative that better communication and cooperation be developed between domestic importers and producers of seed material, in order to establish the best performing and best-adapted seed mixtures for the profile market in the country (Popovici et al., 2008).

In recent years, the number of residential complexes and houses near large cities has steadily increased, which has had the effect of increasing the demand for landscaping.
Under these conditions, horticulturists must meet the challenge of finding the best mixtures to achieve a "lawn carpet" with different requirements for resistance to mechanical action, with minimal maintenance, but which can withstand extreme variations in environmental factors in the face of climate change.
In this context, this paper analyzes a series of plants from the botanical family Poaceae, generically called "turf species", which are most commonly found in mixtures to obtain turf surfaces, in terms of germination and growth conditions in the first vegetation phases, noting that this is the critical stage in obtaining a carpet that meets the requirements of landscapers and beneficiaries.
The seeds were intervened by applying treatments with substances stimulating the seed germination process, the results being compared with those of the control experimental variant, in which the seeds were not subjected to treatments.

MATERIALS AND METHODS

The ability of turf mixtures to cover the soil and form a continuous and uniform carpet is an essential requirement, to which is added the need for uniformity of emergence in the face of climate change.
In this context, a bifactorial experiment was organized within the Faculty of Horticulture in order to establish the behavior of different species found in lawn mixtures, thus:

- **A factor-** the germination temperature: 5°C, 10°C, 15°C, and 20°C in 5 repetitions.
- **B factor-** the applied stimulants: ASFAC, CROPMAX, ATONIK and water.

The experimental variants consisted of seeds belonging to three species considered by most specialists as basic for obtaining qualitative turf surfaces, namely *Festuca arundicancea*, *Lolium perenne* and *Poa pratensis*.
Festuca arundinacea Schreb. belongs to a genus comprising 621 accepted species (Clayton et al., 2006), widespread in the Holarctic region and in the temperate zones of the southern hemisphere (Watson and Dallwitz, 1992). *Festuca* species are characterized by their dorsally rounded lemmas and linear hilum (Catalan et al., 2004). The seeds sprout in approx. 6-12 days after sowing, the plants have a fast growth rate and a good - very good permanence. The plants have a leaf with a medium to coarse texture, dark green. They are very resistant to drought and have a good tolerance to low temperatures. It supports acceptable soil salting. Shadow adaptation has average values.
Lolium perenne L. (perennial ryegrass) is a species native to Europe, Asia and North Africa, with the following main characteristics: the seeds sprout in 5-12 days from sowing, fast growth rate, good perenniality, with medium resistance drought, good cold resistance, good adaptation to medium shade. The plants have a leaf with a fine to the coarse, bright light green texture (Hannaway et al., 1999).
Poa pratensis L. (Kentucky bluegrass) is a species native to Europe, North Asia, and the mountainous areas of Algeria and Morocco. The species is characterized by an emergence duration of 15-25 days from sowing, low to medium growth rate, good perenniality, good drought resistance, cold resistance and medium-good salting, adaptation to medium shade. The plants have a medium coarse, dark green foliage. *Poa* L. species are distinguished by their keeled lemmas and round to oval hilum (Catalan et al., 2004).
These seeds were treated with the application of stimulants (ASFAC, CROPMAX, ATONIK), and without treatment just using water (control variant) following at the same time the germination process under different temperature conditions (5°, 10°, 15° and 20°C).

CROPMAX is a growth stimulator for all types of crops. Its activity is based on the combination of trace elements, amino acids, vitamins and polysaccharides. It is very concentrated, 100% organic and suitable for application to all agricultural and horticultural crops source.

ATONIK is the oldest biostimulator of growth and fruiting in the world (since 1945), stimulates rooting, germination of seeds and pollen, growth of pollen tube and fruit source.

ASFAC BCO - 4 - is the only formula recognized worldwide. It has an influence on seed germination, causing a faster and more uniform growth of plants (it is a biostimulator - based on 4-clor-2 potasium amidosulfonil-fenoxiacetat + microelements and additives; produced in our country by Romchim Protect, Bacău - an invention of Corneliu Oniscu). It is a good stimulating substance for the germination of *Festuca arundinacea* seeds (Delian et al., 2019).

To determine the germination, 200 mm Petri dishes were used, and in each box were placed 100 seeds arranged evenly, on sterile and moist Whatman No. 1 filter paper. ASFAC, CROPMAX, ATONIK and distilled water were used. The dishes were covered with lids, also paper being used, and they were moistened, as well as it was mentioned above.

Germination was performed in a LabTech 320G incubator at 80% humidity.

The evaluation of germination was performed after 14 days of incubation, according to the following formula:

$$G = \frac{n}{N} * 100;$$

where:

n - total number of germinated seeds/ Petri dish;

N - total number of seeds/Petri dish.

The Shapiro-Wilk test was used to verify the normality of the data. Where data were distributed normally we used the Student Test, in other cases the Wilcoxon test was used.

In both cases, we started from the premise that we have paired samples. All these statistical tools were used with the help of Jasp 0.14.3 software (Halter C., 2020, Marsman et al., 2017; Wagenmakers et al., 2020; Navarro D.J. et al., 2019). The test used for each comparison is specified in the appendices.

RESULTS AND DISCUSSIONS

In the statistical interpretation of the experimentally obtained data, the Shapiro-Wilk test was initially applied, following which the normality of the data was observed. Thus, the Student t-test can be applied for paired samples in order to identify significant differences in the average germination (for each species).

1. The influence of stimulant solutions on seed germination.

For this, the average results of seed germination, for each species, were compared at the different temperature values used in the experiments (5, 10, 15, 20°C), by applying the Student Test for paired samples (Figures 1-3).

Poa pratensis - Student's t test (paired samples)

Paired Samples T-Test

Measure 1		Measure 2	t	df	p
Water	-	Cropmax	0.974	3	0.402
Water	-	Atonik	1.044	3	0.373
Water	-	Asfac	0.132	3	0.903
Cropmax	-	Atonik	0.694	3	0.537
Cropmax	-	Asfac	−0.785	3	0.490
Atonik	-	Asfac	−1.632	3	0.201

Note. Student's t-test.

Assumption Checks

Test of Normality (Shapiro-Wilk)

			W	p
Water	-	Cropmax	0.976	0.876
Water	-	Atonik	0.901	0.437
Water	-	Asfac	0.813	0.128
Cropmax	-	Atonik	0.888	0.372
Cropmax	-	Asfac	0.972	0.856
Atonik	-	Asfac	0.939	0.648

Note. Significant results suggest a deviation from normality.

Figure 1. The influence of stimulant solutions on seed germination - *Poa pratensis*

Since for each solution a $p > 0.05$ value was obtained for each comparison, it can be said that the stimulants did not have an impact on the results of seed germination, for none of the grass species among the taken into study.

Festuca arundinacea - Student's t test (paired samples)

Paired Samples T-Test

Measure 1		Measure 2	t	df	p
Water	-	Cropmax	1.147	3	0.335
Water	-	Atonik	0.683	3	0.544
Water	-	Asfac	−1.035	3	0.377
Cropmax	-	Atonik	−0.507	3	0.647
Cropmax	-	Asfac	−1.469	3	0.238
Atonik	-	Asfac	−1.087	3	0.357

Note. Student's t-test.

Assumption Checks ▼

Test of Normality (Shapiro-Wilk) ▼

			W	p
Water	-	Cropmax	0.976	0.879
Water	-	Atonik	0.921	0.541
Water	-	Asfac	0.958	0.764
Cropmax	-	Atonik	0.874	0.312
Cropmax	-	Asfac	0.878	0.332
Atonik	-	Asfac	0.958	0.765

Note. Significant results suggest a deviation from normality.

Figure 2. The influence of stimulant solutions on seed germination - *Festuca arundinacea*

It is known from the literature that the initiation of seed germination is marked by a process of imbibition, which leads to a strong increase in their water content.

Lolium perenne - Student's t test (paired samples)

Paired Samples T-Test

Measure 1		Measure 2	t	df	p
Water	-	Cropmax	0.264	3	0.809
Water	-	Atonik	0.712	3	0.528
Water	-	Asfac	0.212	3	0.846
Cropmax	-	Atonik	0.343	3	0.754
Cropmax	-	Asfac	−0.189	3	0.862
Atonik	-	Asfac	−2.530	3	0.085

Note. Student's t-test.

Assumption Checks

Test of Normality (Shapiro-Wilk)

			W	p
Water	-	Cropmax	0.871	0.302
Water	-	Atonik	0.841	0.198
Water	-	Asfac	0.854	0.240
Cropmax	-	Atonik	0.930	0.593
Cropmax	-	Asfac	0.968	0.827
Atonik	-	Asfac	0.940	0.653

Note. Significant results suggest a deviation from normality.

Figure 3. The influence of stimulant solutions on seed germination - *Lolium perenne*

The transition of the embryo from the state of latency to that of active life therefore begins with imbibition (seed hydration), which will cause an increase in respiration values and an increase in cellular metabolic level.

For the germination of the seeds they use their own reserve substances, stored at their level, without an external supply of substances, in the first phases of germination, so that then, after the seeds have germinated the new plant can feed autotrophic (Burzo et al., 2005). This may probably explain why there are no significant differences between the experimental variants that used the respective water and substance solutions.

2. The influence of temperature on seed germination

The results that express the differences between the germination media at different temperatures depending on the species can be found in Figures 4-7.

Regarding the influence of temperature on the germination of *Poa pratensis* seeds, there were significant differences between the average results obtained from the analysis of seed germination, as follows:

- m5°C>m15°C ($t=6.73$; $p=0.007<0.05$)
- m5°C>m20°C ($t=3.21$; $p=0.049<0.05$).

Poa pratensis - Student's t test (paired samples)

Paired Samples T-Test

Measure 1		Measure 2	t	df	p
V5 °C	-	V10 °C	1.988	3	0.141
V5 °C	-	V15 °C	6.730	3	0.007
V5 °C	-	V20 °C	3.206	3	0.049
V10 °C	-	V15 °C	2.673	3	0.075
V10 °C	-	V20 °C	1.483	3	0.235
V15 °C	-	V20 °C	−0.400	3	0.716

Note. Student's t-test.

Assumption Checks

Test of Normality (Shapiro-Wilk)

			W	p
V5 °C	-	V10 °C	0.939	0.648
V5 °C	-	V15 °C	0.881	0.344
V5 °C	-	V20 °C	0.918	0.525
V10 °C	-	V15 °C	0.916	0.513
V10 °C	-	V20 °C	0.973	0.857
V15 °C	-	V20 °C	0.928	0.585

Note. Significant results suggest a deviation from normality.

Figure 4. The influence of temperature on seed germination - *Poa pratensis*

For *Festuca arundinacea* seeds, the influence of temperature on seed germination materialized in the existence of significant differences between the average germination, as follows:

• m5°C>m20°C (t=6.16; p=0.009<0.05)

Festuca arundicancea - Student t-test (paired samples)

Paired Samples T-Test

Measure 1		Measure 2	t	df	p
V5 °C	-	V10 °C	−1.233	3	0.306
V5 °C	-	V15 °C	−0.581	3	0.602
V5 °C	-	V20 °C	−6.161	3	0.009
V10 °C	-	V15 °C	0.802	3	0.481
V15 °C	-	V20 °C	−2.546	3	0.084

Note. Student's t-test.

Assumption Checks

Test of Normality (Shapiro-Wilk)

			W	p
V5 °C	-	V10 °C	0.923	0.556
V5 °C	-	V15 °C	0.771	0.059
V5 °C	-	V20 °C	0.980	0.902
V10 °C	-	V15 °C	0.917	0.519
V15 °C	-	V20 °C	0.881	0.341

Note. Significant results suggest a deviation from normality.

Figure 5. The influence of temperature on seed germination - *Festuca arundicancea* (whitout V10°C vs V20°C)

On the other hand, when comparing the average seed germination results at 10°C and 20°C respectively, the normality condition is not met.

Festuca arundicancea - Wilcoxon test (paired samples)

Paired Samples T-Test

Measure 1		Measure 2	W	df	p
V10 °C	-	V20 °C	0.000		0.098

Note. Wilcoxon signed-rank test.

Assumption Checks

Test of Normality (Shapiro-Wilk)

			W	p
V10 °C	-	V20 °C	0.691	0.009

Note. Significant results suggest a deviation from normality.

Figure 6. The influence of temperature on seed germination *Festuca arundinacea* (V10 vs V20 °C)

In this case, the Wilcoxon test was used to compare the experimental results on seed germination at 10 and 20 °C.

Following the application of the Wilcoxon test, the existence of insignificant differences is observed (p = 0.098> 0.05).

From the analysis of the experimental results obtained at the germination of *Lolium perenne* seeds, in different temperature conditions, there are significant differences between the average germination, as follows:

• m5°C>m15°C (t=7.35; p=0.005<0.05)

Lolium perenne - Student's t test (paired samples)

Paired Samples T-Test

Measure 1		Measure 2	t	df	p
V5 °C	-	V10 °C	1.815	3	0.167
V5 °C	-	V15 °C	7.346	3	0.005
V5 °C	-	V20 °C	2.502	3	0.088
V10 °C	-	V15 °C	2.524	3	0.086
V10 °C	-	V20 °C	0.775	3	0.495
V15 °C	-	V20 °C	−2.627	3	0.079

Note. Student's t-test.

Assumption Checks ▼

Test of Normality (Shapiro-Wilk) ▼

			W	p
V5 °C	-	V10 °C	0.891	0.387
V5 °C	-	V15 °C	0.950	0.717
V5 °C	-	V20 °C	0.933	0.611
V10 °C	-	V15 °C	0.916	0.516
V10 °C	-	V20 °C	0.993	0.972
V15 °C	-	V20 °C	0.880	0.339

Note. Significant results suggest a deviation from normality.

Figure 7. The influence of temperature on seed germination - *Lolium perenne*

If the germination of the seeds is not influenced by the treatments with stimulants of the type used, the same cannot be said of the temperatures at which the germination process of the seeds was directed, depending on the temperature level there are significant differences, as follows:

• *Poa pratensis*: the highest percentage of seed germination was recorded in the experimental variant with temperatures of 5 °C, significantly higher than the results recorded for the other two experimental variants (15 °C < 20 °C).

• *Festuca arundicancea*: the best results were obtained in the experimental variant in which the seed germination was conducted at 20 °C, followed by the variant at 5 °C but here we have the higher percentage at 20 °C (unlike Poa)

• *Lolium perenne*: the best results in terms of seed germination were obtained in the experimental version with a temperature of 5 °C and the minimum was recorded by the percentage of germination at 15 °C the other experimental variants having intermediate results (20 °C <10 °C).

CONCLUSIONS

As a general trend, it can be said that for *Lolium perenne* seeds the germination percentage is the highest, at the opposite pole being Poa pratensis seeds, with the lowest germination percentage (for both factors taken into account: treatment with substances stimulators respectively temperatures at which seed germination is conducted).

The experimental results confirm the data from the literature, seed germination, for all three grass species having the highest values in the area of optimal temperatures (Beard J.B., 1973).

At the same time, the germination of the seeds of the grass species studied is not influenced by the treatments with stimulants such as those used (or the effect it is not visible at this stage- after 14 days of incubation).

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

URBAN FARMING - OPPORTUNITY FOR FRUIT GROWING DEVELOPMENT IN ROMANIA

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Abstract

We often hurry on the streets, next to parks, among the planted areas of the city without giving them too much importance, perhaps careless or worried about daily worries, most of us do not pay attention to the oases of greenery and trees except when they are cut and replaced by built areas. This study sets out a new way of developing the green spaces, centered on the citizen, wishing to become a way for cities to cope with climate change, food and nutritional security, biodiversity management and human prosperity. The purpose of the present work was to find and implement a tool for the sustainable recovery of degraded lands, by using landscaping that includes spaces dedicated to urban fruit growing orchards. The study was conducted between 2016-2019 and represents a multidisciplinary approach, which took into consideration as many aspects as environment, economic, social, horticultural and biological. The horticultural part is characterized by a great biodiversity and is dominated by fruit growing species. As for the degraded area in which urban orchards are located, they could be the hope of having safe spaces, of improving the landscape, as well as another form of social inclusion. The most important benefits from a social point of view are the maintenance of public spaces at a low cost, the social inclusion, the economy of saving food and the short chain, while under the environmental aspect, the protection of biodiversity and the safety of health become important.

Key words*: urban farming, fruit growing, landscaping.*

INTRODUCTION

Population growth and increasing consumption are placing huge demands on agriculture and natural resources. In the present, approximately 15% of world population is chronically malnourished while our agricultural systems are degrading land, water, biodiversity and climate on a growing scale. To stand up to the world's future food security and sustainability needs, food agriculture production must grow substantially but in the same time to protect the environment (UNDESA, 2014).

This is why we have to find solutions for a cultivated planet and UF could be one.

In the last decade urban farming is expanding into European cities and has recently colonized the roofs of buildings, being part of the large category of roof agriculture, especially due to constraints related to the availability of urban land (Artuso, 2015). The implementation of Roof Agriculture has been done in several ways: from high-tech business-oriented solutions, often promoted by the European Commission's research and innovation programs, to community-oriented agri-environmental and social initiatives (Timpanaro, G., Scuderi, A., Foti, VT and Lo Giudice, V., 2015).

An internet search study led us to a series of existing projects in Europe, which we classified into 4 categories: gardens, farms, engineering and landscape (Kothencz, G., Kolcsár, R., Cabrera- Barona, P. and Szilassi, P., 2017).

The multi-functionality of roof agriculture involves a combination of objectives and benefits, namely food and non-food products, economic and social production services, financial and environmental impact (Torquati, B., Giacchè, G., Marino, D., Pastore, R., Mazzocchi, G., Niño, L., Arnaiz, C., Daga, A., 2018).

The analysis also presented some implementation barriers that constrain the development of RA, limiting the long-term viability of existing projects. How many will stay in the next few years? And how many will replace them, and find a sustainable system? This is the role of research teams to study these initiatives, to test technical policy options and regulations through socio-economic models and scenarios to provide an enabling environment for roof agriculture to become a way for cities to cope with climate change, food and nutrition

security, biodiversity management and human prosperity (Zasada, 2011).
This paper proposes a series of projects/arrangements, analysing solutions to this dilemma, showing that tremendous progress could be made by halting agricultural expansion, closing 'yield gaps' on underperforming lands, increasing cropping efficiency, shifting diets and reducing waste. Together, these strategies could double food production while greatly reducing the environmental impacts of agriculture (Montanaro, G., Xiloyannis, C., Nuzzo, V. and Dichio, B, 2017).

MATERIALS AND METHODS

In order to better understand the ideology (Specht, K., Weith, T., Swoboda, K. and Siebert, R., 2016) of the use of fruit species in public spaces, we resorted to their integration in a proposal to arrange a urban orchards in the city of Iasi.
In the process of identifying the areas with high potential for such territorial systematizations, we discovered different sites of a special kind of picturesque in which the built elements were harmoniously combined with the vegetal ones. These positive aspects of landscape architecture and urban aesthetics of the city led us to identify 3 types of green spaces, ac also were identified by Irvine and collaborators in 2013. The percentage for Iasi is as it follows:
- public parks and gardens, which occupy 14% of the city area;
- forests, located on the outskirts of the city, with the role of stabilizing hilly areas, which do not exceed 11% of the surface of urban green spaces;
- street green spaces which represent the largest percentage of the surface of green spaces of over 70% (Iasi County Directorate of Statistics, 2019).
Also, in the study, we identified the 10 most populated neighborhoods in Iasi, which comprise 56% of the total population of the city and in which green spaces account for only 20% of total green spaces. Among these neighborhoods are the Mircea cel Batran, Nicolina and CUG neighborhoods (Figure 1), where the lack of green spaces was intensified by uncontrolled asphalting in the perimeter of the few areas with vegetation and also did not allow the reintroduction or creation of new green spaces.

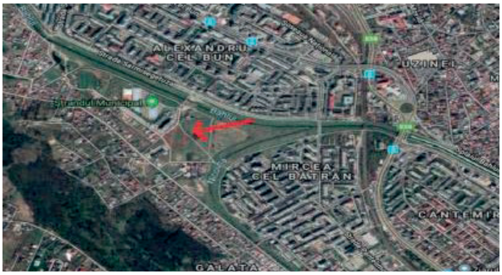

Figure 1. Area between Alexandru cel Bun, Mircea cel Bătrân and Galata Districts, Iasi

Taking into account these considerations, we propose the implementation of the concept of Urban Orchard in an unused space of 6400 sqm, located between the neighborhoods Alexandru cel Bun, Mircea cel Bătrân and Galata (Figure 2.), more precisely to the right of Sofia Nădejde street in front of Arcadia Hospital.

Figure 2. Proposed area for urban orchard project

Figure 3. The existent vegetation at the site

The area proposed for the development of an urban orchard, at the moment does not serve any purpose (Figure 3), it being left in ruins with a rich grassy vegetation. In the realization of the systematization proposal of the space, fruit species were used as a priority, but also dendrological and shrub species (Table 1), according to the requirements of the project and the recommendations for this kind of sites (Montanaro, G., Xiloyannis, C., Nuzzo, V. and Dichio, B., 2017).

In order to achieve a coherent, harmonious, sustainable development proposal that meets all the requirements in the field (Negri, V., Branca, F., and Castellini, G., 2008) and also the needs of the inhabitants of Iasi and those of the three neighborhoods in particular, but also of the people who attend the medical center in the space marked in figure 2, two main tools were used, namely art principles and a structured design process.

Table 1. List of proposed vegetation, species and quantity required

Nr. crt.	Specie	Variety	Pieces
1	*Prunus persica* L., Batsch	**'Dida'**	4
		'Crăița'	4
2	*Pyrus sativa* L.	**'Williams'**	2
		'Williams roșu'	2
		'Untoasă Hardy'	2
3	*Cerasus vulgaris* L.	**'Northstar'**	3
		'Pitic de Iași'	3
4	*Prunus cerasus* L.	**'Rivan'**	4
		'Kordia'	4
5	*Juglans regia* L.	**'Novaci'**	4
6	*Corylus avellana* L.	**'Arutela'**	6
7	*Mespilus germanica* L.	-	4
8	*Citrus limon* L.	**'Bush lemon tree'**	4
9	*Citrus reticulata* Blanco	-	
10	*Cydoni oblonga* Mill.	**'Aromate'**	3
11	*Rubus fruticosus* L.	**'Thornfree'**	20
12	*Rubus idaeus* L.	**'Fertodi Zamatos'**	10
13	*Vaccinium myrtillus* L	**'Blue Gold'**	10
14	*Hippophaë rhamnoides* L.	**'Askola'**	8
15	*Ginkgo Biloba* L.	-	1
16	*Juniperus horizontalis* L.	**'Blue Chip'**	4
17	*Berberis thunbergii* DC	**'Atropurpurea Nana'**	2

The principles used in the proposal for the arrangement of the Urban Orchard not only want to offer solid arguments regarding the efficient change of the landscape on which it is based, but also describe the means by which the fusion between artistic values and the qualities of the created environments is created (Sander, H.A. and Zhao, C., 2015). Thus, within the urban orchard development project, it was decided to suppress the presence of cars in the landscaped space in order to obtain a strictly pedestrian landscape.

Another common technique of spatial planning often found in modernist design principles, namely functional analysis, was used to make the arrangement proposal for easier separation of site functions as a means of resolving conflicts in the landscape.

Functional analysis

As a result of the detailed analysis processes that took place in the area of interest for the project, it was possible to create a list of strengths and weaknesses existing on site, but also outside it. Detailed measurements were made, the components and composition of the area were closely studied, and thus the current needs that will be taken into account in the realization of this urban orchard were identified.

The strengths or advantages of the site are characterized by:

- the presence of institutions of interest to the population, such as the Arcadia hospital and the municipal swimming pool, this being beneficial to the development in terms of the flow of people transiting the area and could benefit from the advantages offered by the urban orchard.
- the geographical location between the three districts, bringing a greater contribution of visitors to the respective area, it being daily crossed by numerous inhabitants, on an alley formed rudimentarily around the analyzed site.
- the presence of access roads for cars, being also a strong point of the area, the access being easier, in all seasons, even in unfavorable weather conditions for walking.
- the construction of a bridge in the vicinity is considered to be a strong point of the analyzed area, because it will increase the flow of people in that area, and the street will also benefit from an adequate lighting system.

The Bahlui River plays an essential role in the development of the site, being considered an ideal water source for irrigating the land at a low cost, even in the driest times of the year.

As disadvantages of the site we can observe:

- the lighting system is not sufficiently developed, in some places being absent, and in

others being in an advanced state of degradation.
- access roads and pedestrian alleys are in an average state of degradation, with potholes and missing parts.
- lack of bicycle tracks,
Following field research, we noticed that the parking lots in the hospital and pool area are not large enough for all visitors to the area, and they park their cars on the nearby green space.
All these detailed observations were made in order to make a coherent proposal in terms of the location of the plants, the systematization of the alleys, the correct zoning of the site functions, so that the arrangement is harmonious, beautiful and sustainable.
Following the analysis, it was determined exactly what are the needs, desires and functions that will have to be fulfilled by the urban orchard development project, as well as how their desired fulfillment could be achieved safely and with minimal effort from all the parties involved in the project implementation process but also in the long term of its maintenance.

RESULTS AND DISCUSSIONS

In this paper, we aimed to design and arrange an urban orchard in Iasi, at the interference of Mircea cel Batran, Alexandru cel Bun and Galata, open to the public, with an essential role in developing the economic and aesthetic potential of the targeted area, by creating new jobs, but also a space designed to relax in a pleasant atmosphere, as close to nature as possible with the family, thus trying to keep the idea of a rustic orchard, but with modernist elements.
Urban orchards are a creative and sustainable solution to many of the urban challenges, providing access to fresh fruit, improving the urban environment and creating habitats necessary to protect local wildlife (especially birds). However, the most important aspect of the project is the opportunity to facilitate connections between the inhabitants of the area, but also for them to benefit from a balanced diet rich in fresh fruits and vegetables (Torquati, B., Viganò, E., and Taglioni, C., 2016). Connecting residents so they can create and view urban orchards as a great way to spend time in the city (most do not have their own garden). There is also a strong educational element: children, in particular, love to learn how to grow and harvest their own food, and early education creates healthy eating habits (Torquati, B., Tancini, C., Paffarini, C., and Illuminati, R., 2015). Harvesting is also an essential part of this goal.
Another aim is to stop wasting the crops produced in the municipality of Iasi, thus ensuring a space for marketing fruits and other products obtained by the most environmentally friendly methods.
The orchard is an element that ensures flavor, freshness and color to each garden. It offers a decorative spring look through flower and color, attracting pollinators and offering delicious and fragrant fruits. In addition, fruit growing can be a relaxing activity practiced with pleasure, which pleases you on all levels. Also, the surplus fruit can be used by processing in different forms: jam, marmalade, compote, etc.
We tried to create an orchard as varied as possible in terms of assortment (Figure 4), especially using qualitative varieties, low vigor (Stănică, F., Dumitraşcu, M., Peticilă, A., 2008) dwarf type (to use the space as efficiently as possible), with medium to high production, with a wide ripening season , from early May to late November.

Figure 4. *Prunus*, *Cydonia* and *Cerasus* genera used in the project

Regarding the location of the trees in the orchard, this was done according to their size from large to small from outside to inside. Perimeter high-species species were used, the specimens being positioned so as to create a natural curtain of protection against proximity, but also to offer privacy to visitors.
The vigor of the planted species was taken into account, ensuring the appropriate planting distances, but also the space necessary for maintenance, the need for water, this being ensured by a well-developed irrigation system.
The planting of fruit bushes (Figure 5) was done near the hedge, on trellis, they also have a fencing purpose, being cultivated in the form of continuous fruit fences.

Figure 5. *Rubus*, *Vaccinium*, *Hippophaë*, *Mespilus* and *Corylus* genera used in the project

The location was made as follows (Figure 6): in yellow we represented the alignment of walnut and hazelnut, with green, peach, quince, sour cherry, cherry and pear, and in red the fruiting shrub species.

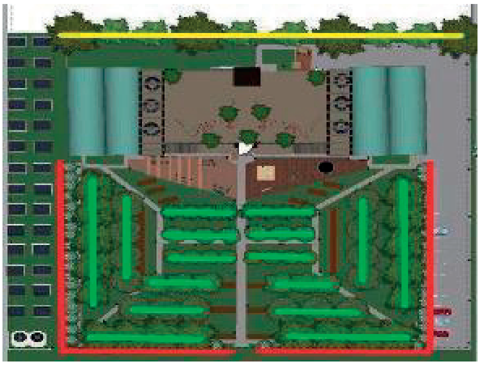

Figure 6. Location of fruit species in the orchard

On the surface of the terrace we placed lemons and mandarins grown in pots, primarily for decorative purposes. Also, in the design theme, the design of an urban orchard was considered, along with a space for capitalizing on products obtained in a way that is as accessible and transparent as possible for the inhabitants of Alexandru cel Bun, Mircea cel Bătrân and Galata neighborhoods.
After making the schemes for planting fruit vegetation, we framed the surface in two main initial areas, the one related to the outside, more precisely the orchard itself, but also the area that will be used to place the ensemble designed for the greenhouses, but first of all the main capitalization and preservation space for the products in a sustainable, modernist manner. The delimitation of the previously mentioned areas was made with two colors, green for the orchard area and blue for the construction area, as can be seen in Figure 7.

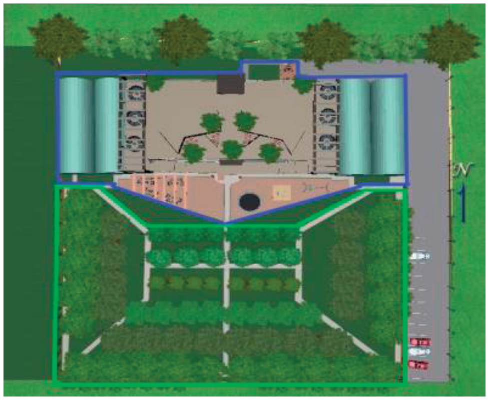

Figure 7. Main zoning

These two areas define a whole, which through its sanogenic, ecological and aesthetic qualities form an oasis of relaxation in the city for the inhabitants of the 3 neighborhoods, but also a place where children can learn new and exciting things in agriculture and horticulture.
The alley frame has been designed so that all areas of the site are as accessible as possible, regardless of positioning, or the access roads used to enter it.
To streamline traffic inside the orchard, as seen in Figure 8, there are two types of alleys, drawn with two different colors, depending on the level of use established.
The most frequently used areas were marked in light blue, and the less frequented areas in dark blue.

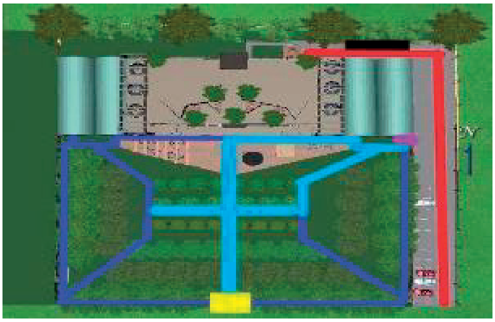

Figure 8. Alley plot and access ways

The area drawn with the red line shows the access way of the visitors' cars to the arranged parking lot, but also of those used for supply in the area marked in black, where the cold storage of the ensemble is located.

We used the color yellow to present the main entrance inside the orchard, and with the secondary purple, from the parking lot.

In order to facilitate the perception of the project, we made a compartmentalization of it, dividing it into eleven areas of interest, trying to respond to all the needs of the inhabitants of these neighborhoods, by creating inside the urban orchard areas destined for recreational, play, social interaction and last but not least of some active relaxation areas, by interacting with the vegetal elements located at the level of the site (Figure 9) (Zlati Cristina, Pașcu Roxana, Bernardis R., (2020).

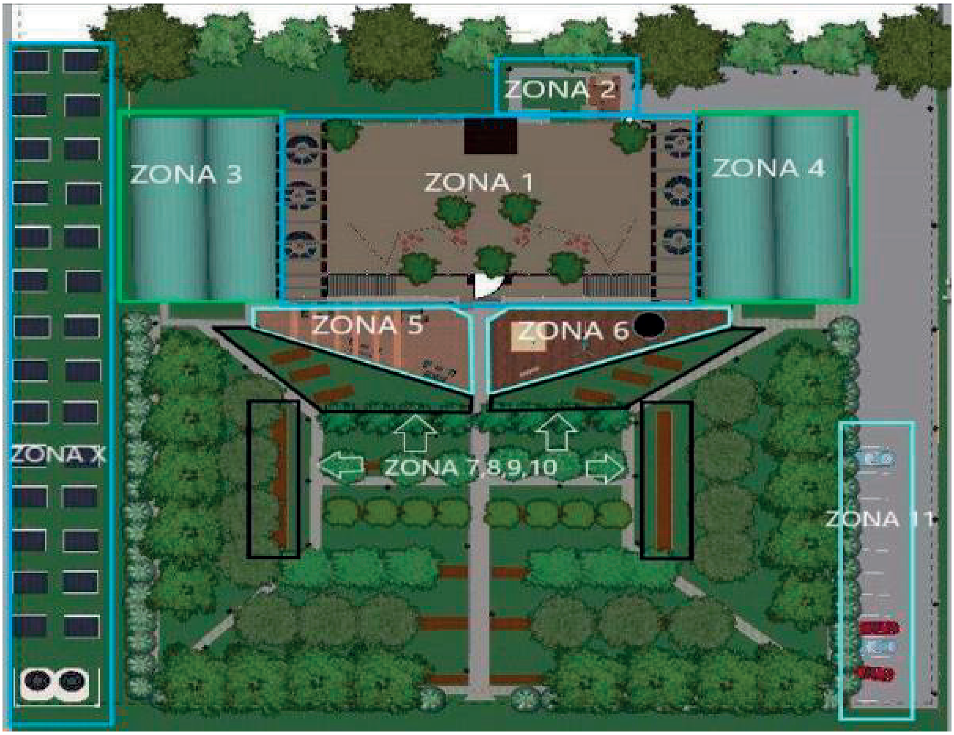

Figure 9. The zoning of the Urban Orchard project, area at the interference of Alexandru cel Bun, Mircea cel Bătrân and Galata neigberhoods

By creating these areas, we did not want to form barriers between users of this space, but instead, we wanted to design a whole consisting of several spaces adapted and created according to the needs of the inhabitants of Iasi, so that they feel as well within the formed ensemble and be as close as possible to nature.

As a result, in Zone 1 we proposed arranging a terrace on the roof of the technical building, with dining areas and an island kitchen. This space was designed specially to host events, not only by the systematization of the elements that make it up, but also by its location at the highest point of the site with a panoramic view

of the orchard, but also the playground for the children.
In order to enliven this area, it is proposed to use several specimens of *Citrus limon* (L.), grown in pots.
In this space we also proposed to introduce an island kitchen, which will be accessible to visitors in the summer months, next to a piece of furniture, located under the two pergolas of the area.
The point of maximum interest of this area are the decorative walls (Figure 10), with the role of compartmentalizing the space.

Figure 10. Zone 1 in detail

Zone 2, of the urban orchard, was designed to offer the ensemble's employees a private space to spend time during the break. The intimacy and shade in this space is to be ensured by the alignment of nuts and hazelnuts, but also by the vegetal compositions of which the specimens of *Ginkgo biloba* are part of.
In **Zones 3** and **4** we suggested to arrange two solariums on each side of the technical building (Figure 11), in which to cultivate a wide range of plants (strawberries, lettuce, greens, seedlings but also various fruiting shrubs), including in the winter months with the help of heating systems based on energy produced by solar panels.
Zone 5 is intended for relaxation and supervision of children aged between 2 and 6 years whose playground we placed in **Zone 6** next to the educational spaces where they have the opportunity to learn the fascinating secrets of agriculture and fruit growing.
For children over 6 years old, we have provided educational spaces in **Zones 7, 8, 9** and **10**, where they can learn through play, how to perform various horticultural operations such as planting, watering and caring for fruit crops.

Figure 11. Green houses from zone 3 and 4

Figure 12. Zone 11-parking detail

The last area planned to be arranged within this urban orchard is Zone 11 (Figure 12), a space where we placed a parking lot with over 15 spaces, which will be available to visitors.

CONCLUSIONS

Considering the importance of green spaces, especially in urban areas, the concept of Urban Orchard is meant to create a space, not only with an aesthetic role, but also with an important sanogenic, economic and social role. This desire can be achieved by introducing an oasis of relaxation, as close as possible to nature for site visitors.

The advantage of using this type of arrangement of fruit species, initially induces visitors the feeling of modernism, but in reality, the landscape created is a rustic, natural and local, being easy to maintain.

The site has been designed so that each space has a well-defined role, both as an orchard, but also as a space for relaxation, trying to please all types of visitors.

The planting of trees and shrubs species will take into account the optimal conditions for these works to ensure the highest possible planting success.

Networking researchers from different fields, agriculture, landscape, biology and others will lead to diverse projects, perspectives, expertise, approaches and solutions, essential for biodiversity in food, agriculture, quality of life and for conserving food plants and will also include their use in our natural landscapes.

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MISCELLANEOUS

EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SUGAR-FREE CONCENTRATED PRODUCTS FROM *ARONIA MELANOCARPA* FRUITS, APPLES AND CARROTS WITH ANTIOXIDANT POTENTIAL FOR PEOPLES WITH DIABETES AND OBESITY

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Abstract

Diabetes mellitus is the most prevalent endocrine disease in the world and is likely to be the major epidemic in human history. The main causes of death due to complications of diabetes are cardiovascular disease and stroke. Due to their complex biochemical composition, especially their high polyphenol content and antioxidant potential, Aronia melanocarpa fruits have many therapeutic properties, such as the ability to fight hyperglycemia-induced oxidative stress and the macrovascular complications of diabetes, including cardiovascular disease. The aim of this work was to obtain of sugar-free concentrated products from Aronia melanocarpa fruits, apples, and carrots with high nutritional value and antioxidant capacity for the prevention and diet therapy of diabetes. These products were analyzed and characterized qualitatively (sensory, physico-chemical and microbiological). The sugar-free concentrated products are characterized by their vitamin C content (24.55-27.28 mg/100g), total polyphenol content (235.48-252.65 mg GAE/ 100 g), potassium (159.65-193.45 mg/100 g), calcium (39.35-55.67 mg/100 g), magnesium (18.85-29.74 mg/100 g), iron (1.85-2.23 mg/100 g) and zinc content (1.16-1.35 mg/100 g). Also, the sugar-free concentrated products have antioxidant capacity. These products are destined for diabetics, obesity and peoples who want to maintain their weight.

