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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS DESIGN AND LANDSCAPE ARCHITECTURE

MISCELLANEOUS

FRUIT GROWING


EX·TERRA·AVRVM

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EFFECT OF INFECTION BY ILAR-VIRUSES ON VEGETATIVE GROWTH OF SWEET CHERRY IN NURSERY AND YOUNG TREES

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Abstract

The effect of infection with Prunus necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV) on vegetative growth of sweet cherry trees was investigated in a nursery and during the first three years in an orchard. The subjects of the research were trees of cultivars 'Van', 'Kozerska', 'Drogans yellow', 'Lambert' and 'Stefania'. In the spring of 2012 virus-free P. mahaleb rootstocks (IK-M9) were budded with virus-free buds of these cultivars. Fourteen days after that, they were artificially infected with isolates of PNRSV, PDV and mixed infection of them, using 'double chip budding' method. Serological analyses were made by DAS-ELISA method. There was a significant negative virus effect on the vegetative growth in most of the single infected with PNRSV or PDV and all mixed infected trees of Van and Lambert in a nursery. As an average for the period of study there were not significant virus effects on the trunk diameter, the height of the trees and the length of the shoots of virus infected trees of the cultivars 'Stefania', 'Kozerska' and 'Van', compared to the healthy young trees.

Key words*: influence, PNRSV, PDV, sweet cherry, vegetative growth.*

INTRODUCTION

The viral infections are one of the main problems in the production of healthy propagated material from stone fruit species and profitable fruit production. The most common and economically important among the viral pathogens infecting the sweet cherry trees are *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV). These viruses, members of the genus *Ilarvirus,* spread not only by infected propagating material, but also by seed and pollen (Amari et al., 2009; Aparicio et al., 1999; Mink, 1993).

Quantitative data on the relationship between virus infection and the effect on the vegetative and reproductive development of sweet cherry trees is limited and most the publications are from the last century.

PNRSV and PDV may cause a significant reduction in taking of buds, varying by percentages dependent on the rootstocks (Nemeth, 1972; Proebsting et al., 1995).

The infection of *Ilarvirus* also has an adverse effect on the development of young trees in the nursery, and retards their growth (Milbrath, 1950; Milbrath, 1957; Nemeth, 1986; Gilmer et al., 1976; Nyland et al., 1976). In the case of combined infections, the damage is always higher than if the viruses infected separately (Cropley, 1968). In the fruit-bearing orchards these viruses induce also growth and crop yield reduction (Parker et al., 1959; Posnette et al., 1968). They can delay maturity in fruit development up to 2 weeks compared to virus-free trees (Howell and Mink, 1984).

The purpose of our study was to investigate the influence of PNRSV and PDV on the some vegetative properties of five sweet cherry cultivars grafted on *Prunus mahaleb* rootstock.

MATERIALS AND METHODS

The investigation was carried out at the Institute of Agriculture-Kyustendil, Bulgaria. In the spring of 2012, virus-free *P. mahaleb* rootstocks (IK-M9) were budded with virus-free buds of cultivars 'Van', 'Kozerska', 'Drogans yellow', 'Lambert' and 'Stefania'. 14 days after that, they were artificially infected with isolates of PNRSV, PDV and mixed infection of them, using 'double chip budding' method (Table 1). Ten trees of each cultivar were infected with PNRSV, PDV and the combination of both. Virus-free trees from each cultivar were used as controls.

Table 1 Virus treatments and source of inoculum from commercial sweet cherry orchards

Virus treatment	Source of virus
PNRSV	naturally infected cherry, cultivar 'Merchant'
PDV	naturally infected cherry, cultivar 'Superstar'
PNRSV+ PDV I	naturally infected cherry, cultivar 'Diana'
PNRSV+ PDV II	naturally infected cherry, cultivar 'Priusadebnaya'

The 2-year-old nursery trees of cultivars 'Van', 'Kozerska' and 'Stefania' artificially infected with PNRSV, PDV and mix combination, as well as - virus free ones were planted in autumn 2013 in randomised complete blocks, at a spacing 4.80 x 4.00 m.

Each variation of each cultivar was represented by three trees.

The performance of these trees in terms of vegetative growth – trunk diameter, height of the tree and length of the shoots on infected and virus free trees was examined for three years. In February 2015, a pruning of healthy and infected trees was carried out.

The viral status of the used rootstocks IK-M9 and sweet cherry cultivars was determined by ELISA testing. All plant materials were subjected to DAS-ELISA using the PNRSV, PDV, PPV, CLRV and RpRSV detection kit of Loewe Biochemica (GmbH, Germany) and Cocktail-ELISA for identification of ACLSV following recommendations of the supplier. The infection of virus free trees by pollen transfer was followed by regular ELISA testing each year.

The data was statistically processed by analysis of variance using F for test significance and LSD for significance of the differences between variant means and control, at level $P<0.05$, 0.01 and 0.001, depending on data dispersion.

The data analysis was performed by computer programs developed by Maneva, 2007 on the base of standard statistical algorithms suitable for small set of data with biological origin (Sokal and Rohle, 1981).

RESULTS AND DISCUSSIONS

In a nursery

The data from the experiment of the influence of PNRSV, PDV and mixed infection with both viruses on the vegetative properties of trees of investigated five cultivars in a nursery are represented in Table 2.

In most of the single and all mixed infected trees of cultivar 'Van' and 'Lambert' was found a significant negative influence of the viruses on vegetative growth of tree.

The development was the least disturbed by PNRSV infection: the rootstock diameter was less by 8.2% in cultivar Lambert and 10.6% in Van, the diameter of scion was less by 17.0% in Van, the height of the young tree was less by 2.3% in Lambert and 12.4% in Van.

The strongest effect on the development was observed in the combined infection of PNRSV and PDV: the diameter of rootstocks was less by 21.4 - 25.3% in Van, the diameter of scion was less by 21.9 -30.4% in Van, the height of the tree was less by 27.8 - 37.0% in Lambert. In the cultivars 'Drogans yellow', 'Stefania' and 'Kozerska' the viruses and the mixed infections of them insignificantly reduced vegetative properties of the trees.

With the exception of the cultivar 'Kozerska', where PNRSV infection induced reduction of the diameter and the height of trees by 32.5%, mixed infection reduced the scion diameter by 24.7%. In cultivar 'Stefania' the combined infection decreased the diameter of rootstocks by 15.5%, which was statistically proven.

In a non – fruit - bearing sweet cherry orchard:

The effect of virus infections on the vegetative properties of the investigated cultivars in the orchard is shown in Table 3. As an average for the period of study, there were not significant virus effects on the trunk diameter, the height of the trees and the length of the shoots of virus infected trees of cultivars 'Stefania', 'Kozerska' and 'Van', compared to the healthy young trees.

Although it was noted that virus infections insignificantly decreased the trunk diameter by 2.2% in trees of cultivar 'Van' infected with PNRSV to 27.8% in trees 'Stefania' with PNRSV+PDV, the height of the young trees by 3.8% in cultivar 'Stefania' infected with PNRSV to 13.9% in 'Stefania' with PDV, the shoot length by 3.2% in trees of 'Stefania' with PNRSV to 28.9% in trees of 'Kozerska' with mix infection PNRSV+PDV.

Table 2. Vegetative properties of virus-free and virus infected trees in a nursery

Cultivar	Virus treatments	Rootstock diameter, cm	Scion diameter, cm	Height of tree, cm
'Drogans yellow'	virus free	1.82	1.40	146.8
	PNRSV	1.66 ns	1.28 ns	132.6 ns
	PDV	1.76 ns	1.11 ns	126.4 ns
	PNRSV+ PDV I	1.82 ns	1.36 ns	134.6 ns
	PNRSV+ PDV II	1.78 ns	1.32 ns	124.6 ns
Sd		0.1146	0.1660	17.5374
F		0.6574	0.9819	0.4995
LSD 0.05		0.243	0.3518	37.17
'Stefania'	virus free	1.94	1.40	160.4
	PNRSV	1.84 ns	1.52 ns	157.6 ns
	PDV	1.80 ns	1.34 ns	152.8 ns
	PNRSV+ PDV I	1.64 *	1.40 ns	149.2 ns
	PNRSV+ PDV II	1.96 ns	1.36 ns	164.4 ns
Sd		0.1129	9.2518	11,1132
F		2.5830	1.1403	0.5873
LSD 0.05		0.2394	0.1961	23.56
'Kozerska'	virus free	1.92	1.78	185.8
	PNRSV	1.90 ns	1.20 **	125.4 *
	PDV	1.76 ns	1.58 ns	182.0 ns
	PNRSV+ PDV I	1.84 ns	1.34*	145.0 ns
	PNRSV+ PDV II	1.86 ns	1.58 ns	180.8 ns
Sd		0.1843	0.1782	22.4815
F		0.2285	3.2544	2.9007
LSD 0.05		0.3906	0.3778	47.66
'Lambert'	virus free	2.18	1.46	163.6
	PNRSV	2.00 ns	1.48 ns	159.8 ns
	PDV	1.84 *	1.40 ns	143.2 ns
	PNRSV+ PDV I	1.80 *	1.18 *	118,0*
	PNRSV+ PDV II	1.72 *	1.10 *	101.8 **
Sd		0.1582	0.1259	16.0780
F		2.6772	3.7680	5.5424
LSD 0.05		0.3354	0.2669	34.08
'Van'	virus free	2.06	1.64	175.5
	PNRSV	1.84 ns	1.36 *	153.8 ns
	PDV	1.68 **	1.36 *	145.8 *
	PNRSV+ PDV I	1.62 **	1.28 *	129.6***
	PNRSV+ PDVII	1.54***	1.14 **	136.0 **
Sd		0.1278	0.1263	11.1178
F		5.2043	4.1705	5.1885
LSD 0.5		0.2709	0.2678	23.56

During the period of the investigation, each spring the virus free trees of the investigated cultivars were retested for the present of viruses - PNRSV and PDV. The results of the serological analyzes confirmed that all control trees were virus free.

Sweet cherry trees in Bulgaria are grown primarily on Mahaleb (*P. mahaleb*) or Mazzard (*Prunus avium*) seedlings. These rootstocks are generally tolerant to infection by the pollen-borne ilarviruses PDV and PNRSV (Lang and Howell, 2001). It should be noted that in our experiment, it was used only *P. mahaleb* rootstock and this may be one of the reasons for the established insignificant influence of these viruses on the vegetative growth of infected young sweet cherry trees in an orchard.

However, recent research revealed that trees on some new dwarfing rootstock like Gisela 6, Damil, Inmil and others exhibited detrimental reactions to infection by PDV and PNRSV (Lang et al., 1998; Andersone et al., 2002; Lankes, 2007).

Table 3. Vegetative properties of virus-free and virus infected trees in orchard

Cultivar	Virus treatments	Trunk diameter, cm	Height of tree, cm	Length of one-year shoots, cm
		Average (2014-2016)		
'Stefania'	virus free	1.53	228.3	41.89
	PNRSV	1.36 ns	219.6 ns	40.52 ns
	PDV	1.20 ns	196.6 ns	35.08 ns
	PNRSV+ PDV	1.11 ns	200.3 ns	33.99 ns
Sd		0.2503	33.2811	7.5832
F		1.0957	0.4180	0.5341
LSD 0.05		0.6133	81.53	18.57
'Kozerska'	virus free	1.82	260.6	56.95
	PNRSV	1.44 ns	241.6 ns	42.67 ns
	PDV	1.726 ns	248.0 ns	48.00 ns
	PNRSV+ PDV	1.473 ns	238.3 ns	40.46 ns
Sd		0.3005	19.6718	6.8374
F		0.7765	0.5017	2.3030
LSD 0.05		0.7362	48.19	16.75
'Van'	virus free	1.52	249.6	49.34
	PNRSV	1.486 ns	239.3 ns	45.47 ns
	PDV	1.386 ns	232.3ns	40.03ns
	PNRSV+ PDV	1.36 ns	223.3 ns	38.21sn
Sd		0.0879	29.7069	5.853
F		1.5340	0.2808	1.5135
LSD 0.05		0.2155	72.78	14.34

CONCLUSIONS

There was a significant negative virus effect on the vegetative growth in most of the single infected with PNRSV or PDV and all mixed infected trees of cultivars 'Van' and 'Lambert' in a nursery.

As an average for the period of study there were not significant virus effects on the trunk diameter, the height of the trees and the length of the shoots of virus infected trees of the cultivars 'Stefania', 'Kozerska' and 'Van'

grafted on *Prunus mahaleb* rootstock, compared to the healthy young trees.

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MECHANICAL PROPERTIES OF THE FRUITS OF SOME PERSPECTIVE SWEET CHERRY CULTIVARS

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Abstract

It was performed a research on the resistance of sweet cherry fruits to mechanical pressure and detachment force from their fruits stem for the cultivars 'Merchant', 'Pobeda Krimska', 'Summit', 'Vanda', 'Stella', 'Sunburst', 'Rainier', 'Bing', 'Mizia', 'Kozerska', 'Vasinika', 'Alexton' and control cultivar 'Van'. The survey was conducted during harvesting maturity of the fruits. These indicators characterize the transportability of the sweet cherry fruits and suitability of the cultivars for mechanized harvesting. The results show that the fruits of all investigated cultivars had from „good" to „very good" transportability, with the exception of 'Pobeda Krimska' and 'Sunburst'. The cultivar 'Vasinika', created at the Institute of Agriculture - Kyustendil, Bulgaria stands out with easily removable fruits, without leakage of juice. This cultivar was the most suitable for mechanized harvesting from all investigated cultivars.

Key words*: cultivar, mechanized harvesting, sweet cherry, transportability.*

INTRODUCTION

Along with the size and flavor, the density of sweet cherry fruits is one of the main criteria determining the quality of fruits for fresh consumption (Guyer et al., 1993; Bernalte et al., 1999; Vursavuş et al., 2006).

In some cultivars, the fruit flesh can be soft, in others - tight in different degree (Georgiev et al., 2007).

According to Zhivondov et al. (2011), the later ripening cultivars are characterized by good density, crispness, tender skin, juiciness and separability of the stone, while the early ones always have a soft or semi-thick texture.

In general the market preferences are targeted at sweet cherry cultivars having hard-fleshed fruits, mainly because of their better transportability, storage and resistance to manipulation.