Key words: *Aronia melanocarpa, apple, carrot, diabetes, sugar-free products.*

INTRODUCTION

Diabetes mellitus (DM) is among the most prevalent chronic diseases in the world, as stated by the International Diabetes Federation (IDF). In 2017, there were approximately 451 million diagnosed patients and 5 million deaths worldwide were related to DM (Cho et al., 2018). DM refer to a group of metabolic disorders characterized by chronic hyperglycemia, resulting from impaired insulin production by pancreatic β-cells and/or insulin resistance by peripheral tissues (Goyal & Jialal, 2018). Genetic, environmental and lifestyle (diet and exercise) factors are among the different causes that can promote or prevent DM development. Moreover, oxidative stress has been strongly associated with DM pathogenesis and the progression of its comorbidities (Fernández-Ochoa et al., 2020).

In diabetes, diet has a therapeutic role being adapted to the metabolic needs of each individual.

The development of sugar-free products, but with appropriate sensory characteristics, without changing the glycemic balance of diabetic patients, is of real interest, both for engineers from food industry and for nutritionists (Catană et al., 2019).

Black chokeberry (*Aronia melnocarpa*) is a source of many bioactive compounds with a wide spectrum of health-promoting properties. Fresh fruits are rarely consumed due to their astringent taste, but they are used in the food industry for the production of juices, nectars, syrups, jams, wines, tinctures, fruit desserts, jellies, fruit teas and dietary supplements. Polyphenols are components that determine the high bioactivity of chokeberries, some of the richest sources of polyphenols, which include anthocyanins, proanthocyanidins, flavonols and phenolic acids (Sidor and Gramza-Michałowska, 2020). *Aronia melanocarpa* fruits have antioxidant, anti-inflammatory and anti-aging effects because their fruits have very high content of anthocyanins. Jeon et al. (2018) studied the effect of *Aronia melanocarpa* fruits on type 1 diabetes *in vivo* and *in vitro.*

The authors reported that these fruits decrease the blood glucose level when it's high and also has the protection effect of pancreas β cell. This study confirms the anti-diabetic effects of *Aronia* berry and is of real interest to increase its utilization in diet therapy and the treatment of diabetes. Mu et al. (2020) studied the influence of *Aronia melanocarpa* berry extract on hepatic insulin resistance in type 2 diabetes mellitus rats. The results of this study indicated that extract from the berries of *Aronia melanocarpa,* exert hypoglycemic effects in animal models of diabetes. So, it reduced levels of blood glucose, improved glucose tolerance, increased hepatic glycogen content, and regulated activity of enzymes involved in glucose metabolism.

The aim of this work was to obtain sugar-free concentrated products from *Aronia melanocarpa* fruits, apples, and carrots with high nutritional value and antioxidant capacity for the prevention and diet therapy of diabetes.

MATERIALS AND METHODS

Samples

The fruits of *Aronia melanocarpa*, which were provided by University of Agronomic Sciences and Veterinary Medicine of Bucharest (Faculty of Horticulture). *Jonathan* variety apples and carrots, were purchased from the market.

As sweetening agent, a mixture of *Stevia rebaudiana* extract and erythritol, allowed in diabetic diet, was used.

The *Sea Buckthorn* juice was used as acidifier, but also for the vitamin C and β-Carotene fortification of sugar-free jams. Low methoxyl pectin, was used to gel the sugar-free jams (the total soluble solids = 15-45°Brix).

The achieved products were sensory, physico-chemical and microbiologically analysed (Figures 1 and 2).

Figure 1. Sugar-free concentrated product from *Aronia* and apples (left: general appearance; right: product appearance)

Figure 2. Sugar-free concentrated product from *Aronia,* apples and carrots (left: general appearance; right: product appearance)

Methods

Statistical Analysis

The sugar-free concentrated products were analyzed in triplicate. Mean and standard deviation are reported for each analytical parameter studied.

Sensory analysis

Sensory analysis (appearance, colour, taste and flavor) was performed by descriptive method and by *"Comparison method with unitary score scales"* method. Sensory quality of the fortified product was established based on the total average score by comparison with a scale from 0 to 20 points (18.1-20 - "very good"; 15.1-18 - "good"; 11.1-15 - "satisfactory"; 7.1-11 - "unsatisfactory"; 0-7 **-** "inadequate").

Measurement of the colour parameters (*L**, *a** and *b**) of samples was performed using a CM-5 colorimeter (Konica Minolta, Japan) and SpectraMagic NX software.

The textural properties (firmness, cohesiveness, adhesiveness and gumminess) of the sugar-free concentrated products were performed using an Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) and Bluehill 3.13 software.

Physico-chemical analysis
Total soluble solids were performed with refractometer. Chemical composition was determined by AOAC Methods: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content).
The samples were mineralized by calcination. The minerals potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe) were determined by atomic absorption spectrophotometry. Phosphorus was determined by spectrophotometric method (McKie & Mccleary, 2016).
The crude fibre content of the samples was determined by gravimetric method (Fibretherm-Gerhardt equipment).
Determination of vitamins C and B content was performed by HPLC-HRMS (Asănică et al., 2019).
Determination of β-carotene content was performed by liquid chromatography with diode array detection (Catană et al., 2020).

Total polyphenol content
Total polyphenol content was performed by Folin-Ciocalteau method according to Horszwald and Andlauer (2011) using UV-VIS Jasco V 550 spectrophotometer, at a wavelenght λ = 755 nm. The calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The polyphenol extraction solvent was a mixture of methanol and water (methanol: water = 1: 1). Total polyphenol content was expressed as mg of Gallic Acid Equivalents (GAE) per 100 g product.

Antioxidant capacity
Antioxidant capacity was performed by DPPH (1,1diphenyl-2-picryl hydrazyl) radical scavenging assay, according to Horszwald and Andlauer (2011),) using UV-VIS Jasco V 550 spectrophotometer, at a wavelenght λ = 517 nm. The calibration curve of Trolox achieved in the concentration range 0-0.4375 mmol/L. Antioxidant capacity was expressed as μmol Trolox Equivalents per g product.

Microbiological analysis
For the microbiological analysis of canned food SR 8924:1995 was used. Canned food was analized for *Enterobacteriaceae* presence according to SR EN ISO 21528-2:2017 method, *Escherichia coli* according to SR ISO 16649-2:2007 method and *Salmonella* according with method decribed in SR EN ISO 6579-1:2017.

RESULTS AND DISCUSSIONS

Sensory analysis
Sensory analysis revealed that the sugar-free concentrated products from *Aronia* and apples are well-gelled, it has a reddish-brown color in which purple *Aronia* fruits are distinguished. Also, Sensory analysis revealed that the sugar-free concentrated product from *Aronia,* apples and carrots are well-gelled, has a reddish-brown color with purple Aronia fruits and orange carrot noodles.
Colour instrumental analysis (Figure 3) revealed that sugar-free concentrated product from *Aronia* and apples is the darkest, recording the minimum value of luminance (L* = 10.97), and that obtained from *Aronia,* apples and carrots is the lightest (L* = 12.18).

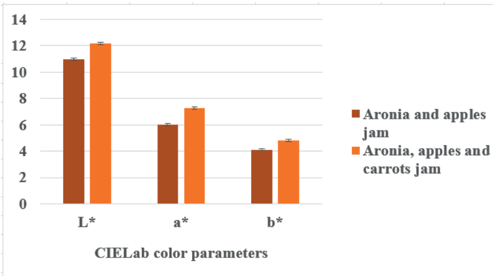

Figure 3. Colour parameters of the sugar-free concentrated products from *Aronia,* apples and carrots (the data are presented as mean ± standard deviation)

Also, the maximum positive value of parameters a* (red colour coordinate) and b* (yellow colour coordinate) were recorded for the sugar-free concentrated products from *Aronia,* apples and carrots (a* = 7.27; b*= 4.8). Following the sensory evaluation, using "*Comparison method with unitary score scale*", the sugar-free concentrated products obtained "*very good*" qualifying, recording the following scores: 19.68 points (*Aronia* and apples jam) and 19.84 points (*Aronia,* apples and carrots jam) (Figure 4).

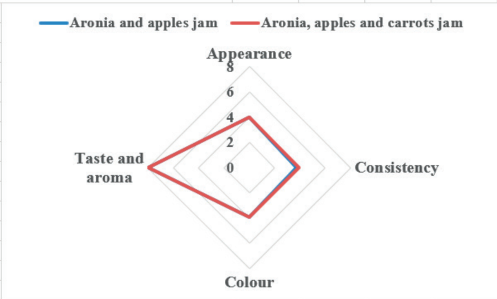

Figure 4. Sensory evaluation of the sugar-free concentrated products from *Aronia,* apples and carrots

The textural properties (firmness, cohesiveness, adhesiveness, gumminess) of the sugar-free concentrated products are presented (as mean± standard deviation) in Table 1 and the compression curves in Figures 5 and 6.

Table1. Texture properties of the sugar-free concentrated products from *Aronia,* apples and carrots (the data are presented as mean ± standard deviation)

Sugar-free concentrated product	Firmness (N)	Cohesiveness	Adhesiveness (N)	Gumminess (N)
Aronia and apples jam	0.21±0.01	0.70±0.02	3.29±0.10	0.16±0.02
Aronia, apples and carrots jam	0.32±0.02	0.83±0.06	5.15±0.65	0.27±0.03

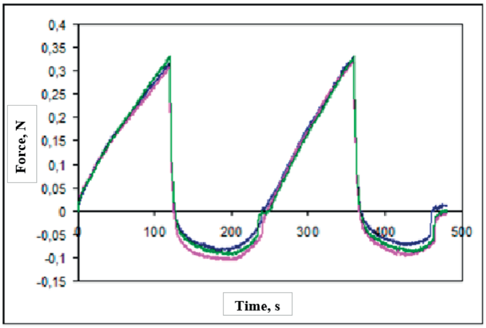

Figure 5. Compression curves of the sugar-free concentrated product from *Aronia* and apples

The sugar-free concentrated product from *Aronia,* apples and carrots had the highest values of firmness (0.32N), cohesiveness (0.83), adhesiveness (5.15) and gumminess (0.27), compared to that of the product achieved from *Aronia* and apples.

The firmness of the products (pectic gel formation) was ensured by the native pectic substances from *Aronia*, apples and carrots, by the optimal acidity (0.75-0.77 g citric acid/ 100 g) and by the addition of low methoxyl pectin.

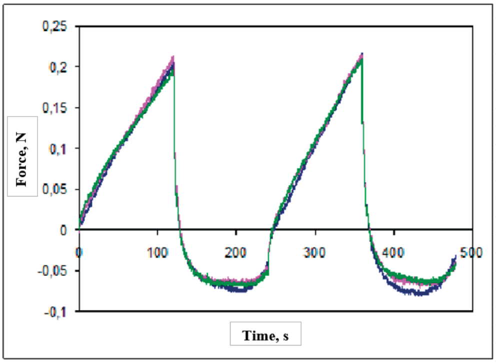

Figure 6. Compression curves of the sugar-free concentrated product from *Aronia,* apples and carrots

To obtain the compression curve (Force = f (time)) for each sample, three determinations were made. The curves obtained had a high accuracy (they were almos overlapping).

Physico-chemical analysis

Composition of the sugar-free concentrated product from *Aronia,* apples and carrots is presented (as mean ± standard deviation) in Table 2. Physico-chemical parameters of the *Aronia,* apples and carrots jam have higher values compared to those of the *Aronia* and apples jam.

Table 2. Physico-chemical composition of the sugar-free concentrated products from *Aronia,* apples and carrots

Parameters	Sugar-free concentrated product	
	***Aronia* and apples jam**	***Aronia,* apples and carrots jam**
Total soluble solids (°Brix)	30.8±0.02	32.6±0.02
Acidity (g citric acid/100g)	0.75±0.01	0.77±0.01
Total sugar (%)	6.08±0.02	6.87±0.03
Protein (%)	1.21±0.01	1.39±0.01
Fat (%)	0.49±0.006	0.54±0.008
Crude fibers (%)	2.82±0.05	3.23±0.06
Ash (%)	1.25±0.012	1.44±0.014
Pectic substances (% calcium pectate)	3.45±0.10	3.90±0.11

The total soluble solids (TSS) content of sugar-free concentrated products in Aronia, apples and carrots was higher than that reported by Sutwal et al. (2019) in the case of low-calorie apple jam (natural sweetener Stevia - concentration 0.6%; TSS = 23.30°Brix), but

comparable to that reported by Catană et al. (2019) in the case of sugar-free jams from artichoke tubers and apples (sweeteners *Stevia rebaudiana* and erythritol; TSS = 31.5-32.5°Brix).
Acidity content of the sugar-free concentrated products from *Aronia,* apples and carrots is higher than those reported by Catană et al. (2019) in case of sugar-free jams from Jerusalem artichoke tubers and apples (Acidity = 0.67-0.70%) and by Sutwal et al. (2019) in the case of low-calorie apple jam (Acidity = 0.57%). The total sugar content of the sugar-free concentrated products from *Aronia,* apples and carrots achieved in this experimental study is comparable to that reported by Catană et al. (2019) in case of sugar-free jams from artichoke tubers and apples from Jerusalem artichoke tubers and apples. Also, this chemical parameter is about 2.6 times lower than that obtained by Sutwal et al. (2019) in the case of low-calorie apple jam (Total sugar = 16.64%).
The low sugar content of the products is ensured by the raw materials (*Aronia* and apples, *Aronia,* apples and carrots, respectively) and the natural sweetener used.
Proteins content of the sugar-free concentrated products achieved in this study recorded lower values about 2 times in the case of proteins compared to those obtained by Perumpuli et al. (2019) for hypoglucidic Beetroot (*Beta vulgaris* L.) jam (Protein = 2.6 ± 0.03%). Also, crude fibres content is comparable to that reported by this authors (Crude fibres = 3.5 ± 0.04%).
Also, the sugar-free concentrated products achieved in this study, stand out through their content in pectic substances (*Aronia* and apples jam = 3.45% calcium pectate; *Aronia,* apples and carrots jam = 3.90% calcium pectate).
Pectins have many beneficial effects on the body: immunomodulatory, anti-inflammatory, antitussive, anticoagulant, anticancer, gastro-protective, antidiabetic, hypolipidemic, antioxidant effects. The effect of pectin is due to its good gelation, non-toxicity, high stability, biocompatibility, bioavailability (Zaitseva et al., 2020).
The sugar-free concentrated products from *Aronia,* apples and carrots have a high content of minerals (K, Ca, Mg, P, Zn and Fe) (Figures 7 and 8). Mineral content of the sugar-free concentrated product from *Aronia,* apples and carrots is higher than that recorded for the product obtained from *Aronia* and apples.
Sugar-free jams were noted for their potassium content (159.65 ± 13.25 mg/100 g for *Aronia* and apples jam; 193.45 ± 16.06 mg/100 g for *Aronia,* apples and carrots jam), calcium content (39.35 ± 4.06 mg/100 g for *Aronia* and apples jam; 55.67 ± 5.74 mg/100 g for *Aronia,* apples and carrots jam) and their magnesium content 18.85 ± 2.99 mg/100 g for *Aronia* and apples jam; 29.74 ± 4.71 mg/100 g for *Aronia,* apples and carrots jam).

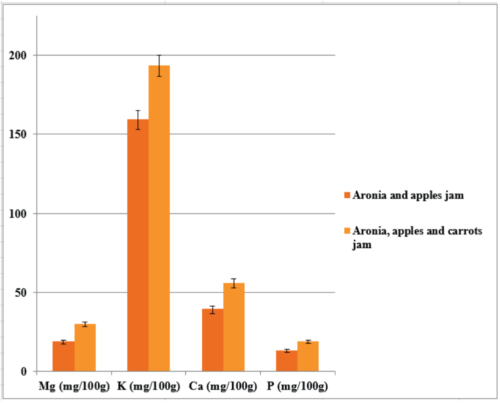

Figure 7. Mineral content (K, Ca, Mg and P) of the sugar-free concentrated products from *Aronia,* apples and carrots (the data are presented as mean ± standard deviation)

The potassium content of the sugar-free concentrated products is 1.6-1.9 times smaller than that reported by Catană et al. (2019) in case of sugar-free jams from Jerusalem artichoke tubers and apples and 7-8 times higher than that obtained by Perumpuli et al. (2019) for hypoglucidic Beetroot (*Beta vulgaris* L.) jam (22.378 ± 0.232 mg/100 g).
These values of the potassium content can be explained by the high potassium content of raw materials used in experiments (*Aronia melanocarpa* fruits, apples and carrots) compared to those of Beetroot (*Beta vulgaris* L.).
Adequate potassium intake can reduce blood pressure and the risk of kidney stones and osteoporosis (Freitas, 2017).
The Dietary Guidelines Advisory Committee (DGAC, 2010) recommended a potassium intake, ideally up to 4700 mg per day for adults.

The sugar-free jams recorded an iron content of 1.85 ± 0.056 mg/100 g for *Aronia* and apples jam and 2.23 ± 0.067 mg/100 g for *Aronia,* apples and carrots, respectively. Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour et al., 2014).
The zinc content of the products was lower than their iron content.

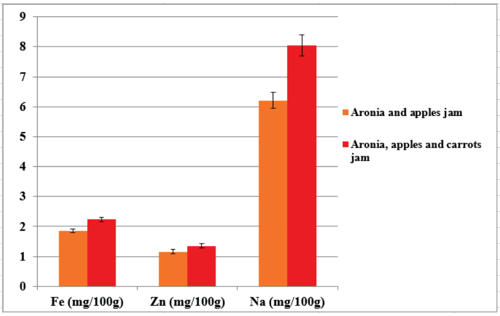

Figure 8. Mineral content (Fe, Zn, and Na) of the sugar-free concentrated products from *Aronia,* apples and carrots (the data are presented as mean ± standard deviation)

The sodium content of the sugar-free concentrated products was low (6.21 ± 0.268 mg/100 g for *Aronia* and apples jam and 8.05 ± 0.347 mg/100 g for *Aronia,* apples and carrots, respectively). Zn is an important element of the immune system. Also, Bashandy et al. (2016) showed that the protective effect of zinc can be attributed to its antioxidant and anti-inflammatory properties.

Bioactive compounds content
Sugar-free concentrated products are sources of bioactive compounds: total polyphenols, vitamin C and β-Carotene (Table 3).
The total polyphenol content of products is 1.9-2.2 times higher than that reported by Catană et al. (2019) in case of sugar-free jams from Jerusalem artichoke tubers and apples.

Many studies showed the role of phenolic compounds in the reduction of cardiovascular diseases risk factors. Thus, phenolic compounds may be considered natural inhibitors of platelet aggregation, contributing to reducing the individual risk of developing cardiovascular diseases which causes thrombosis (Lutz et al., 2019).
Also, the vitamin C content of these products is 3.5-3.9 times higher than that obtained by Sutwal et al. (2019) for sugar-free apple jam (Vitamin C = 6.90 mg/100 g). These differences can be explained by fortifying the product with *Sea Buckthorn* juice and also by the fact that for the concentration of these products, short cooking times (3-4 minutes) and long diffusion times (30-40 minutes) were applied.
Vitamin C is an important antioxidant. It functions as a redox buffer which can reduce, and thereby neutralize, reactive oxygen species.

Table 3. Bioactive compounds content of the sugar-free concentrated products from *Aronia,* apples and carrots

Bioactive compounds	**Sugar-free concentrated product**	
	***Aronia* and apples jam**	***Aronia*, apples and carrots jam**
Total polyphenols (mg GAE/100g)	235.48±5.42	252.65±5.81
Vitamin C (mg/100g)	27.28±0.68	24.55±0.61
Vitamin B5(mg/kg)	1.853±0.019	1.924±0.019
Vitamin B6 (mg/kg)	0.683±0.006	0.695±0.006
β-Carotene (mg/100g)	0.125±0.002	2.432±0.039

It is a cofactor for enzymes involved in regulating photosynthesis, hormone biosynthesis, and regenerating other antioxidants; which also regulates cell division and growth, is involved in signal transduction, and has roles in several physiological processes, such as immune stimulation, synthesis of collagen, hormones, neurotransmitters, and iron absorption, has also roles in detoxifying the body of heavy metals (Pehlivan, 2017). Also, vitamin C stimulates the production and activation of immune cells, so perhaps supplementation could be used to improve the immunity of the cancer patients (Gorkom et al., 2019).
The vitamin B content (vitamin B5 and vitamin B6) of sugar-free concentrated products is low.
The *Aronia,* apples and carrots jam it stands out by β-Carotene content (2.432 ± 0.039 mg/ 100 g).
Carotenoids play an important role in disease prevention caused by oxidative stress, due to their antioxidant activity (Queiroz et al., 2020).
International studies show that the beneficial effect of carotenoids on the human body is due to their ability to neutralize free radicals. (Goyal, & Jialal, 2015).

Antioxidant capacity

Due to their content in antioxidants (total polyphenols, vitamin C, β-Carotene etc.) sugar-free concentrated products from *Aronia,* apples and carrots have antioxidant capacity.

The antioxidant capacity of *Aronia* and apples jam is 27.4 μmol Trolox Equivalents/g and 31.12 μmol Trolox Equivalents/g, for *Aronia,* apples and carrots jam, respectively.

Antioxidant capacity of sugar-free jams from *Aronia,* apples and carrots is similar with that reported by Abolila et al. (2015) for hypoglucidic orange jams sweetened with various sweeteners (fructose, stevioside and sucralose), which varied in the next range: 17.63-39.15 μmol Trolox Equivalents/g

Microbiological analysis

Results of the microbiological analysis of the sugar-free concentrated products from *Aronia,* apples and carrots are presented in Table 4.

Table 4. Microbiological analysis of the sugar-free concentrated products from *Aronia,* apples and carrots

Microbiological indicators	**Sugar-free concentrated product**	
	***Aronia* and apples jam**	***Aronia*, apples and carrots jam**
Yeast and mold (CFU/g)	Absent	Absent
Salmonella (CFU/25 g)	Absent	Absent
Enterobacteriaceae (CFU/g)	< 10	< 10
Escherichia coli (CFU/g)	< 10	< 10
Total viable count (CFU/g)	< 10	< 10

After incubation at 37°C for 14 days, the products did not show any changes of outer part of the jars and/or leakage of content, odour and/or other changes caused by a microbial activity. Microbiological analysis shown that the products are within the legislation into force. At the same time, on the basis of microbiological and sensory analysis, the shelf life of the products was established (18 months). Sugar-free products should be stored in a dark, dry, cool places at a maximum temperature of 25°C. After opening, the products should be stored at 2-8°C and consumed within 12 days.

CONCLUSIONS

The sugar-free concentrated products from *Aronia,* apples and carrots achieved in this experimental study using as sweeteners *Stevia rebaudiana* and erythritol had superior sensory characteristics (appearance, colour, flavor, taste and texture) like the jams obtained with sugar.

The sugar-free products obtained within this study are important sources of bioactive compounds (polyphenols, vitamin C, β-Carotene) and minerals (K, P, Fe, Mg, Ca).

Also, these products are noted by total polyphenol content (235.48 ± 5.42 mg GAE/100 g for *Aronia* and apples jam; 252.65 ± 5.81 mg GAE/100 g for *Aronia,* apples and carrots jam), vitamin C content (27.28 ± 0.68 mg/100 g for *Aronia* and apples jam; 24.55 ± 0.61 mg/100 g for *Aronia,* apples and carrots jam) and β-Carotene (2.432 ± 0.039 mg/100 g for *Aronia,* apples and carrots jam).

The sugar-free concentrated products from *Aronia,* apples and carrots have antioxidant capacity and their consumption in a healthy diet can prevent diseases caused by oxidative stress.

The sugar-free concentrated products from *Aronia,* apples and carrots presented in this paper can be successfully introduced in the diet of diabetics, obese and people who want to maintain their weight.

ACKNOWLEDGEMENTS

This work was achieved through Core Programme (PN 19 02), supported by the Ministry of Education, Research and Innovation, contract 22N/2019, project no. PN 19 02 02 01.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

BIOCHEMICAL COMPOSITION AND ANTIOXIDANT CAPACITY OF A FUNCTIONAL INGREDIENT OBTAINED FROM ELDERBERRY (*SAMBUCUS NIGRA* L.) POMACE

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Abstract

The elderberry (Sambucus nigra L.) is cultivated worldwide for the production of fruits and flowers. The elderberry fruits are not consumed fresh but are mainly processed into longer shelf-life food products, such as juice, concentrates, syrups, jams, jellies, colorants, and wines. In this paper are presented the results of the research performed to achieve a functional ingredient (powder) from elderberry (Sambucus nigra L.) pomace resulting in the fruit juice industry. For this purpose, Aronia pomace (from the conventional and organic culture of our country) was subjected to a convective drying process at 50 °C or lyophilisation at -55 °C to protect the bioactive compounds to moisture content to allow their milling and turning them into powder and their stability in terms of quality. Powder achieved from elderberry pomace is characterized by total dietary fiber content (46.28-50.50%), minerals (3.13- 3.50%), total sugar (9.73-12.37%), pectic substances (6.88-8.32%), vitamin C (5.21-16.14 mg/100 g), vitamin E (1.50- 3.05 mg/100 g), and total polyphenols (97.84-146.76 mg GAE/g). At the same time, powder achieved from elderberry pomace has antioxidant capacity. Due to its complex biochemical composition and antioxidant potential, the functional ingredient achieved from elderberry pomace can be used to fortify bakery and pastry products.

***Key words**: dietary fiber elderberry, pomace, powder, total polyphenols.*

INTRODUCTION

European black elderberry is grown in Europe and in many parts of the world, for fruits and flowers production. Elderberry fruits are rich in nutrients such as carbohydrates, proteins, lipids/fatty acids, organic acids, minerals, vitamins, but also essential oils.

Polyphenols are bioactive compounds known for their antiradical activity, which are found in high concentrations in elderberry fruits. The biochemical composition of elderberry fruits depends on the variety, cultivation area, climatic conditions and their maturation degree (Młynarczyk et al., 2018).

The carbohydrates in elderberry, according with recent research results, contains 7.86-11.50% sugar of total sugar and 2.8-8.55% of reducing sugar. According to Veberic et al. (2009), the total sugar content of elderberry fruits varied between 68.53 and 104.16 g/kg fresh substance (f.s.), depending on the variety and cultivation area.

The main sugars identified were glucose (33.33-50.23 g/kg f.s.) and fructose (33.99-52.25 g/kg f.s.), sucrose being present only in low concentrations in fruits (0.47-1.68 g/kg f.s.).

Carbohydrates found in elderberry fruits (*Sambucus nigra* L.) also include dietary fiber, especially pectin, pectic acid, protopectin, calcium pectate and cellulose. Elderberry is an important source of proteins: 2.7-2.9% in fruits, 2.5% in flowers and 3.3% in leaves. Proteins include sixteen amino acids, nine of which are essential amino acids.

The total content of essential amino acids in elderberry is 9% in flowers and 11.5% in

leaves. Glutamic acid, aspartic acid and alanine have been reported as the main amino acids.
Fats are mostly accumulated in elderberry fruit seeds (fat content: 22.4%) and in elderberry seed flour (fat content: 15.9%). The organic acid content of elderberry fruits is 1.0-1.3%. Veberic et al. (2009) detected four organic acids in elderberry fruits: citric acid (3.08-4.81 g/kg f.s.), malic acid (0.97-31.31 g/kg f.s.), shikimic acid (0.14-0.93 g/kg f.s.) and fumaric acid (0.10-0.29 g/kg f.s.).
The mineral elements are located in both fruits and flowers. The mineral content of elderberry fruits represents 0.90-1.55% of their mass. According to Vulić et al. (2008) the mineral content of the elderberry is: Potassium 391.33 mg/100 g; Phosphorus 54.00 mg/100 g; Ca 28.06 mg/100 g; Magnesium 25.99 mg/100 g; Iron 1.86 mg/100 g; Zinc 0.36 mg/100 g; Manganese 0.27 mg/100 g; Copper 0.14 mg/ 100 g.
Several studies have shown the beneficial effects of elderberry, used in antiviral drugs (Zakay-Rones et al., 1995; Roschek et al., 2009; Barak et al., 2019) efficient supplements in the treatment and prevention of diabetes, cardiovascular diseases, and cancer (Ciocoiu et al., 2009; Jing, et al., 2008). Thus, various nutraceutical *Sambucus nigra* berries products (syrups, drops, tablets, emulsions, suspensions, extracts) are commercially available (Młynarczyk et al., 2018; Barak et al., 2019).
Sambucus nigra fruits are not consumed fresh but are mainly processed into longer shelf-life food products, such as juice, concentrates, syrups, jams, jellies, colorants, and wines (Kaack et al., 2008; Schmitzer et al., 2012). In the case of solid juice processing by-products amounts 20-40% of total elderberry biomass (Seabra et al., 2010). Data on global production of elderberry pomace is not available. Syrups and beverages are indicated as important elderberry products (e.g., the share of beverages was 17.5% in 2017). So, the processing of elderberry fruits generates large amounts of pomace (Technavio, 2019).
The aim of this work was to obtain a functional ingredient (powder) from elderberry (*Sambucus nigra* L.) pomace, a by-product of fruits juice processing.

MATERIALS AND METHODS

Samples

The elderberry (*Sambucus nigra* L.) pomace resulted by processing the elderberry fruits with a juicer extractor (Philips) in the laboratory at IBA Bucharest.
Elderberry fruits used in the study come conventional (II – *Ina* variety) and bio (I- *Nora* variety and III- *Bradet* variety) culture. Till processing, elderberry pomace was stored under refrigeration conditions (at 3°C), for a maximum of 36 hours. Fresh pomace was subjected to hot-air dehydration (at 50°C) or freeze-drying (at -55°C) and then grinding and turned into powder.
The elderberry pomace samples I and III were hot-air dehydrated and freeze-drying. Sample II was not lyophilized, it was only hot-air dehydration.
The milling of the dry semi-finished products was performed using the Retsch mill, at a temperature of 20-22°C, to ensure the quality parameters of the final product.
Figure 1 shows elderberry pomace powders obtained by hot-air dehydration (at 50°C), and Figure 2 those obtained by freeze-drying (at -55°C)

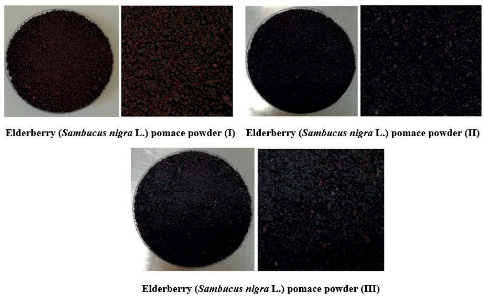

Figure 1. Elderberry pomace powder
(hot-air dehydration at 50°C)

Figure 2. Elderberry pomace powder
(freeze-drying at -55°C)

Methods

Statistical Analysis

Elderberry pomace powder samples were analyzed in triplicate. Mean and standard deviation are reported for each analytical parameter studied.

Sensory analysis

Sensory analysis (appearance, taste and smell) was performed by descriptive method.

Physico-chemical analysis

Measurement of the colour parameters (L^*, a^* and b^*) of samples was performed using a CM-5 colorimeter (Konica Minolta, Japan) and SpectraMagic NX software.

The water content was determined by infrared moisture balance (Ohaus Moisture Analyzer MB45), a temperature of 105°C.

Chemical composition was determined by AOAC Methods: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content).

The samples were mineralized by calcination. The minerals (potassium, calcium, magnesium and iron were determined by atomic absorption spectrophotometry. Phosphorus was determined by spectrophotometric method (McKie & Mccleary, 2016).

Schoorl method was used to establish the total sugar content.

Enzymatic method using the assay kits: K-TDFR ‘‘Total dietary fibre’’ (AOAC Method 991.43) was used for determining the total dietary fibre (TDF). Gravimetric method was used for determining pectic substances (expressed as calcium pectate).

Determination of vitamin C content was performed by by HPLC-HRMS (Catană et al., 2017).

Determination of vitamin E (α-tocopherol) content was performed by liquid chromatography with diode array detection (Popović et al., 2015).

Total polyphenol content

Total polyphenol content was performed by using Folin-Ciocalteau method according to Horszwald and Andlauer (2011) by means of UV-VIS Jasco V 550 spectrophotometer, at a wavelenght λ = 755 nm. The calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The polyphenol extraction solvent was a mixture of methanol and water (methanol: water = 1: 1). Total polyphenol content was expressed as g of Gallic Acid Equivalents (GAE) per 100 g elderberry pomace powder.

Antioxidant capacity

Antioxidant capacity was performed by DPPH (1,1diphenyl-2-picryl hydrazyl) radical scavenging assay, according to Horszwald and Andlauer (2011), using UV-VIS Jasco V 550 spectrophotometer, at a wavelenght λ = 517 nm. The calibration curve of Trolox achieved in the concentration range 0-0.4375 mmol/L. Antioxidant capacity was expressed as mg Trolox Equivalents per g elderberry pomace powder.

Microbiological analysis

Aquaspector AQS-2-TC, Nagy, equipment was used to establish the water activity (*A*w). The measurements were performed at 25ºC. SR ISO 21527-1:2009 method was used to check for the yeasts and moulds. SR EN ISO 21528-1:2017 method was used to check for the presence of *Enterobacteriaceae.* SR ISO 16649-2:2007 method was used to check for the presence of *Escherichia coli*. The method SR EN ISO 6579-1:2017 was used to determine the *Salmonella* presence.

RESULTS AND DISCUSSIONS

Sensory analysis

As a result of the sensory analysis, it was found that the powders achieved from elderberry pomace, have colours from dark cherry to dark brown and, respectively, dark and have pleasant taste and smell, characteristic of elderberry fruits.

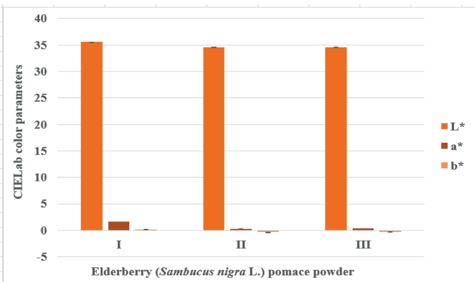

Figure 3. Colour parameters of the powders achieved from elderberry pomace (hot-air dehydration at 50°C)

As a result of the instrumental color analysis, it was found that the powders obtained from elderberry pomace by freeze-drying at -50°C, are lighter in color compared to those obtained by dehydration at 50°C, registering higher luminance values, respectively, L * = 39.75, in the case of sample I (Figures 3, 4).

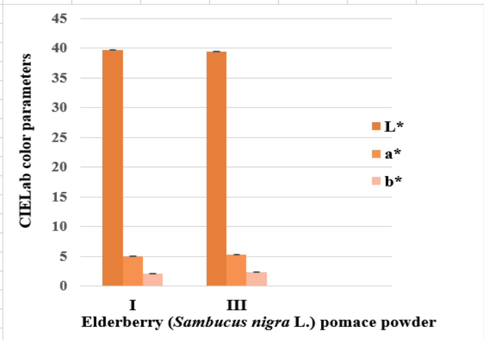

Figure 4. Colour parameters of the powders achieved from elderberry pomace (freeze-drying at -55°C)

Also, the maximum values of the parameters a* (red colour coordinate) and b* (yellow colour coordinate) were recorded for powders obtained from elderberry pomace, by freeze-drying at
-55°C (a* = 5.35 and b* = 2.35, values recorded in the case of sample III).

Physico-chemical analysis

The physico-chemical indicators of the powders obtained from elderberry pomace are presented in Table 1.

Table 1. The physico-chemical indicators of powders achieved from elderberry pomace

Functional ingredient	Water (%)	Ash (%)	Protein (%)	Fat (%)	Total sugar (%)	Total fibre (%)
Dehydration with hot air at 50°C						
Sample powder I	5.03±0.13	3.23±0.04	10.23±0.09	19.18±0.21	10.48±0.052	49.75±0.92
Sample powder II	5.44±0.14	3.13±0.04	11.01±0.10	18.47±0.20	12.37±0.062	47.50±0.88
Sample powder III	4.96±0.14	3.42±0.05	10.31±0.09	19.44±0.21	9.73±0.049	46.28±0.86
Lyophilization at - 55°C						
Sample powder I	3.42±0.09	3.30±0.04	10.47±0.09	19.55±0.21	10.71±0.053	50.50±0.93
Sample powder III	3.85±0.10	3.50±0.04	10.51±0.09	19.74±0.22	9.88±0.049	46.82±0.87

Sample II was not lyophilized, it was only hot-air dehydration. The results show that these powders are distinguished by their content in protein (10.23-11.01%), fat (18.47-19.74%), total ash (3.13-3.50%), total sugar (9.73-12.37%) and total fiber (46.28-50.5%). Water content of the powders obtained from elderberry pomace in this study, is lower compared to that of the elderberry pomace (residual moisture content 6.2%) achieved by Kitrytė et al. (2020).

Powders achieved from elderberry pomace (sample III) has the highest total ash content (3.42% in the case of powder obtained by dehydration at 50°C and 3.50% in the case of that obtained by freeze-drying at -55°C), fat (19.74% in the case of powder achieved by freeze-drying, at -55°C). The powder from elderberry pomace (sample II), by dehydration at 50°C, had the highest content of total sugar (12.37%), protein (11.01%). Also, the powders from elderberry pomace (sample I) has the highest total fiber content (49.75% for the powder obtained by hot-air dehydration at 50°C and 50.50% in the case of that obtained by freeze-drying at -55°C).

The elderberry pomace powders had a high content of pectic substances in the following range: 6.88-8.32 % pectate of calcium (Table 2).

The content in pectic substances of the powders achieved from elderberry pomace, within this study is like that reported by Sidor and Michałowska (7.52%), in case of *Aronia melanocarpa* powder.

Table 2. Content in pectic substances of powders achieved from elderberry pomace

Functional ingredient	Pectic substances (% calcium pectate)
Dehydration with hot air at 50°C	
Sample powder I	7.85±0.20
Sample powder II	6.88±0.17
Sample powder III	7.97±0.20
Lyophilization at - 55°C	
Sample powder I	8.15±0.20
Sample powder III	8.32±0.21

Several studies showed that pectic substances are physiologically active substances with immunomodulating properties and anti-inflammatory activity, with the capacity to lower cholesterol and triglyceride in the blood serum, normalize glucose metabolism, bind and remove toxins and radionuclides from the body. Also, pectin polysaccharides can provide protection for the gastrointestinal tract and have anticarcinogenic and antimetastatic effects.

Powders achieved from elderberry pomace, are important sources of minerals (K, Ca, Mg, Fe, Zn). The mineral content of the elderberry pomace powders obtained by hot-air dehydration at 50°C is shown in Figures 5 and 6.

Elderberry pomace powder from sample III, had the highest potassium content (548.85 mg/100 g).

The calcium content of the elderberry pomace powders was lower than that of potassium and varied in the range of 180.25 - 220.42 mg/100 g (the maximum value being recorded in the case of sample III and the minimum value, in the case of sample II).

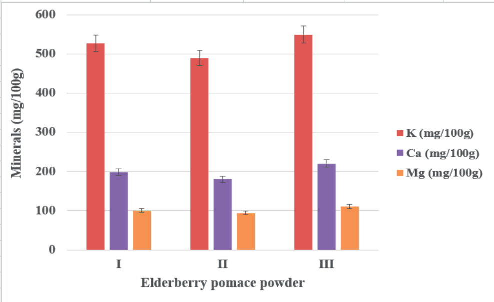

Figure 5. Mineral content (Na, K, Ca, and Mg) of the powders achieved from elderberry pomace (hot-air dehydration at 50°C)

The magnesium content of the powders was lower than that of calcium, representing about 50.21-52.19% of their calcium content.

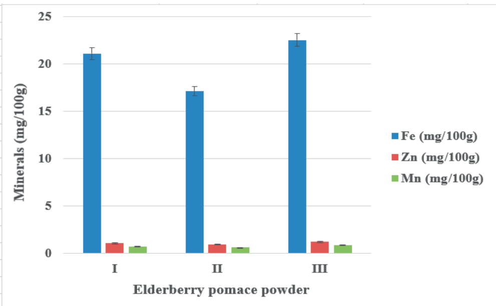

Figure 6. Mineral content (Fe, Zn, and Cu) of the powders achieved from elderberry pomace (hot-air dehydration at 50°C)

The elderberry pomace powders differed by their iron content, which varied in the range 17.17-22.56 mg/100 g (the maximum value being recorded in the case of sample III and the minimum value, in the case of sample II).

The zinc and manganese content of the elderberry pomace powders recorded low values, in the range 0.64-1.25 mg/100 g.

Content in calcium and iron of elderberry pomace powders achieved by dehydration at 50°C, is higher compared to that of the elderberry pomace achieved by Różyło et al. (2019) (calcium: 177 mg/100 g; iron: 4.78 mg/100 g), and the potassium and magnesium content is lower.

The mineral content of powders (samples I and III) obtained by lyophilization at -55°C is similar to that of powders obtained by dehydration at 50°C (Table 3). Sample II was not lyophilized, it was only hot-air dehydration. Very small differences can be explained by the lower water content of the powders obtained by lyophilization.

Table 3. Mineral content of the powders achieved from elderberry pomace (lyophilisation at -55°C)

Minerals	Powders achieved from elderberry pomace	
	Sample powder I	Sample powder III
K (mg/100g)	535.10 ±18.73	555.26±19.43
Ca (mg/100g)	201.48±7.05	222.99±7.80
Mg (mg/100g)	101.95±3.57	111.96±3.92
Fe (mg/100g)	21.51±0.65	22.82±0.68
Zn (mg/100g)	1.11±0.06	1.29±0.07
Mn (mg/100g)	0.74±0.02	0.91±0.03

Bioactive compounds content

The powders obtained in this study were noted for their content in bioactive compounds: total polyphenols, vitamin C and vitamin E (α-tocoferol) (Table 4). Sample II was not lyophilized, it was only hot-air dehydration.

Table 4. Bioactive compounds content of the powders achieved from elderberry pomace

Functional ingredient	Total polyphenols (g GAE/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)
Dehydration with hot air at 50°C			
Sample powder I	10.36±0.26	7.84±0.26	1.62±0.09
Sample powder II	10.09±0.25	5.21±0.17	1.50±0.08
Sample powder III	9.78±0.24	9.22±0.30	1.85±0.10
Lyophilization at - 55°C			
Sample powder I	13.99±0.35	12.39±0.41	2.62±0.14
Sample powder III	14.68±0.23	16.14±0.53	3.05±0.17

The total polyphenol content of the elderberry pomace powders was very high and varied in the range 9.78-14.68 g GAE /100 g.

Also, the elderberry pomace powders are distinguished by their vitamin C (5.21-16.14 mg/100 g) and vitamin E (α-tocopherol: 1.50-3.05 mg/100 g).

Elderberry pomace powders obtained by lyophilization at -55°C recorded a higher retention of bioactive compounds, compared to those obtained by dehydration at 50°C. Thus, their content in vitamin C was 1.58-1.75 times higher and that of total polyphenols 1.35-1.50 times higher, respectively).

Total polyphenol content of the elderberry pomace powders obtained by lyophilisation at -55°C is higher compared to that of the elderberry pomace achieved by Tańska et al. (2016) (13.86 g GAE/100 g D.M.)

Antioxidant capacity

The high antioxidant content (total polyphenols, vitamin C, vitamin E etc.), of the powders obtained in this study, gives them antioxidant capacity (Figure 7).

The highest values of antioxidant capacity were recorded in the case of powders obtained by lyophilization at -55°C: 11.45 mg Trolox Equivalents/g (sample I) and, respectively, 11.95 mg Trolox Equivalents/g (sample III).

The powders obtained by dehydration at 50°C recorded lower values of antioxidant capacity in the range 5.90-6.57 mg Trolox Equivalents/g (the minimum value being recorded in the case of sample III, and the maximum in the case of sample I).

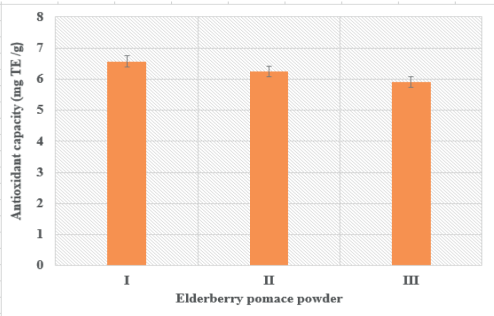

Figure 7. Antioxidant capacity of the powders achieved from elderberry pomace (hot-air dehydration at 50°C)

Microbiological analysis

The microbiological indicators of the elderberry pomace powders are presented in Table 5. Sample II was not lyophilized, it was only hot-air dehydration.

Microbiological analysis shown that the powders are within the legislation into force.

Also, these powders recorded low values of water activity, in the range of 0.285-0.305, which gives them microbiological stability.

Table 5. The microbiological indicators of powders achieved from elderberry pomace

Functional ingredient	Yeast and mold (CFU/g)	*Enterobacteriaceae* (CFU/g)	*Escherichia coli* (CFU/g)	*Salmonella* (in 25 g)	Water activity
Dehydration with hot air at 50°C					
Sample powder I	< 10	< 10	< 10	absent	0.300
Sample powder II	< 10	< 10	< 10	absent	0.305
Sample powder III	< 10	< 10	< 10	absent	0.294
Lyophilization at - 55°C					
Sample powder I	< 10	< 10	< 10	absent	0.267
Sample powder III	< 10	< 10	< 10	absent	0.285

CONCLUSIONS

This study reports valorization of elderberry (*Sambucus nigra* L.) pomace, a by-product of fruits juice processing.

Powders achieved from elderberry pomace are distinguished by their content in minerals (K, Fe, Mg, Ca and Zn), total dietary fibres, pectic substances and bioactive compounds (total polyphenols, vitamin C, vitamin E).

The process of lyophilization of elderberry pomace at -55°C C ensures a higher retention of bioactive compounds compared to hot-air dehydration at 50°C.

Due to its high content of bioactive compounds, these powders have antioxidant capacity (5.90-11.95 mg Trolox Equivalents/g) and their consumption is beneficial in a healthy diet to prevent diseases caused by oxidative stress.

Due to its high nutritional value, bioactive compounds content and antioxidant potential, the powders achieved from elderberry pomace are functional ingredients.

An important practical application of these functional ingredients is the fortification of food products (especially for bakery and pastry products), in order to increase the nutritional value, the content of bioactive compounds, dietary fiber and antioxidant potential.

ACKNOWLEDGEMENTS

This work was achieved through Core Programme (PN 19 02), supported by the Ministry of Education and Research and Innovation, contract 22N/2019, project no. PN 19 02 02 03.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECT OF ENTOMOPATHOGENIC FUNGI (*BEAUVERIA BASSIANA*) ON SOIL NUTRIENTS CONTENT

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Abstract

The sustainable management of agroecosystems and its resistance to pests in the context of climate change strongly depend on soil health. The soil is a living and dynamic ecosystem, maintaining nutrient cycling, providing water, oxygen, nutrients, and root support for crop plants, controlling plant diseases, insect and weed pests. One of the eco-friendly alternatives for sustainable production is the use of entomopathogenic fungi, providing multiple ecosystem services, as plant protection, promotion of plant growth, improving the nutrient uptake etc. Entomopathogenic fungi are also considered as indicators of soil health. In the present study we aim to evaluate the effect of inoculants based on Beauveria bassiana on micro- and macroelements content and soil mycoflora, in an organic tomato crop, in Buzău, România. The soil samples were taken in 2020, from 0-20 cm depth, and analyzed microbiologically and for nutrients content. The results showed that fungi inoculation has a variable, minor, influence on nutrients accumulation on short term, but higher impact on soil fungi communities. Further studies are required to establish the implications on medium and long term.

***Key words**: entomopathogenic fungi, microbial inoculants, Beauveria bassiana, nutrients content, organic soil,*

INTRODUCTION

Soil is one of the most vulnerable resources that we have, but also one of the most precious. According to FAO, 2015, the soil delivers ecosystem services that enables life on Earth, as provision of food, fibre and fuels, provision of construction materials, nutrient cycling, water purification and soil contaminant reduction, source of pharmaceuticals and genetic resources, habitat for organisms, climate regulation, carbon sequestration, flood regulation, foundation for human infrastructures, cultural heritage etc. Unsustainable intensification of agriculture, including crop, livestock and forest-based systems has negatively impacted environmental and human health. While the need of increasing food production and its nutritional quality exists, this must be done without affecting the environmental health of the agroecosystems. The soil biota is particularly important in maintaining soil functions. Sustainable alternatives to synthetic fertilizers and pesticides are one of the means.

The use of microorganisms, especially entomopathogenic fungi (EPF) as biological control, is a practice that has been used more and more in recent years, to protect soil health. In natural environments, entomopathogenic fungi occur in soils and are a very important soil health indicator. They also play a role in nutrient cycling, plant growth, nutrient uptake, yield, in controlling pests and disease, plant adaptation to biotic (due to insect herbivores, plant pathogens, or parasitic nematodes), and abiotic stresses (drought or mechanical damage), interact with plant rhizosphere colonizers and biofertilizers, they are important also for environmentally friendly agriculture and sustainable management of agroecosystems (Senicovscaia 2012; Espinoza et al., 2019; Barra-Bucarei et al., 2020; Blanco-Pérez et al., 2020; González-Guzmán et al., 2020; Rajula et al., 2020; Alves et al., 2021; Li et al.,2021; Sharma et al., 2021). In soils with high organic matter content, a high biological activity and presence of antagonistic organisms can lead to a reducing EPF content, but a higher diversity and density of insects in soils with high organic matter content, implies a higher diversity of hosts for EPF having a positive effect. Also, the carbon: nitrogen-ratio can influence the EPF in soils (Uzman et al., 2019). These fungi can produce phytohormones (auxins) that improve water transport, are involved in the solubilization of phosphate, potassium, and siderophores production, activeting biologic protection mechanisms and inducing systemic resistance to phytopathogens. *B. bassiana* colonise the soil or plants as a saprophyte or an endophyte. *B. caledonica* can produce organic acids, such as oxalic and citric acids and *B. bassiana* formic, lactic, orotic, oxalic and citric acids. These organic acids change the pH of the medium and inorganic phosphorus is released. *Beauveria bassiana* also produce siderophores, which play an important role against the cellular stress caused by iron deficiency, iron being necessary for fungal cell growth and metabolism (Raya-Díaz et al., 2017; Barra-Bucarei et al., 2020; Harith-Fadzilah et al., 2021).

It is necessary to understand soil properties' relationship with EPF, soil ecology and diversity of EPF and their relationship with the plants.

Sharma et al., 2021 showed that soils that have a high content of organic matter have a higher biological activity having as results the increased EPF antagonists. For example, calcium ions promote *B. bassiana* and inhibit *Metarhizium robertsii,* another very important EPF in biocontrol, while soils with higher acidity inhibit *B. bassiana*, and higher C:N, and magnesium ions inhibit *M. robertsii* (Sánchez-Rodríguez et al., 2015; Sharma et al., 2021). Fungal microbes influence significantly plant tissue macrontrients like calcium and magnesium (Moloinyane & Nchu, 2019).

The tomato crop has great importance worldwide for its nutritional composition and its antioxidant content. Barra-Bucarei et al., 2020 demonstrated that the greatest plant heights and biomass in the tomato crop was obtained after treatments with *B. bassiana*. Besides its activity in growth promotion, this fungus also provides important protection levels against whiteflies, one of the most spead tomato pests.

Sánchez-Rodríguez et al., 2015 showed that *B. bassiana* influences the availability of microelements as Fe, Cu, and Ag when it is directly applied to a metallic surface. On tomato and wheat crops the fungus can install, but without a negative effect on height, plant dry weight, root development in tomato or grain production in wheat. In both crops, *B.bassiana* had a positive role in preventing chlorosis, but the effect was influenced by the Fe availability. Further, a modification in plant nutrition with macroelements, mainly K, was found in wheat and tomato crops. González-Guzmán et al., 2020 showed that wheat crops inoculated with *B. bassiana* have an increase yield, being positively correlated with soil available phosphorus and with small content of Fe oxides. The increase in aerial dry matter was negatively correlated with soil available Zn in crops inoculated with *B. bassiana*, the fungi enhancing grain Zn uptake.

Purchase et al., 2009 showed that *Beauveria bassiana* grown in modified glycerol asparagine medium containing elevated levels of Zn and Pb at 30°C, accumulated up to 0.64% of available Zn and 8.44% of Pb. They also found that metal tolerance was not affected by a decrease in temperature and the mechanism of resistance in *B. bassiana* can be related to the precipitation of Pb (possibly in the form of oxalates).

Considering all the above-mentioned interrelations between EPF and chemical elements, the main objective of the present study was to elucidate the effects of *B. bassiana* on micro and macroelements accumulation in an experimental plot cultivated with tomatoes.

MATERIALS AND METHODS

Soil preparation

The soil samples were taken from topsoil (0-20 cm) in June in 2020, from two fields of an experimental plot planted with tomato, from Buzău, Romania. One field was inoculated in 2019 and the second one in 2020, respecting crop rotation scheme, the first one passing the winter in the normal conditions. The experimental variants were the following: uncultivated soil - control (C), soil cultivated with tomato, but without *B. bassiana* inoculation - NBb and soil cultivated with tomato, and treated with *B. bassiana* - Bb. Sample's preparation, physico-chemical and microbiological analyses have been performed in the Research centre for study of food quality and agricultural products, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Analysis of soil micro- and macroelements

Dried milled, homogenized, and sieved through a 250 µm soil samples were used for microwave-assisted digestion. 0.1 g soil and Suprapur solvents: 65% HNO_3 and 37% HCl were used. Elemental analysis of the samples with ICP-MS was performed, according to Dobrin et al. (2020), using an Agilent 7700x ICP-MS instrument with Mass Hunter 4.3 Workstation software for ICP-MS, version C.01.03 (Agilent Technologies, Tokyo, Japan). A single and multi-element ICP-MS calibration standard was used for the calibration curve between 0-8 µg/l for microelements and 0-800 µg/l for macroelements.

An amount of 5-10 mg of sample was used for quantification of total soil nitrogen using CHNS elemental analyser (EA3100 Elemental Analyzer). Cystine was used as standard reference material. All determinations were performed in three repetitions.

Microbial Analysis

The count of the bacteria and fungi was carried out by spread plate technique. This was done by inoculating 0.5 ml of diluted samples on PDA suitable for microbiology from Scharlau, prepared according to manufacturers' instruction. The agar plates were sealed with Parafilm® M then incubated at room temperature (28 ± 2 °C) for 24–48 h. Observed colonies were counted and expressed as colony forming units (CFUs) per gram of soil (cfug^{-1}) (Ameh & Kawo, 2017; Enuneku et al., 2020).

Statistical analysis

All the results for the effects of *Beauveria bassiana* on soil macro and microelements, and for bacteria and fungi count were expressed as the mean values. For statistic results we used Statistical package IBM SPSS Statistics for Windows (Version 27.0, SPSS, Ireland), using Duncan's multiple range test at significance level P≤0.05 (5%)

RESULTS AND DISCUSSIONS

Macroelements

Nitrogen content of the soil it is an important parameter that can indicate the availability and abundance of fungal species (Hallouti et al., 2020)

Regarding the influence of *B. bassiana* on distribution and accumulation of nitrogen (N) in soil, there were no significant differences between soils inoculated in 2019 and those treated in 2020 and also we didn't find any statistical differences between control, without inoculation and with inoculation experimental variants (Figure 1).

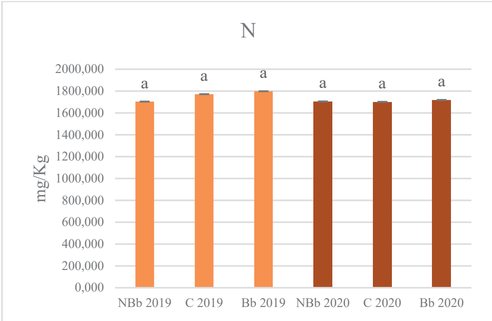

Figure 1. Effect of *B bassiana* on N soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

In the plot treated in 2019 we found that between untreated variants and the one inoculated with *B. bassiana* there are not statistical differences regarding phosphorus (P) content in soil, but in 2020 we found an increase by 639.922 mg/Kg in treated variant compared to untreated variant and by 430.723 mg/Kg compared to control variant in 2020 (Figure 2). Our results are in accordance with Alves et al., 2021 who also find that the *B. bassiana* application increased soil P.

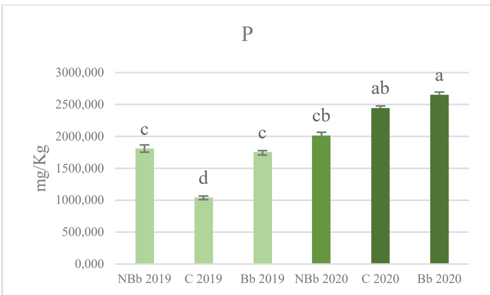

Figure 2. Effect of *B bassiana* on P soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Potassium (K) content in soil from inoculated variants had no significant variation in 2020 compared with those treated in 2019 and with untreated variants from 2019, but in 2020 it was observed an increase in K content in inoculated variants compared with control and untreated variants (Figure 3).

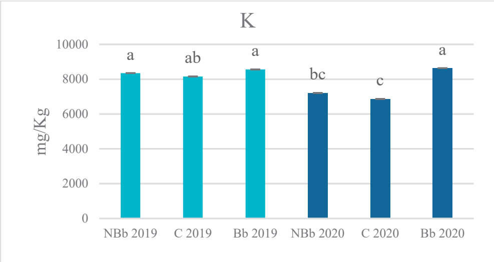

Figure 3. Effect of *B bassiana* on K soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Like K, sodium (Na), also did not had an increase in 2020 in inoculated experiments compared with those from untreated variants from 2019. In 2020 we found that there were not statistical differences between inoculated variants and control variants. Na content had a similar evolution with magnesium (Mg), in 2019 having higher values than in 2020 (Figure 4).

Figure 4. Effect of *B bassiana* on Na soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Mg is an important element for the process of photosynthesis, being a building block for chlorophyll. It also activates certain plant enzymes involved in plants growth and contributes to protein synthesis and secondary metabolite production (Moloinyane & Nchu 2019; Uzman et al., 2019). Mg had a similar accumulation in soil like Na. No statistical differences between control, untreated and treated variants for the 2020 experimental plot were found. For 2019 inoculated plots, a significant difference was found between the uncultivated control and the plots cultivated with tomatoes, regardless of their *B. bassiana* amendment (Figure 5).

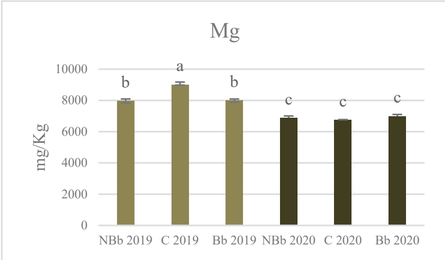

Figure 5. Effect on *B bassiana* on Mg soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Calcium (Ca) is an important element for the development of new plant tissues. It strengthens cell walls and promotes cell elongation. It has also a role in plant protection against pathogenic fungi and bacteria (Moloinyane & Nchu 2019). Ca had a similar evolution with N without significant differences between inoculated variants, control and untreated ones. Also we don't find differences between 2019 and 2020 experimental years regarding control and inoculated variants (Figure 6).

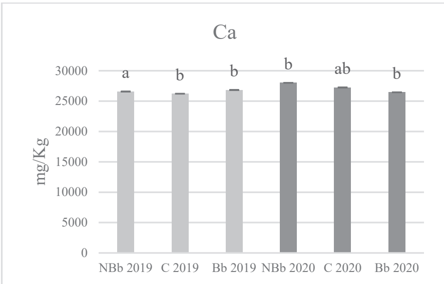

Figure 6. Effect of *B bassiana* on Ca soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Microelements

The analysed microelements are iron, copper, zinc, manganese, molybdenum, chromium, and cobalt. Iron content have also the same accumulation in experimental variants like Ca, with no differences between years and experimental variants (Figure 7).

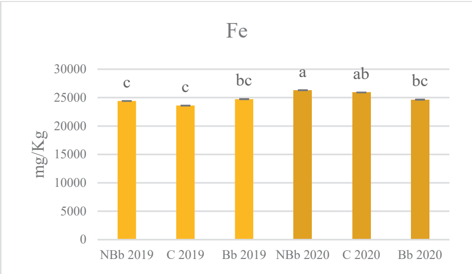

Figure 7. Effect on *B bassiana* on Fe soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

For the other analysed microelements, as Cu, Zn, Mg, Mo, Cr, Co, (Table 1), the following discussions could be made: for Cu there is difference between inoculation years, but not between experimental variants, following the same trend like Mg, higher content in 2019 than in 2020; Zn content in 2019 hadn't a significant difference between treated and untreated variant, but in 2020 the inoculated sol had the highest content, similar to P; Mn and Mo content had no statistically assured variations; Cr content was higher in treated variants compared with control and untreated variants in both years.

Table 1. Effects of *B.bassiana* treatments on microelements content in soil. Data are means ± SE. Values with different letters are significantly different according to Duncan's multiple range test (P≤0.05)

		Cu (mg/Kg)	Zn (mg/Kg)	Mn (mg/Kg)	Mo (mg/Kg)	Cr (mg/Kg)	Co (mg/Kg)
2019	NBb	39.673±0.542 b	99.720±3.436 ab	717.427±7.940 bc	2.866±0.088 a	51.417±2.145 ab	11.468±0.427 bc
	C	46.199±0.665 a	83.837±3.323 b	698.384±4.359 c	2.753±0.062 a	54.991±1.829 a	12.747±0.291 a
	Bb	41.388±0.931 b	98.846±2.354 ab	729.133±7.167 abc	2.719±0.070 a	52.483±1.004 ab	11.561±0.097 b
2020	NBb	33.668±0.931 c	93.524±3.473 ab	757.681±9.524 a	2.887±0.111 a	46.935±0.315 a	11.060±0.338 bcd
	C	32.058±0.809 c	85.633±1.206 b	739.272±10.779 ab	2.860±0.046 a	46.659±0.604 a	10.593±0.237 cd
	Bb	34.415±0.684 c	113.556±3.969 a	717.009±11.896 bc	3.051±0.164 a	50.759±0.869 b	10.319±0.260 d

Regarding the soil biota, in soil samples from 2020 inoculated plot, both the number of bacteria and fungi from inoculated variants were higher than in control ones, and higher compared with soil from 2019 plots (table 2). The total bacterial counts are in accordance with Ameh & Kawo, 2017, that found 5.94 x 10^6 cfu g^{-1} in untreated soils, and Jaskulska et al., 2020 found that bacteria CFUs ranging between 4.3-12.9 × 10^6 cfu g^{-1} soil, and for fungi ranging between 26.3-35.0 × 10^4cfu g^{-1}.

Table 2. Effect of inoculation treatments on total bacteria and fungi (cfu/g) in soil. Data are means ± SE. Values with different letters are significantly different according to Duncan's multiple range test (P≤0.05)

	Bacterial count (10^6 cfu g^{-1})		Fungi counts (10^4 cfu g^{-1})	
	2019	**2020**	**2019**	**2020**
NBb	5.651±0.070 b	6.962± 0.238 ab	8.679 ±0.450 c	21.858±1.859 b
C	8.245±0.579 a	5.778±0.487 b	6.195±0.605 c	12.079±0.269 c
Bb	4.579±0.077 b	6.468± 0.138 ab	6.889±0.110 c	42.945±0.995 a

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CONCLUSIONS

B. bassiana had an influence on P, Na and Zn. Also, the highest CFU of fungi was found in the inoculated variants, in both years. These soil indicators can be used to predict the soil microbial quality based on soil chemical analysis, useful for integrated pest management, in the context of sustainable agriculture.

Further investigations are necessary to understand the ecology of the EPF, the relationships of bacteria and fungi with the habitat, as well as among coexisting species. Further studies are foreseen on other beneficial soil microbes.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0301/ 28PCCDI, within PNCDI III.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTS OF MELATONIN ON ROOT KNOT NEMATODE: *IN SITU* ESTIMATION OF PHYSIOLOGICAL RESPONSES IN TOMATO

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Abstract

This research was conducted in 2020 to investigate the influence of melatonin (10, 50 and 100 µM) given in three methods (immersion, irrigation and foliar spraying), on some physiological aspects of tomato seedlings exposed to root knot nematodes. The seedlings were inoculated with 1000 infective juveniles of Meloidogyne incognita [(Kofoid and White) Chitwood]. Dualex® optic sensor was used to in situ measure total chlorophylls, flavonols and anthocyanins contents and nitrogen balance index (NBI). Results indicated that while no significant effects were observed on chlorophyll content, melatonin ameliorated the adverse effects of M. incognita on chlorophyll depending on the concentration and mode. Flavonols were at the highest in the irrigated plants and the lowest in the immersed ones, between 06-1.1, exhibiting a significant difference. NBI was affected by the method the melatonin was applied, and immersing boosted it, while irrigation caused a significant decrease. Irrigation resulted in significantly higher anthocyanins compared to the other two methods. One observation was that applying melatonin in the low and medium concentrations to the nematode containing soil increased the anthocyanins. Melatonin merits a value in developing a response against the nematode but needs further elucidation.

Key words: *anthocyanins, chlorophyll, flavonoids, Meloidogyne incognita, NBI.*

INTRODUCTION

Pathogen attacks are one of the growth- and quality-limiting factors plants face throughout their lives. Ameliorating or completely evading these stress factors rely on plant's innate resistance capacity as well as the favourable support from environment. Nematodes, especially root knot nematodes (RKN) *Meloidogyne* spp., are one of the major life-threatening pests that use hundreds of plants as hosts. Four predominant species of *Meloidogyne* species are *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*. Among the symptoms plants indicate after infection are chlorophyll loss in leaves, nutrient insufficiency resulting in growth loss and if persists, plant death. Due to damage in vascular tissues of root, water and nutrients can fail to move through the plant (Ralmi et al., 2016).

Despite that RKNs could be managed with the use of chemical nematicides, increasing crop production in an ecologically friendly manner is both a necessity and a challenge in a world with increasing awareness in human and environment health (Collange et al., 2011). In the recent years, melatonin, a naturally existing compound in plants, has attracted an interest in boosting sustainable crop production due to its beneficial effect on human health and environment (Reiter et al., 2015; Qiao et al., 2019). Melatonin functions as an antioxidant against reactive oxygen and nitrogen species (Moustafa-Farag et al., 2020).

Recent years in melatonin research have focused on its effects on plant growth and development along with its protective role against abiotic stress factors. Readers have an opportunity to follow some excellent reviews on the subject (Sharif et al., 2018; Yu et al., 2018). However, its effects on helping plants cope with biotic stress factors have not been explored in as much detail. Yin et al. (2013), Wei et al. (2017), Zhang S. et al. (2017), Aghdam et al. (2017) and Liu et al. (2019) have presented improving effects of melatonin against fungal pathogens in apple, banana, potato, strawberry, and tomato, respectively. It was reported that protection comes from increase in defence gene expression, scavenging reactive oxygen species, raise in nitric oxide production and cell wall thickening (Shi et al., 2015; Wei et al., 2018; Zhao et al., 2019). However, to our best knowledge, there is not a study that involves influence of melatonin against root knot nematode attacks on plants.

Early assessment of stress symptoms is possible with non-destructive monitoring of crops using optical or thermal sensors. *In situ* and *de novo* changes in the physiological aspects of plant development can enable to have the results quickly and periodically with ease (Padilla et al., 2014). Among these sensors is Dualex® (Orsay, France), a leaf-clip chlorophyll-meter which estimates chlorophyll (CHL) and leaf epidermal flavonoids as well as anthocyanins using CHL fluorescence screening method (Agati et al., 2016). Non-destructive optical tools use spatial and temporal dimensions to assess adaptability levels of plants under stress conditions (Barnes et al., 2015). Dualex® have been used for many abiotic stress related studies for early detection of symptoms, for instance, heat (Zhou et al., 2017), chilling (Oustric et al., 2017) and UV (dos S. Nascimento et al., 2020). In more recent years, it has been also utilized for grapevine leaf stripe disease (Di Gennaro, 2016), root-knot nematodes in eggplant (Silva-Sánchez et al., 2019), reniform nematode in cotton (Singh et al., 2020) and wheat stripe rust (Emebiri et al., 2020).

This study was planned to assess the effects of melatonin given through immersion, irrigation, and spraying on some physiological characteristics measured with the Dualex® of tomato plants exposed to *M. incognita.*

MATERIALS AND METHODS

Seedlings of commercially processing tomato cultivar (H2274; *Lycopersicon esculentum* L.) with 3-4 established leaves were used as the plant material. Seedlings were separated in three groups according to the method the melatonin solutions were given: root-immersion, root-irrigation, and foliar spraying. Melatonin (Mel) obtained from Merck (M2250) was prepared in three concentrations (10, 50 and 100 μM). The trial also had one negative control (only distilled water), and one positive control (distilled water plus nematode inoculation). Mel was applied to the seedlings with and without nematode inoculation of 1000 infective juvenile stage 2 (IJ_2) per ml of *Meloidogyne incognita* reproduced from a single egg mass. Seedlings were planted in 1.4-liter plastic pots containing sterilized soil and sand mixture (approx. 450 g). Pots later were placed in a growth chamber under the conditions of 26 ± 1°C and 18/6 hours day/night light periods. Plants were only irrigated on the basis of need at fixed amount (50 ml).

Root-immersion: After the growth medium around the roots of the seedlings were cleared under tap water, the roots were tap dried with a paper towel and they were kept in the melatonin solutions for 10 minutes. Control groups were kept in the distilled water for the same duration.

Root-irrigation: The seedlings were irrigated with the melatonin solutions at an amount of 10 ml/pot. Control groups were irrigated with 10 ml distilled water. Second (20 ml/pot) and third (40 ml/pot) applications were done 1 week apart.

Foliar-spraying: After planting in the pots, the leaves of the seedlings were sprayed with 10 ml Mel solutions until the runoff. Control groups were sprayed with distilled water. Second (20 ml/pot) and third (40 ml/pot) applications were done 1 week apart.

Optical measurements

At the end of 8 weeks (on the 56th day), optical measurements with the Dualex Scientific+ Chlorophyll and Polyphenol-Meter (Force-A, Centre Universitaire Paris-Sud, France) were performed on the third youngest leaf. The portable meter Dualex® allowed simultaneous readings from the abaxial and adaxial surface of the leaves and provided a mean value for each reading. Three readings per leaf away from the midrib were made. Features measured were chlorophyll (μg per cm^2), relative absorbance units of flavonols (0 to 3) and anthocyanins (0 to 1.5), and the nitrogen balance index, determined by the relationship between chlorophyll and flavonols.

Statistical analysis

The experiment was arranged in a completely randomized design with three replications. The data of chlorophyll, flavonols, anthocyanins and nitrogen balance index (NBI) were analyzed and corresponding graphs were plotted R version 4.0.2 (2020-06-22). There were 6 levels of Mel concentrations with and without nematode application, 2 control groups and 3 levels of mode. Every treatment consisted of three replicates of one plant per pot.

RESULTS AND DISCUSSIONS

Melatonin given in three modes (i.e., root-immersion, root-irrigation and foliar-spraying) resulted different responses in chlorophyll, flavanols, anthocyanins and nitrogen balance index in tomato plants inoculated with *Meloidogyne incognita.*

Although chlorophyll amounts were not affected by none of the treatments (Figure 1), generally immersed plants had more CHL than the irrigated or sprayed ones. Comparing with the negative controls showed that nematode addition caused 40-50% decrease in the positive control plants.

Treating plants with Mel resulted in as much CHL, depending on the concentration, as in the DW treated ones. It was observed that Mel in the nematode inoculated plants ameliorated the adverse effects on CHL depending on the concentration and type of administration, for instance immersing in 100 μM Mel or spraying with 50 μM Mel, respectively, provided increased levels of CHL.

It is believed that primary site for melatonin production is chloroplasts (Martinez et al., 2018) and Weeda et al. (2014) stated that melatonin can act as a protectant of CHL content. Wang et al. (2013) also reported decreased levels of chlorophyll-degrading enzyme, pheide-a-oxygenase with melatonin. Zhang et al. (2014) expressed that melatonin, when applied exogenously, protected CHL in the apple leaves exposed to abiotic stress. *Malus hupehensis* Rehd seedlings had les chlorophyll degradation when root-treated with melatonin (Tan et al., 2007a). Yin et al. (2013) showed that apple plants irrigated with melatonin had comparably close contents of chlorophyll to the control plants when infected with Marssonina apple blotch. Sun et al. (2019) indicated that melatonin treated cucumber against downy mildew had increased levels of chlorophyll. Similar activity was observed in this research where tomato plants exposed to nematode damage.

Plants respond to stress factors by synthesizing flavonols (Brunetti et al., 2013). R analysis indicated that the mode had a significant influence on flavonols in the tomato plants (Figure 2a). They were the highest in the irrigated plants, ranging from 0.9-1.5 and the lowest in the immersed ones, between 06-1.1. Flavonoids are formed when plants are exposed to pests (Brunetti et al., 2013). Bali et al (2018) stated that flavonoid contents raised in the seedlings of tomato plants treated with jasmonic acid against root knot nematode. Although no significant effects of melatonin were detected in the present study, how it is received by the intact plants showed a clear importance. One possible reason for spraying to have had the lowest amount of flavonols might be the anatomical structure of the leaves (i.e. the trichomes and undulation on the surface), owing to the studies have shown that flavonoids are placed in epidermal layers or in the cuticle of leaves (Tattini et al., 2004), which might have been a barrier for the solution to infuse,

In the current study, NBI level was affected by the method melatonin was applied to the plants, and immersing boosted it while irrigation caused a significant decrease (Figure 2b). It is considered a general acceptance that plant nitrogen status of a plants can be estimated through chlorophyll and flavonoid contents (Agati et al., 2016). Because chlorophyll is incorporated in chlorophyll (Evans et al., 2001), and flavonoids contents act oppositely to N contents in the plant, the ratio of CHL to flavonols is shown to be a more sensitive indication of plant nitrogen status (Longchamps and Khosla, 2014; Padilla et al., 2014). Kautz et al. (2014) indicated that under saline conditions the tomato leaves had elevated levels of NBI. Sun et al. (2019) reported increased levels of enzymes in N metabolism in cucumber seedlings treated with melatonin against *Pseudoperonospora cubensis*. The plots of both CHL and NBI show that root-immersion and spraying were better at abating the adverse effects of *M. incognita*. Scientific evidence indicates that growth and development in plants are closely regulated by auxin and melatonin sharing a precursor with auxin might aid same processes (Arnao and Hernandez-Ruiz, 2007). Direct contact with

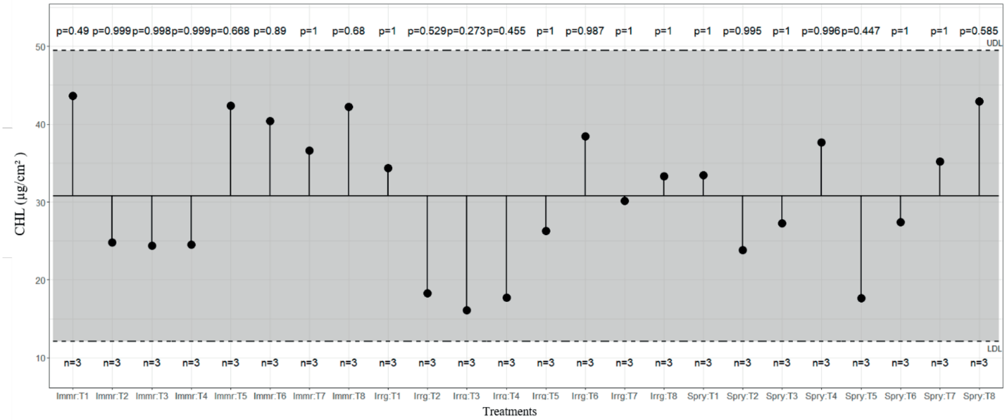
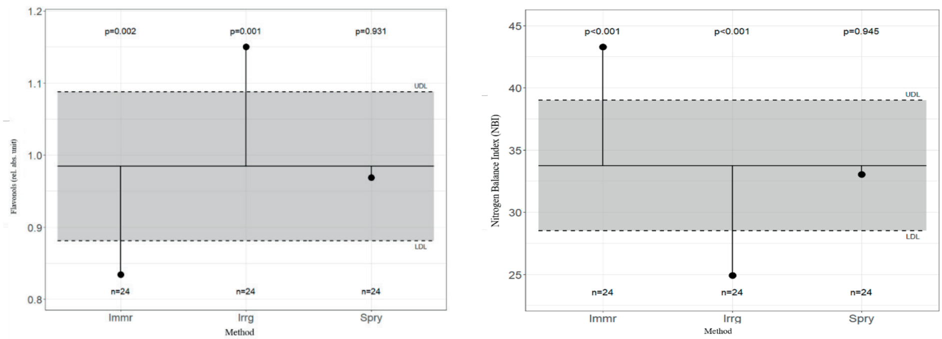


Figure 1. Effects of melatonin given in three methods to the tomato plants exposed to *Meloidogyne incognita* on chlorophyll content. Abbreviations; imm: immersion, irr: irrigation, spr: foliar spraying, T1: distilled water, T2: distilled water+ nematode, T3: 10 µM mel + nematode, T4 50 µM mel + nematode, T5: 100 µM mel + nematode, T6: 10 µM mel, T7: 50 µM mel, T8: 100 µM mel

melatonin in the roots through immersion for 10 min. might have induce level of auxin and improved root activity (Chen et al., 2009) and efficiency in nitrogen uptake and metabolism (Zhang R. et al., 2017).