The strength of attachment of the fruits to the fruit stems is directly related to the suitability of the cultivar for mechanized harvesting. When detached, the fruit stem may tear the fruit flesh or skin, and cause leakage of juice.

The cultivars, which fruits are easily detached from the stem, without tearing the skin and flesh and no juice leakage, are suitable for mechanized harvesting (Georgiev et al., 2007).

In the investigation carried out by the same authors was found that the cultivar 'Volsko' had the weakest detachment force of the fruits from the stems-less than 250 g.

It was most difficult to detach the fruit stems from fruits in cultivars 'Badaconi' and 'Poznanska' - from 531 to 740 g.

The purpose of this study was to establish the theoretical transportability and suitability for mechanized harvesting of some promising sweet cherry cultivars.

MATERIALS AND METHODS

The research was conducted during the period 2014-2016 at the experimental sweet cherry plantation of the Institute of Agriculture - Kyustendil, Bulgaria.

The object of the study were cultivars 'Merchant', 'Pobeda Krimska', 'Summit', 'Vanda', 'Stella', 'Sunburst', 'Rainier', 'Bing', 'Mizia', 'Kozerska', 'Vasinika' and 'Alexton'. The cultivar 'Van' was used as control.

The trees were planted in the spring of 2002 at distance of 6 x 5 m in the rows and formed in free growing crown.

They are grafted on *P. mahaleb* rootstock IK-M9, grown without irrigation. Each sweet cherry cultivar was represented by 5 trees.

The soil in the experimental plantation is highly leached, slightly sandy-clay cinnamon forest soil with a neutral reaction.

The fruit transportability was experimentally determined by measuring the resistance of the fruits to mechanical pressure until cracking of the skin, and by measuring the detachment force from their fruit stems.

The measurements were carried out during physiological maturity of the fruits, with a device made for this purpose, similar to the apparatus AC 2 (Georgiev et al., 2007).

The mechanical resistances most closely imitate the transport pressures and the force of detachment of the fruits from their fruit stems during harvesting.

The indicators are measured in grams. After detachment of the fruit stems, it has been reported whether there was observed any tearing of the skin and leakage of juice. The indicator is presented as a percentage of the total number of fruits recorded.

There were investigated 3 replicates of 50 fruits per each cultivar.

The obtained experimental results were processed by the method of analysis of variance, using the LSD-test to prove statistical significance of the differences found between the control and the variants. The evaluation was made at levels of significance $P < 0.05$, $P < 0.01$ and $P < 0.001$ (Maneva, 2007).

RESULTS AND DISCUSSIONS

The transportability of sweet cherry fruits is defined to a large extent by the biological characteristics of the cultivar. The rainfalls and their amount during the ripening period, as well as the applied agro-technology, also have an impact.

The results of our study show that, with the exception of ‘Pobeda Krimska’ and ‘Sunburst’, the fruits of other sweet cherry cultivars had good to very good transportability (Table 1). As an average for the period of study with the highest endurance of mechanical pressure, exceeding the value of the control ‘Van’ (2151 g), were the fruits of the cultivars ‘Mizia’ (2278.3 g) and ‘Summit’ (2214.1 g). "Very good" transportable qualities, and resistance to pressures from 1812.9 to 2036.3 g had also the fruits of ‘Bing’, ‘Alexton’, ‘Kozerska’, ‘Rainier’ and ‘Vanda’. The cultivars ‘Vasinika’ (1675.7 g), ‘Stella’ (1495.0 g) and ‘Merchant’ (1461.0 g) are characterized by "good" transportability.

Relatively low resistance to pressure and "low" transportability, respectively, were found for fruits of the cultivars ‘Pobeda Krimska’ (977.1 g) and ‘Sunburst’ (1277.7 g).

The strength of attachment of the fruit to the fruit stem has a great practical importance.

The cultivars, which fruits are easily to detach from the fruit stems, without tearing the skin and no leakage of juice, are suitable for mechanized harvesting.

The data showed that during the study period the smallest force of detachment of the fruits from their fruit stem was found in cultivar ‘Pobeda Krimska’ - from 220.5 to 240.7 g, followed by ‘Vasinika’ - from 335.0 g to 459.3 g. With "easy to medium hard" detachment of the fruits were the cultivars ‘Merchant’ (from 409.6 g to 505.3 g), ‘Van’ (from 359.1 g to 578.0 g), ‘Sunburst’ (from 399.0 g to 595.3 g) and ‘Stella’ (from 447.0 g to 571.3 g). For the cultivars ‘Alexton’ (from 379.3 g to 610.0 g) and ‘Rainier’ (from 450.2 g to 590.0 g), detachment of the fruits was "medium hard". An average of 580.9 g to 622.3 g of force was needed for cultivars with "hard" detachment of the fruits – ‘Vanda’, ‘Summit’ and ‘Mizia’. "The most hard" was detachment of the fruits in a cultivar ‘Kozerska’ from 689.5 to 871.7 g (Table. 1).

The lower the value of this indicator, the more suitable the cultivar for mechanized harvesting.

The statistical analysis of the results showed that proven differences were established between the cultivars ‘Mizia’ and ‘Summit’, and the control in regard to resistance of the fruits to mechanical pressure.

For the other cultivars the statistical proof is negative or insignificant.

The force of detachment of the fruits from their stems was less than the control for cultivars ‘Pobeda Krimska’, ‘Vasinika’ and ‘Merchant’.

The difference between ‘Sunburst’ and the control ‘Van’ was insignificant, and for other cultivars - positively proven (Table 1).

Table 1. Mechanical properties of sweet cherry fruits, 2014-2016

Cultivar	Mechanical properties							
	The force of mechanical pressure of the fruits to crack				The force of detachment of the fruits from the fruit stems			
	g				*g*			
	2014	2015	2016	x*	2014	2015	2016	x*
'Merchant'	1520.7 − − −	1427.9 − − −	1421.7 − − −	1461.0 − − −	505.3 −	409.6 −	358.0 n.s.	424.3 −
'Pobeda Krimska'	1020.2 − − −	967.2 − − −	944.0 − − −	977.1 − − −	240.7 − − −	230.9 − − −	220.5 − − −	230.7 − − −
'Summit'	2820.5 + + +	2031.8 − − −	1790.0 − − −	2214.1 +	784.2 + + +	581.1 + + +	396.0 n.s.	586.7 + + +
'Vanda'	1871.9 − − −	1801.9 − − −	1765.0 − − −	1812.9 − − −	753.1 + + +	560.4 + + +	429.2 + +	580.9 + + +
'Stella'	1650.0 − − −	1469.9 − − −	1365.0 − − −	1495.0 − − −	571.3 n.s.	465.0 n.s.	447.0 + + +	494.4 +
'Sunburst'	1168.7 − − −	1249.5 − − −	1415.0 − − −	1277.7 − − −	595.3 n.s.	420.0 n.s.	399.0 n.s.	471.4 ns
'Vasinika'	1680.1 − − −	1640.0 − − −	1707.0 − − −	1675.7	459.3 − − −	351.6 − − −	335.0 n.s.	382.0 − − −
'Alexton'	2148.2 − −	1970.0 − − −	1833.0 n.s.	1983.7 − − −	610.0 n.s.	514.9 + + +	379,3 n.s.	501.4 +
'Mizia'	2700.5 + + +	2249.5 n.s.	1885.0 n.s.	2278.3 + + +	750.2 + + +	569.8 + + +	547.0 + + +	622.3 + + +
'Rainier'	1926.5 − − −	1990.0 − − −	1897.0 n.s.	1937.8 − − −	590.0 n.s.	450.2 n.s.	504.0 + + +	514.7 + +
'Kozerska'	2116.0 − − −	2000.0 − − −	1800.0 − −	1972.0 − − −	871.7 + + +	710.0 + + +	689.5 + + +	757.0
'Bing'	2222.6 −	1999.0 − − −	1887.3 n.s.	2036.3 − − −	610.1 n.s.	541.7 + + +	462.0 + + +	537.9 + + +
'Van' (control)	2372.0	2195.5	1880.0	2149.1	578.0	445.0	359.1	460.7
F	133.6	275.3	222.9	384.1	53.9	106.2	63.4	124.2
SD	65.7	33.1	27.4	28.2	31.0	16.4	20.2	16.0
LSD (0.05)	135.4	68.2	56.4	58.1	63.9	33.9	41.6	32.9

* Average for the period 2014-2016

Figure 1 shows the average data for the presence of skin tearing after detachment of the fruit stem from the fruit, as well as leakage of juice. For the cultivars with "medium hard" and "hard" detachment of the fruits, the value of the indicators was low, and in five of them - 'Summit', 'Misia', 'Kozerska', 'Bing' and 'Van' control - zero.

An exception were 'Sunburst' and 'Rainier', where it was found that about 20% of the fruits had torn skin and leakage of juice. From the cultivars with easily detached fruits, 'Vasinika' stood out with only 5.0% tearing of the skin and without leakage of juice. As a result of the biological cultivar characteristics, the differences between the cultivars included in the experimental work are significant.

The variation of the indicators over the years is in a relatively narrow range. The absence of big variations in the values means that the studied cultivars are stable in terms of these important mechanical parameters.

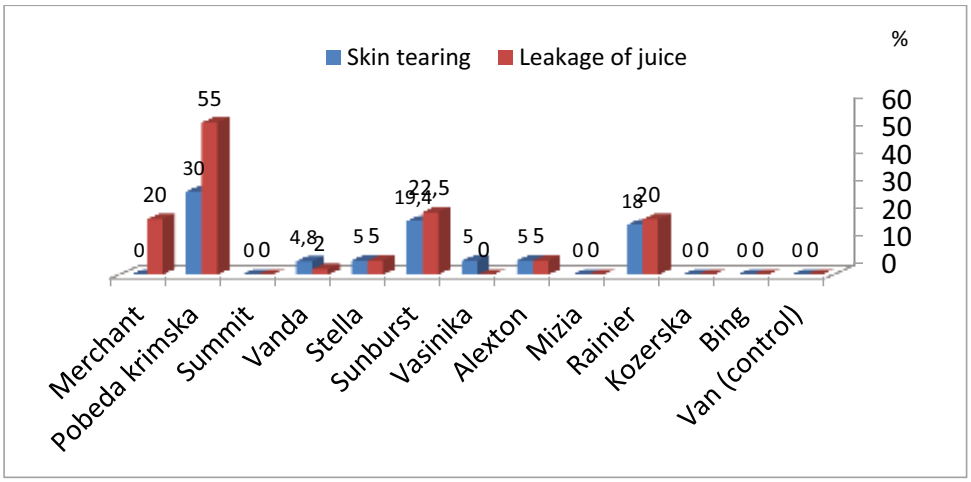

Figure 1. Condition of the fruits after detachment from the fruit stems

CONCLUSIONS

The transportability of sweet cherry fruits is defined to a large extent by the biological characteristics of the cultivar. The fruits of the investigated cultivars 'Mizia', 'Summit', 'Bing', 'Alexton', 'Kozerska', 'Rainier' and 'Vanda' had a "very good" transportability. With "good" transportable qualities were characterized the fruits of 'Vasinika', 'Stella' and 'Merchant', and with "low" - 'Pobeda Krimska' and 'Sunburst'.

A relatively "easy" picking of the fruits from their stems was found for the cultivars 'Pobeda Krimska' and 'Vasinika'. "Easy to medium hard" for 'Merchant', 'Van', 'Sunburst' and 'Stella'. "Medium hard" for 'Alexton' and 'Raineer'. "Hard" detachment was found for the cultivars 'Vanda', 'Summit' and 'Mizia', and "the most hard" - for the 'Kozerska'.

The absence of big variations in the values means that the studied cultivars are stable regarding these important mechanical parameters.

There are statistically proven differences between the cultivars 'Mizia' and 'Summit' and the control in regard to resistance of the fruits to mechanical pressure.

The force of detachment of the fruits from their stems is lower for the cultivars 'Pobeda Krimska', 'Vasinika' and 'Merchant' than in the control 'Van'.

The sweet cherry cultivar 'Vasinika', created at the Institute of Agriculture - Kyustendil, Bulgaria stands out by "easily" detached fruits from stems, without leakage of juice. These qualities define it as the most suitable cultivar for mechanized harvesting.

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RESEARCH ON THE VARIETY INFLUENCE ON VEGETABLE GROWTH AT APRICOT SPECIES IN SANDY SOILS CONDITIONS IN SOUTHERN OLTENIA

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Abstract

The growth of shoots is a basic element in the relationship between growth and fructification, on which depends the fruit trees equilibrium respectively the production provision for year and next years. Cuttings made during the spring and green works on annual increases are a very important link in agrotechnics of intensive plantations, but at the same time an element that loads the production cost. In conditions the sandy soils from Dăbuleni, fourteen apricot varieties were studied, with different maturation periods, but also with differences in growth processes. Of the three experimental years, the highest annual growth rates were recorded in year 2015 (158.21 cm average varieties) year in which both the air temperature and the amount of rainfall were at normal values during the intensive growth of the shoots. The rhythm of most intense growth was recorded in June and July, after which it diminished slightly in August and September as a result of the ending vegetative increases of vegetative growth and the beginning of preparing the trees for the winter dormancy. The year 2016, following the measurements made, showed smaller growth increases (124.21 cm), due to the thermohydric stress conditions in the sandy soil area. In year 2017, on the background of optimum soil humidity the growth of shoots was very intense as early as May. Due to the fact that in 2017 the trees presented fruit, the growth rate decreased in June and July, and after the harvesting production, the vegetative growths again showed an intense rhythm. The average value of varieties was 121.60 cm. The varieties that showed the highest annual increases in the three years of study were 'Crystal' (130.2-190.2 cm) and 'Orizont' (132.8-199.2 cm).

Keys words*: dynamic, annual growths, shoots, works in green.*

INTRODUCTION

The changes expected in the climate regime in Romania fall within the global context, but with particularities specific to the geographic region in which it is located. Compared with northwestern Europe, for example, where the warmest heating is expected in winter, for Romania, heating is expected to be more pronounced during the summer (Sunley et al., 2006).

In the intensive growth process, the synthesized substances are consumed almost entirely, the accumulations being poorly represented. In the phenophase of intense growth, trees have high nitrogen and water needs to form new tissues to increase the volume and size of the crowns.