Anthocyanins were affected both by the treatments and the methods they were given to the plants (Figure 2c, d). Irrigation resulted in significantly higher anthocyanins compared to the other two (Figure 2c). Plants having nematode but no melatonin (T2) produced the highest amounts of anthocyanins (Figure 2d). The rest of the treatments fell in the same group showing similar values. One observation was that applying Mel in the low and medium concentrations to the nematode containing soil increased the anthocyanins. On the other hand, Mel alone seemed not supportive of the anthocyanin production. Although response of anthocyanin accumulation under stress conditions differed in plants, melatonin appeared to have an increasing effect on nematode-inoculated tomato in the current study. Similar observation was confirmed by Zhang et al. (2016) on cabbage seeds. Bali et al. (2018) reported increased levels of anthocyanins in the tomato plants after jasmonic acid application, indicating that signalling molecules have a stimulating effect on antioxidative defence system.



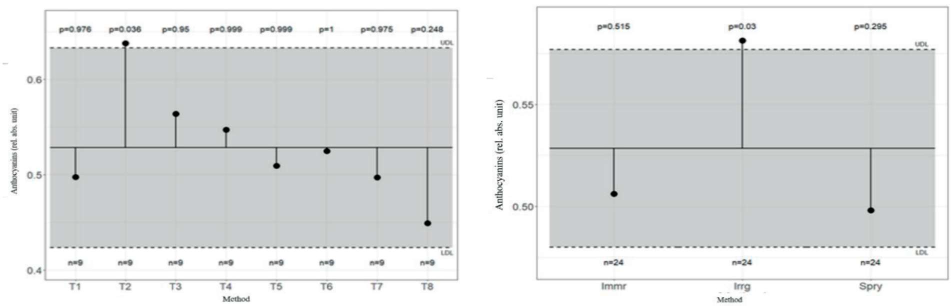


Figure 2. Effects of melatonin given in three methods to the tomato plants exposed to *Meloidogyne incognita* on flavonols (a), NBI (b), and anthocyanins (c and d). Abbreviations; imm: immersion, irr: irrigation, spr: foliar spraying, T1: distilled water, T2: distilled water+ nematode, T3: 10 µM mel + nematode, T4 50 µM mel + nematode, T5: 100 µM mel + nematode, T6: 10 µM mel, T7: 50 µM mel, T8: 100 µM mel

CONCLUSIONS

Root knot nematodes have been a subject of plant survival studies due to their countless number of hosts and above ground symptoms costing a loss in yield and in extreme cases, plant's life. Using synthetic chemical-based compounds for protection has raised environmental and public health safety, therefore a tendency to utilize more friendly approaches increases. Melatonin, being a safe molecule both present in humans and plants, is becoming a centre of studies for its regulatory and supporting roles in plant's growth and development. Results of this study indicates that it merits a value in developing a response against the nematode but needs further elucidation. This response might come from protection of chlorophyll content, therefore preserving nitrogen balance in the plant, as well as stimulating defence mechanisms. Different concentrations with varying exposure time are needed to elucidate these responses.

ACKNOWLEDGEMENTS

This research was funded by The Scientific and Technological Research Council of Turkey (Project number: 119O660)

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF THE MICROFUNGAL COMMUNITY FROM SOIL TO ONION CROP IN AN INTEGRATED PROTECTION SYSTEM

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Abstract

Soil is a reservoir of microorganisms including microfungi who play a key role as saprotrophs, plant mutualists, symbionts, decomposers, pathogens and excellent bioindicators of soil quality. The diversity of soil fungi communities is influenced by crop protection products. This study aimed to evaluate the diversity of soil fungal community in onion crop. Two plant protection methods were applied - i) diatomite in three different doses: 52.5 kg ha^{-1} (T1), 105 kg ha^{-1} (T2), and 210 kg ha^{-1} (T3) and ii) biological control agent Trichoderma asperellum Td85 strain (T4). Of 58 operational taxonomic units isolated from all treatments (including control) only 7 operational taxonomic units were found in common. The highest value of colonization frequency was observed in T2 (167%), followed by control (125%), T3 (58%), T4 (50%) and T1 (42%). Results indicate that the degree of soil colonization with Trichoderma is related with the dose of diatomite.

Key words: *soil microorganisms, integrated pest management, biological control agent.*

INTRODUCTION

Besides bacteria, soil fungi constitute an essential component of biological characteristics in soil ecosystems playing a key role as saprotrophs, plant mutualists, symbionts, decomposers, pathogens (Peay et al., 2016; Victorino et al., 2021) and being an excellent bioindicators of soil quality (Orgiazzi et al., 2012). A growing number of studies show that conventional farming leads to lower soil quality and less biological activity (i.e. microbial populations and microbial respiration rate) than organic farming to different crops (Droogers and Bouma, 1996; Girvan et al., 2004; Mader et al., 2002) even for onion crop (Knerr et al., 2020). It is important to reveal and understand the interactions of fungal diversity in soil when different organic amendments are applied to select the best option for ecology (Swer et al., 2011).
In European agriculture, the trend is to increase areas organically cultivated using biological means for plant protection or organic substances from natural sources. *Trichoderma* spp. has many roles in soil ecology such as suppress soil-borne pathogen fungi (Harman et al., 1989; Harman, 2000), increase N and P nutrient contents in soil, degrade nutrients produced by photosynthesis into a state in which they can be used for plant growth, increase in the soil enzyme activity of the rhizosphere soil of seedlings, expand the contact area between the rhizosphere and soil (Halifu et al., 2019), improve the rhizosphere microbial community structure (Elena et al., 2015; Zhang et al., 2013). Diatomaceous earth (diatomite) in agriculture mitigates plant biotic and abiotic stresses (Camargo et al., 2017; Liang et al., 2015) and increases yield acting as a fertilizer (Pati et al., 2016). In Romania, onion is one of the most cultivated vegetable crop with 30.000 ha in 2018 (https://www.madr.ro/horticultura/fructe-si-legume.html). Many studies of onion crop targeted the arbuscular mycorrhizal fungi (AMF) (Bolandnazar, 2009; Charron et al., 2001; Galván et al., 2009) but soil fungal communities were less addressed. Onion crop may also enhance soil microorganisms communities of other plants when cultivated in intercropping system (Li et al., 2020). We expected *Trichoderma* strain will supress some

soil fungi and a less genera will be found in the plot treated with it. The aim of this study was to determine i) the diversity of the soil fungal communities in onion crop and ii) if relative abundance of *Trichoderma asperellum* is influenced by treatments.

MATERIALS AND METHODS

Field experiment. The experiment was carried out in 2020 at the Research and Development Station in Vegetables Buzau, Romania. In the trial onion variety 'De Buzau' was seeded at 4th March in soil with pH 8.2, 2.57% organic matter content and 4.3% $CaCO_3$. Previous crop was dwarf bean. Onion crop was fertilized with 366 kg ha^{-1} of a complete fertilizer mixture of 20-20-0-13 N-P_2O_5-K_2O-S. The herbicide Cerlit (333 g/L fluroxypyr) was applied at 0.3 L ha^{-1}. Treatments employed were i) diatomite 52.5 kg ha^{-1} (T1), ii) diatomite 105 kg ha^{-1} (T2), iii) diatomite 210 kg ha^{-1} (T3), iv) bioinoculant *Trichoderma asperellum* Td85 strain three grams of inoculated calcium alginate per plant (1×10^7 ml^{-1}) (T4) and v) not-treated plots (control). Plots were established at 7 m^2 (1.4 m x 5 m), with four repetitions per treatment with a total of 20 experimental units in a complete randomized block design.

Field sampling. Soil samples were collected from each plot at 5 cm depth with a soil sampling probe at the harvest time. The samples were placed in sterile polyethylene bags, transferred to the laboratory, and stored at low temperature (4°C) until tested.

Isolation of fungi. Soil samples per treatment (each with 4 repetitions) were manually blended and 3 g of each soil sample repetition was divided into three replicates, each with 1 g, finally having 12 samples per treatment or control. Each sample was introduced into a sterile tube with 10 ml sterile distilled water and vortexed for 30 seconds at 2000 rpm. Samples were diluted in series (1:10 and 1:100) and the lowest one was dispensed in 9 cm-Petri dishes with potato dextrose agar (PDA) nutrient medium containing a mix of antibiotics chloramphenicol + ampicillin (0.2 mg/L) + tetracycline (0.2 mg/L). Each plate corresponds to 1 g of soil sample. Plates were incubated at +25°C in darkness for 7 days. Fungal colonies were counted and only the fungi with visible different morphological characteristics were subcultured. Eventually, when an endophyte was acquired in pure culture it was cultured on PDA, malt extract agar (MEA) and oatmeal agar (OA) medium for colony characterization. Fungal colonies were morphologically separated in morphotypes (Cosoveanu et al., 2018) classified according to colour and shape of mycelium, pigmentation of medium, and morphological characteristics of asexual/sexual organs (Bankina et al., 2017) resulting 58 operational taxonomic units (OTUs). To separate OTUs by microscopically characters a microscope at 40x magnification was used. For the mycological collection (long-term conservation), OTUs isolates were maintained in glycerol (20%) and mineral oil at -38°C and 5°C, respectively.

Diversity indices. Colonization frequency (CF%) was calculated as the total number of isolates of one OTU in all treatments (each with four repetitions and three samples per repetition) or per treatment divided by the total number of dispensed plates; where each plate contained 1 g of soil sample. CF% = (number of colonies of an OTU/total number of Petri dishes sampled) x 100.

For the diversity of soil fungi, Margalef index, Shannon index and Simpson's dominance index were used. Margalef index (Cosoveanu et al., 2018) measures species richness while Shannon index combines richness and evenness. The Margalef index was calculated using formula:

$d = (S-1)/\ln N$, where S is the number of OTUs and N is the number of isolates in the sample.

The dominance of Simpson (Cosoveanu et al., 2018) was calculated according to the formula:

$D = 1 - \sum [ni\,(ni-1)/N(N-1)]$, where ni is the number of isolates belonging to i OTUs and N is the total number of isolates.

The Shannon diversity index was calculated according to the formula:

$$H' = -\sum_{i=1}^{S} pi \ln pi,$$

where, p is the proportion (n/N) of isolates of one particular OTU found (n) divided by the total number of isolates found (N), ln is the natural log, $\sum$ is the sum of the calculations and S is the number of OTUs.

Effective number of OTUs were calculated according to Jost (2006) for Shannon diversity index and Simpson index. The number of equally-common species required to give a particular value of an index is called the "effective number of species". This is the true diversity of the community in question. For the diversity indices, PAST software version 3.15 (copyright Hammer & Harper, Natural History Museum, University of Oslo, Norway) was used. Venn diagrams were performed using the web-based tool InteractiVenn (Heberle et al., 2015).

RESULTS AND DISCUSSIONS

The first morphological inspection resulted in 484 colonies clustered in 58 OTUs.

Only six OTUs were found with values of colonization frequency per one gram of soil in all treatments, higher than 50% while 15 OTUs registered values of 10% and 50%. The majority of soil fungi OTUs were found with CF < 10% per gram of soil (Figure 1).

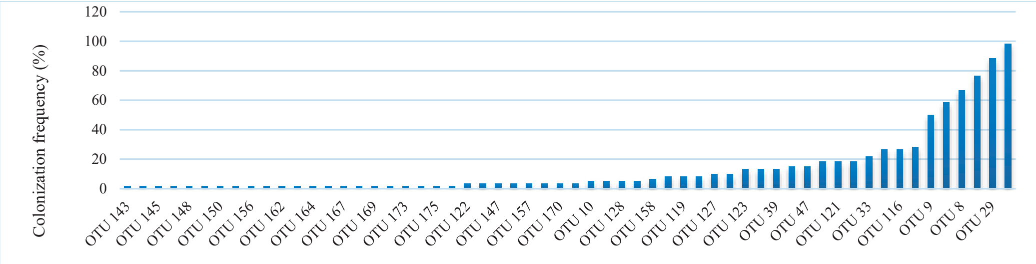

Figure 1. Mean values of colonization frequency of each OTU per 1 gram of soil in all plots (all five treatments including control, each with four repetitions and three replicates)

Although the bioinoculant *T. asperellum* strain T85 was applied in T4 plots, the highest value of colonization frequency was observed in T2 (167%), followed by not treated plots - control (125%), T3 (58%), T4 (50%) and T1 (42%) (Figure 2). Present identification does not rely on molecular data, therefore OTU *Trichoderma* might gather different strains and species of *Trichoderma* naturally present in the soil. Experimental design also might have played a role in the registered values as not-treated plots (control) was set up in two repetitions next to T4 plots. Yet, plots of T2 were not placed nearby control.

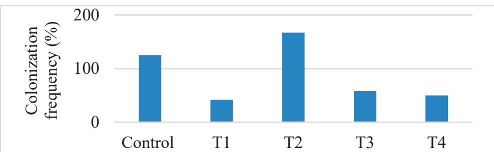

Figure 2. Colonization frequency % of *Trichoderma* strains per gram of soil across treatments: T1-diatomite 52 kg ha^{-1} ; T2- diatomite 105 kg ha^{-1} ; T3- diatomite 210 kg ha^{-1} ; T4- *T. asperellum* Td85 strain (1×10^{7} ml^{-1})

OTUs number per treatment slightly varied with the lowest value registered for T4 (25 OTUs) and the highest value registered for T1 (30 OTUs). Of 58 OTUs isolated in all treatments, including not-treated plot (control), only 12% were found in common. Single OTUs per treatment varied from five in T4 to eight in T2 and control. Generally, only one OTU was found common for at least two treatments. The highest number of common OTUs between at least two treatments was four (Figure 3). Different dosages of organic compost (low versus high), nitrogen fertilizer and untreated control were found to shift the selection of bacterial species and their abundance (Enebe et al., 2020). Therefore, in this study it comes easy to speculate that singleton OTUs were isolated due to treatments applications which restricted their habitat.

Control plots registered highest values individual counted 115 colonies, followed by T1 (106 colonies) and T4 (93 colonies). Shannon diversity index was used to indicate both the richness and the evenness of the soil fungal community being sensitive to changes in rare species. Results indicate the highest value in T3 (H = 2.99) and the lowest in T2 (H = 2.76). Thus, it comes easy to speculate that diatomite has an effect on richness of fungal soil community, as T3 was amended with the highest dose of diatomite (210 kg ha^{-1}).

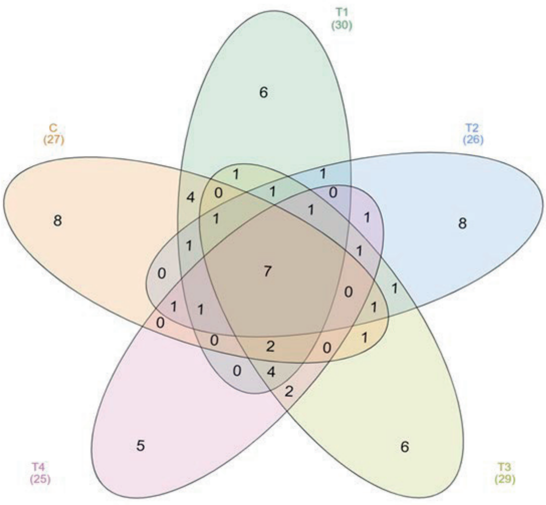

Figure 3. Venn diagram showing common OTUs among treatments: C - control; T1 - diatomite 52 kg ha^{-1}; T2 - diatomite 105 kg ha^{-1}; T3 - diatomite 210 kg ha^{-1}; T4 - *T. asperellum* Td85 strain (1×10^7 ml^{-1})

Application of large amounts of vermicompost and mushroom residues enhanced the biodiversity of soil bacterial communities in *Leymus chinensis* grasslands (Shang et al., 2020). Converting to effective number of species, which is the true diversity of species, it can be observed that the differences between the values of this index are not that high (T2 = 16 OTUs versus T3 = 20 OTUs). It is noteworthy to underline that T3 resulted in the highest diversity of fungal soil community (H = 2.99), also having high value of evenness (Simpson 1-D = 0.93) and highest species richness index (Margalef = 6.32). Relative abundances of several bacterial species were positively correlated with increasing organic fertilizer in the rhizosphere soil of grapes (Wu et al., 2020). Lowest values for species richness, diversity and increased dominance were found in not-treated plots (Table 1).

Table 1. Diversity indices per treatment: C-control; T1-diatomite 52 kg ha^{-1}; T2-diatomite 105 kg ha^{-1}; T3-diatomite 210 kg ha^{-1}; T4-*T. asperellum* Td85 strain (1×10^7 ml^{-1})

	C		T1		T2		T3		T4	
	Diversity indices	Effective species	Diversity indices	Effective species	Diversity indices	Effective species	Diversity indices	Effective species	Diversity indices	Effective species
Taxa_S	27		29		26		29		25	
Individuals	115		106		82		84		93	
Simpson_1-D	0.90	10.13	0.93	14.27	0.90	10.10	0.93	15.08	0.94	15.65
Shannon_H	2.79	16.22	2.94	18.93	2.76	15.83	2.99	19.81	2.94	18.90
Margalef	5.48		6.00		5.67		6.32		5.30	

CONCLUSIONS

This study suggests that application of diatomite in high dose is positively correlated with higher diversity and evenness of soil fungal communities. Further analysis is to be considered to identify the isolated strains of *Trichoderma* and to evaluate the scarce colonization of soil of the bionoculant T. *viridae* T85.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovations, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0659 PCCDI, within PNCDI III. The first two co-authors are deeply indebted to Andreea Cosoveanu (RDIPP) for her laboratory guidance and polishing this manuscript.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

BIOLOGICAL ACTIVITY OF NATURAL AROMATIC PRODUCTS FROM THREE *NICOTIANA* SPECIES AGAINST BACTERIAL PHYTOPATHOGENS

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Abstract

The objective was to assess the biological activity of the natural aromatic products concrete and resinoid obtained from different Nicotiana species (N. tabacum L., N. alata Link & Otto and N. rustica L.), against some bacterial (Xanthomonas spp., Pseudomonas spp., Bacillus spp.) phytopathogens. Test pathogens were isolated from infected plant materials and soil. The aromatic products were obtained by twofold extraction with following solvents (concrete with petroleum ether and resinoid with 95% ethanol) at high temperature and subsequent concentration on a rotary vacuum evaporator. Their biological activity was tested under in vitro conditions by the diffusion method/sterile zone diameter (mm)/. Products-specific inhibition has been found at the different species of phytopathogens. The antimicrobial activity of aromatic products was general better expressed under the influence of the resinoids. The results reveal opportunities for the development of biological plant protection products based on natural extracts of the genus Nicotiana with antibacterial action.

Key words: *Nicotiana species, aromatic products, antibacterial activity, phytopathogen.*

INTRODUCTION

The potential and benefits of using plant-derived chemicals as bioactive and therapeutic agents have been the focus of extensive research in the last decades. Those potent phytochemicals almost always exclusively embody secondary plant metabolites resulting from alkaloid, terpenoid and flavonoid biosynthesis and transformations (Aravindaram & Yang, 2011). In this view, many of the species of genus *Nicotiana* (*Solanaceae*) have revealed extensive potential as basis of various isolated bioactive substances or complex extracts (Budzianowski, 2014). In fact, *Nicotiana* species are one of the richest sources of biologically active metabolites and different aspects of their therapeutic or protective potential in human medicine have been reported (Jassbi et al., 2017; Mostafa et al., 2018; Anumudu et al., 2019). Some tobacco varieties accumulated polyphenols in amounts higher than those in traditionally proven medicinal plants with antimicrobial activity. Extracts derived from cultured tobacco (*N. tabacum* L.) have been shown to show insecticidal and antimicrobial activities (Bakht et al., 2013; Kekuda et al., 2017). Although historically most research has focused on economically important types of tobacco for cigarettes - mainly on *N. tabacum,* not as much on *N. rustica* L. and relatively less on the over 70 non-commercialized *Nicotiana* species, there are sufficient evidences that many of them accumulate specific metabolites involved in plant defense mechanisms (Jassbi et al., 2017; Bally et al., 2018). The species *N. alata* Link & Otto was found to synthesize protease inhibitors against a wide spectrum of pathogens, as well as plant defensins, specialized proteins with antifungal and antibacterial effect (Bleackley et al., 2016). These inhibitors are thought to be involved in the genetic mechanism of plant protection and the isolated plant defensins were used to generate pathogen resistant transgenic plants (Ghag et al., 2016). The specificity of the phenological development of *Nicotiana* plants makes them extremely suitable for various bioengineering procedures, as well. Some of them have been used as model plants in the experimental production of vaccines and other areas (Budzianowski, 2014; Bally et al., 2018). The species *N. tabacum* L., *N. benthamiana* Domin., *N. glutinosa* L. and *N. alata* Link & Otto, along with other plant species, have performed successfully in tests for the production of valuable immunoglobulins and other recombinant proteins (Budzianowski, 2014). Different extracts of these and several other *Nicotiana* species have revealed expressed antibacterial and antifungal activities against human and plant pathogens, as well as antioxidant and insecticidal properties (Ru et al., 2012; Nwachukwu, 2017; Al-Lahham et al., 2020). On the other hand, *N. tabacum* and some of the rest *Nicotiana* species can be regarded as genuine aromatic plants, and fresh or dried leaf and flowers are processed to obtain traditional aromatic products, used in perfumery, cosmetics and phytopharmacy. Those natural aromatic products include: tobacco essential oil (obtained by hydro-distillation from an acidified medium); extraction concentrates tobacco concrete (by extraction of dry material with non-polar organic solvents, such as petroleum ether, n-hexane, and concentration by complete removal of the solvent); tobacco resinoid (by extraction with polar solvents, mostly ethanol and concentration); tobacco absolute (obtained by re-extraction of concretes, resinoids or other extracts with ethanol at cooling and separation of precipitates), and some others (Bauer et al., 2001). Aromatic products of *N. tabacum* are commercially available, have been produced on a large scale by established technologies and used in industry. Tobacco remains one of the economically important crops, and leaf production and processing technology generates significant amounts of available biomass (Dyulgerski, 2020). Several studies elucidated the chemical composition of these tobacco aromatic products (Nedeltcheva-Antonova et al., 2016; Popova et al., 2015; 2018; 2019; 2020a; 2020b). The data in these studies clearly identified the presence of various volatile and semi-volatile compounds reported to be active in bacterial and fungal inhibition processes (alkaloids, terpenoids phenylpropanoids) (Patil et al., 2015; Sharma et al., 2016). In fact, according to some of our previous works, the natural aromatic products used in this study demonstrated antimicrobial activity against some bacterial strains, representing common human skin pathogens being relevant to the development of cosmetic formulations. Promising results were achieved for several *Nicotiana* species as resources for obtaining biologically active extracts and natural aromatic products (Popova et al., 2015; 2017; 2018; 2019; 2020a; 2020b). However, to the best of our knowledge, natural aromatic products from *Nicotiana* spp. have not been studied in plant protection aspects, but the above suggests the potential for their use in this direction.

Therefore, the objective of this work was to assess the biological activity of the natural aromatic products concrete and resinoid obtained from three *Nicotiana* species (*N. tabacum* L., *N. alata* Link & Otto and *N. rustica* L.), in a direct comparison, against some bacterial pathogens, causing diseases of various crops.

MATERIALS AND METHODS

Plant material

Three *Nicotiana* species (*Solanaceae*) were used as primary plant material for the obtaining of concentrated natural extraction products: *N. tabacum* L., *N. rustica* L., and *N. alata* Link & Otto. *N. tabacum* L. (Common tobacco) is one of the most important cash crops worldwide, cultivated in over 110 countries for the production of various types of tobacco raw materials used by the cigarette industry. In the present study, Oriental-type tobacco of the Plovdiv 7 variety, widely grown in the region of Central Southern Bulgaria, was used. *N. rustica* L. (Aztec tobacco) is also common to a wide geographical zone, although leaf production nowadays is concentrated within a number of countries in Asia, mainly. It is the only other species used for manufactured tobacco products and is one of the most high-alkaloid species of the genus, with nicotine content as high as 8-10% or more (Sisson & Severson, 1990). *N. alata* Link & Otto (also known as Jasmin tobacco, Persica or Winged tobacco) is a popular ornamental plant worldwide, recognized for its abundant and highly fragrant flowers. In contrast to *N. rustica* and *N. tabacum*, it is one of the most low-alkaloid species of the genus (Sisson & Severson, 1990). Two *N. alata* genotypes were used in the study - one with white flowers and one with pink flowers.

All plants were grown side-by-side on the experimental field of the Tobacco and Tobacco Products Institute, Bulgarian Agricultural Academy, situated in the region of Plovdiv, Central South Bulgaria (42°04'55.2"N 24°42'16.8"E), with humus-carbonate/Rendzina/ soil type (Figure 1a - d). The plant materials were collected during the active growth and flowering of the tobacco plants and were dried. Soil characteristics, production practices and leaf curing technology were described previously (Popova et al., 2017; 2018; 2020a).

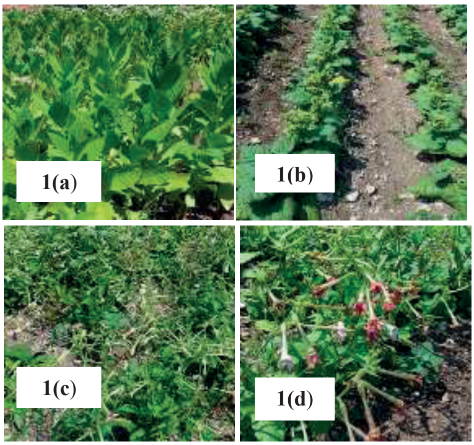


Figure 1. *Nicotiana* plants on the field: 1(a) *N. tabacum* (var. 'Plovdiv 7'); 1(b) *N. rustica*; 1(c) *N. alata* genotype with white flowers; 1(d) *N. alata* genotype with pink flowers (photos by authors)

Natural aromatic products

Two natural aromatic products (Bauer et al., 2001) obtained from the dried leaves of the three *Nicotiana* species, concrete and resinoid, were used in the study. The extraction procedure was performed as described previously (Popova et al., 2017; 2018; 2020a). In brief, concrete was obtained by twofold extraction with petroleum ether (FILLAB, Bulgaria), for 60 and 30 min, respectively, at a temperature of 30ºC and solid-to-liquid ratio of 1:10 (w/v). The solvent was completely evaporated from the combined extracts on a rotary vacuum evaporator at water bath temperature of 35ºC. The yield of concrete (on a dry weight basis) was: *N. tabacum* 1.9 ± 0.07% DW (w/w) (Popova et al., 2018), *N. alata* 1.68% (white flower genotype), 1.35% (pink flower genotype) (Popova et al., 2017) and *N. rustica*, 1.50 ± 0.01% (w/w) (Popova et al., 2020a). Resinoid was obtained by twofold extraction with 95% ethanol for 2.5 h and 2h, at a temperature of 70ºC and solid-to-liquid ratio of 1:10 (w/v). The combined extract was concentrated on a rotary vacuum evaporator, at a temperature of 55ºC. The yield of resinoid was: *N. tabacum*, 20.4 ± 0.19% DW (w/w) (Popova et al., 2018), *N. alata*, 10.27% (white flowers genotype) and 9.50% (pink flowers genotype) (Popova et al., 2017), and *N. rustica*, 15.62 ± 0.09 % (w/w) (Popova et al., 2020a). All extraction products represented semi-solid waxy masses, light to dark brown in color, with specific odor.

Isolation of phytopathogens

The phytopathogens with which this study was conducted are the causes of bacterial diseases on agricultural crops. Four pathogens were isolated and tested. Two of them were isolated from plants with characteristic disease symptoms and two from soil as potential pathogens. Classical methods accepted in phytopathology and microbiology for their initial isolation had been used (Grudeva et al., 2006; Sinclair et al., 2019). The procedure for isolation from plant tissues was as follows: Fresh leaves of infected plants - mulberry (*Morus alba* L.); pepper (*Capsicum annuum* L.) were washed with tap water, the surface was disinfected sequentially for 1 min with sodium hypochlorite (10%), with ethyl alcohol (70%) and washed three times with sterile distilled water. Leaf pieces taken from the boundary between the living and symptomatic tissue of the infectious spots were placed on the surface of Petri dishes with potato-dextrose agar (PDA, Sigma Aldrich Ltd.) and incubated for three days at a temperature 28°C.

Isolation of potentially present phytopathogens in the soil was performed by inoculating of ten-fold dilutions soil suspensions homogenized in sterile saline (0.9% NaCl) on solid nutrient media starch-ammonia agar (SAA). The cultures were incubated under the same conditions as the plant isolates. After incubation from the developed colonies with characteristic morphological features for the presumed pathogens, new cultures were made on the respective nutrient media and series of purification streaks was performed until pure cultures from each isolate were obtained, by Drigalski method (Grudeva et al., 2006). Pure cultures of the isolates were analyzed by macro-morphological characteristics of the colonies and observations under a light microscope, at a magnification of 900x; Gram staining was performed (used: crystal violet as the main dye, lugol's solution as a fixative and 0.5% safranin as the second dye) and a rapid catalase test with H_2O_2 was made.

***In vitro* testing of the biological activity of *Nicotiana* aromatic products**

The biological activity of the aromatic extraction products was determined *in vitro* by the diffusion method in agar wells (Valgas C. et al., 2007). From 24-hour pure cultures of the isolated phytopathogens, suspensions were prepared in 5 ml of sterile saline and incubated at 28°C. Surface inoculations by 24-hour pure cultures were performed on Mueller-Hinton Agar (MHA, Sigma Aldrich Ltd.) and wells (d - 6 mm) were made in the nutrient medium. The density of the bacterial suspensions is approximately 1.5 x 10^8 CFU /ml/ turbidity: 0.5 McFarland - standard/ (Balouiri et al., 2016). Volumes of bacterial inoculums were 0.1 µl on the Petri dish (9 mm).

The concentrated aromatic products, concretes and resinoids were dissolved in 5 ml of 90% ethanol and 5 ml of sterile distilled water is added. The concentration of the tested products in the obtained solutions was 2.5% (w/v). The solutions were prepared immediately before testing. An amount of 100 µL of each extract solution was dropped to the test-wells; the dishes were left for 1 hour at 4°C in order to allow the diffusion of the solution into the media and then incubated for 24h at 37°C. The extract solutions were pre-sterilized with a sterilized through Millipore filter (0.22 mm). The diameter of the sterile zone (mm) was measured. The size of the wells (6 mm) was also included in the presented dimensions of the inhibitory zone. The solution in a ratio of 1:1 of 90% ethanol and sterile distilled water was used as a control. All tests were performed in five reps. Results were presented as mean values ± standard deviation (n = 5).

RESULTS AND DISCUSSIONS

In the present study based on the phenotypic characteristics made, the isolated phytopathogens were identified by genus. The manifestation of the characteristic symptoms of the diseases caused by them, the macro-morphological and microscopic diagnostics were not sufficient for accurately determine of their species affiliation. The results obtained were as follows:

The symptomatic picture of pepper was characteristic of the disease “bacterial scab”. Bacteria have been isolated from infected leaf lesions. The morphology of the colonies allows them to belong to the genus *Xanthomonas*. The colonies were round, with smooth edges, convex, mucoid, creamy-yellow and did not produced fluorescent pigment into the nutrient medium. Bacteria were Gram (-), movable rods; the catalase test was positive. Species of this genus cause a variety of bacteriosis across a wide range of hosts (Figure 2 a - b). Multiple strains, races, and pathovars, depending on the host were differentiated (Jones et al., 2000). Until recently, the main cause of the disease "bacterial scab" on pepper and tomato was considered to be the species *X. campestris* pv. *vesicatoria*, but in recent years its taxonomic status has been revised (Jones et al., 2004). Based on an in-depth study on the species and intraspecific differentiation of bacteria of the genus *Xanthomonas* for Bulgaria, four species were indicated as causative agents of pepper and tomato disease - *X. euvesicatoria*, *X. vesicatoria*, *X. perforans* and *X. gardneri* (Kizheva et al., 2013).

The symptoms of mulberry leaf (spots with yellow halos and necrotic dark-brown spots) as well as the morphological characteristics of the isolate allow the conditional identification of the pathogen as the bacterium *Pseudomonas* *syringae* pv. *mori* (Young, 2010). The colonies were round, smooth, with a full edge, convex, pearly whitish or with a pale yellowish tint in the coloration, with weak fluorescence. Microscopic observation showed Gram (-) movable rods. The catalase test performed was positive (Figure 2 c - d). In plant pathology, phytopathogens related to *P. syringae* are classified on the basis of the host into a general phylogenetic group "*P. syringae* - complex", whose taxonomy is controversialy. According to some authors, the group includes about 15 species with more than 60 pathogens (Gomila et al., 2017). According to others, bacterial diseases were caused by an extremely universal and adaptive to hosts and environmental conditions single species (Mansfield et al. 2012; Popović et al., 2021). The species *P. syringae* pv. *mori*, widespread in different parts of the world was considered to be the causative agent of mulberry bacteriosis (Mansfield et al., 2012). It was first registered in 2017-2018 in mulberry plantations in Poland for the conditions of Europe (Krawczyk et al., 2020).

The third bacterial species with which the present study was conducted was isolated from soil and can be attributed to spore-forming bacteria of the genus *Bacillus*. The choice for its initial isolation was made in order to evaluate the biological activity of the tested aromatic products from *Nicotiana* and on representatives of Gram (+) bacteria. The morphological characteristics of the isolated colonies show two bacterial morphotypes, which can be conditionally referred to the group of *B. cereus* - medium-sized, raised colonies, whitish with smooth or slightly curly edges and to the group of *B. subtilis* - relatively large creamy columns with rough texture, with raised wrinkles. Gram staining indicates the presence of G (+) spore-forming rod-shaped bacteria. Both isolates were catalase positive (Figure 2 e - f). Species of this genus were widespread in nature, heterotrophic, with a huge role in the carbon cycle, and have been used successfully in organic farming as part of PGPRB (Beneduzi et al., 2012). At the same time, members of the group *B. cereus* refer to the so-called opportunistic pathogens that cause certain gastrointestinal and skin diseases in humans (Mendes et al., 2013). In the phytopathological aspect, pathogens of *B. subtillis*-complex have been reported, which can cause rot of bulbous plants (Stoyanova et al., 2011).

The bacterial pathogens examined in this study cause serious infectious diseases in a number of crops, leading to deterioration in quality, stability of yields and production, as well as substantial economic losses in the producing countries. Species of the genus *Xantomonas* and the genus *Pseudomonas* are included in the top-10 most important plant pathogenic bacteria (Mansfield et al., 2012).Their ubiquity as part of the epiphytic (plants and seeds) and soil microflora, high virulence and adaptability to various environmental factors, the development of resistance, make the control extremely difficult, mainly with chemicals (Mansfield et al., 2012).

Figure 2. Isolation of bacterial pathogens in the study: 2(a) and 2(b) *Xanthomonas* sp., isolated from spots on pepper leaves; 2(c) and 2(d) *Pseudomonas* sp., from spots on mulberry leaves; 2(e) and 2(f) *Bacillus* sp., isolated from soil - morphotype - 1 (mt-1) defined as *B. cereus* and morphotype - 2 (mt-2) defined as *B. subtillis* (photos by authors)

The results of the *in vitro* application of the aromatic products obtained from the three *Nicotiana* species to the isolated bacterial pathogens showed that all tested natural aromatic products achieved an inhibitory effect on the bacterial pathogens included in the study. Specific differences in activity were observed, depending on the type of product, the species of *Nicotiana* and the species of pathogen. As a general observation, resinoids were the more effective inhibitors of the four pathogens studied, than the concretes. The strongest inhibition of the gram-negative bacteria (*Xanthomonas* spp. and *Pseudomonas* spp.) was accomplished by the resinoids of *N. rustica* and *N. tabacum*. The zone of inhibition under the influence of these resinoides was over 20.00 mm. The concrete obtained from *N. tabacum* also showed a relatively high inhibitory effect at *Xantomonas* spp. (IZ - 20.00 mm). The effect of the concretes of the other *Nicotiana* species against *Xanthomonas* spp. was lower, in the range of 15.5-13.5 mm. Against *Pseudomonas* spp. the concretes of all four species of *Nicotiana* have a weak effect as the reported inhibitory zone was 11.50-14.50 mm (Figures 3 and 4).

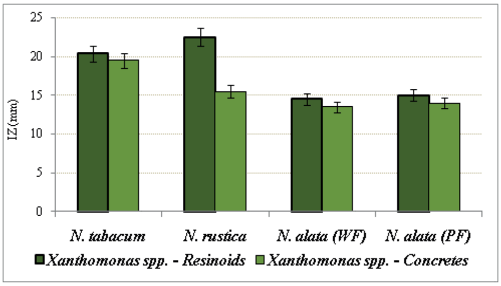


Figure 3. *In vitro* biological activity of *Nicotiana* aromatic products (concretes and resinoids) against the pathogen identified as G (-) bacterium *Xanthomonas* spp.

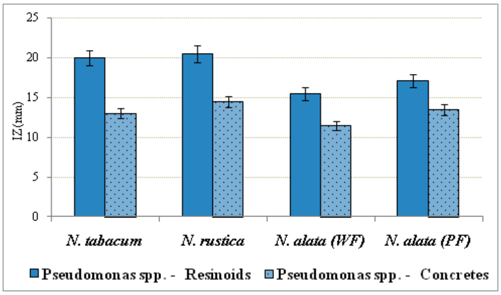


Figure 4. *In vitro* biological activity of *Nicotiana* aromatic products (concretes and resinoids) against the pathogen identified as G (-) bacterium *Pseudomonas* spp.

The resinoids of *N. alata* genotype with pink flowers (PF) and *N. rustica* had the highest bactericidal activity against Gram-positive bacteria *Bacillus* spp. At morphotype 1 (mt-1), conditionally defined as *B. cereus* with diameters of the zone of inhibition 24.50 mm and 21.00 mm, respectively, and at morphotype - 2 (mt-2) defined as *B. subtillis* the inhibition zone was 22.50 mm and 20.50 mm. The effect of resinoids from the other *Nicotiana* species was low the diameter of the inhibition zone was less than 20.00 mm (Figures 5 and 6). Bacteria referred to as *Bacillus* spp. were relatively susceptible to *N. rustica* concrete (IZ 19.00 mm and 16.50 mm), also. The bactericidal action of the concretes of the other *Nicotiana* species was low (IZ at mt-1 was 11.50-10.00 mm, and at mt-2 between 8.00-9.50 mm).

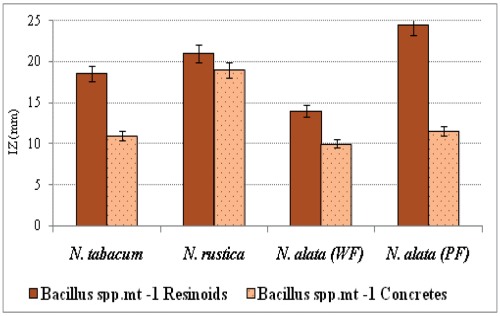


Figure 5. *In vitro* biological activity of *Nicotiana* aromatic products (concretes and resinoids) against the pathogen identified as G (+) bacterium *Bacillus* spp. - morphotypes - 1

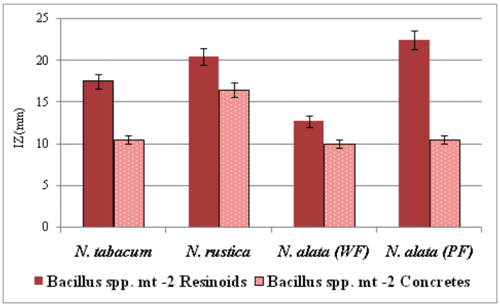
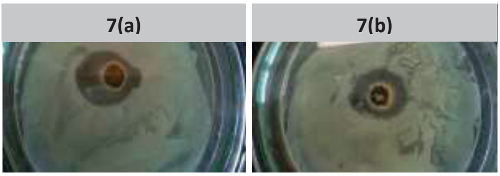
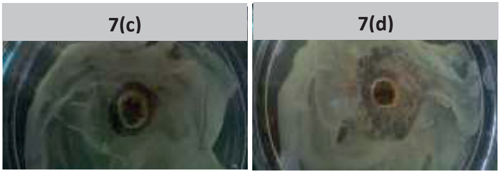


Figure 6. *In vitro* biological activity of *Nicotiana* aromatic products (concretes and resinoids) against the pathogen identified as G (+) bacterium *Bacillus* spp. - morphotypes - 2

The results on the specificity of action of aromatic products depending on the Nicotiana species show that the products with *N. rustica* had the strongest general bactericidal action against both G (-) and G (+) bacteria, followed by the products obtained from *N. tabacum*. The data confirmed the antimicrobial activity of *N. rustica* and *N. tabacum* extracts found by other authors (Bakht, J. & Shafi, 2013). The overall bactericidal activity of the products derived from *N. alata* was less, but differences in activity have been registered between the two genotypes. Higher activity similar to that reported in *N. tabacum* had the products of the pink genotype with a focus mainly on G (+) bacteria, and those of the white genotype - against G (-) pathogens. The highest inhibition effects achieved in the study are presented on Figure 7.



Xanthomonas spp.



Pseudomonas spp.

Bacillus spp.

Figure 7. Maximal inhibition of bacterial pathogens by *Nicotiana* aromatic products: 7(a) resinoid of *N. tabacum*; 7(b) concrete of *N. tabacum*; 7(c) resinoid of *N. rustica*; 7(d) resinoid of *N. tabacum*; 7(e) resinoid of *N. alata* genotype with pink flowers; 7(f) concrete of *N. rustica* (photos by authors)

The results were consistent with recently published data on the antimicrobial activity of *Nicotiana* species in both medical and phytopathological aspects (Anumudu et al., 2019; Al-Lahham et al., 2020). A number of authors have established the efficacy of *N. tabacum* extracts against human pathogenic bacterial strains of *B. cereus* (Bakht et al., 2013; Patil et al., 2015; Sharma et al., 2016), *P. aeruginosa* (Ameya et al., 2017; Pramono et al., 2018; Al-Lahham et al., 2020). Biological activity of extracts of *N. plumbanginifolia* Viv. against *B. subtilis, B. cereus, B. fusiformis*, and *P. aeruginosa* had been reported (Singh et al., 2010; Kekuda et al., 2017).

Efficacy studies against phytopathogens were fewer in number, but one had been reported on extracts from leaves and roots of *N. tabacum* against *P. solanacearum* and *Xanthomonas axonopodis* pv. *malvacearum*, causative agents of bacteriosis on potatoes and cotton (Singh et al., 2010).

These first results were encouraging, from the perspective of practical use of tobacco aromatic products in phytoprotection, considering the significantly higher yield and better solubility of resinoids, compared to concretes. The results were in full agreement with previous findings about the influence of the used organic solvent on the antimicrobial activity of plant extracts obtained from *Nicotiana* and other species. Typically, extracts obtained with solvents with higher polarity (methanol, ethanol, 1-butanol) demonstrated better biological activities in antibacterial and antifungal tests, compared to those obtained with less polar or non-polar solvents, such as n-hexane (Singh et al., 2010; Patil et al., 2015; Sharma et al., 2016; Ameya et al., 2017; Al-Lahham et al., 2020). The variation patterns between the activities of the two aromatic products were obviously bound to differences in their chemical composition, reflecting not only the effect of solvent polarity, but also that of other extraction factors such as temperature and duration. Therefore, our results were consistent with previously published data about the GC-MS composition of the tested aromatic products (Popova et al., 2017; 2019; 2020a), as well as with the findings about the individual contribution of their major components to the overall biological activity or the synergistic/ antagonistic interactions within the complex mixture (plant essential oils and extracts). Several studies connected the antibacterial, antifungal and antioxidant activities of *Nicotiana* and other plant EOs and extracts with the decisive role of alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, coumarins, and some other classes of chemical compounds or their individual representatives (Patil et al., 2015; Sharma et al., 2016; Nwachukwu, 2017). Therefore, the variation of the antimicrobial activity of the tested concretes and resinoids agreed very well with the different chemical profiles of the two products. Regardless of species, the profiles of *Nicotiana* resinoids, found to be more active in this study, were dominated by nitrogenous compounds, mainly

alkaloids (over 50% of the identified content), followed by diterpenes (over 13%), and phenylpropanoids (over 9%) (Popova et al., 2017; 2019; 2020a); all those represent classes with distinct biological activity as already stated.
In turn, the concretes contained mostly oxygenated aliphatics (over 64% of the identified composition), nitrogenous compounds (about 22%) and oxygenated diterpenes (about 10% share). In general, phenolic and terpenoid compounds were found to be more efficient pathogen inhibitors compared with esters, alcohols and aldehydes; higher activity was attributed to more hydrophobic molecules, such as phenolics and aromatic aldehydes (Raveau et al., 2020); those observations were supported by our results, too. Nicotine, in particular, cited as a key inhibitor, equally effective on G (+) and G (-) bacteria and other pathogens (Bakht et al., 2013), was extracted in high concentrations into the resinoids (by a polar solvent, ethanol), but was a minor compound in the n-hexane extracted concretes. The differences in the antibacterial activity recorded depending on the type of *Nicotiana* in the present study were also in line with the opinions of a number of authors that in addition to nicotine, the individual composition of aromatic products reveals the presence in high concentrations of other potent microbial inhibitors (alkaloid derivatives oxynicotine and cotinine; limonene and others), as well as a number of minor active compounds (e.g., the terpenoids 1,8-cineole, farnesyl acetone and α-pinene) all probably involved in a synergistic pathogen inhibition. The mechanisms of action of these metabolites is not completely cleared yet, but several pathways have been suggested, depending on microorganism cell structure and metabolism specifics Only a few of the studies investigated the direct activity of pure extract components against the respective pathogens (Nazzaro et al., 2017; Pramono et al., 2018; Raveau et al., 2020). Therefore, our results create grounds for further investigation in this aspect, which is set as a possible objective of future research.

CONCLUSIONS

This study examined the biological activity of two natural ready-to-use concentrated aromatic products, resinoid and concrete, derived from the leaves of three species of the genus *Nicotiana* (*N. tabacum, N. rustica* and *N. alata*, in two genotypes) against phytopathogens, causes of bacteriosis in many cultivated plants, fruits and vegetables.
The pathogens with which the tests were performed from the leaves of infected plants (pepper and mulberry) with symptoms characteristic of bacteriosis and from soil were isolated. Based on the macromorphological characteristics of the colonies and microscopic diagnosis of the isolates, they were assigned to the genus *Xantomonas* G (-), to the genus *Pseudomonas* G (-) and two morphotypes to the genus *Bacillus* G (+).
All extraction products were showed inhibitory potential activity against the test pathogens, but there were variations in both species and products. *Nicotiana* resinoids had significantly stronger bactericidal activity (Gram-positive and Gram-negative), compared to the respecttive concretes. The resinoids derived from *N. rustica* and *N. tabacum* had the strongest action against G (-) bacteria defined as *Xantomonas* spp. and *Pseudomonas* spp. The extracts obtained from *N. alata* (genotype with pink flowers) showed the highest bactericidal activity against G (+) bacteria from genus *Bacillus*.
To the best of our knowledge, the aromatic products concrete and resinoid obtained from different *Nicotiana* species have not been studied previously in terms of their phytoprotective potential and these are the first data in this aspect. The outcomes from the study are promising and provide grounds for the development of natural or combined products with bio-protective activity as the next step, as well as for further investigations on the biological activity of *Nicotiana* species.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Agricultural Academy, Bulgaria, Projekts: PDZM – 220; TH9

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCHES REGARDING THE INFLUENCE OF VERMICOMPOST ON HORTICULTURAL PLANT SPECIES

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Abstract

Vermicompost is a new generation of organic fertilizer which is obtained by processing organic matter with the help of earthworms. The use of vermicompost contributes to the improvement of the physico-chemical properties of the ground through a better structuring of the soil, a decrease of the apparent density and an increase of the humus content in the soil. Vermicompost can replace chemical or organic fertilizers being very rich in nutrients and with a beneficial effect on the development of cultivated plants. The research was conducted in 2020 and aimed at the influence of vermicompost on the germination and development of tomato, pepper, eggplant and cucumber plants. There were used different variants: V1 - control, V2 - control treated with Tecamin Raiz, V3 - vermicompost 1 l/200 l water, V4 - vermicompost 1 l/150 l water and V5 - vermicompost 1 l/100 l water. The results obtained led to the determination of vermicompost doses for each species, being known that each group of plants has a certain need for nutrients.

Key words: *dose application, fertilisation, organic matter, soil fertility, vermicompost.*

INTRODUCTION

Vermicompost is a new generation of organic fertilizer that is produced with the help of earthworms. It is a concentrated, mineral-organic fertilizer, with no preservative in its composition (Munroe, 2007).
Earthworm humus is the best fertilizer as it contains high concentrations of beneficial bacteria and other microorganisms, many biologically active stimulants for plants, vitamins, amino acids, fulvic and humic acid, all added during the digestive process of the earthworm (Atiyeh et al., 2007). Earthworm humus completely replaces any chemical or organic fertilizer and contains 100 times more nutrients and microorganisms beneficial to plants (Subler, Scott et al., 1998; Indian Journal of Biotehnology, 2007).
Research carried out over several years in laboratories in several countries has concluded that earthworm humus is excellent in preventing diseases such as: *Alternaria* spp. (Alternariosis), *Botrytis* spp. (Gray mold), *Fusarium* spp. (Fusariosis), *Peronospora* spp (manna), *Phytophthora cinnamomi*, *Pythium* spp. (Root rot), *Pseudomonas syringae* (bacterial burning in peas), *Rhizoctonia solani* (rhizoctoniosis), *Septoria* spp. Inequalis, *Thielaviopsis basicola* (black tobacco rot), *Venturia inequalis* (apple rot) (Gershuny, 2011; Sudha, 2000).
Vermicompost does not contain or provide living conditions for *E. coli*, *Salmonella* or other pests (Journal of Agricultural Science, 2003).
Vermicompost is the only fertilizer, accepted in the EU as an amendment for organic farming, according to *Regulation (EC) no. 834/2007*.
Other benefits of using this organic fertilizer are: rapid germination, better developed and stronger root system, better water retention in plants, accelerated budding, faster growth and resistance to various bacteria and diseases. Liquid vermicompost prevents the appearance and growth of pathogenic micro-flora, reduces the nitrate content in fruits and vegetables, blocks the absorption of heavy metals from the soil by plants, increases the content of fructose, proteins and vitamins. Stimulates flowering, fertility, enriches the taste and resistance of products.

MATERIALS AND METHODS

In order to obtain the vericompost in 2020, a platform with a thickness of 25 cm was built on which the substrate was prepared for the

earthworm bed. This substrate was prepared from cattle manure that was kept soaked for 4-5 months and loosening wheat straw. After the bed was prepared, the earthworms were brought and placed in the nest. After introduction, another layer of manure of about 10 cm was placed over the earthworms (Ilie, 2019).
The first feeding was done one month after the worms were introduced into the nest, after which they were fed 100 kg of manure and straw every 2 weeks. They had to be watered almost daily, especially during the summer. The aeration was done once a month and consisted of loosening the first 10 cm layer.
After 6 months the earthworms were moved to another nest, but the vermicompost was left for another 3 months after which it was removed, sifted to be crushed and loosened. After the previously mentioned actions, the vermicompost extract was turned into liquid.
After obtaining the solid vermicompost and making the liquid vermicompost extract, there was performed the physico-chemical and biological analysis of this pH-6.5, Humus 6.35, Soluble sprouts 0.303, N-NO3 1662.5 mg/kg, N-NH4-19.125 mg/kg, P-19.05 mg/kg, Ca 22 mg/kg and Mg 20 mg/kg.
The proposed variants for testing the liquid vermicompost for the vegetable seeds germination were: Liquid vermicompost dilution 1:30, Liquid vermicompost dilution 1:40. The seeds were kept in solution for 24 hours after which they were dried for 24 hours and then sown.
The variants that were used for vermicompost testing in seedling production: V1 - untreated control; V2 - control treated with Tecamin Raiz biostimulator; V3 - treated with vermicompost 1 l/200 l water; V4 - treated with vermicompost 1 l/150 l water; V5 - treated with vermicompost 1 l/100 l water. Tomato, pepper, cucumber and eggplant seeds were used and the substrate was Kekkila DSM 3W peat.

RESULTS AND DISCUSSIONS

The influence of vermicompost on seed germination.

It can be seen in Figure 1 that after 5 days, the percentage of germinated tomato seeds is higher in the vermicompost variant. In the other days, the germination percentage is decreasing compared to the other variants.
In Figure 2 it can be seen that every day the percentage of germinated seeds is higher than in the other variants. From this it can be concluded that the best dose of vermicompost for the germination of tomato seeds is 1:40.

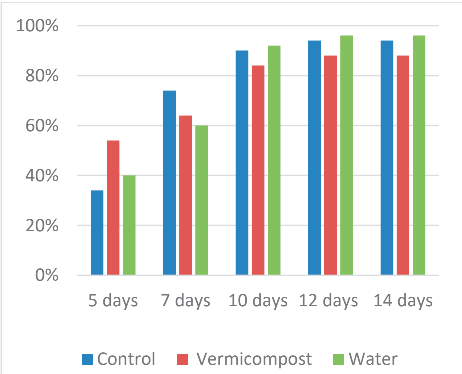

Figure 1. Germination of tomato seeds using vermicompost in dilution 1:30

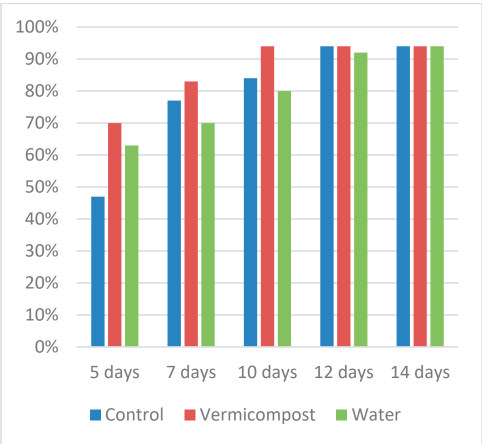

Figure 2. Germination of tomato seeds using vermicompost in dilution 1:40

The influence of vermicompost on pepper seed germination.

The were used 50 pepper seeds from each variant (Figures 3 and 4).

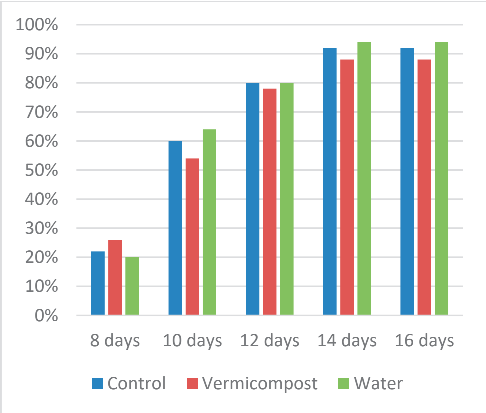

Figure 3. Germination of pepper seeds using vermicompost in dilution 1:30

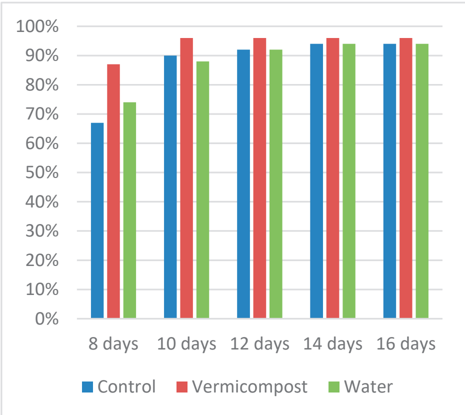

Figure 4. Germination of pepper seeds using vermicompost in dilution 1:40

In Figure 3 it can be observed that after 8 days, the percentage of germinated pepper seeds is higher in the vermicompost variant. In the other days, the germination percentage is decreasing compared to the other variants.

In Figure 4 it can be observed that after 8 days, the percentage of germinated pepper seeds is higher in the vermicompost variant and remains the same in the other days. It can be concluded that the best dose of vermicompost for the germination of pepper seeds is 1:40.

In Figures 5 and 6 can be seen the germination of cucumber seeds which have been kept for germination in different dilutions of vermicompost.

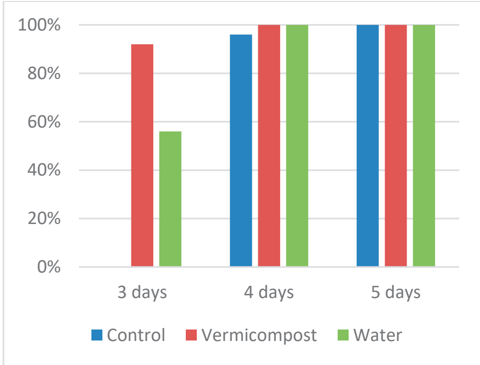

Figure 5. Germination of cucumber seeds using vermicompost in dilution 1:30

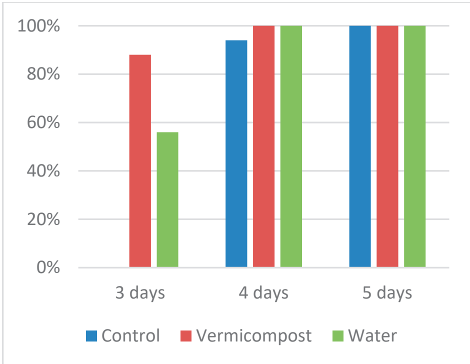

Figure 6. Germination of cucumber seeds using vermicompost in dilution 1:40

In Figure 5 can be seen that the variant in which vermicompost was used, the seeds germinated faster, on day 3 the percentage of germinated seeds was 92% compared to the control, in which the percentage was 0%.

Figure 6 shows that the variant in which vermicompost was used but in a concentration of less than 1:40, the percentage of seeds germinated on day 3 was 88% compared to the control, in which the percentage was also 0%. It can be concluded that for cucumber seeds germination the best dose of vermicompost is 1:30, but the dose of 1:40 is not bad being only 4% lower.

Figure 7 shows that the percentage of seeds germinated in the vermicompost version is lower than in the water version every day, but in recent days it is also lower than the control version.

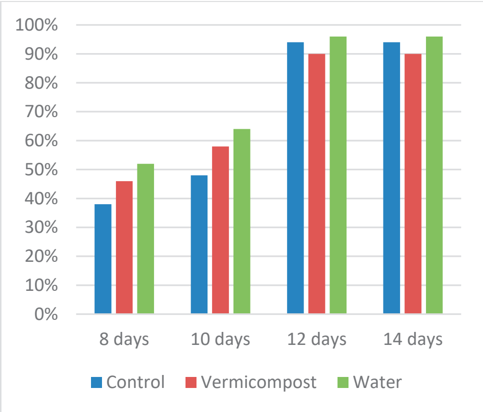

Figure 7. Germination of eggplant seeds using vermicompost in dilution 1:30

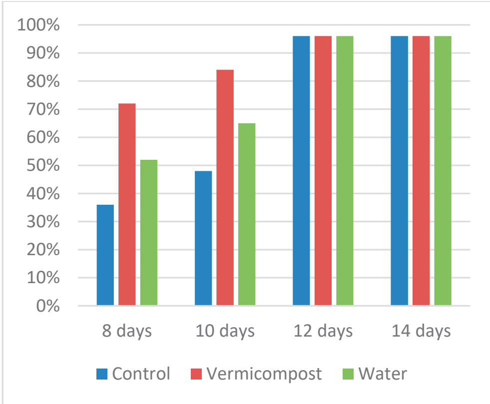

Figure 8. Germination of eggplant seeds using vermicompost in dilution 1:40

In Figure 8 it can be observed that the percentage of germinated seeds in the vermicompost variant is higher in the first days than in the other variants. It can be concluded that for the germination of eggplant seeds the best dose of vermicompost is 1:40, because the dose of 1:30 inhibits the germination of seeds.

The influence of vermicompost on the growth and development of tomato, pepper, cucumber and eggplant seedlings.

In Figure 9 it can be seen which variant was better for each plant.

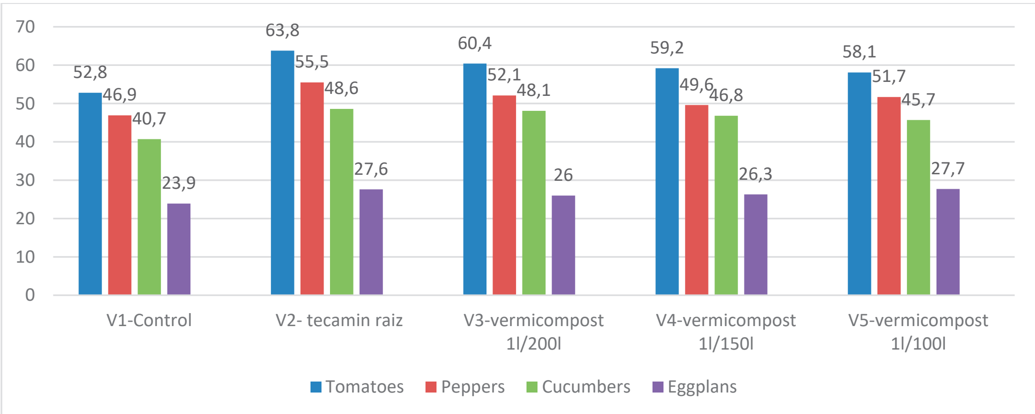

Figure 9. Total length of plants (cm)

Of the 3 variants with vermicompost, the best variant was: V3 for tomatoes with 60.4 cm; V3 for peppers with 52.1 cm; for cucumbers V3 with 48.1 cm and for eggplants V5 with 27.7 cm. Figure 10 shows in which variant the root system was better developed for each plants category.

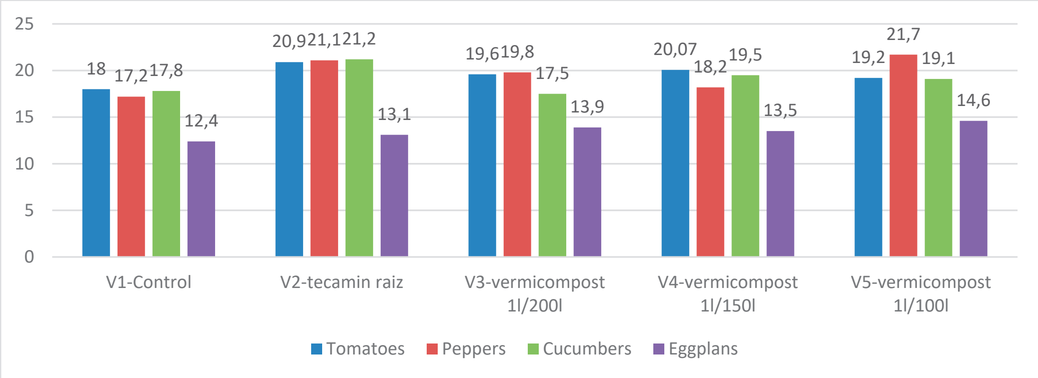

Figure 10. Plant root length (cm)

Among the variants with vermicompost, the best variant was: V4 for tomatoes with 20.07 cm; for peppers it was V5 by 21.7 cm; in cucumbers it was V4 with 19.1 cm and in eggplants V5 with 14.6 cm. In Figure 11, the average stem length can be observed for each tested variant and for each category of plants.

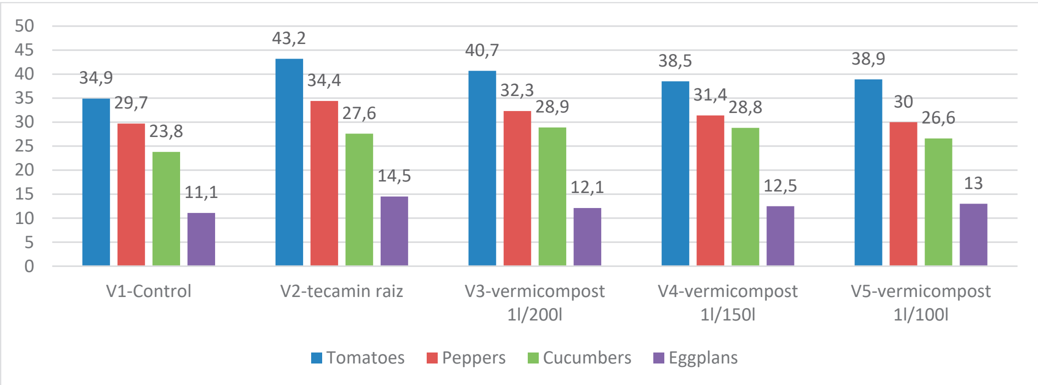

Figure 11. Length of plant stem (cm)

Among the variants with vermicompost, the best variant was: V3 for tomatoes with 40.7 cm; in peppers it was V3 by 32.3 cm; in cucumbers it was V3 with 28.9 cm and in eggplants it was V5 with 13 cm. In Figure 12 you can see the thickness of the plant package for each variant.

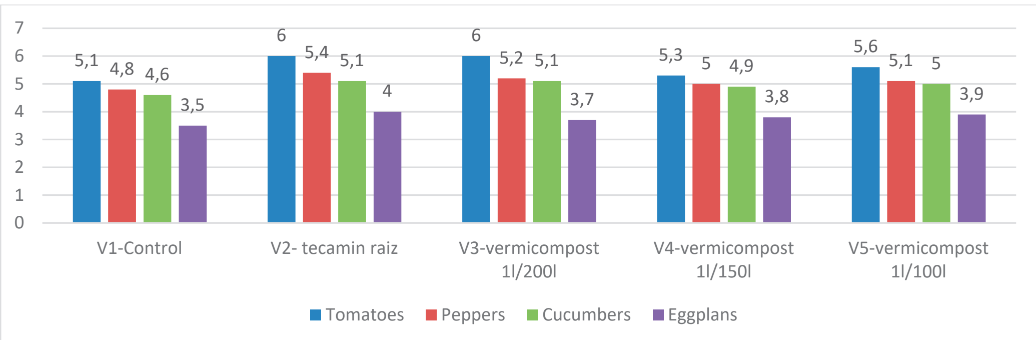

Figure 12. Plant package thickness (mm)

Among the vermicompost variants, the variant with the thickest package was: V3 for tomatoes with 6 mm; V3 for peppers with 5.2 mm; V3 for cucumbers with 5.1 mm and V5 for eggplants with 3.9 mm. In Figure 13 it can be seen how the weight of the plants differs from one variant to another.

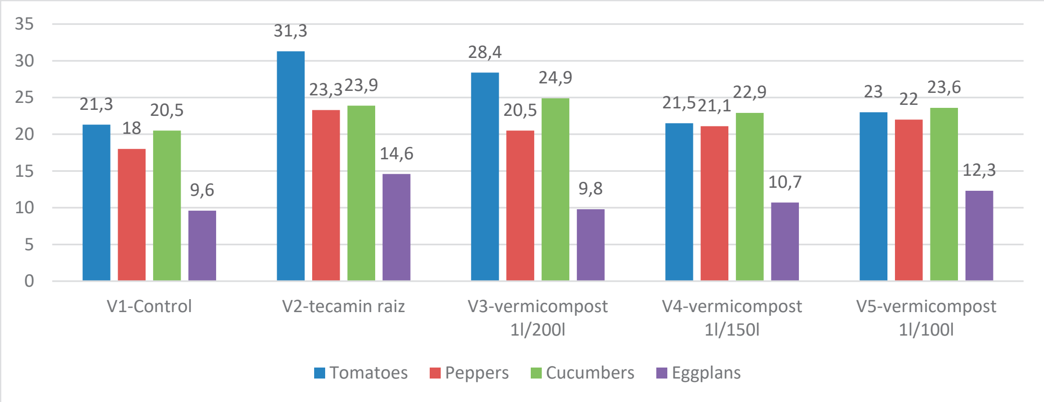

Figure 13. Total weight of plants (g)

Among the vermicompost variants, the variant with the best results was: V3 for tomatoes with 28.4 g; V5 for peppers with 22 g; V3 for cucumbers with 24.9 g and V5 for eggplants with 12.3 g. In Figure 14 the weight of the plant stem can be seen in all the studied variants.

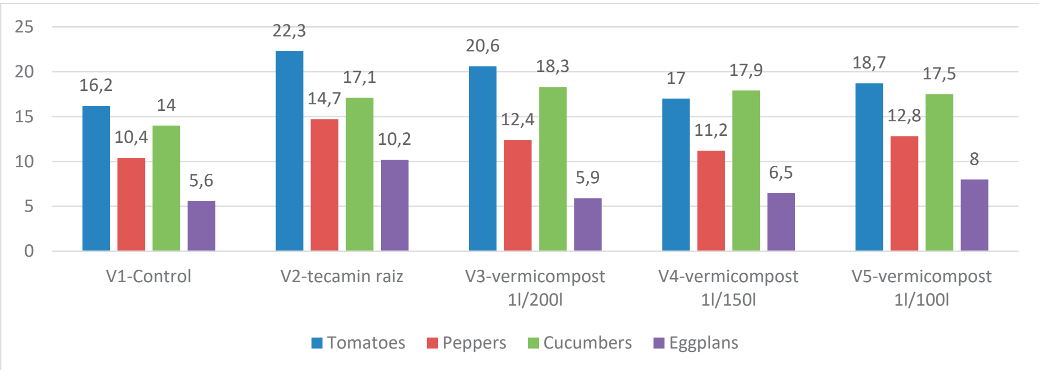

Figure 14. Weight of plant stem (g)

Among the variants with vermicompost, the variant with the best results was: V3 for tomatoes with 20.6 g; V5 for peppers with 12.8 g; V3 for cucumbers with 18.3 g and V5 for eggplants with 8 g. In Figure 15 the weight of the plant root can be seen in each variant studied.

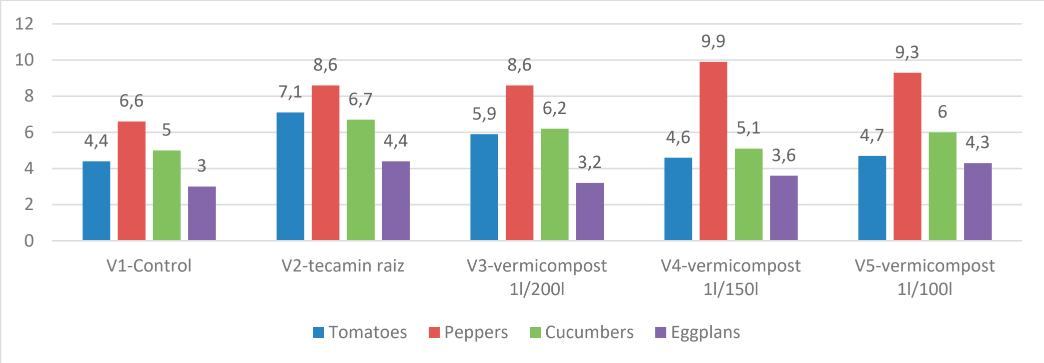

Figure 15. Weight of plant root (g)

Among the variants with vermicompost, the variant with the best results was: V3 for tomatoes with 5.9 g; V4 for peppers with 9.9 g; V3 for cucumbers with 6.2 g and V5 for eggplants with 4.3 g. In Figure 16 the weight of the dried plants can be seen for each variant.

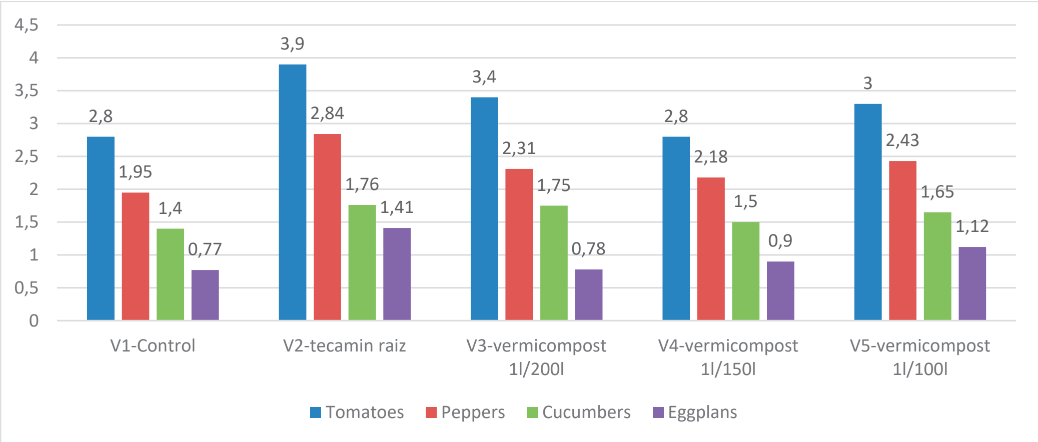

Figure 16. Weight of dry plants without roots (g)

Among the variants with vermicompost, the variant with the best results was: V3 for tomatoes with 3.4 g; V5 for peppers with 2.43 g; V3 for cucumbers with 1.75 g and V5 for eggplants with 1.12 g.

Table 1 shows the values obtained for V1 variant - untreated control, for V2 variant - control treated with Tecamin Raiz and for vermicompost, the variant with which were obtained the best results.

From the table it results that the variants with vermicompost are at a great distance from the variant V1 - untreated control, so the vermicompost has good results.

In the case of V2 variant - control treated with Tecamin Raiz, the variants with vermicompost are at close values but lower than the value of V2 variant.

Out of the total of 32 measurements performed on the 4 groups of plants, the variants with vermicompost exceeded the value of the treated V2 control in 7 cases, in 2 cases they were equal and in 23 cases they were lower than the V2 variant.

The variant with vermicompost with the best results in total, was V3 - vermicompost 1 l/200 l - out of the total of 32 measurements in 17 was on the first place.

On the 2nd place was the variant V5 - vermicompost 1 l/100 l - with 12 measurements and on the 3rd place, the variant V4 - vermicompost 1 l/150 l - with 3 measurements.