The intense growth phenophase of shoots is due to the production and spread of neoformed internodes, an activity that is growing more and more accelerating. As a result of this intense activity, the shoots grow in length, the number of leaves and the foliar surface grow rapidly, the leaves being of normal size (Baciu Adrian A., 2005).

In the juvenile period the interval that is sufficient for the tree not to suffer is between 10-20 branches on the tree. As trees grow, their number may increase, suggesting that 40% of the branches are vegetative (Mitrea, and Tudosie, 2011).

MATERIALS AND METHODS

The research was carried out at the, Research-Development Station for Plant Crop on Sands Dăbuleni in the apricot species, with fourteen varieties.

The trees were planted at a distance of 4 m x 4 m, and the crown shape was flattened.

Determinations have been made on the growth rate of annual shoots from May to September during the period 2015-2017.

RESULTS AND DISCUSSIONS

Measurements of the growth rate of shoots in apricot varieties were carried out in vegetation phases from May to the end of September. The results obtained are presented in table 1. The measurements included a group of fourteen varieties with different ripening time and highlighted very differentiated growth depending on the variety and climatic conditions.

In the climatic conditions of year 2015, the length of the shoots was of 77.8 cm at the 'Goldrich' variety, and 101.2 cm at the 'Cristal' variety in May, and reached the end of the growth at values ranged between 128.5 cm at the 'Goldrich' variety and 190.2 cm to the 'Cristal' variety (Table 1).

Compared to the 'Olimp' control variety, the annual growth rates were of 158.4 cm in September, higher values been determined to the next varieties: 'Histria' (169.2 cm), 'Cristal' (190.1 cm), 'Auraş' (161.4 cm), 'Ceres' (179 cm) and 'Orizont' (199.2 cm).

The growth rate was very intense in June and July, after that it slightly diminished in August and September as a result of the interruption of vegetative growth and the start of preparing trees for winter dormans (Figure 1).

Table 1. The dynamics growth of shoots at apricot cultivars in 2015

Variety	Mean length of shoots on tree to species apricot (cm)				
	May	June	July	August	September
'Fortuna'	65.2	96.9	118.7	131.5	142.3
'Dacia'	58.3	97.5	125.5	142.7	145.7
'Goldrich'	50.7	89.6	115.3	122.1	128.5
'Harcot'	66.7	110.7	145.5	161.3	165.8
'Amiral'	62.3	98.7	129.9	132.4	134.6
'Mamaia'	51.1	94.5	126.6	136.8	141.3
'Olimp' (Control)	82.5	111.6	139.9	156.9	158.4
'Augustin'	68.5	102.8	126.6	137.8	141.5
'Histria'	54.7	116.4	145.5	168.7	169.2
'Cristal'	89.0	145.9	172.1	189.6	190.2
'Auraş'	73.8	129.9	141.0	157.8	161.4
'Euxin'	70.8	118.7	140.0	153.5	157.6
'Ceres'	71.3	133.6	162.1	176.7	179.3
'Orizont'	71.4	110.7	188.8	196.9	199.2

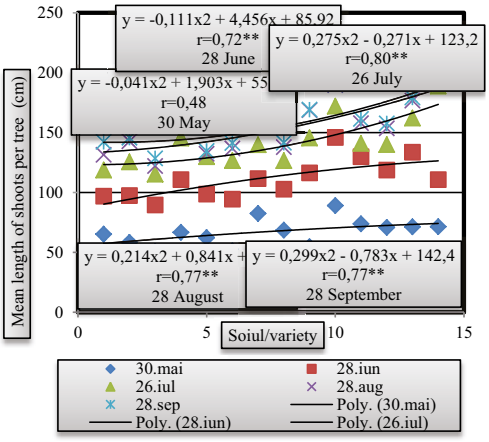

Figure 1. The correlation between the average length of apricot shoots and the growth dynamics of apricot varieties studied in the year 2015

Compared to 2015, in 2016, the measurements showed smaller growth increases between 50.7 cm for 'Mamaia' variety and 76.5 cm for 'Orizont' variety in May, and at the end of the increases, the length of the shoots was 104.6 cm in the 'Goldrich' variety and 146.8 cm in the 'Orizont' variety, but the rhythm of growth was much more intense (Table 2). The correlation factors determined were significant as early as May, from 0.73** to 0.82** in August, after then fell to 0.77** (Figure 2).

Table 2. The dynamics growth of shoots at apricot species in year 2016

Variety	Mean length of shoots on tree to species apricot (cm)				
	May	June	July	August	September
'Fortuna	55.7	73.7	94.7	106.7	115.5
'Dacia'	46.8	66.8	91.6	108.8	114.8
'Goldrich'	51.9	68.7	87.9	98.9	104.6
'Harcot'	58.8	77.5	100.8	116.2	125.1
'Amiral'	60.6	81.3	103.5	113.2	119.4
'Mamaia'	48.4	71.4	98.8	107.5	113.8
'Olimp' (Control)	70.1	88.9	112.9	125.7	131.7
'Augustin'	61.4	81.3	104.1	117.2	123.1
'Histria'	49.9	72.9	91.9	107.3	112.1
'Cristal'	79.8	102.7	121.3	132.4	139.2
'Auraş'	67.8	92.8	113.7	124.8	131.5
'Euxin'	65.8	87.8	110.4	122.6	126.5
'Ceres'	72.6	95.4	118.6	129.9	134.8
'Orizont'	76.5	98.7	125.8	139.7	146.8

The growth rate of shoots depends on the variety, the climate conditions, the amount of water in the soil and the supply of nutrients.
In the early years of planting the apricot has a rapid growth rate, forming shoots of 60-120 cm and numerous early shoots.

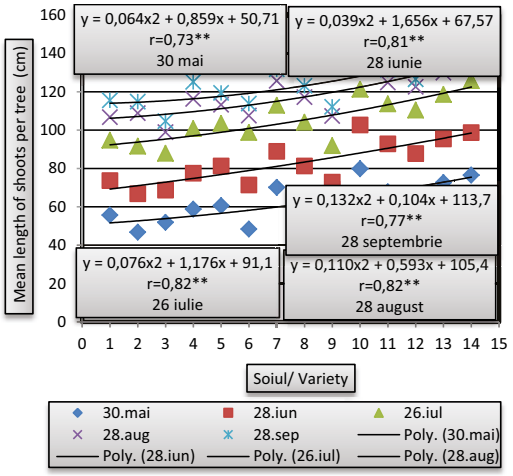

Figure 2. The correlation between the average tree length of apricot shoots and the growth dynamics of the apricot varieties studied in the year 2016

In 2017, on the background of optimum soil humidity the growth of shoots was very intense as early as May.
Values were included between 45.6 cm in the 'Goldrich' variety and 74.3 cm in 'Ceres' variety.
Compared to the 'Olimp' control variety which in may the annual increases were 66.7 cm, the 'Cristal', 'Auraş', 'Euxin', 'Ceres' and 'Orizont' varieties were recorded higher values between 68.1 cm in the 'Euxin' variety and 74.3 cm in the 'Ceres' variety (Table 3).
Due to the fact that in 2017 the trees presented fruit, the growth rate decreased in June and July, and after harvesting production, the vegetation growths again showed an intense rhythm.
The correlation factors shown in (Figure 3) show the intensity of this process.
The highest annual growth rates were recorded in 2015, in which both the air temperature and the amount of rainfall were at normal values during the intensive growth of the shoots.
The highest values were determined for the varieties: 'Cristal' (190.2 cm), 'Orizont' (199.2 cm) and 'Ceres' (179.3 cm).