Table 1: Determining the best variant with vermicompost in relation to the untreated control variant and the treated control variant

Test performed	Plant	Untreated witness V1	Witness treated with Tecamin Raiz V2	Vermicompost variant with the best results
Total length of plants	Tomato	52.8 cm	63.8 cm	V3 – 60.4 cm
	Pepper	46.9 cm	55.5 cm	V3 – 52.1 cm
	Cucumbers	40.7 cm	48.6 cm	V3 – 48.1 cm
	Eggplants	23.9 cm	27.6 cm	V5 – 27.7 cm
Plant root length	Tomato	18 cm	20.9 cm	V4 – 20.07 cm
	Pepper	17.2 cm	21.1 cm	V5 – 21.7 cm
	Cucumbers	17.8 cm	21.2 cm	V4 – 19.5 cm
	Eggplants	12.4 cm	13.1 cm	V5 – 14.6 cm
The length of the plant stem	Tomato	34.9 cm	43.2 cm	V3 – 40.7 cm
	Pepper	29.7 cm	34.4 cm	V3 – 32.3 cm
	Cucumbers	23.8 cm	27.6 cm	V3 – 28.9 cm
	Eggplants	11.1 cm	14.5 cm	V5 – 13 cm
The thickness of the plant package	Tomato	5.1 mm	6 mm	V3 – 6 mm
	Pepper	4.8 mm	5.4 mm	V3 – 5.2 mm
	Cucumbers	4.6 mm	5.1 mm	V3 – 5.1 mm
	Eggplants	3.5 mm	4 mm	V5 – 3.9 mm
Total weight of plants	Tomato	21.3 g	31.3 g	V3 – 28.4 g
	Pepper	18 g	23.3 g	V5 – 22 g
	Cucumbers	20.5 g	23.9 g	V3 – 24.9 g
	Eggplants	9.6 g	14.6 g	V5 – 12.3 g
The weight of the plant stem	Tomato	16.2 g	22.3 g	V3 – 20.6 g
	Pepper	10.4 g	14.7 g	V5 – 12.8 g
	Cucumbers	14 g	17.1 g	V3 – 18.3 g
	Eggplants	5.6 g	10.2 g	V5 – 8 g
The weight of the plant root	Tomato	4.4 g	7.1 g	V3 – 5.9 g
	Pepper	6.6 g	8.6 g	V4 – 9.9 g
	Cucumbers	5 g	6.7 g	V3 – 6.2 g
	Eggplants	3 g	4.4 g	V5 – 4.3 g
The weight of dried plants without roots	Tomato	2.8 g	3.9 g	V3 – 3.4 g
	Pepper	1.95 g	2.84 g	V5 – 2.43 g
	Cucumbers	1.4 g	1.76 g	V3 – 1.75 g
	Eggplants	0.77	1.41	V5 – 1.12 g

CONCLUSIONS

Based on the research conducted with different doses of vermicompost on tomato seeds, peppers, cucumbers and eggplants and then on seedlings, the following emerged:

The best dose of vermicompost for the germination of tomato seeds, eggplant peppers and cucumbers is 1:40, because at a higher concentration it inhibits germination;

The height of the plants was influenced by the doses applied as follows: V3 for tomatoes by 60.4 cm; V3 for peppers with 52.1 cm; V3 for cucumbers with 48.1 cm and for eggplants V5 with 27.7 cm;

Regarding the length of the plant root - among the variants with vermicompost the best variant was: V4 for tomatoes with 20.07 cm; for peppers it was V5 by 21.7 cm; for cucumbers it was V4 with 19.1 cm and for eggplants V5 with 14.6 cm;

At the length of the plant stem - among the variants with vermicompost the best variant was: V3 for tomatoes with 40.7 cm; in peppers it was V3 by 32.3 cm; for cucumbers it was V3 with 28.9 cm and for eggplants it was V5 with 13 cm and for the thickness of the plant package - among the vermicompost variants, the variant with the thickest package was: V3 for tomatoes with 6 mm; V3 for peppers with

5.2 mm; V3 for cucumbers with 5.1 mm and V5 for eggplants with 3.9 mm;

The total weight of the plants - among the variants with vermicompost, the variant with the best results was: V3 for tomatoes with 28.4 g; V5 for peppers with 22 g; V3 for cucumbers with 24.9 g and V5 for eggplants with 12.3 g and related to the weight of the plant stem - among the variants with vermicompost, the variant with the best results was: V3 for tomatoes with 20.6 g; V5 for peppers with 12.8 g; V3 for cucumbers with 18.3 g and V5 for eggplants with 8 g;

From the obtained results it shows that the variants with vermicompost are at a great distance from the V1 variant - untreated control, so the vermicompost has good results;

In the case of V2 variant - control treated with Tecamin Raiz, the variants with vermicompost are at close values, but lower than the value of V2 variant. Out of the total of 32 measurements performed on the 4 groups of plants, the vermicompost variants exceeded the value of the treated control V2 in 7 cases, in 2 cases they were equal and in 23 cases they were lower than the V2 variant.

The variant with vermicompost with the best results on the total, was V3 - vermicompost 1 l/ 200 l followed by the variant V5 - vermicompost 1 l/100 l. For cucumbers - V3 variant with 7 measurements out of the total of 8 performed;

From the results obtained it can be concluded that each group of plants has different nutritional needs and that it is not possible to establish a single dose of vermicompost that is useful in all cases, but one can choose the dose that had the best results. As can be seen in the case of the variant treated with another product - although it is better, it is still not perfect, surpassing the variants with vermicompost in 7 cases.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DETERMINATION OF AGRO-TECHNICAL INDICATORS IN SOIL TREATMENT IN ORCHARDS

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Abstract

A field experiment was conducted with three tillage machines (disc harrow, soil tiller and cultivator) in an orchard in the area of Asenovgrad, Plovdiv region. The following agrotechnical indicators were observed - Soil loosening, pruning of weeds in the row spacing and maintenance of the set depth of work. It was found that the degree of loosening of the soil (85.5%) is highest in the milling machine. The disc harrow achieves maximum weed control (88.9%) and best maintains the set working depth (deviation 0.44 cm). An analysis of the obtained results was performed.

Key words: *disc harrow, tiller, cultivator, orchard, agrotechnical requirements.*

INTRODUCTION

The main task that has always faced science in fruit growing is to increase yields and improve the quality of fruit production. One of the factors that create the necessary conditions for solving this task is the system for maintaining the soil in orchards. The main goal is to destroy weeds in the order and row spacing of orchards and improve the structure of the surface soil layer.

Traditional for our country is tillage with disc harrows or cultivation. Each of these operations is applicable depending on the condition of the soil and especially on its moisture.

The main requirements for the operation of these machines, from the point of view of the agrotechnics of the cultivated crops, are crushing the soil and pruning the weeds (St. Dimov et al., 1969). Regarding these indicators, there are many studies of domestic and foreign scientists.

P. Panchev, L. Manov, Hr. Nankov (1983) observed the operation of V- and X-shaped disc harrows. They found that soil aggregates up to 50 mm in size represented 86.0 to 88.9% of the soil volume after twice passing through the cultivated field. Weed control varies from 66.8% in the first pass to 92.3% after the second pass with the working bodies of the harrow. Establish a relationship between the working width of the machine and the degree of fragmentation of the soil. With a smaller width of the used machine there is a better copying of the microrelief and from there better crushing of the soil and weeds control.

Significant is the contribution in this direction of St. Tirovska (1981), who studies the operation of a disc harrow with forced drive of the working bodies. Comparing the work in different operating modes, it was found that this machine is very effective when working on wetter soils. Under such conditions, the necessary agro-technical parameters of the cultivated soil are achieved with only one working stroke

R. Pal, J.P. Bhimwal, S. Choudhary (2015) investigated the operation of an offset soil tiller in the cultivation of orchards. They found that with increasing the depth of processing and changing the kinematic indicator increases fuel consumption and energy consumption

Bychkov V., Kadykalo G., Shevkun V. (2011) have analyzed the existing and future designs of machines for work in orchards. They have identified the trends that must be met by the technical means for processing the inter-row strips of perennial crops and their working bodies: the possibility to change the depth of cultivation; ensuring complete extraction of weeds from the soil from elements of the working body; preservation of the root system of the cultivated plant; automatic maintenance of the working depth; reversible drive; ability to work in orchards and berry fields with different row spacing.

Gorovoy and Kharchenko (2018) found that the use of combined machines in tillage in the aisle of the garden allows to minimize labor costs and funds, to increase the culture of agriculture. The possibilities for combining technological operations are determined by the used systems of agriculture and soil cultivation, the pollution of the fields with weeds and their species composition, meteorological conditions, parameters of energy resources, agronomic, technical and economic and other factors. Tillage with simultaneous application of fertilizers along the contour of the root system of fruit trees in the rows of the garden reduces the number of technological operations from 5-7 to 1-2 during the growing season. Reducing the number of tillage leads to a reduction in fuel consumption for their implementation; reduces soil compaction; targeted fertilization increases yields.

In their study, M.JUGOVIĆ, T.JAKIŠIĆ, O.PONJIČAN (2020) observed and compared the operation of a harrow, a disc harrow and a milling machine. Comparing the work of the three machines, they found that the milling machine sprayed the soil more strongly.

Dallev M., I. Ivanov (2012) develop and study a machine that combines the principles of operation of a disc harrow and a milling machine. The authors found that depending on the inclination of the discs and the speed of movement, soil aggregates with a size of less than 25 mm, during one working stroke with the machine vary from 52.9 to 76.7%.

Kostadinov et al. (2016), G. Parkhomenko G. S. Tverdohlebov, A. Ponomarev (2016) systematize the machines used for tillage in fruit growing. The authors recommend that the control of the working bodies of the machines be performed automatically with a hydraulic tracking device. Only under this condition has a positive economic effect

MATERIALS AND METHODS

In the present work the object of observation are disk harrow BDTS-2,5; tiller FN-1,6 and vineyard machine UNLM-3,5 when working in orchards in the area of Asenovgrad municipality. The Table 1 provides a brief description of the plantation and soil condition:

Table 1 Characteristics of the cultivated field

№	Indicators	Value
1	Field dimensions, m length width	 560 400
2	Row spacing, m	4.5
3	Soil type	Alluvial-meadow
4	Soil moisture, %	22.5
5	Hardness, HB	44.8

The main indicators for the operation of the machines are:

Soil loosening (η) - is defined as the percentage of aggregates up to 50 mm in size relative to the total volume of the soil sample.

Destroyed weeds (δ) - The requirement is to destroy at least 90% of the weeds, and the inspection is performed in 5 places.

Maintaining set working depth (Δa) - In the present work, the machines are pre-set to work at a depth of 10 cm. The actual working depth is measured at 5 places along the working stroke of each machine. The difference between the average value and the set working depth gives the deviation from the working depth.

In order to determine whether the work of each of the three machines differs from the work of the others, a comparison of the average values is performed for each of the monitored indicators.

RESULTS AND DISCUSSIONS

The results of the experiments are systematized and arranged in the Tables 2-4.

Table 2. Soil loosening, %

Machine	Experience		Aggregates ≤ 50 mm, kg	Loosening, %
	№	Mass kg		
BDTS-2,5 disc harrow	1	4,25	2,68	63
	2	4,79	2,83	59
	3	5,06	3,59	71
	4	4,51	3,07	68
	5	3,98	2,83	71

average		**4,518**	**3,126**	**66,40**
mill FN-1,6	1	3,25	3,02	83
	2	4,81	4,09	85
	3	4,75	4,23	89
	4	3,99	3,17	79,5
	5	5,16	4,70	91
average		**4,392**	**3,842**	**85,50**
vineyard machine UNLM-3,5	1	5,01	2,40	48
	2	4,11	2,14	52
	3	3,86	2,08	54
	4	3,54	1,73	49
	5	4,83	2,80	58
average		**4,27**	**2,23**	**52,20**

The best for this indicator are the data for the soil tiller, and the worst - those for the vineyard machine. This is completely logical, since the working bodies of the cutter are active, with forced drive. During operation, they cut small particles of soil, which are further broken down into smaller units, by hitting the machine cover. Regarding weed control, the following results were obtained:

Table 3. Weed control

Машина Machine	Experience	Weeds before processing, number / m2	Weeds after processing, number / m2	Weed control, %
BDTS-2,5 disc harrow	1	34	4	88,23
	2	16	2	87,5
	3	27	5	81,48
	4	21	2	90,48
	5	30	1	96.67
average				**88,87**
mill FN-1,6	1	16	3	81,25
	2	25	4	84,00
	3	31	1	96,77
	4	30	6	80,00
	5	18	4	77,78
average				**83,96**
vineyard machine UNLM-3,5	1	27	6	77,78
	2	43	6	86,05
	3	22	8	63,64
	4	8	1	87,50
	5	16	3	81,25
average		**23,2**	**3,2**	**79,24**

As with loosening, the data for machines with active implements are better than those for cultivators. However, we should not forget the fact that in the presence of rhizome weeds it is not recommended to use disk and milling machines because by cutting the roots of weeds we increase their reproduction.

To maintain the set working depth, the following results were obtained:

Table 4. Deviation from the set working depth

Experience / Machine	1	2	3	4	5	average	Deviation from the depth, cm
БДТС-2,5	10,5	11	10	10	10,7	10,44	**0,44**
ФН-1,6	9,5	8	11	8,5	10,2	9,44	**1,04**
УЛНМ-3,5	11	12	10	11	10	10,8	**0,8**

The cutter has the largest deviation at the set working depth. It has the least weight and micro-irregularities more easily affect its vertical stability. Conversely, the disc harrow has the greatest weight, which contributes to its minimal deviation from the set working depth.
The comparison of the performance indicators for the used machines is graphically presented in Figures 1, 2 and 3.

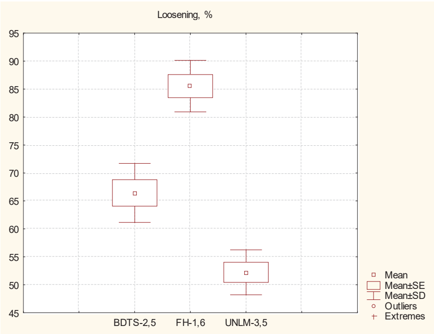

Figure 1. Loosening, %

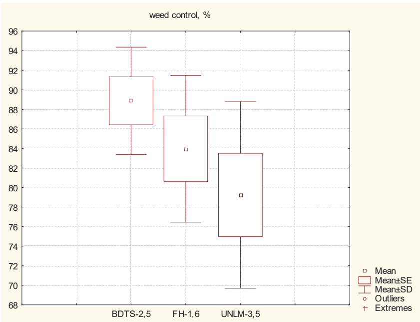

Figure 2. Weed Control, %

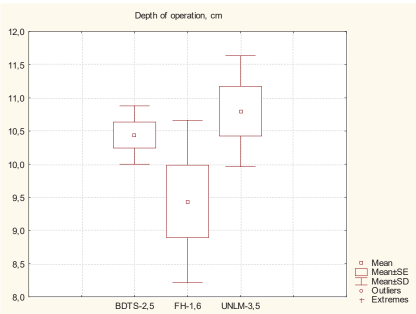

Figure 3. Depth of operation, cm

It can be seen that only with the indicator of soil loosening there is a proven statistical difference in the operation of the three machines, with the best value is the cutter. For the other two indicators, despite the difference in the average values, there is no proven statistical difference due to their large variation.

CONCLUSIONS

The following conclusions can be drawn from the performed experiments, the performed analyzes and summaries:
All three machines meet the agro-technical requirements for surface tillage in orchards. Only with the indicator of soil loosening there is a proven statistical difference in the operation of the three machines, with the best value is the cutter. For the other two indicators, despite the difference in the average values, there is no proven statistical difference due to their large variation.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CLIMATE TRENDS IN OLTENIA. CASE STUDY: CRAIOVA - BANU MĂRĂCINE

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Abstract

Climate is the result of the interaction of biotope factors. It is important for cultivated plants because it ensures the passage of the vegetation phases. Climate change may require the adaptation of cultivation technology, the change of varieties or the modification of the cultivation area. For this reason, the paper studies the evolution of the climate in Oltenia, a historical region located in the south-west of România. This region is important from an agricultural point of view (cereals, grapevine, vegetables). This study is based on weather records (e.g. hours of sunshine, annual rainfall, monthly precipitation, average monthly temperature, average annual temperature) over a long period of time, obtained from the Craiova weather station. In order to show the climate trend in Oltenia, the statistical indices of the data series were calculated (e.g. arithmetic mean, standard deviation, coefficient of variation). The thermic and water resources of a territory can be important for characterizing the existing climate. As a result, the values of these climatic factors were used to calculate two ecological indicators: the Martonne`s aridity index and the Lang`s humidity index. The obtained results were included in the tables and represented graphically and show the evolution of the climate in Oltenia over the last fifty years.

Key words*: climate, monitoring, trend, agroecology.*

INTRODUCTION

The climate is the result of the interaction of biotope factors. The change in the composition of the atmosphere, highlighted by the change of the concentration of some gases, such as carbon dioxide, disturbs the existing equilibrium. Studies on this topic show that climate change is not observed only at local level, but it affects the entire planet, with different intensities. For example, aridization phenomena have been reported in the area bordering the Black Sea, namely in the Republic of Moldova (Ivanov, 2017), in the Dobrogea region of Romania (Ionac et al., 2015), in Turkey (Deniz et al., 2011) or in Serbia (Ruml et al., 2016). The increase of the influence of the Mediterranean climate was highlighted in the Oltenia region (Romania) (Marinică et al., 2016).
The consequences of such evolutions manifest on many levels. From an economic point of view, the agriculture is one of the most affected sectors. For the horticultural sector, reductions in the content of substances responsible for the quality of wine production were reported, as well as a quantitative reduction in production (Fourment et al., 2013, 2020; Matei et al., 2009). In this regard, Marković et al., 2015, show that the grapes flavour accumulates in smaller quantities in the years when the average temperature in the growing season is higher compared to the multiannual average. The viticultural climate, as a whole, is evolving, with a tendency to increased temperatures in the northern viticultural area of Romania, for example (Bucur et al., 2016).
For other types of agricultural ecosystems, severe climatic risks have been identified (Roussos, 2020) caused by the early start of the vegetative stage of plants (Cojocaru, 2020).
In this context, there are studies that show that, as the temperature increases, evapotranspiration increases, the water deficit increases and the drought intensifies in irrigated areas (Vizitiu et al., 2019).

MATERIALS AND METHODS

The paper analyses the evolution of some climate indicators calculated based on the records from the Craiova weather station, where there is also located the Banu Mărăcine wine growing centre. The period under study is long enough to identify the climate trend in this horticultural area (1962-2019).

Specific indicators from meteorology, climatology (average annual temperature, annual amount of precipitation, duration of sunshine, etc.) and biogeography like Martonne aridity index, Lang index, Gams index, Dantin - Ravenga index, simple continentality index (Ivanov, 2017; Satmari, 2010; Vlăduț et al., 2017) were taken into account.

A series of statistical indices were calculated (arithmetic mean, rolling average, absolute amplitude, relative amplitude, root mean square deviation, standard deviation, coefficient of variation) and graphs were made that highlight the trend of the studied climatic factors. The Excel program ensured the fast and correct processing of the meteorological data. The interpretation of the obtained values and their correlation with the nature of the climate was made in accordance with the information from the literature (Patriche, 2009; Satmari, 2010; Vlăduț et al., 2017).

RESULTS AND DISCUSSIONS

The analysed time interval is large enough to ensure the characterization of the climate in Craiova - Banu Mărăcine and to indicate, at the same time, the dynamics and the trend. Figure 1 shows the evolution of the average annual temperature, the average temperature in July and the average temperature in January.

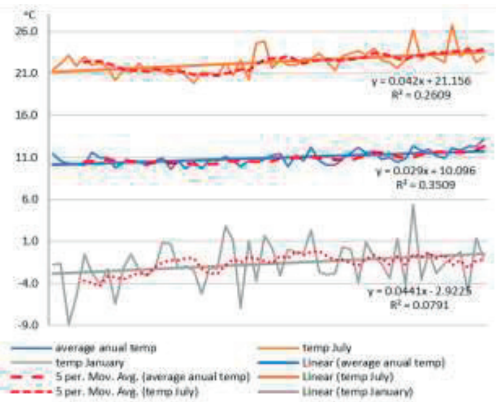

Figure 1. The temperature evolution in Craiova - Banu Mărăcine

Regarding the three series of values, the general evolution in time is highlighted, being noticed an upward trend. The data show a warming in the cold season (January) and in summer (July); the growth trend is lower at the annual level.

The dynamics of the hours of sunshine and annual rainfall recorded in the studied period is shown in Figure 2.

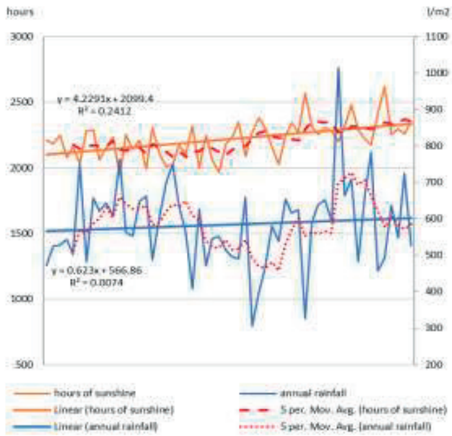

Figure 2. The evolution of sunshine hours and rainfall in Craiova - Banu Mărăcine

The trend is similar to that identified in the case of temperature. The data indicate a growth trend of helio-thermal resources, probably with a moderate general trend of increasing the annual amount of rainfall.

Taking into account all these data, it is advisable to consider the interaction of biotope factors, among which we can mention temperature and humidity, as important factors for the genesis and characterization of the climate.

As a result, Figure 3 shows the evolution of bioclimatic indicators calculated on the basis of the two factors mentioned above, namely the Martonne aridity index and the Lang index. They are important because they make a connection between the nature of the climate and the specific vegetation.

For Craiova, the trend shows slightly reduced values. This means a slow evolution of the climate, in the sense of moving from the warm temperate climate (Lang index: 100 - 60) to the lower limit of the semi-arid climate (Lang index: 60 - 40). It should be mentioned that in the time interval we are referring to, values characteristic for the steppe climate was also recorded (Lang index: 40 - 20) in a proportion of 8.9% of cases. All of them were reported mainly in the second part of the interval, starting with 1983.

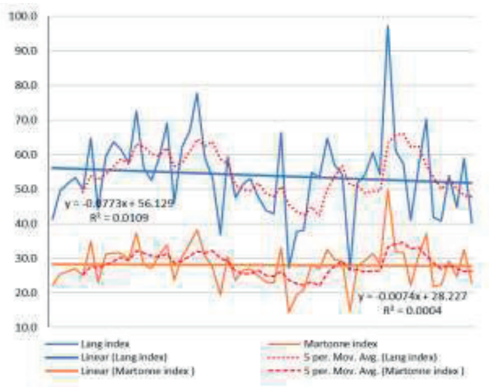

Figure 3. The evolution of the Lang and Martonne indices, in Craiova - Banu Mărăcine

The Martonne aridity index fluctuates from values that characterize the steppe and Mediterranean climate (Martonne index: 10 - 20) to values specific to the humid climate (Martonne index: 30 - 60).

The advantage of this indicator is that it ensures a more nuanced interpretation of the climate and its correlation with the type of vegetation. The data series that is dominant characterize the semi-humid climate (Martonne index: 20 - 30), closely followed by the values characteristic of the humid climate (Martonne index: min 30). As in the previous case, the semi-arid (Martonne index: 15 - 20) and arid (Martonne index: 10 - 15) years were reported when there are high helio-thermal resources amid low rainfall.

The cases were mainly reported in the second part of the considered interval, starting with 1983.

In addition, two more indices used for such studies were calculated, namely: the Gams index and the Dantin-Ravenga index (Figure 4).

The Gams Index has a tendency to change values upwards and maintain zonal favourability for thermophilic species. There are rare cases (3.6%) in which the value of this index falls below 2 but they are common cases when they exceed 3 (50%).

The Dantin - Ravenga index also marks an upward trend, with higher values, which indicates a tendency of aridity of the area.

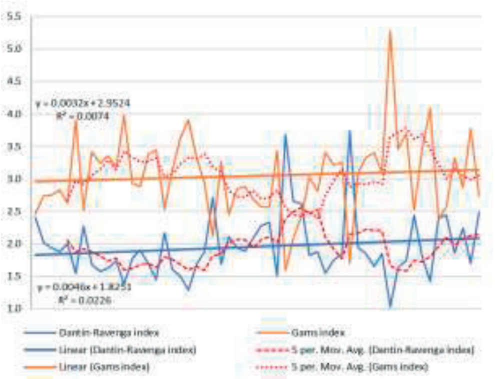

Figure 4. The evolution of the Gams and Dantin - Ravenga indices, in Craiova - Banu Mărăcine

Although the dominant one, in this case, is represented by the values that characterize the humid climate (Dantin-Ravenga index: 0 - 2), there is a trend of the last 25 years oriented towards the prevalence of higher values, specific to the semi-arid climate (Dantin-Ravenga index:> 2) and arid climate (Dantin - Ravenga index:> 3).

Summarizing the previous information provided by these indicators, in the form of average values of the analysed period, we can characterize the current climate; some indicators are also of viticultural importance (Table 1).

Table 1. The climate characteristics in Craiova - Banu Mărăcine

Indicator	Value	Characterization
Average annual temperature (°C)	10.9	Characteristic for temperate climate
Average monthly temperature July (°C)	22.4	Favourable area for obtaining wines with a controlled designation of origin
Average monthly temperature January (°C)	-1.7	-
Hours of sunshine	2219.9	Favourability for viticulture
Annual rainfall (l/m^2)	583.7	Favourability for viticulture
Lang index	53.9	Semi-arid climate
Martonne index	28.0	Semi-temperate climate, forest-steppe vegetation
Gams index	3.04	Climate favourable for thermophilic species
Dantin – Ravenga Index	1.96	Humid climate but at the limit of the semi-arid climate

For further characterization of the data, the variation indicators of the data series were calculated (Table 2).
If we refer to the relative amplitude, we find a similarity of the data to three out of the four climatic parameters. For annual rainfall, the variability is about four times higher than that of temperatures and hours of sunshine.

Table 2. The statistical indicators of the data series

Climate parameter / Statistical indicator	Average annual temperature (°C)	Average monthly temperature (°C) July	Annual rainfall (l/m^2)	The sum of the hours of sunshine (hours)
Absolute amplitude	3.50	6.96	707.84	679.8
Relative amplitude (%)	32.05	31.16	121.27	30.62
Alternative	0.64	1.82	14197.56	19724.30
Standard deviation	0.80	1.35	119.15	140.44
Coefficient of variation (%)	7.30	6.03	20.41	6.33

In fact, the coefficients of variation have close values, if we refer to the average annual temperature, the average temperature of July and the hours of sunshine, while, for humidity, the coefficient of variation has a value of about three times higher.
For this reason, we appreciate that in Craiova - Banu Mărăcine, the volume of annual rainfall varies within much wider limits than the temperature and hours of sunshine.

CONCLUSIONS

The analysis highlighted the dynamics of climate indicators in the studied area and the amplification of helio-thermal and water resources, but in a different way. The trend of the period is more pronounced, if we refer to temperature and hours of sunshine and it is lower, if we consider the volume of rainfall.
The dispersion indices show the temporal variation of the climatic elements, which in Craiova - Banu Mărăcine is higher in the case of rainfall, compared to the temperature and the sum of the hours of sunshine.
The interaction of biotope factors, expressed through the Lang, Martonne, Gams and Dantin - Ravenga indices, confirms the trend of climate aridization. In this context, there are climate challenges to which farmers will have to find adaptation solutions, in the short term, as well as in the medium and long term.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECT OF PERLITE AND NATURAL BIOSTIMULATORS AND FERTILIZERS ON MICROBIAL ACTIVITY IN OIL-POLLUTED SOIL

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Abstract

The presence of hydrocarbons and salts in oil-polluted soils is responsible for inappropriate water, air and nutrients regimes, with negative consequences for plants growth. The aim of this paper is to present the results of research carried out to improve soil conditions for microbial communities, using a mix of oil-polluted soil from Icoana farm, Olt county with Perlite and natural stimulators and fertilizers (AMALGEROL, VERMIPLANT, POCO, IGUANA and FORMULEX) in greenhouse experiments with bean (Phaseolus vulgaris L. cultivar UNIDOR). The paper presents the total counts of bacteria and fungi (estimated by dilution plate) and soil respiration (by substrate-induced respiration method). All the natural products and perlite significantly increased bacterial populations and reduced the fungal counts, especially pathogenic species. Biodiversity was stimulated in bacterial communities, generally dominated by Pseudomonas fluorescens, Bacillaceae and Actinomycetes. The dominance of antagonistic fungi Trichoderma viride and Fusarium oxysporum was recorded in myco-coenoses from variants with perlite and in variants treated with VERMIPLANT, IGUANA or POCO. Soil respiration was stimulated by the better substrate aeration with perlite and the natural stimulators and fertilizers FORMULEX and IGUANA.

Key words: *natural fertilizers, biostimulators, perlite, microbial activity, oil-polluted soil.*

INTRODUCTION

Oil hydrocarbons are considered as persistent organic pollutants with negative effect on environment and human health (Varjani, 2017). The presence of hydrocarbons and salts in oil-polluted soils is responsible for inappropriate water, air and nutrients regimes, with negative consequences for plants growth. Microbial life from soil is also affected by the increased quantities of carbon that cause imbalances in C:N ratio. Edaphic microorganisms (bacterial and fungal species) contribute to soil decontamination by the biodegradation of hydrocarbons depending on nutrient availability, moisture content, temperature and soil pH (Abed et al., 2015; Lahel et al., 2016; Zhao et al., 2018; Galitskaya et al., 2021).
The effect of fertilizers and soil aeration on oil degradation was evidenced (Odu 1984; Margesin and Schinner, 2001; Amadi & Ukpaka, 2016; Tangahu et al., 2017; Fagnano et al., 2020). Many results reported their beneficial impact on biodegradative activity of edaphic microorganisms such as fluorescent bacteria from genus *Pseudomonas*, Bacillaceae or fungi from genera *Aspergillus* (Scarlat et al., 2015), *Penicillium* (Techaoei et al., 2007), frequently used as bioinoculants in bioaugmentation technologies for decontamination of oil-polluted soils (Bonilla et al., 2012; Patowary et al., 2017). Literature reported the antagonistic capacity of various bio-fertilizers against seed-borne myco-pathogens of tomato plants (Mogle & Mane, 2010; Jaiswal et al., 2017; Bonanomi et al., 2020) or stimulation of plant growth and resistance to pathogens for *Eruca sativa* L. plants cultivated on polluted soil under bioinoculation with commercial products based on *Trichoderma harzianum* Rifai (Al-Rajhi, 2013). Research demonstrated that biosurfactants with various chemical composition produced by the consortium of bacterial strains from soil contaminated with hydrocarbon in Cepu area, Central Java, Indonesia, were involved in decomposition of oil residues (Sumiardi et al., 2012). Xu et al.

(2005) reported bioremediation of oil-contaminated sediments on an inter-tidal shoreline using a slow-release fertilizer and chitosan.
Research has been carried out to assess the quantitative and qualitative changes in microbial communities from soil contaminated with hydrocarbons under the influence of perlite added to dilute the soil for improving aeration and with various natural biostimulators and plant fertilizers.

MATERIALS AND METHODS

Soil for greenhouse experiment was collected from a private farm located to Icoana, Olt county, accidentally contaminated with total petroleum concentration of 72.87g x kg^{-1} dry soil by spillage from deteriorated pipes. Microbial communities in polluted soil have been characterized comparatively with those from non-polluted soil (Matei & Matei 2017).
In order to improve soil conditions for microbial communities, a mix in proportion of 50% each (v/v) of surface (A horizon) oil-polluted soil from Icoana with perlite (provided by PROCEMA SRL) was used in the greenhouse experiment with bean (*Phaseolus vulgaris* L. cultivar UNIDOR) as test plant. Expanded perlite obtained by thermic treatment has twenty times increased volume comparatively with initial material. It is a white, hard and very porous material utilisable as additive for soil. Perlite for horticultural utilization 5 (0-5 mm in diameter) has the density 0.1-0.9 $gxcm^{-3}$, humidity 0.5%, pH 6.5-8, refraction index 1.5, thermic conductivity at 24ºC 0.04-0.06 W/m*K, solubility in water <1%, weak acids, contains 3% bound water, silicon 33.8%, aluminium 7.2%, potassium 3.6%, sodium 3.4%, iron 0.6%, calcium 0.6%, magnesium 0.2%, microelements 0.2% (Drăghici et al., 2016a). Available water in expanded perlite was 36.5-43.2% from its volume. This water can be released in time helping plants to survive during drought conditions. Five natural stimulators and fertilizers (AMALGEROL, VERMIPLANT, POCO, IGUANA and FORMULEX) were added to.
AMALGEROL (Hechenbichler, Austria) is a natural product with vegetal oils and hormones that stimulates plant growth, mycorrhizal symbiosis, N_2-fixing, microbial activity, vegetal debris decomposition, improves soil structure and fertility (Retrieved from https://www.amalgerol.com/).
VERMIPLANT (Doctor Plant Morile Mătieș, Romania) is a biofertilizer enriched in natural nutrients from earthworms, containing microelements (barium, iron, zinc, manganese) and amino acids that stimulate microbial activity and plant growth (Retrieved from https://doctorplant.ro/ingrasaminte/201-304-vermiplant-ingrasamant-foliar.html).
POCO (Wise Use International BV, Netherlands) is a natural product of herbs and plant extracts (utilized for pollution control), stimulating and accelerating the growth and metabolic activity of microorganisms by micro-nutrients and trace elements (Retrieved from https://www.wiseuse.nl./wiseuseeng/pocoeng.html).
IGUANA (Advanced Nutrients, Canada) is a natural organic product of algae with macro and microelements plus other co-factors necessary for improving soil conditions, stimulating plant growth and yields (Retrieved fromhttps://www.advancednutrients.com/secret-menu/iguana-juice-organic-oim/).
FORMULEX (Growth Technology, England) is a natural complete, balanced and stabilized nutrient solution of all macro and microelements for optimum plant growth and rooting in horticulture (Retrieved from https://www.growthtechnology.com/product/formulex/).
Microbiological analyses were performed by soil dilution method on specific culture media with agar-agar (Topping for aerobic heterotrophic bacteria and PDA for fungi).
After 7 days incubation at dark, colonies were counted and microbial density was reported to gram of dry soil.
Taxonomic identification was done using morphologic criteria, according to Bergey's manual (Bergey & Holt 1994) for heterotrophic bacteria and to Domsch & Gams (1970) and Watanabe (2002) determinative manuals for fungi.
The total number of species in community (S) was recorded for each experimental variant. The ratio between microbial effectives and the number of species in communities expressed species richness (SR_2 index).

The global physiological activities of microflora were determined by substrate induced respiration method (SIR) and results were expressed as mg CO_2 x 100 g^{-1} soil (Matei, 2011).

All assays were carried out in triplicate. Results were interpreted by one-way analysis of variance (ANOVA). The value $p<0.05$ was considered statistic significant (Student test).

RESULTS AND DISCUSSIONS

The results showed that all the natural products added and perlite significantly increased bacterial populations (Figure 1) and reduced the fungal counts (Figure 2), especially pathogenic species that dominated in untreated control (polluted soil).

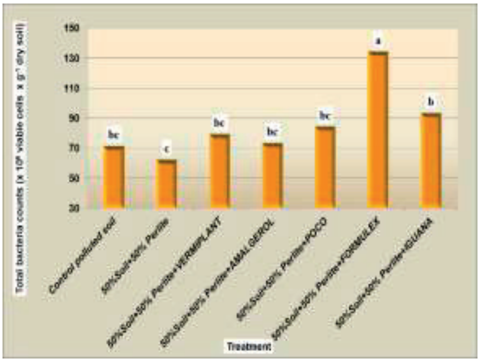

Figure 1. Influence of perlite and natural stimulators and fertilizers on bacteria

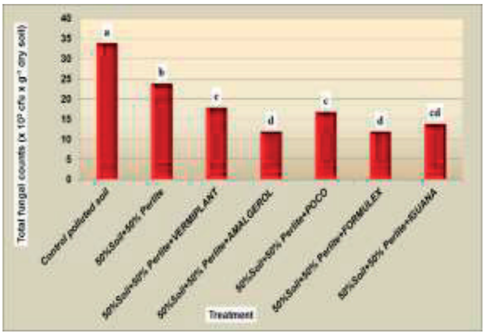

Figure 2. Influence of perlite and natural stimulators and fertilizers on fungi

Biodiversity was stimulated in bacterial communities, generally dominated by *Pseudomonas fluorescens*, Bacillaceae and Actinomycetes, with a maximum of 15 species for the variant with 50% polluted soil + 50% perlite (Fig. 3).

It is well-known the complex role of siderophore-producing fluorescent bacteria (*Pseudomonas*) and Actinomycetes in plant growth promotion, biocontrol of pathogens and bioremediation (Verma et al., 2011; Sah & Singh, 2020).

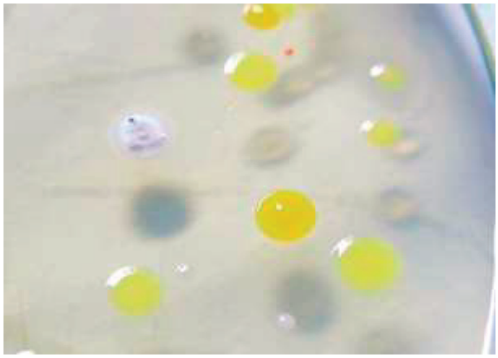

Figure 3. Bacteria from the variant with 50% polluted soil + 50%Perlite

The dominance of antagonistic fungi *Trichoderma viride* (Figure 4) and *Fusarium oxysporum* was recorded in myco-coenoses from variants with 50% perlite and in variants treated with VERMIPLANT, IGUANA or POCO.