Table 3. The dynamics growth of shoots at apricot species in year 2017

Variety	Mean length of shoots on tree to species apricot (cm)				
	May	June	July	August	September
'Fortuna	49.3	67.6	86.4	96.1	104.5
'Dacia'	50.1	73.1	97.4	109.9	118.1
'Goldrich'	45.6	64.6	82.5	104.6	110.5
'Harcot'	56.2	79.2	95.5	105.6	113.4
'Amiral'	58.5	79.8	104.1	113.9	120.6
'Mamaia'	52.3	70.2	90.3	101.5	108.8
'Olimp' (Control)	66.7	98.0	112.3	122.1	128.0
'Augustin'	63.2	90.2	108.6	121.5	128.4
'Histria'	52.5	84.5	100.0	112.7	122.3
'Cristal'	71.4	93.4	108.8	120.4	130.2
'Auraş'	72.4	97.4	110.3	123.6	128.8
'Euxin'	68.1	87.1	105.4	116.0	126.5
'Ceres'	74.3	101.3	114.1	123.0	129.3
'Orizont'	69.7	92.2	105.4	118.9	132.8

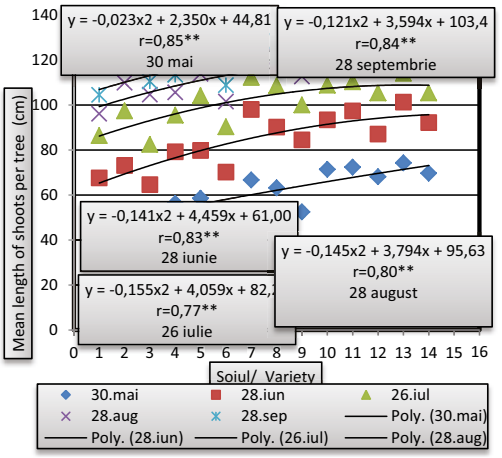

Figure 3. The correlation between the average tree length and the growth dynamics of apricot varieties studied in the year 2017

If we compare the three years of study, the highest annual growth rates were recorded in 2015, when both the air temperature and the amount of rainfall were at normal values during the intensive growth of the shoots.
The highest values were determined for the varieties: 'Cristal' (190.2 cm), 'Orizont' (199.2 cm) and 'Ceres' (179.3 cm) (Figure 4).

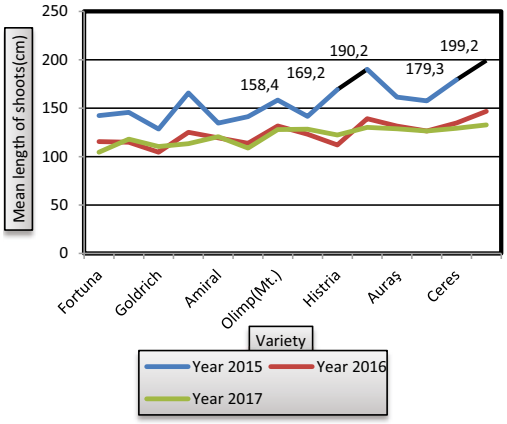

Figure 4. Annual vegetative increases in apricot varieties studied in the period 2015-2017

CONCLUSIONS

In three years of experiments (2015-2017), climatic conditions in 2015 have inprinted an overlapping of phenophases of growth and fructification earlier by about a week, compared to 2017.

The highest annual growth rates were recorded in 2015, when both the air temperature and the amount of rainfall were sitated at normal values during the intensive growth of the shoots.

The highest values were determined for the varieties: 'Cristal' (190.2 cm), 'Orizont' (199.2 cm) and 'Ceres' (179.3 cm).

The growth rate of shoots depends on the variety, climatic conditions, the amount of water in the soil and the supply the tree with nutrients.

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STUDIES REGARDING THE INFLUENCE OF PRE -HARVEST AND POST - HARVEST TREATMENTS UPON THE QUALITY OF SOME APPLE FRUIT VARIETIES

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Abstract

Losses during storage of fruits are still considerable in some cases: about 20 - 30% of all produced harvested worldwide is not consumed because of fungal or physiological disorders. In the present paper, we show the research results of the pre-harvest treatments using the products: Rover - 0.2%, Sumilex - 0.1% and Topsin - 0.1%, as well as the post-harvest treatments using: Rover - 0.2% and Sumilex - 0.1%. The treatments performed in the orchard before the harvesting period have had a major effect to reduce the percent of rotten fruits in the storehouse. The reduction was above 50% in the case of Rover - 0.2%, as compared with the untreated control. The studied apple varieties ('Jonathan', 'Generos' and 'Golden Delicious') originated from the private farmers in Voinesti - Dambovita.

Key words*: pre-harvest, post-harvest, rotten fruits.*

INTRODUCTION

The biggest part of apples production is intended for storage, which allows the commercialization for long periods after harvest (Hackbarth C. et al., 2017).
In the apple growth technology, the most important link is constituted by the phytosanitary treatments performed in the orchard, as well as during the storage period. The losses due to the fungus impact during the storage period are considerable, being up to 20% from the total yield.
Improving the cultural practices and choosing the best varieties has an important contribution to yield increase and to the fruit quality.
Pre-harvest and post-harvest phytosanitary treatments represent an indispensable link for apple culture.
Optimal postharvest treatments for fresh produce seek to slow down physiological processes of senescence and maturation, reduce / inhibit development of physiological disorders and minimize the risk of microbial growth and contamination (Mahajan et al., 2014).
Economical losses caused by parasite fungus justify the phytosanitary treatments during the vegetation period, but at the same time, imply a special care of diminishing the pesticide residuum on fruits (Bompeix G., 1985).
During storage period, apples can be attacked by a high number of fungus pathogens that cause their diseases. Infection can begin from the orchard or during transport and storage period (Franchet J., 1991).

MATERIALS AND METHODS

The experience was carried out at Voinesti, in the private orchards of some members of The Dambovita Fruit Growing Association.
The purpose of this experience was that of evaluating the apple fruits storage capacity and the maintenance of quality, following the phytosanitary treatments applied in the orchard and after harvest, in the autumn of the year 2017. Fruits samples were also analysed, with a view to appreciate the physicochemical characteristics, at the end of the storage period, for 'Jonathan', 'Golden Delicious' and 'Generos' varieties.
In our country the traditional variety is 'Jonathan' (20%), but in the last decades new and valuable varieties have been introduced,

such as 'Generos', 'Florina' and so on. These feature a genetic resistance to scab (Stanica Fl., 2011).

It is necessary to mention that in the orchard the treatments were performed on 0.5 ha/ farmer, and after harvest the fruits were exposed to phytosanitary treatments, 100 kg fruits on each variety.

The fungicides used pre-harvest were Rover 0.2%; Sumilex 0.1% and Topsin 0.1%.