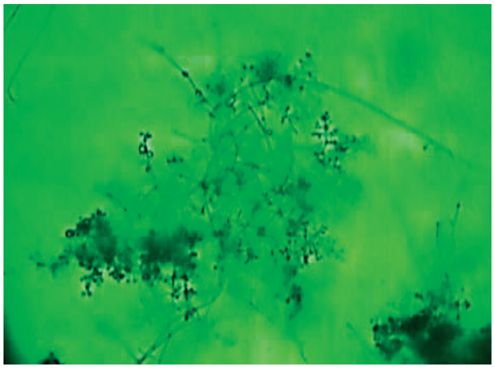

Figure 4. *Trichoderma viride* from the variant with 50% polluted soil + 50% Perlite + VERMIPLANT (150x)

The presence of natural biostimulators and fertilizers induced increased biodiversity in microbial communities, with maximum SR_2 values of 0.814 for bacteria at variant with FORMULEX.

Similar values of this index (0.500) were recorded for fungi at variants with AMALGEROL, POCO and IGUANA comparatively with 0.083 and respectively 0.294 in non-treated control (Table 1).

Physiological activities of microbiota were stimulated by the better substrate aeration with perlite and the natural stimulators and fertilizers FORMULEX and IGUANA (Figure 5).

Table 1. Taxonomic composition and biodiversity indices of bacterial and fungal microflora in greenhouse experiment

Experimental variant	**Bacterial species**	**Fungal species**
Control Polluted soil	*Pseudomonas fluorescens,* *Bacillus polymixa,* *Bacillus circulans,* *Bacillus cereus* Actinomycetes Series Albus, Fuscus	*Fusarium verticillioides,* *Aspergillus ochraceus,* *Fusarium avenaceus,* *Aspergillus flavus,* *Aspergillus terreus,* *Fusarium* sp., Non-identified, *Rhizopus stolnifer,* *Eurotium herbariorum,* *Fusarium sporotrichioides*
	S=6 SR_2=0.083	S=10 SR_2=0.294
50% polluted soil+ 50% Perlite	*Bacillus megaterium,* *Pseudomonas fluorescens,* *Arthrobacter globiformis,* *Pseudomonas aeruginosa,* *Bacillus circulans,* *Pseudomonas striata,* *Arthrobacter citreus,* *Arthrobacter simplex,* *Bacillus subtilis,* *Bacillus polymixa,* *Micrococcus* sp. Actinomycetes Series Albus, Griseus, Fuscus, Coeruleo-griseus	*Fusarium oxysporum,* *Aspergillus niger,* *Trichocladium* sp., *Penicillium* sp., *Eurotium herbariorum,* *Mortierella minutissima,* *Acremonium chartarum*
	S=15 SR_2=0.237	S=7 SR_2=0.291
50% polluted soil+ 50% Perlite + VERMIPLANT	*Pseudomonas fluorescens,* *Bacillus cereus var. mycoides,* *Bacillus megaterium,* *Bacillus circulans,* *Pseudomonas pseudogleyi,* *Bacillus* sp., *Pseudomonas* sp., *Arthrobacter oxydans*	*Trichoderma viride,* *Aspergillus flavus,* *Aspergillus fumigatus,* *Fusarium* sp., *Penicillium vermiculatum,* *Fusarium equisetii*
	S=8 SR_2=0.100	S=6 SR_2=0.333
50% polluted soil+ 50% Perlite + AMALGEROL	*Pseudomonas fluorescens,* *Bacillus megaterium,* *Bacillus cereus* var. *mycoides,* *Pseudomonas aeruginosa,* *Arthrobacter globiformis,* *Bacillus cereus,* *Arthrobacter citreus,* *Pseudomonas* sp., *Micrococcus* sp. Actinomycetes Series Albus	*Cunninghamella elegans,* *Trichocladium* sp., *Fusarium oxysporum,* *Acremonium* sp., *Fusarium* sp., *Monocillium indicum*
	S=10 SR_2=0.135	S=6 SR_2=0.500
50% polluted soil+50% Perlite + POCO	*Bacillus megaterium,* *Pseudomonas fluorescens,* *Bacillus cereus* var. *mycoides,* *Pseudomonas* sp., *Bacillus cereus,* *Pseudomonas striata,* *Bacillus circulans,* *Arthrobacter globiformis* Actinomycetes Series Fuscus, Albus, Ruber	*Fusarium* sp., *Trichocladium* sp., *Penicillium vinaceus,* *Aspergillus niger,* *Trichoderma* sp., *Fusarium culmorum,* *Myrothecium catenulatum*
	S=11 SR_2=0.129	S=7 SR_2=0.500

Experimental variant	Bacterial species	Fungal species
50% polluted soil+50% Perlite + FORMULEX	*Pseudomonas fluorescens,* *Arthrobacter globiformis,* *Bacillus cereus* var. *mycoides,* *Arthrobacter citreus,* *Bacillus circulans,* *Arthrobacter simplex,* *Micrococcus* sp. Actinomycetes Series Albus, Griseus, Fuscus, Luteus	*Trichoderma viride,* *Fusarium oxysporum,* *Trichocladium* sp., *Paecilomyces marquandii,* *Neosartoria fischeri* (sin. *Aspergillus fischeri*)
	S=11 SR_2=0.814	S=5 SR_2=0.416
50% polluted soil+ 50% Perlite + IGUANA	*Pseudomonas fluorescens,* *Bacillus megaterium,* *Bacillus subtilis,* *Pseudomonas pseudogleyi,* *Arthrobacter citreus,* *Bacillus circulans,* *Bacillus cereus* Actinomycetes Series Fuscus	*Fusarium oxysporum,* *Myrothecium roridum,* *Aspergillus candidus,* *Trichocladium* sp., *Cladosporium cladosporioides,* *Verticillium leccani,* *Neosartoria fischeri* (sin. *Aspergillus fischeri*)
	S=8 SR_2=0.084	S=7 SR_2=0.500

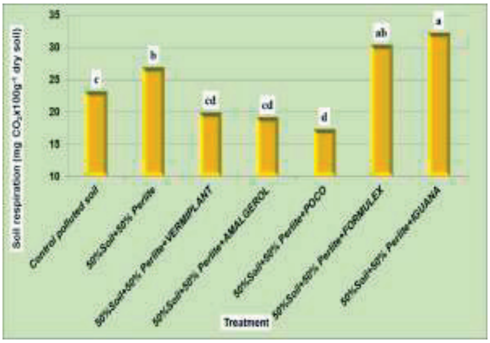

Figure 5. Influence of perlite and natural stimulators and fertilizers on soil respiration

Total plant biomass increased when used 50% perlite and in the variant with AMALGEROL as compared to non-treated control (Figure 6).

Figure 6. Bean plants grown in polluted soil (control), in the variant with perlite and with perlite and AMALGEROL

Results from the present research are in concordance with data from literature reporting bioremediation of a crude oil-polluted soil by application of fertilizers (Chorom et al., 2010). Margesin and Schinner (2001) found increased microbial counts and soil respiration, as well as enzyme activity in fertilized soil that induced a 70% hydrocarbon loss comparatively with 50% in unfertilized alpine soil polluted with Diesel oil hydrocarbons. Other research revealed that combined use of organic soil amendments (poultry dung) and phytoremediation with five plant species significantly improved the activity of microbial community, promoting the restoration of ecosystem (Nwaichi et al., 2015). Rahman et al. (2003) reported enhanced bioremediation of n-alkane in petroleum sludge using a bacterial consortium amended with rhamnolipid and micronutrients.

Previous research evidenced the beneficial effect of fertilizers, aeration condition and inoculation with hydrocarbon degrading microbial strains on the decontamination of oil-polluted soil and restoration of microbial diversity and physiological activity of edaphic microbiota (Dumitru et al., 2004). This data evidences the importance of the technology for stimulating microbial biodiversity and activity of natural hydrocarbon degraders and accelerating decontamination process (Xu & Lu, 2010; Chandran et al., 2020;).

In conditions of contaminated soil from experiment, we recommend management of physical and chemical conditions to improve the microbial activity and hydrocarbon biodegradation by using mixtures with perlite and natural biostimulators and plant fertilizers.

Perlite was chosen to be used in mixture with polluted soil because it is a porous material, with both excellent water retention and drainage capabilities, provides proper aeration, also acting as an efficient insulator or protector against temperature changes.

Perlite is an inert and sterile medium, can be used without fear of tracking in pests or plant pathogenic microorganisms, it is guaranteed to last for years, inexpensive and environment friendly.

Previous research on application of perlite in oil-contaminated sandy soil evidenced its beneficial effect on improving humidity conditions, water uptake and stimulating potato growth during decontamination process by including it in phytoremediation technology (Drăghici et al., 2016b).

In the present study, the process of biostimulation involved the supplying of polluted soil-perlite mix with nutrients as various natural organic or inorganic fertilizers that stimulated microbial proliferation and activation of hydrocarbon degraders from indigenous microflora. The effect on bean plants utilised for phytoremediation was presented elsewhere (Matei et al., 2018).

It is assumed that the hydrocarbons can be more rapidly degraded by the higher microbial counts induced by the nutrients added to soil as compared with natural attenuation process (Wu et al., 2019).

Similar results were obtained by Ruperto et al. (2003) using biostimulation and bioaugmentation to increase the bioremediation of a hydrocarbon contaminated Antarctic soil comparatively with effectiveness of natural microflora or phytoremediation and bioaugmentation with oil-degrading strains for remediating saline soil contaminated by heavy crude oil (Cai et al., 2016).

As in the present experiment, recent results on remedial efficiency of bioaugmentation with microbial consortia and biostimulation for improving diesel-contaminated soils evidenced specific response of bacterial diversity, metabolic activity and biodegradation pathway as a function of fertilizers or amendment variants and suggested that holistic approach including both consortia bioaugmentation and biostimulation was the most adequate option (Wu et al., 2016a; Chaudari et al., 2021).

CONCLUSIONS

All the natural products and perlite significantly increased bacterial populations and reduced the fungal counts.

The presence of natural biostimulators and fertilizers induced increased biodiversity in microbial communities, with maximum SR_2 values of 0.814 for bacteria at variant with FORMULEX and 0.500 for fungi at variants with AMALGEROL, POCO and IGUANA comparatively with 0.083 and respectively 0.294 in non-treated control.

Microbial communities were dominated by *Pseudomonas fluorescens*, bacillaceae and actinomycetes, and antagonistic fungi *Trichoderma viride*, *Fusarium oxysporum*, accompanied by *Aspergillus* or *Penicillium*.

Physiological activities of microbiota were stimulated by the perlite and the natural stimulators and fertilizers FORMULEX and IGUANA.

Total plant biomass increased when used the mixture with perlite and in the variant with AMALGEROL.

Management of soil physical and chemical conditions with perlite and natural biostimulators and plant fertilizers to improve hydrocarbon biodegradation by microbial communities is recommended for reclaiming the contaminated soil from Icoana.

ACKNOWLEDGEMENTS

This research work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number PN-III-P2-2.1_PTE-2016-0084, (40-PTE), acronym ECOREMTEH and also was financed from Project PN 19 34 04 01 /2019.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE USE OF SYNTHETIC SEED IN CONSERVATION OF *KALANCHOE BLOSSFELDIANA*

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Abstract

Synthetic seed technique can be a useful tool in plant conservation, as it combines the advantages of vegetative and generative propagation: uniformity of the material, lower costs for storage and transportation and the production of pathogen-free planting material. Kalanchoe blossfeldiana in vitro derived explants were used to examine the influence of sucrose concentration in the encapsulation maxtrix and the influence of salt concentration in the storage medium on the conservation of this species. Nodal segments and shoot tips were encapsulated in alginate solution containing two different sucrose concentrations (2% and 4%) and stored for 7,30 and 60 days in liquid medium with different MS salt concentrations (normal, halved and quartered). Statistical tests did not reveal an influence of the sucrose concentration in the alginate solution. On the other hand, tests indicated that the variants with the low salt concentrations (halved and quartered) showed better growth in terms of average shoot length and average number of leaves.

***Key words**: artificial seed, Kalanchoe blossfeldiana, micropropagation, plant conservation, synthetic seed.*

INTRODUCTION

Kalanchoe blossfeldiana is one of the most economically important ornamental pot plants grown worldwide. *Kalanchoe blossfeldiana* originally has 2n=34 chromosomes, but modern varieties obtained by inter-specific hybridization have higher levels of ploidy (2n=51 triploid, 2n=68, tetraploid) (Van Voorst and Arends, 1982). Seed propagation was a common propagation method for early cultivars, but vegetative propagation is commonly used nowadays because it offers uniformity in plant growth, growing habit and flower colour (Sanikhani et al., 2006). *In vitro* propagation of *Kalanchoe blossfeldiana* was achieved by numerous authors (Dickens & Staden, 1990; Ioannou & Ioannou, 1992; Sanikhani et al., 2006; Frello et al., 2002; Kaviani et al., 2014; Kale et al., 2018).

Synthetic seeds are represented by somatic embryos, axillary or terminal buds, nodal segments, cell aggregates or other types of artificially encapsulated tissues that can be used for sowing and have the ability to transform into plants, and that can retain this ability even after short term and medium term storage (Hussain et al., 2000; Micheli & Standardi, 2016; Magray et al., 2017).

The concept of synthetic seed was first introduced in 1977, by Murashige. Initially, the term referred to encapsulated somatic embryos (Murashige, 1977), but later, the concept was extended to non-embryogenic tissues as well. (Bapat et al., 1987). Synthetic seeds were created as a means to make the somatic embryos more similar to zygotic ones, by creating a protective layer aiming to make manipulation and storage easier, so that they can be used in propagation and germplasm conservation (Magray et al., 2017). The use of somatic embryos has been studied on several plant species, by numerous authors: *Rotula aquatica* (Chithra et al., 2005), *Oryza sativa* (Kumar et al., 2005), *Pinus radiata* (Aquea et al., 2008), *Quercus suber* (Pintos et al., 2008), *Litchi chinensis* (Das et al., 2016). The advantage of using somatic embryos over other types of tissue for encapsulation, is their bipolar structure, thus their ability to regenerate roots and shoots simultaneously, without any specific treatment (Hussain et al., 2000). However, a main disadvantage of somatic embryos is that their structures do not possess an endosperm and protective tissues, and they are dependent on the culture media and their manipulation and storage is difficult (Hussain et al., 2000; Magray et al., 2017; Lambardi et al., 2006).

Because of the limitations that somatic embryos possess (asynchronous development, lack of protective tissues, difficulty of creating a protocol for each species), in 1987, Bapat proposed the use of non-embryogenic tissues to make synthetic seeds, especially in species that are recalcitrant to somatic embryogenesis. Generally, those type of explants (shoot tips, nodal segments) are easier to obtain compared to somatic embryos, the risk of somaclonal variations is reduced and can be applied to most species (Standardi and Micheli, 2013).

Regarding the technology used, there are two types of synthetic seeds: dehydrated and hydrated. Dehydrated synthetic seeds are produced from somatic embryos, either not encapsulated or encapsulated in polyethylene glycol, and then dehydrated. Dehydrating the somatic embryos increases their storage period and survival rate, but this technology can only be applied to embryos that tolerate the dehydration process (Magray et al., 2017). Hydrated synthetic seeds designates/ represents the encapsulation of explants (somatic embryos, shoot tips, nodal segments, callus) in different hydrogel solutions: sodium alginate, potassium alginate, sodium pectate, carrageenan, gelrite (Hussain et al., 2000).

The technology of synthetic seeds has many applications: it can be used to asexually propagate endangered species, and valuable genotypes of species that normally don't produce seeds (Qahtan et al., 2019). The technique can also be used for exchange between laboratories and institutions and for short and medium conservation of germplasm (Standardi and Micheli, 2013).

Long term conservation of synthetic seeds can be achieved by cryopreservating the plant material. Otherwise, these can be stored on short and medium time in the fridge, at 2-8°C , depending on the species (Micheli & Standardi, 2016). Synthetic seeds can be stored up to 90 days in the fridge, but the optimum storage period is dependent on the species, but generally, most species cand be stored in the fridge, at 4-6°C (Qahtan et al., 2019). Some species can be stored at room temperature, in dark conditions (Standardi & Micheli, 2013). High humidity and low temperature are essential for storing the synthetic seeds (Mallikarjuna et al., 2016).

The advantage of synthetic seeds is that it combines the advantage of asexual propagation with the seed propagation: easy storage, easy transportation, the possibility of using sowing machinery, protection against pests and disease, the production of virus free planting material (Lambardi et al., 2006).

Plants developed from synthetic seeds are phenotypically identical with the plant that was used as the explant source. Synthetic seeds can be produced in a short period of time (one month), compared with natural seeds, which are the result of a complex reproductive process and that extends over a longer period of time. Also, they can be produced in any season of the year, as they production is not dependent on season and on field conditions. Theoretically, synthetic seeds don't require the acclimatization phase that is mandatory for *in vitro* obtained cultures (Gantait and Kundu, 2007). These can be sown directly in soil or in different substrates such as sand, perlite or vermicompost. Direct sowing was achieved successfully in numerous species, such as *Medicago sativa* (Fuji et al., 1992), *Erythrina variegata* (Javed et al., 2017), *Dalbergia sissoo* (Chand & Singh, 2004), *Phyllanthus amarus* (Singh et al., 2006), *Musa* spp. (Ganapathi et al., 1992), *Morus indica* (Bapat & Rao, 1990), *Sphagneticola calendulacea* (Kundu et al., 2018).

One of the main limitations of using synthetic seed as a practical technology is the difficulty of sowing them directly in soil or in non-sterile substrates such as vermiculite, perlite, compost, etc (Rihan et al., 2012). The use of somatic embryos for large scale production is limited because of their asynchronous development, precocious germination and structural anomalies. However, this inconvenient can be overcome by using other type of explants for encapsulation, such as shoot tips, nodal segments and axillary buds.

In this context, the paper presents a study on the influence of sucrose from the encapsulation matrix, the effect of the salt concentration from the storage medium and the influence of sodium alginate concentration on the storage capacity and viability of *Kalanchoe blossfeldiana* synthetic seeds.

MATERIALS AND METHODS

The material used for encapsulation was represented by nodal segments and shoot tips of *in vitro* grown *Kalanchoe blossfeldiana* plants, explants that were exercised after several proliferation subcultures on MS medium containing cytockinins (6-benzylaminopurine), auxins (indole-3-butyric acid) and gibberelines (gibberellic acid). Encapsulation was done in aseptical conditions, under the laminar hood.
For encapsulation, two variants of sodium alginate solution were used, both prepared in MS basal salt solution: V1 (with 2 % sucrose, v/w) and V2 (with 4 % sucrose, v/w), both prepared in MS basal salt solutions, in order to observe the influence sucrose has on the storage capacity of the explants. For the $CaCl_2$ solution, only one variant was used, in concentration of 100 mM. The pH of the sodium alginate solutions was modified to 5.8 and then the alginate and $CaCl_2$ solutions were sterilised in the autoclave at 121°C and 1.1 Bar atmospheric pressure for 20 minutes.
Explants with a small quantity of sodium alginate were dipped in the $CaCl_2$ solution using a glass pipette. Two immersion times were used for each variant: 10-15 minutes and 30-35 minutes. During the ion exchange time, the encpasulated explants were constantly stirred on the magnetic stirrer, at aprox. 10 rpm. After the ion exchange time ended, the encapsulated explants were rinsed three times with bidistilled sterile water, to remove any remaining traces of $CaCl_2$. The capsules formed after 30-35 minutes of immersion were isodiametrical and compact (Figure 1). Decreasing the immersion time to 10-15 minutes resulted in the formation of capsules that were fragile and more difficult to handle.
Synthetic seeds were stored in 3 variants of liquid MS storage medium. The liquid storage medium consisted of MS salts in different concentrations, with 3 % sucrose (v/w), în 3 variants: X (normal salts concentration), X/2 (halved salts concentration) and X/4 (quartered salts concentration). After preparation, the storage medium was sterilized in the autoclave, using the same protocol used for the alginate and $CaCl_2$ solutions.

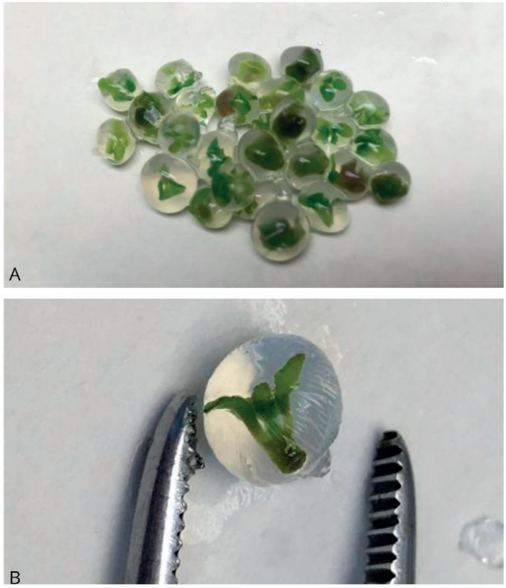

Figure 1. A, B: *Kalanchoe blossfeldiana* synthetic seeds after encapsulation (source: personal archive)

The encapsulated explants were kept în the liquid medium at 6°C and under, dark conditions. After the storage period (7, 30 and 60 days), the synthetic seeds were sown in *in vitro* conditions on basal hormone-free basal MS medium containing macronutrients, micronutrients, vitamins (Murashige & Skoog, 1962) and 3% sucrose (w/v) and solidified with 0.7% agar (w/v). Media pH was adjusted to 5.8 after the addition of sucrose and agar and was autoclaved at 1.1 Bar and 120°C for 20 minutes.
After inoculation on MS medium, synthetic seeds were transferred in the growing room at 22-25°C, with a 16 hours light/8 hours dark photoperiod and a 9.280 lx light intensity.
Observations reffering to rate of development, rooting percentage and average growth were taken at 7, 14 and 60 days of culture on hormone-free MS medium. Statistical analasys was applied in order to evidentiate any statistical differences in growth regarding the two factors analysed: the concentration of sucrose from the encapsulation, matrix and the concentration of MS salts from the storage medium. Bifactorial ANOVA test was applied to compare the means across the two independent variables:

the concentration of sucrose in the encapsulation matrix and the concentration of salts to evidentiate differences on average leaf number, followed by Tuckey's HSD test to see if the means significantly differ from each other. In case of growth, Kruskall Wallis (factor A - sucrose) and Mann-Whitney (factor B - MS salt concentration) tests were applied instead of two-factor ANOVA. Tukey HSD Post Hoc test was used to conduc a separate comparison between the variants.

Acclimatization started after 70 days of culture, using rooted shoots obtained from the synthetic seeds stored for 30 days in the frigde, at 6°C. Rooted shoots (Figure 2) were removed from the *in vitro* environment and the agar was removed from the roots. These were placed in small pots, in sterilised substrate composed of peat and sand. The pots were sheltered with glass covers in order to keep the humidity level high.

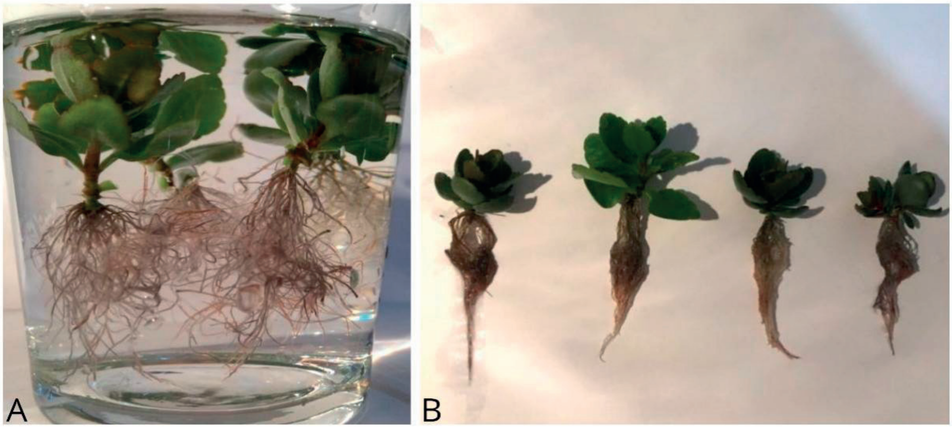

Figure 2. Rooted shoots of *Kalanchoe blossfeldiana* obtained from synthetic seeds, used for the acclimatization phase (source: personal archive)

RESULTS AND DISCUSSIONS

In vitro development of synthetic seeds

After seven days of storage: Observations on the development percetage were made 14 days after the innoculation of the synthetic seeds stored for seven days.

On average, synthetic seeds that were kept for 30 minutes in the $CaCl_2$ solution had a higher development rate (96.6 %) and viability, compared to the ones that were immersed for only 10 minutes (92.5 %).

All variants recorded 100 % development rate, except for variants V1 X/4 (10 minutes), V2 X/2 (30 minutes) and V1 X/4 (10 minutes), where the development varied between 75-80 % (Figure 3).

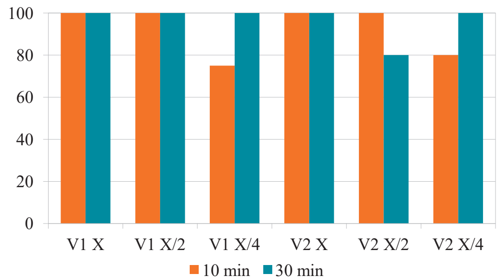

Figure 3. Development percentages of synthetic seeds stored for 7 days at 6°C.

After 30 days of storage: Development percentage after 14 days from sowing dropped from 96.6 % to 48.3 % when modifing the storage time from 7 to 30 days (Figure 4).

The highest development rates were recorded in the variants that were stored the medium with the quartered salt concentration (V1 X/4 and V2 X/4).

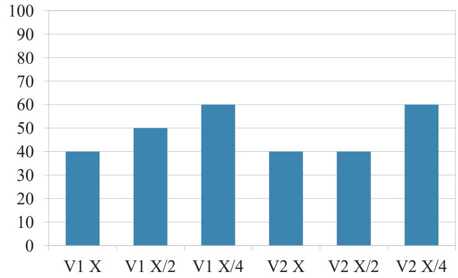

Figure 4. Development percentages of synthetic seeds stored for 30 days at 6°C

Rooting of synthetic seeds

Rooting took place simultaneously with the shoot growing, in the absence of hormonal treatments. Observations were made after 14 days of culture on hormone - free MS medium. The percentages of rooted explants were between 83 and 100% (Figure 5).

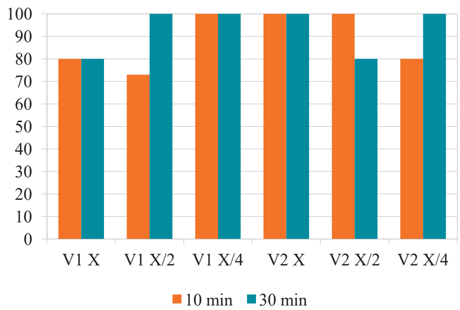

Figure 5. Percentages of rooted explants, after 14 days of culture on MS medium

Growth of synthetic seeds

Observations on growth were taken after 60 days of culture. This were recorded for the synthetic seeds immersed for 30 minutes in 100 mM $CaCl_2$ solution and stored for 30 days at 6°C. Mann-Whitney test revealed that modifing the sucrose concentration from the encapsulation matrix from 2% to 4% did not have a statistically significant influence on ulterior growth.

On the other hand, Kruskal-Wallis (Figure 6) test revealed significant differences between the stem growth regarding the salt concentration from the storage medium. The most favorable growth were recorded on the variants with the lowest salt concentrations: 1/4: 15.8 mm for V1 X/4 and 13.8 for V2 X/4, followed by the variants with the halved concentrations: 13 mm for V1 X/2 and 7.8 for V2 X/2. The variants with the normal salts concentration recorded the lowest growth: 5.4 mm for V1 X and 5 mm for V2 X (Figure 7).

	MS X	MS X/2	MS X/4	
median	5	10.5	15	
rank sum	66.5	163	235.5	
count	10	10	10	30
r^2/n	442.225	2656.9	5546.025	8645.15
H-stat				18.55032
H-ties				18.76321
df				2
p-value				8.43E-05
alpha				0.05
sig				yes

Figure 6. Kruskal-Wallis test results. Statistically significant differences between variants

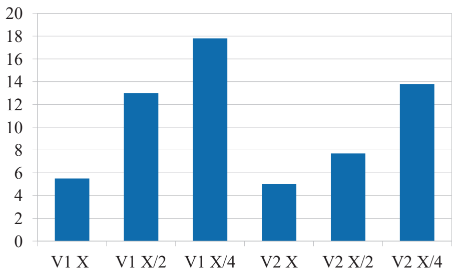

Figure 7. Average growth of synthetic seeds (mm), after 60 days of culture on MS medium

Average leaf number

Observations on average leaf number were recorded after 60 days of *in vitro* culture of the seeds immersed for 30 minutes and stored for 30 days at 6°C. Statistically, no differences between the average leaf number were recorded, for synthetic seeds encapsulated în V1 and in V2.

Statistically, significant differences were recorded between the storage mediums. Bifactorial ANOVA gave a significant result (p value =0.000198, <0.05), for factor A (concentration of MS salts in the storage medium), as a result the analysed means are not equal (Figure 8).

But in case of factor B (concentration of sucrose in the encapsulation matrix), p value (p=0.084445, >0.05) shows that there were no differences between the means (Figure 8).

ANOVA				Alpha	0.05	
	SS	*df*	*MS*	*F*	*p-value*	*sig*
Rows	83.46667	2	41.73333	12.52	0.000189	yes
Columns	10.8	1	10.8	3.24	0.084445	no
Inter	5.6	2	2.8	0.84	0.444012	no
Within	80	24	3.333333			
Total	179.8667	29	6.202299			

Figure 8. ANOVA test results

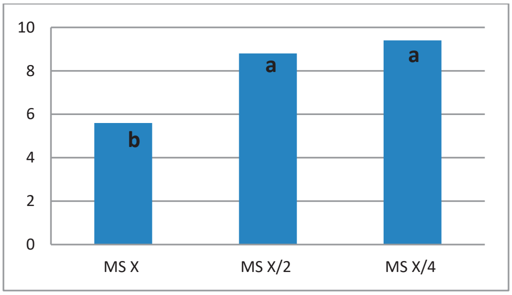

Figure 9. Tukey HSD results. Significant differences between MS X and MS X/2 & MS X/4 variants

The highest values were recorded in the variants with the quartered concentration,
followed by the ones with the halved concentration, with no significant differences between them, according to the results of Tukey HSD test (Figure 9).

Acclimatization of explants.
After two weeks of transferring the rooted shoots from *in vitro* to *in vivo* environment, the percentage of survival was 100 % for all encapsulation tested variants (Figure 10).

Figure 10. *In vitro*-grown acclimatized *Kalanchoe blossfeldiana* plants

CONCLUSIONS

Synthetic seeds stored for 1 week in MS salts liquid medium had development percentages between 75 and 100 %. On average, synthetic seeds that were kept for 30 minutes in the $CaCl_2$ solution had a higher development rate (96.6 %) and viability, compared to the ones that were immersed for only 10 minutes (92.5%).
Viability and development percentage decreesed from 96.6% to 48.3%, after increasing the storage time from 7 days to 30 days. Higher development percentages were recorded for the seeds that were stored in the medium with the quartered salt concentration (V1 X/4 and V2 X/4). The sucrose concentration did not influence the viability and growth of the synthetic seeds.
The concentration of MS salt in the storage medium did have a significant influence on the stem growth and leaf growth of synthetic seeds, as revealed by ANOVA and Kruskal-Wallis tests. Tukey's HSD test indicated that synthetic seeds stored in the medium with the lowest salt concentrations (X/2 & X/4) showed the highest average leaf growth.
No hormonal treatment is required in order to achieve rooting for synthetic seeds of *Kalanchoe blossfeldiana.* The development of the root system took place simultaneously with the shoot development. The percentages of rooted explants were between 73 and 100 % after 14 days of culture, but all shoots developed roots eventually, after 30 days of culture.
Acclimatization of the plants obtained from synthetic seeds was achieved successfully for all encapsulation tested variants.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INVASIVE AND POTENTIALLY INVASIVE ALOGEN PLANTS IN THE AGRICULTURAL CROPS OF OLTENIA

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Abstract

Following the research carried out in the recent years in Oltenia it was found out that there is an affinity between certain invasive allogenic and potentially invasive plant species and cultivated land. The analysis of the presence of these plant species in agricultural crops highlights their presence in large numbers in weeding crops and less in cereal crops. Data on the existence of these plants in agricultural crops in Oltenia territory are sporadically found in several specialized works. The analysis of the floristic spectrum of invasive and potentially invasive allogenic plants in Oltenia`s agricultural crops highlights the presence of some taxa that are on the list of alarming for the European Union (eg. Ailanthus altissima – in vine crops in Dolj and Mehedinți counties and Asclepias syriaca in corn crops in Gorj county). Among the species with a strong impact on the agricultural crops of Otenia we mention: Sorghum halepense (for corn crops), Ambrosia artemisiifolia (for corn, sunflower and watermelon crops) and Galinsoga parviflora (for watermelon crops).

***Key words**: allogenic plants, crops, invasive, Oltenia, Romania.*

INTRODUCTION

Invasive species are one of the biggest threats of biodiversity. In 2002 the United Nations Convention on Biological Diversity (CBD) mentioned the need for a global approach on invasive species.

A set of tools with best prevention and and management practices for these plants is presented by Wittenberg et Cock (2001).

The study of the segetal plant species from the agricultural crops of Oltenia attracted the attention of the numerous botanists who roamed the lands in this part of Romania.

The analysis of the research carried out so far shows the presence of invasive and potentially invasive taxa in agricultural crops (Buia, 1939; Păun, 1966; Păun & Pop, 1970; Păun et al., 1975, 1979; Păun & Popescu 1983; Chirilă et al., 1998; Ianovici & Sârbu, 2007; Anastasiu & Negrean, 2005, 2007; Rădutoiu et al., 2010). The species mentioned in the work papers are part of the floristic inventory of each researched region. In the paper prepared by Niculescu & Cismaru (2013) is presented an inventory of invasive species without mentioning the invasive energy and its effect.

Data on the study of segetal weed species are known from different regions of the country (Bujorean et al., 1956; Anghel et al., 1972; Ciocârlan & Chirilă, 1982; Chirilă, 2001, Chirilă et al., 2002; Ciocârlan et al., 2004), but information on invasive and potentially invasive plants in the segetal weed species group is sporadic (Costache & Rădușoiu, 2005, 2006; Rădușoiu & Costache, 2006, 2008; Rădușoiu, 2011; Niculescu & Cismaru, 2013; Rădușoiu et al., 2010; Rădușoiu & Stan, 2013; Rădușoiu & Ștefănescu, 2016; Rădușoiu & Popescu, 2020 - under print).

In addition to invasive and potentially invasive species, various pathogens are part of the category of biotic stress factors for Oltenia agricultural crops (Paraschivu et al., 2014, 2015, 2017, 2019), which have recently grown due to climate change. That is why we consider paying more attention to both factors of biotic stress that cumulatively cause significant damage for crops.

At European level there is a list of invasive species of interest to the EU. Among them are taxa that have an area also in Oltenia (*Asclepias syriaca*, *Elodea nuttallii* and *Impatiens glandulifera*). Globally, there is a database of invasive species.

MATERIALS AND METHODS

Oltenia is one of the most interesting regions of Romania in terms of natural conditions. To the south is placed Oltenia Plain, part of the Romanian Plain which continues to the north with the Getic Piedmont represented by Motru, Jiu and Olteț hills and by the beautiful hilly depressions of Desnățui and Teslui rivers to the south. At the upper part of the Getic Piedmont is the sub-Carpathian depression area that continues with the Meridionali Carpathians (Parâng and Căpățânii Mountains).

The agricultural crops in Oltenia are present from the level of plain region to the sub-Carpathian hills. The substratum of these areas is represented by quaternary loess, alluvium of gravel and sands (at the plain level), sedimentary deposits with very different character (at the level of the piedmont and sub-Carpathians hills).

Soils in areas favorable to agricultural crops in Oltenia are represented by many types and varieties (Popescu, 1975). These varied conditions led to the installation of a varied spontaneous flora.

Data included in this paper were obtained following numerous trips made in different agricultural areas of Oltenia, in Olt, Vâlcea, Mehedinți, Gorj and Dolj counties (127 localities).

There were studied the invasive and potentially invasive allogenic weeds from the following crop groups: straw cereals (wheat, barley, rye), weeding crops (corn, potato, beans, peas), vines, vegetable gardens and tree orchards.

The study was carried out on the itinerary, by geographical regions: Oltenia Plain, Getic Piedmont and the sub-Carpathian depression to cover a wider range of anthropogenic habitats. The itineraries for floristic studies targeted the areas where agricultural crops were well represented. In order to assess the impact of this category of plants on certain areas, stationary studies were also conducted to analyse the populations of identified invasive and potentially invasive species. The diagnosis of the species has been made according to Ciocârlan (2009).

A classification of the most invasive species in the research territory is also made according to the number of localities in which the population typology was found.

RESULTS AND DISCUSSIONS

Following the analysis of the allogenic invasive and potentially invasive species in Oltenia`s agricultural crops regarding the number of localities in which there were identified and their population typology by geographical regions we can say which are the most aggressive of them. The table analysis highlights the large spread in the plain and Getic Piedmont level (Table 1).