These were applied 20 days before harvesting and are recommended to prevent and control the major apple fruits storage diseases, produced by fungus: *Penicillium* sp.*; Botrytis cinerea* and *Gloeosporium album*.

The fruits were stored in a unit with natural ventilation, with the following conditions: temperature 14 - 15^{0}C and air relative humidity 70 - 75%.

Spraying was performed as a post-harvest treatment, using the products: Rover 0.2% and Topsin 0.1%. For the two experimental variants both the fruits and the packaging were treated.

RESULTS AND DISCUSSIONS

As far as the pre-harvest treatment is concerned, from the data presented in Table 1 it can be noticed that for all varieties, the best results were obtained with the product Rover at 0.2%.

The attack percent was 6.8% in the case of 'Jonathan'; 6.9% for 'Generos' and 9.2% for 'Golden Delicious', but after different storage period, depending on the variety.

From the tested products, the poorest results were obtained in the case of Topsin, the apple fruits being attacked in a percent of 8.6% - 'Jonathan'; 9.4% - 'Generos' and 11.3% - 'Golden Delicious'.

The Sumilex product was more efficient than the fungicide Topsin, but less efficient than the Rover product.

Table 1. Pre-harvest treatments efficacy during storage period

Variety	Variant	Concentration (%)	Storage period (days)	Rotten fruits (%)
'Jonathan'	Control	-	80	17.2
	Rover	0.2	80	6.8
	Sumilex	0.1	80	7.0
	Topsin	0.1	40	8.6
'Generos'	Control	-	60	14.0
	Rover	0.2	60	6.9
	Sumilex	0.1	60	7.9
	Topsin	0.1	60	9.4
'Golden Delicious'	Control	-	95	19.0
	Rover	0.2	95	9.2
	Sumilex	0.1	95	10.4
	Topsin	0.1	95	11.3

We can state that the treatments performed in the orchard before harvest period have had a major effect on reducing the percent of rotten fruits in the storehouse. The reduction was above 50% in the case of Rover 0.2%, as compared with the untreated control.

Also, it was observed that 'Jonathan' cv. the principal pathogen was *Penicillium* sp. which produce the moist rot, while for 'Golden Delicious', the most important was the lenticelary rot produced by the fungus *Gloeosporius album*.

If we consider the storage period, that was 80 days for 'Jonathan', 60 days for 'Generos' and 95 days for 'Golden Delicious', we can say that the last variety had a very good behaviour during storage, in relation with the major pathogens.

As concerning the post-harvest treatment, as it can be observed in Table 2, these were more efficient than those performed during the vegetation period, on the same product and at the same concentration.

The Rover product in a concentration of 0.2% stood out again and it gave the best results. 'Golden Delicious' had a high percent of rotting fruits, as a consequence of the longest storage period.

Table 2. Pre-harvest treatments efficacy during storage period

Variety	Variant	Concentration (%)	Storage period (days)	Rotten fruits (%)
'Jonathan'	V1-Control	-	80	13.2
	V2-Rover (fruits)	0.2	80	5.9
	V3 –Rover (wraps+fruits)	0.2	80	1.6
	V4 –Sumilex (fruits)	0.1	80	6.6
	V5 – Sumilex (wrap+fruits)	0.1	80	3.4
'Generos'	V1-Control	-	60	11.2
	V2-Rover (fruits)	0.2	60	5.8
	V3 –Rover (wraps+fruits)	0.2	60	1.2
	V4 –Sumilex (fruits)	0.1	60	6.6
	V5 – Sumilex (wrap+fruits)	0.1	60	2.8
'Golden Delicious'	V1-Control	-	95	14.2
	V2-Rover (fruits)	0.2	95	7.2
	V3 –Rover (wraps+fruits)	0.2	95	1.9
	V4 –Sumilex (fruits)	0.1	95	8.5
	V5 – Sumilex (wrap+fruits)	0.1	95	3.8

From the present data it can be noticed that the pre-harvest and especially the post-harvest treatments - including wraps disinfecting - are efficient to control pathogens during storage period. Finally, at the end of the storage period, physicochemical tests were run, with a view to characterise the fruit quality. Results are presented in Table 3 and Table 4.

It was emphasized that during the storage period, the water contents decreased and there was a higher soluble carbohydrate content, a diminishing of fruits weight as a consequence of water losses, and a decrease of fruits firmness because of pectin's enzymatic breakdown.

In the case of fruit originated from the treated variants, the fruit storage capacity was better and the qualitative characteristics were higher as compared to the untreated control.

Table 3. Fruits chemical analysis

Variety	Water content (%)	Total dry weight (%)	Soluble dry weight (%)	Total acidity (%)	Acid ascorbic (mg/100 g fw.)	Minerals (%)
'Jonathan'	81.40	18.60	15.60	0.27	3.96	0.25
'Generos'	80.50	19.50	14.90	0.43	4.76	0.27
'Golden Delicious'	78.60	21.40	15.10	0.18	4.25	0.23

Table 4. Fruits physical analysis

Variety	Mean weight (g)	Specific weight (g/cm^3)	Firmness (kgf/cm^2)
'Jonathan'	120	0.744	3.9
'Generos'	135	0.780	3.7
'Golden Delicious'	138	0.760	3.6

CONCLUSIONS

The treatments performed in the orchard before the harvesting period have had a major effect to reduce the percent of rotten fruits in the storehouse. The reduction was above 50% in the case of Rover 0.2%, as compared to the untreated control.

For all varieties, the lower rotting percentage has been registered for the variant were both the fruits and the wraps were treated, because these are an important source of pathogen infection.

In the case of fruit originated from the treated variants, the fruit storage capacity was better and the qualitative characteristics were higher as compared with the untreated control.

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SYSTEMS FOR SHAPING AND PRUNING OF PLUM TREES USED IN BULGARIA

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Abstract

The article presents an overview of the formation systems that have been imposed in the plum production in Bulgaria. Crowns and pruning systems are directly related to the intended use of the fruit production, the planting density, the degree of mechanization of the planting processes and harvesting. For fresh consumption (manual harvesting), at stem height 50 -60 cm and planting distances are 5.0-5.5 m between the rows and 4.0 - 4.5 m in the rows, the trees are formed as oblique palmette trees or fruit hedge (free palmette). For fruit processing such as dried, marmalade, mousse, juice etc., mechanized harvesting requires stem height 90 - 100 cm at planting distances 6.5-7.0 m between the rows and 4.5-5.0 m in the rows, trees are formed as modified central leader, semi-flat free crown and applying contour pruning. For brandy and fruit spirit - the peculiarities of formation and pruning are described.

Key words*: plum, pruning, forming systems.*

INTRODUCTION

Plum is an economically valuable fruit species with traditions of production in our country. It occupies the second place after sweet cherry, which is 6,705 ha, or 18 % of areas under permanent crops in our country (MAF, 2017).

Pruning and pruning systems in Bulgaria are constantly evolving, due to the necessity to increase yield and increase the quality of fruit production (Bozhkova 2006; Djouvinov and Vitanova, 2002; Djouvinov et al., 2012; Iliev et al., 1977; Vitanova et al., 2010).

The shaping and pruning of plum trees depends on the variety and rootstock growth characteristics, the application of fruit production (for fresh consumption or processing), and the need to adapt the crown to some of the mechanized agro-technical activities (soil treatments, pruning, harvesting, etc.) (Djouvinov et al., 2012; Domozetov et al., 2014; Petrov et al., 1979; Velkov, 1965; Vitanova et al., 2010).