Table 1. Invasive and potentially invasive plant species in agricultural crops from Oltenia

Scientific name	Population typology			Crop	Way of introduction	Loc. nr.	Loc. nr. (percent from total)
	Plain	Getic Piedmont	Sub-Carpathians hills				
Ambrosia artemisiifolia L. (Figure 3)	5	5	3	Pb, V, Gr, Oz, Sec.,Leg., Sunflower	accidentally	127	100%
Sorghum halepense (L.) Pers. (Figure 4)	5	5	3	Pb, V, Gr, Oz, Leg., Sunflower	accidentally	116	100%
Xanthium italicum Moretti	5	5	4	Pb, C, V, Leg.	accidentally	112	97.32%
Conyza canadensis (L.) Cronq.	5	5	4	Leg., Trees	accidentally	97	86.59%
Amaranthus powellii S.Watson	5	5	4	V	accidentally	84	92.85%
Erigeron annuus (L.) Pers. subsp. *strigosus* (Muhl. ex Willd.) Wagenitz (Figure 5)	5	4	5	Gr, Oz, V, Sec.	accidentally	72	90.27%
Amaranthus retroflexus L.	4	4	2	V, Trees	accidentally	71	87.32%
Bassia scoparia (L.) A. J. Scott	4	2	1	Leg.	accidentally	28	50%
Galinsoga parviflora Cav. (Figure 6)	4	5	2	Pb, Leg., Lub.	accidentally	124	91.93%
Cuscuta campestris Yunck. (Figure 7)	4	3	2	Pb	accidentally	66	81.81%
Xanthium spinosum L.	3	2	1	Sunflower	accidentally	42	83.33%
Ailanthus altissima (Mill.) Swingle (Figure 8)	3	3	1	V	accidentally	25	100%
Abutilon theophrasti Medik.	3	3	1	Pb, Leg.	accidentally	102	87.25%
Amaranthus hybridus L.	3	2	1	V	accidentally	24	75%

Scientific name	Population typology			Crop	Way of introduction	Loc. nr.	Loc. nr. (percent from total)
	Plain	Getic Piedmont	Sub-Carpathians hills				
Veronica persica Poir.	3	3	2	Gr, Oz, Pb, C, Leg., Sec.	accidentally	98	92.85%
Datura stramonium L.	2	2	1	Leg.	accidentally	45	80%
Amaranthus albus L.	2	1	-	Pb, V	accidentally	23	91.30%
Acer negundo L.	1	1	-	V, Trees	accidentally	68	79.41%
Bidens frondosa L.	1	-	-	Lub.	accidentally	76	71.05%
Galinsoga quadriradiata Ruiz et Pav.	2	2	3	Leg., Lub.	accidentally	54	77.77%
Oxalis corniculata L.	2	2	2	Leg.	accidentally	52	86.53%
Oxalis dillenii Jacq.	3	2	1	V, Pb, Sec.	accidentally	85	95.29%
Phytolacca americana L.	2	2	1	Lub., Leg., V	accidentally	75	85.33%
Asclepias syriaca L. (Figure 9)	2	1	4	Pb.	accidentally	4	75%
Lycium barbarum L.	2	2	-	Lub., V	ornamental	34	82.35%

Loc. nr. – number of localities where the species was identified.
Population typology: 1 - solitary individuals; 2 - rare populations, on areas < 10 m^2; 3 - rare populations, on areas > 10 m^2; 4 - dense populations, on areas < 10 m^2; 5 - dense populations, on areas > 10 m^2.
Used shortcuts: V - vine; Pb corn; Leg. – Vegetables (tomatoes, paper, cabbage, onions, cucumbers); Lub. – watermelon; C – Potato; Gr – Wheat; Oz – Barley, Sec. – Rye.

At the level of sub-Carpathians hills some species are represented by solitary individuals or by rare populations, rarely by dense populations on large areas.

If it is made a residence time analysed, we can observe that all the species are neophytes.

The taxonomic analysis highlights the leading place occupied by the Asteraceae family. It is followed by Amaranthaceae, Solanaceae and Oxalidaceae (Table 2). The best represented genera are: Amaranthus (4 species), Xanthium, Galinsoga and Oxalis (with 2 species each).

Table 2. Taxonomic analysis

Family	Nr. genera	Nr. species
Asteraceae	6	8
Amaranthaceae	1	4
Solanaceae	2	2
Oxalidaceae	1	2
Poaceae	1	1
Chenopodiaceae	1	1
Cuscutaceae	1	1
Simaroubaceae	1	1
Malvaceae	1	1
Scrophulariaceae	1	1
Aceraceae	1	1
Phytollacaceae	1	1
Asclepiadaceae	1	1

Cytotaxonomic analysis of invasive and potentially invasive allogenic plant from Oltenia`s agricultural crops highlight the large number of tetraploid species followed by diploid and polyploid ones (Table 3).

Table 3. The analysis of cytotaxonomic

Ploidy degree	Nr. species
Tetraploid	15
Diploid	6
Polyploid	4

The analysis of bioforms highlights the predominance of annual species (Table 4). Although the geophytes have only one representative, it has a good representation in the crops placed at plain and Getic Piedmont level where it causes significant damages.

Table 4. The analysis of life forms

Bioform	Nr. species
Therophyta	18
Phaneropyta	3
Hemicryptophyta	3
Geophyta	1

Of the total invasive and potentially invasive allogenic species in Oltenia`s agricultural crops, 72% originate from America, 20% from Asia and 8% from the Mediterranean (Figure 1).

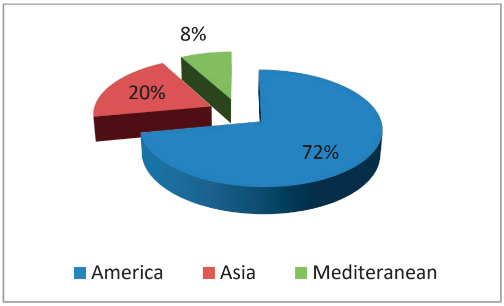

Figure 1. Geoelements spectrum

A good part of these plants (around 70%) are less demanding species compared to the water factor (Figure 2). This explains their development and expansions in this part of Romania, considering the characteristic climatic conditions in the Olteni area,

especially those in the southern part where the water regime is defective.
The large area occupied by agricultural lands in Oltenia are present in the plain region (over 50% of the agricultural area).

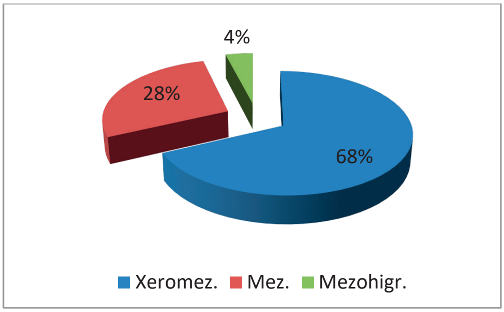

Figure 2. The humidity index spectrum

In some areas a mutual contamination between these species could be observed (ex. *Ambrosia artemisiifolia* installed in a wheat crop was strongly parasitized by *Cuscuta campestris*, aspect mentioned also by Sârbu et al. (2015).

Figure 3. *Ambrosia artemisiifolia* in watermelon culture at the edge of Ghindeni locality

Figure 4. *Sorghum halepense* in cereal culture at the edge of Castranova locality

Figure 5. *Erigeron annuus* in vine culture at the edge of Scăești locality

Figure 6. *Galinsoga parviflora* in watermelon culture in Rojiște locality

Figure 7. *Cuscuta campestris on Ambrosia artemisiifolia* after wheat culture at the edge of Scăești locality

Figure 8. *Ailanthus altissima* in vine culture at the edge of Șimnicul de Sus locality

Figure 9. *Asclepias syriaca* in corn culture at the edge of Tg. Cărbunești locality

CONCLUSIONS

An analysis of the number of individuals of certain invasive allogenic species weed species in a certain crop and the degree of weeding of these crops highlights the predominance of the species belonging to *Poaceae* family (both for straw and hoe crops) and the predominance of the species belonging to *Amaranthaceae* and *Asteraceae* family (for hoe and vegetable crops).

Annual weeds are common in vegetable, hoe and straw crops; the perennials are better represented in autumn crops, vines and tree orchards.

The most harmful and widespread segetal weeds belonging to allogenic species or potentially invasive are: *Sorghum halepense*, *Xanthium italicum*, *Ambrosia artemisiifolia*. In addition, there are species that have a limited spread imposed by ecological conditions (eg. *Abutilon theophrasti* – invasive in corn crops in the Danube meadow and almost absent in the sub-Carpathians piedmonts and hills).

The presence of these plants in agricultural crops brings multiple disadvantages manifested by high yield losses and high costs for control.

ACKNOWLEDGEMENTS

This paper is a segment of the research undertaken within the POIM/178/4/1/120008 project.

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Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MORPHO-ANATOMICAL CHARACTERS AND PRELIMINARY PHARMACOLOGICAL EVALUATION OF *PLUMERIA* SP.

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Abstract

Plumeria (fam. Apocynaceae), known as frangipani is a semi-deciduous shrub, native to tropical areas of the Pacific Islands, South and Central America. The flower offers a genuine delight to the visual and olfactory senses, due to its shape & colour attractiveness and to its amazing fragrance. The bio-compounds of Plumeria sp. are used for treating various ailments, while the petals are edible - they are used in different dishes, for cosmetic purposes and spiritual ceremonies. In our country, eight varieties of Plumeria sp. are in the process of acclimatization; frangipanis can resist outdoor up to 10°C. The size of analyzed frangipanis ranged between 42-88 cm in height and 36-75 cm in width. Plumeria sp. leaves are bright green, simple, alternative, elliptic-obovate, 18.8-32 cm long, 8.2-12.7 cm large, clustered at the end of the branches. The petiole's length varied between 4.5-6.7 cm and its diameter was averagely of 0.65 cm. Microscopically, the leaf showed the presence of thick lamina, adaxial & abaxial epidermis, adaxial phloem, palisade mesophyll, trichomes, air chambers, laticifer cells, tannins, starch granules, oil glands, collateral vascular strand. Preliminary pharmacological screening pointed out the presence of polyphenols and flavones at the analyzed frangipanis.

Key words: *frangipani, leaf & pedicel anatomy, plant morphology, therapeutic proprieties.*

INTRODUCTION

Plumeria or frangipani is a genus of flowering plants in the dogbane family, *Apocynaceae*.
The genus *Plumeria* is named in the honor of French botanist, Charles Plumier, but the first who gave the name "plumeria" was Francisco de Mendoza, a Spanish priest, in 1522. The common name "frangipani" comes from an Italian noble family, "Marquess" which produced a *Plumeria* scented perfume (Sudharani et al., 2012). The genus contains primarily deciduous shrubs and small trees (Goyal et al., 2012). Frangipanis are native to Pacific Islands, Central America, Caribbean and South America and can be grown in tropical and sub-tropical regions (Henry et al., 1987). Due to their ease of propagation, especially through cuttings, many heirlooms and hybrids of *Plumeria* sp. are widely cultivated and distributed in the faraway lands of the world (Omata et al.,1991). Frangipani is the most celebrated of all tropical flowers, but it is a very valuable medicinal plant (Devprakash et al., 2012; Rotblatt & Ziment, 2002), as well. Moreover, frangipani's petals are edible and they are used in different dishes, for cosmetic purposes and spiritual ceremonies (Newall et al., 1996). Medicinal plants belong to the oldest known health care products (Mills, 1993). Written records of the use of herbal medicine, such as Ayurveda, Chinese, Tibetan, Siddha and Unani traditional medicine date back more than 5,000 years and they consider the ailments arising from a lack of equilibrium between mind, body and environment (Schneeman, 2005; Wikers et al., 2001). The World Health Organization recently estimated that 80 percent of people worldwide rely on herbal medicines for some part of their primary health care (Tilburt & Kaptchu, 2008; Zamiska, 2006). After years of overmedicating, facing resistant bacteria in the microbiome and treating the illness rather than the cause of the problem, people are beginning to pay more attention to natural, herbal medicine (Swerdlow, 2000; Linde & Jonas, 1999) and even the worldwide researchers started to steer

away from conventional drug development and look towards more alternative and natural forms of treatment (In resources for authors: Why more people opt for herbal medicine (n.d.). Draxe. Retrieve from https://draxe.com/health/herbal/ medicine; Gopal, 2013; Narayana et al., 2008). It has been estimated that one third to one half of currently used drugs were originally derived from plants (Schaffner, 2002; Barrett et al., 1999). Latest technological development has led to increased accuracy in estimation, purification, separation and determination of principle and therapeutically active constituents of crude drugs (Mukherijee, 2002).

Plumeria species have strong anti-microbial activity (Zahid et al., 2010; Egwaikhide et al., 2008), antioxidant (Begum et al., 2010) and anti-inflammatory proprieties (Choudhary et al., 2014), antifungal effects (Radha et al., 2008), antiparasitic (Sharma et al., 2011), hepato-protective (Chowdhury et al., 2012), hypo-lipidemic (Begum et al., 2010) & hypo-glycemic (Bihani, 2021), antinociceptive (Gupta et al., 2007) and anti-mutagenic activity (Dobhal et al., 1999; Guevara et al., 1996). Due to their active constituents, plumerias are widely used as a purgative, remedy for diarrhea (Nadkarni, 1976), cure of itch, bronchitis, cough (Sura et al., 2018) asthma, fever (Misra et al., 2012), bleeding piles & wounds (Bura, 2018), dysentery, ulcer (Singh et al., 2012), blood disorders, HIV-1 (Tan, 1991), cancer (Banu & Jayakar, 2011; Radha et al., 2008).

Ethanolic and methanolic extracts of *Plumeria sp*ecies were tested for anti-microbial activity against Gram-positive bacteria (*Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Micrococcus luteus*), Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium*) and fungi (*Aspergillus niger* and *Candida albicans*) by disc diffusion method (Gupta et al., 2008; Radha et al., 2008; Ramalingam et al., 2008). The extracts did not show any toxic symptoms against the tested mice (Rasool et al., 2008).

From ancient time till nowadays, frangipanis are a divine gift for humans, through their healing proprieties, nutraceutical compounds, amazing fragrance and highly ornamental value.

MATERIALS AND METHODS

Eight varieties of *Plumeria* sp. were cultivated at the Institute of Research and Development for Processing and Marketing of Horticultural Products - Horting, Bucharest and microscopically analyzed at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Botany Department.

The varieties of *Plumeria* sp. which have been the subject of the present study were: *California Sunset*, *Star White*, *Exotica*, *Inca Gold*, *Jubilee*, *Divine*, *Thumbalina and Mini White.*

Three main species of frangipanis are commonly found: *Plumeria obtusa, Plumeria acuminata* and *Plumeria rubra* (Corner, 1952). Of the analyzed varieties of *Plumeria* sp*., Star White (2 plants)* and *Mini White* (3 plants) *belong to P. obtusa* species*,* Inca Gold (3 plants) belongs to *P. acuminata* species and *California Sunset* (3 plants), *Exotica* (2 plants), *Jubilee* (3 plants), *Divine* (3 plants) and *Thumbalina (3 plants)* are varieties of *P. rubra* species.

The microscopical analyses performed at the Botany laboratory were used to highlight the anatomical characteristics of frangipanis. The cross-sections were clarified with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine. (Luchian et al., 2019).

The pictures and measurements were made using the optical microscope Novex Holland and Sony photo camera.

Voucher specimens of *Plumeria obtusa, Plumeria acuminata* and *Plumeria rubra* varieties were deposited at the University of Agronomic Sciences and Veterinary Medicine of Bucharest's Herbarium.

The bio-chemical analyses were performed at Chem-Analyst Laboratory, 101 L Timisoara Avenue, District 6, Bucharest.

RESULTS AND DISCUSSIONS

The morphological and anatomical studies performed on *Plumeria* species - leaf, petiole and pedicel cross-sections provided for the first-time valuable information regarding the frangipani's cultivation in our country.

Macroscopic characteristics

In the current study were analyzed 22 plants from 8 varieties of *Plumeria* species, known also as frangipani. The plants were multiplied by cuttings, using stems fragments originating from Italy and their age was equal with their mother-plants age, ranging between 3 and 5 years old. *Plumeria*'s were grown in containers filled with peat, forest earth, perlite and salt free sand. They are medium drought resistant, due to their milky sap (latex). Roots are thick, but sensitive at breakage and, for this reason, the potting and re-potting must be carefully done. *Plumeria*'s request fertilization twice a month, but the key for their optimum growth is the soil quality (fertility). The fertilizers used to the analysed plants were Vitaflora and Cropmax (foliar fertilizer), both containing macro and micro-nutrients essential for the wellness of flowering plants, applied alternatively. High attention must be done on plants misting, in order to create a relative humidity of air, close to that of *Plumeria*'s native climate. Water spraying in hot summer (avoided in case of insolation) is a wise method to prevent red spider mite's attack, as well. Mealy bugs, white & grey flies and aphids are other pests which need an attentive monitorization. Plumerias are medium sensitive to fungi (wet rot) and bacteria (black spot).

All 8 varieties of *Plumeria* sp., analyzed in this study, are in the process of acclimatization in our country. Observing the plants' behaviour and adaptation to our climate, the outcomings pointed out that frangipanis can resist outdoor May till October, when the external temperature is not going below 10°C.

Acclimatization work on other tropical species, highly valuable for their medicinal properties is conducted successfully in Romania at *Psidium guajava*, known as guava (Toma & Luchian, 2019) and at *Murraya koenigii*, known as curry leaf tree (Toma et al., 2020).

Plumerias are famous for their colour and fragrance attractivity. Of the frangipanis analyzed in this study, *Star White* (Figure 1) is recognized for its white colour with small yellow centre, sharp petals, spicy perfume and large cymes, while *Mini White* (Figure 2) has a smaller size, white round petals with pastel edges, large yellow centre, sweet spicy perfume, abundant blooming. *Inca Gold* (Figure 3) has a tall habitus, bright yellow petals, fresh perfume. *California Sunset* (Figure 4) has yellow-salmon colour of petals with white edges and an amazing peach fragrance. *Exotica* (Figure 5) has a large habitus, big flowers coloured in various shades of pink with a charming citric-floral perfume. *Jubilee* (Figure 6) is recognized by a strong pink colour with salmon coloured centre,

medium large flowers and very pleasant perfume. *Divine* (Figure 7) is a very prolific type of *Plumeria species.* Its petals are spirally arranged, coloured in a beautiful melange of pink, yellow and peach colours with an unbelievable fragrance. *Thumbalina* (Figure 8) has a smaller size, but very abundant blooming; its flowers are mainly white with pink & salmon intrusions and vanilla perfume.

All *Plumeria* species are shrubs or small trees with thick, fleshy, stout branches (having high or low branching capacity) and they produce a milky juice when the leaves or branches are cut down.

Plumeria obtusa, the standard frangipani, has white flowers with small brilliant yellow centre, up to 9 cm in diameter; the leaves are dark green, glossy, obovate and obtuse - at both ends. The shrub can grow to about 6-9 m tall and it is partly deciduous at different times of the year.

Plumeria acuminata, syn. *Plumeria lancifolia* is an evergreen or partly deciduous shrub up to 6 cm high; its leaves are light green in colour, elliptic in shape with acuminate tips and the colour of the flower can vary from white to yellow (Chinn & Criley, 1983).

Plumeria rubra, commonly known as *temple tree*, has flowers in various shades of red, pink, orange and yellow; its leaves have different sizes, shapes and colours (Chin & Enoch, 1988). This species is a deciduous shrub which can exceed 10 m tall at its origin places (Walker, 1992).

Macroscopically, frangipani has a thick succulent trunk and branches covered with a thin grey bark. The leaves are simple, alternative, having entire undulate margin, elliptic, obovate or oblanceolate shape (Figure 9), pinnate, with very defined midrib, primary & secondary veins, bright green in colour (Figure 10) and clustered at the end of the branches. The branches are brittle and when broken, ooze a white latex, that can be irritating to skin and mucous membrane. Frangipani flowers are large, waxy, fragrant, arranged in terminal or lateral stalked cluster or peduncled cyme (Figure 10). *Plumeria* sp. fruits are elongated, cylindrical, with 20-60 flat seeds contained in a pod of~ 17.5 cm. The fruits are persistent on the plant and they do not attract wild life (Chin, 1993).

Figure 1. *Star White*

Figure 2. *Mini White*

Figure 3. *Inca Gold*

Figure 4. *California Sunset*

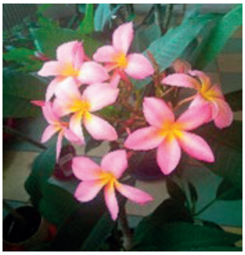

Figure 5. *Exotica*

Figure 6. *Jubilee*

Figure 7. *Divine*

Figure 8. *Thumbalina*

At the analyzed plants of this study, the leaf ranged between 18.8-32 cm long and 8.2-12.7 cm large. The petiole's length varied between 4.5-6.7 cm and its diameter was averagely of 0.65 cm.
The veins increase in number according with the leaf's length, as is represented in the Figure 11. The size of analyzed *Plumeria* sp. have ranged between 42 cm (*California Sunset*) and 88 cm (*Inca Gold*) in height and 36 cm (*Inca Gold*) and 75 cm (*Exotica*) in width. Large canopy was also observed at *White Star* (71 cm), medium at *Jubilee* (57 cm) & *Divine* (55 cm) and small-medium at *Thumbalina* (47) & *Mini White* (44 cm).
High branching capacity was observed at *Exotica* and *Star White*, since *Inca Gold* branched very less, as is detailed in the Figure 12.

Figure 9. Types of *Plumeria* sp. leaves

Figure 10. Peduncled cymes at *Plumeria* sp.

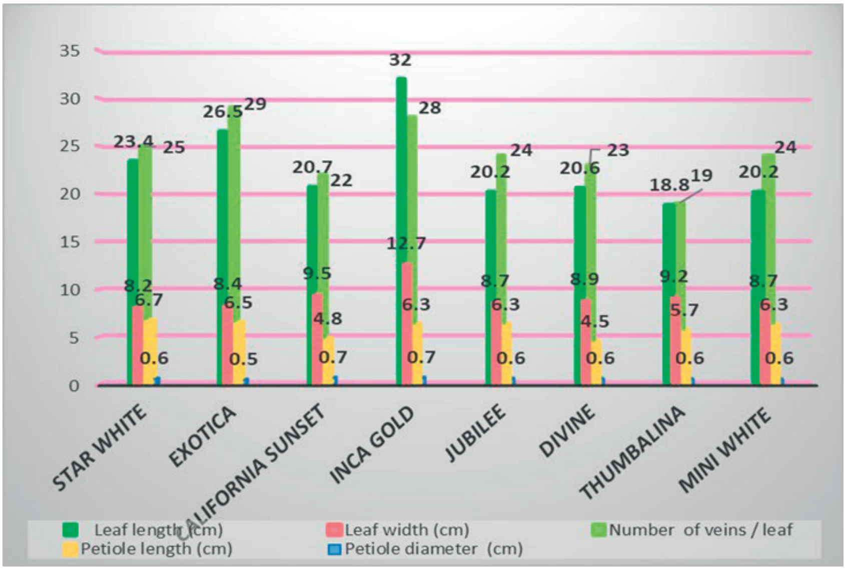

Figure 11. Growth dynamics of analyzed frangipanis in December 2020

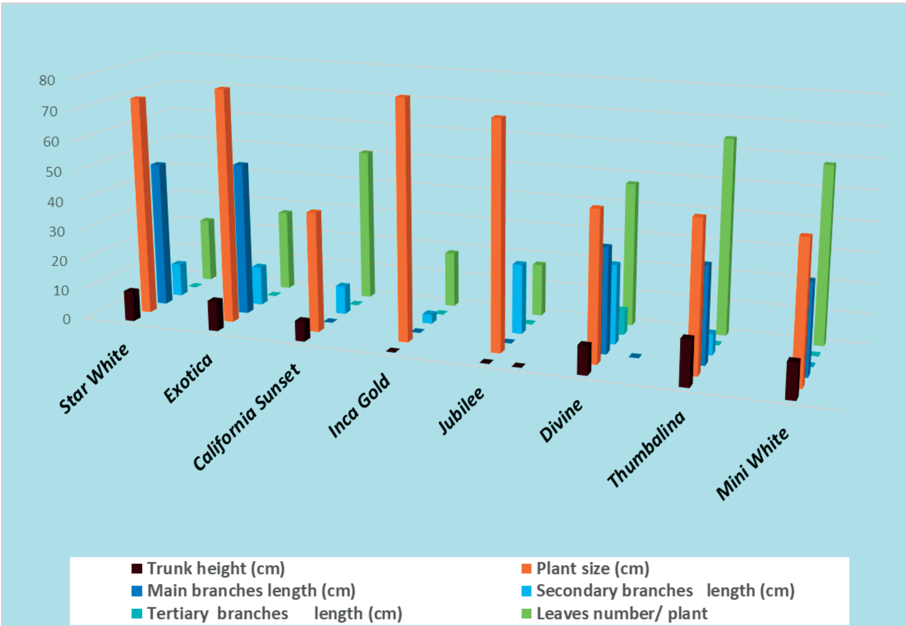

Figure 12. Leaf and petiole measurements of *Plumeria* varieties in 2020

Microscopic characteristics

Leaf anatomy. The lamina (Figures 13-15) is uniform and thick. The marginal part of the lamina is conical and blunt. The adaxial epidermis of the lamina is thick and prominent with fairly wide semi-circular cells (Figures 19-21). The abaxial epidermis is thin with narrow cylindrical cells (Figures 22-24). The mesophyll tissue is distinctly dorsoventral, differentiated into adaxial band of cylindrical, compact palisade cells and spongy parenchyma (Figure 17). Dorso -ventral mesophyll, single or multiple layered hypoderms and bi-collateral vascular bundle (Figures 16 and 18) in the midrib, are common in *Apocynaceae* (Metcalfe & Chalk, 1950), since the internal phloem may not extend into the smaller veins in leaves (Esau, 1977). The laticifers observed at *Plumeria* species, are larger than the neighbouring cells and have polygonal or circular transverse section, conspicuous nucleus, dense cytoplasm and no starch (Murugan & Inamdar, 1987; Appezzato-da-Glória & Estelita, 1997). The lower part of the lamina has many wide irregular air chambers formed by reticulate filaments of spongy mesophyll cells. The epidermal layer of the margin consists of small highly thick-walled cells. Inner to the marginal epidermis layer comprising compact, thick-walled cell. The inner part of the leaf margin has similar structure as the lamina.

Anatomical analyses of lamina showed the presence of palisade mesophyll, adaxial phloem, adaxial epidermis, paracytic stomata, laticifer cells, ground tissue with angular parenchymatic cells, angular laticifers cell and a collateral vascular strand, as they are also noticed in the study of Venkatachalam et al. (2018).

The midrib of the leaf is plano-convex and fairly thick. The adaxial epidermis of the midrib is flat with vertically elongated slightly papillate cells. The abaxial epidermis is thin with small, thick walled papillate cells.

At the analyzed plants, stomata are mainly observed on the abaxial epidermis. Starch granules, tannins and oils glands are identified (Figure 24), as they are also mentioned in the study of Bent (2008).

The ground tissue is parenchymatous with compact, angular parenchymatous cells. Some of the ground cells can be slightly wider and angular representing the laticifers or latex secreting canals. The vascular strand is single small and collateral. It includes a few groups of xylem units with 3 or 4 xylem elements arranged in radial rows. The xylem elements are highly thick walled and angular in outline. Phloem elements occur both on the lower and upper part. In the midrib's closeness were identified trichomes, as a characteristic feature of *Plumeria* sp. (Araújo et al., 1984).

The trichomes are important to the plant's survival and by regulating evapotranspiration, shielding from harmful rays and deterring insects and predators (Duke, 1994; Thomas, 1991).

Petiole anatomy. The petiole's cross-sections of analyzed frangipani leaves pointed out an epidermis, collenchymatous hypodermis and paren-chymatous ground tissue with a single typically bi-collateral vascular bundle, arc-shaped. (Figures 25-30). Other two smaller vascular bundles exist at the parenchyma's level.

At *Plumeria rubra - Jubilee* variety, the petiole exhibits through its typical cells, some different cells of dark pink colour - subject of a further study.

In addition, the distinctive arrangement of the external and internal phloem, as well as the tracheary elements, aligned in rows, were previously mentioned at dogbane family by Metcalfe & Chalk (1950) and Cronquist (1981).

Pedicel anatomy. In cross-sections (Figures 31-36), the pedicels of the clustered cyme are differentiated into epidermis, hypodermis and parenchy-matous ground tissue with bi-collateral ring shape vascular bundle.

At the level of epidermis were observed numerous non-glandular trichomes, especially at *Plumeria rubra - Divine* and *Thumbalina* varieties. The presence of trichomes on pedicels represents the plant way to defend the organs of reproduction which ensure the perpetuation of the species.

The trichomes play a key role in plant defence, especially with regard to phytophagous insects, avoiding insect feeding & oviposition and the larvae's nutrition (Fahn, 2000).

Preliminary pharmacological evaluation

The bio-chemical analyses were done on *Plumeria sp.* fresh leaves collected in February 2021 (Table 1).

The polyphenols content was determined using Folin-Ciocalteu method (Johansen, 1940), as modified by Yi & Wetzstein (2010) and further modified by Vaidya et al. (2013) and Meena et al. (2017).

The analyses showed a mean content of polyphenols of 66 mg GAE/100g, the highest polyphenols content was found at *Plumeria acutifolia* (106 mg GAE/100 g), represented by *Inca Gold* variety.

The mean flavones content was of 2.15 mg rutin/100 g, the highest flavones content was found at *Plumeria obtusa* (4.4 mg rutin/100 g), represented by *White Star* and *Mini White* varieties.

The polyphenols and flavones are bio-compounds with antioxidant and anti-microbial capacity. These compounds are beneficial to the body by protecting the skin, supporting digestion and the immune system (Das et al., 2011). The anti-oxidants reduce the risk of cancer and heart diseases (García-Lafuente et al., 2009).

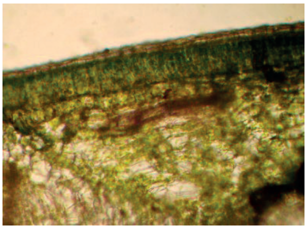

Fig. 13. Leaf lamina - *Mini White*

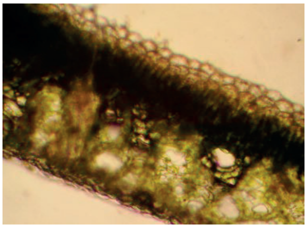

Fig. 14. Leaf lamina - *Inca Gold*

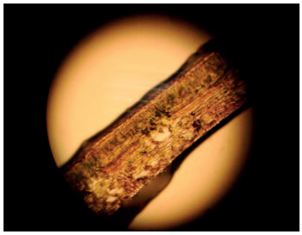

Fig. 15. Leaf lamina - *Jubilee*

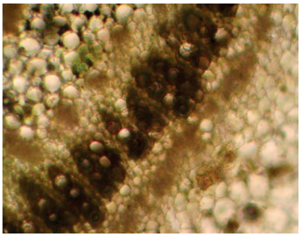

Fig. 16. Midrib vasc. bundle - *Mini White*

Fig. 17. Bifacial mesoph. - *Inca Gold*

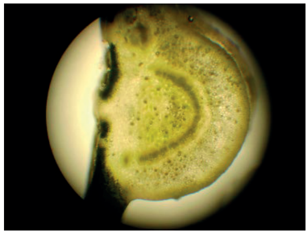

Fig. 18. Midrib's vasc. bundle - *Jubilee*

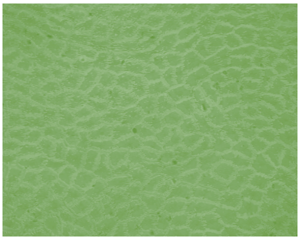

Fig. 19. Adaxial epidermis - *Mini White*

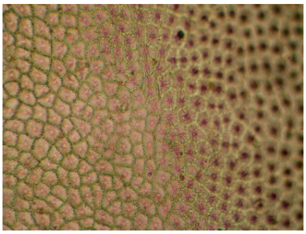

Fig. 20. Adaxial epidermis - *Inca Gold*

Fig. 21. Adaxial epidermis - *Jubilee*

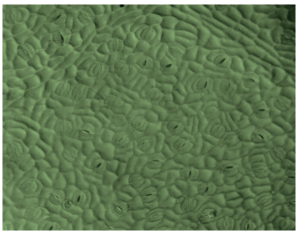

Fig. 22. Abaxial epidermis - *Mini White*

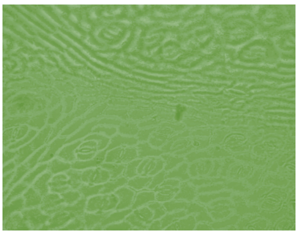

Fig. 23. Abaxial epidermis - *Inca Gold*

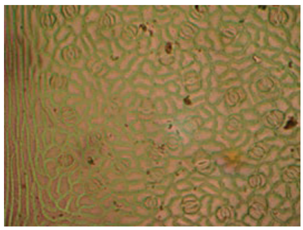

Fig. 24. Abaxial epidermis - *Jubilee*

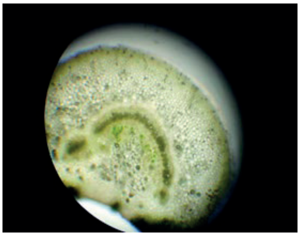

Fig. 25. *Inca Gold*'s petiole

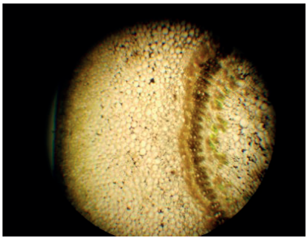

Fig. 26. *Inca Gold*'s petiole

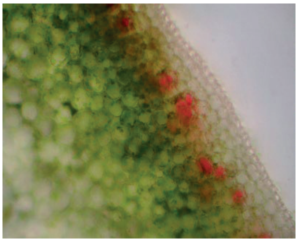

Fig. 27. *Jubilee*'s petiole - pink cells

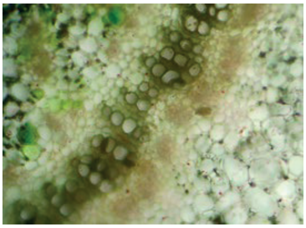

Fig. 28. Vascular bundle - *Inca Gold*

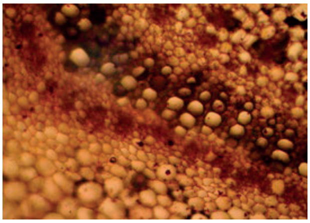

Fig. 29. Vascular bundle - *Inca Gold*

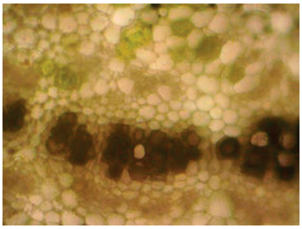

Fig. 30. Vascular bundle - *Jubilee*

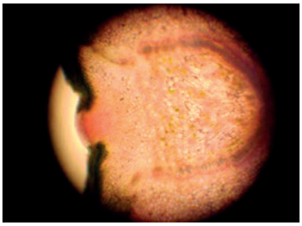

Fig. 31. U-shape vasc. bundle - *Inca Gold*

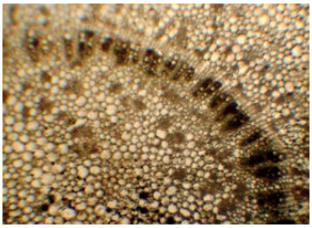

Fig. 32. Rays of vasc. bundle - *Divine*

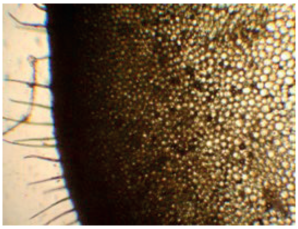

Fig. 33. Trichomes - *Divine*

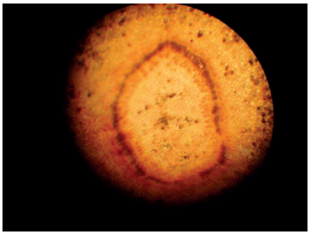

Fig. 34. Ring vasc. bundle - *Thumbalina*

Fig. 35. Trichomes - *Thumbalina*

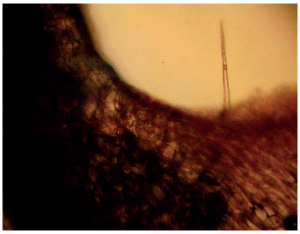

Fig. 36. Trichome - *Divine*

Table 1. Determination of *Plumeria* sp. bio-compounds with antioxidant capacity

Analyzed bio-compounds	**CLASSES OF *PLUMERIA* SPECIES**			**Test method**
	***Plumeria obtusa* (white colour)**	***Plumeria acutifolia* (yellow colour)**	***Plumeria rubra* (melange colours)**	
Polyphenols [mg GAE /100 g]	43	106	48.83	Ph. Eur. 8-th Edition/2013
Flavones [mg rutin/100 g]	4.4	0.3	1.75	

CONCLUSIONS

Plumeria's cultivation in Romania represents a novelty and a great challenge in the horticultural field, as well as the preliminary pharmacological evaluation of containerized frangipanis represents an Avant-guard research. In our country, eight varieties of *Plumeria* species, subject of the current study are in the process of acclimatization. They resisted outdoor May till October, when external temperature is not going below 10°C.

The morphological and anatomical studies performed on *Plumeria* sp. - leaf, petiole and pedicel cross-sections provided for the first-time valuable information regarding the frangipani's cultivation in Romania.

The size of analyzed frangipanis ranged between 42-88 cm in height and 36-75 cm in width. *Plumeria* sp. leaves varied between 18.8-32 cm long, 8.2-12.7 cm large, clustered at the end of the branches. The petiole's length was between 4.5-6.7 cm and its diameter was averagely of 0.65 cm.

Microscopically, the leaf showed the presence of thick lamina, adaxial & abaxial epidermis, palisade mesophyll, trichomes, air chambers, laticifer cells, tannins, starch granules, oil glands, collateral vascular strand.

The preliminary evaluation of analyzed frangipanis bio-compounds showed a mean polyphenol content of 66 mg GAE/100 g and the mean flavones content was 2.15 mg rutin/100 g.

The amazing ornamental potential of *Plumeria* sp., based mainly on its flowers and leaves attractiveness gathered in one very large collection of varieties with countless colours & fragrances, opens a good opportunity for our local florists and flower designers to extend their collaborations enhancing their profit.

The results of this study can serve as source of information for researchers, horticultural engineers, providing suitable standards in future investigations and applications in bio-medicine, pharmaceutics, cosmetics, perfume industries, bio-technology, etc.

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ISSN 2285 – 5653
ISSN-L 2285 – 5653