The shaping and pruning of plum trees in the production plantations must facilitate mechanized harvesting of fruit and contour pruning (Djouvinov and Vitanova, 2002; Petrov et al., 1979; Sotirov et al., 2015).

The purpose of the currant study is to present the most widely used systems for shaping and pruning plum trees in Bulgaria.

MATERIALS AND METHODS

An in-depth analysis of the shaping and pruning systems used in Bulgaria was made.

Documents and literary, agrobiological and technological sources have been researched in order to provide more complete information about the applied system formations in Bulgaria.

A parallel is made between the systems used for shaping and pruning and are presented the most suitable for our country depending on the way of harvesting and the purpose of the obtained fruit production.

RESULTS AND DISCUSSIONS

Different types of crowns are known in practice, which are divided into 3 main groups, depending on the purpose of the resulting fruit production:

Hand fruit harvesting is used for fresh consumption. The trees are formed as oblique palmette trees or fruit hedge (free palmette) and the planting distances are 5.0 to 5.5 m between the rows and 4.0 to 4.5 m in the rows.

For processing fruits such as dried, marmalade, mousse, juice etc., mechanized harvesting is

recommended, which requires a height of the stem 90-100 cm. Trees formed as modified central leader, semi-flat free crown and applying contour pruning. Planting distances are 6.5-7.0 m between the rows and 4.5-5.0 m in the row.

For brandy production and the production of fruit spirit - the shaping and pruning of trees for these production lines are related to the simplification and reduction of the cutting operations in order to low quality requirements of the resulting fruit production.

Plum trees used for the production of fresh fruit in our country are formed as oblique palmette trees or fruit hedge (free palmette). These are semi-flat and flat crowns, which are suitable for dessert varieties, the orientation of the skeleton branches is in a plane in the direction of the row or with very little deviation from it. The total height of the tree is 3.5-4 m and the stem 60-70 cm.

For manual harvesting the planting distances are 5.0-5.5 m between the rows and 4.0-4.5 m in the row. Different systems of tree formation are used. Formation and pruning of oblique palmette plum trees. It is a flat artificial crown with a leader on which 2-3 floors are formed. Each floor consists of two scaffold branches located opposite that are directed in the direction of the row (Figure 1). Thus, a continuous wall of scaffold and lateral wood with a width of about 1.5-2 m is formed in the row.

The distances between the scaffolds and the number of branch set groups are determined depending on the growth of the trees and the growing wood. In the case of highly growing trees, three floors are formed at 110-120 cm apart.

Figure 1. Oblique palmette plum trees

Scaffold branches of the oblique palmette tree formed only subscafold wood, evenly located sideways and outwards.

It is used in varieties grafted on dwarf rootstocks. This system requires a lot of manual work, strong pruning and the cost of materials to build a supporting structure. It provides better crown lightening, more effective plant protection, convenient for harvesting of high quality fruit.

First dormant pruning (pruning after planting). Newly planted trees are cut to 80-90 cm above the soil surface. Two opposing early twigs are selected to form the first floor, the strong ones cut to 15-20 cm and the weak to 4-5 buds.

In the first year, young trees should be grown so that the leader and scaffolds grow longer than the inter-floor distance.

Second dormant pruning. Suitable for scaffolds are opposite branches. It is desirable for scaffolds to select shoots aligned in length, thus saving extra measures to balance their growth. Scaffolds are not abbreviated during the second dormant pruning, as well as throughout the formation period. Head back leader to form the second floor. Cut at a height of 10-15 cm longer than inter-floor distance. If the leader has not reached that height by more than 10 cm, he does not shorten.

Third dormant pruning. Two strong and aligned lengths for scaffolds on the second set of branches are also choosen. Starters that make a larger angle with the leader are preferred, and their competitors are eliminated. The other lower ones on the leader, with slight and moderate growth, are not cut.

The leader shorten to 120-130 cm to form third set of scaffolds.

The scaffolds on the first floor tilt if they are not inclined, the competitors are removed as well as the stronger vertically growing branches.

Next pruning to complete the formation. In next years trought the summer and dormant pruning the third floor is shaped and the growth of the already formed scaffolds is guided.

After the formation of the last floor, the leader is not shorten. It is left without prunning for a few years until the fruit grows, and growth is significantly reduced, then reduced to 50-60 cm above a moderately growing lateral branch with fruit buds.

Until the trees come into full fruiting, the pruning must be as weak as possible. Primary the

competitors of the leader ans scaffolds are removed. Some very strong and upright growing branches, grown from the inside side of scaffolds, are also removed. Forming is easier and faster when unnecessary shoots is done by summer pruning. Shortening of one-year-old twigs is not recommended except for the length of the leader to obtain branches for scaffolds.

Pruning for shaping and growing of fruit hedge (free palmette). This system is a free palmette with a larger volume of the skeletal part. It is used with moderate rootstocks.

First dormant pruning (pruning after planting). Trees cut down to 80 cm above the soil surface. Weak twigs are shortened to 5-6 buds and the strong ones to 15-20 cm, and those in the stem area are cut to the base.

Second dormant pruning. Slight pruning is done. Oppositely, disposed branches are selected, with wide angle of deflection (above 45°) and grow in the direction of the scaffolds.

There is a head back the leader to form the second floor. Cut at a height of 10-15 cm greater than the specified inter-floor distance. If the leader don't exceeded this height by more than 10 cm, it won't be shortened.

Upright and upward-facing shoots are removed. For branching varieties ('Gabrovska', 'Pop Hariton' etc.), the leader and scaffolds are not cut, and those with weak branching ('Stanley' etc.) Require a moderate reduction in length.

Next pruning to complete the formation. Using summer and dormant pruning in the following years the remaining floors are formed and the growth of the already formed scaffolds is guided.

After the formation of the last floor, the leader is not abridged. It is left un pruned for a few years until the fruit grows, and growth is significantly reduced, then reduced to 50-60 cm above a moderately growing lateral branch with fruit buds.

After the 5^{th}-6^{th} year, crown regulation is performed, which can be done manually on contours or mechanized with RAC-6, in 2-3 years.

Contour pruning can only be on the top, on both sides, with a triangular contour accompanied by a slight manual pruning even in the middle years.

The aim is to keep width of 3.0-3.5 m and a height of 4.0-4.5 m of fruit hedge.

For mechanized harvesting of plum fruits, the trees are formed as modified central leader, semi-flat free crown. The planting distances are 6.5-7.0 m between the rows, 4.5-5.0 m in the row and the height of the stem 90-100 cm.

Modified central leader is a naturally rounded crown with a leader and 4-6 scaffolds, the first three forms the first floor and the next are located individually on the leader (Figure 2). It is easy to create, the trees are vital and give very high yields.

Figure 2. Modified central leader

First dormant pruning. The trees is cut to 100-130 cm. Premature twigs in the area of floor formation are shortened - weak to 4-5 buds, and strong to 15-20 buds and lower located on the stem part are removed to the base.

Second dormant pruning. Appropriate twigs are selected for the main skeleton parts and the branches of the main floor, observing the basic principles of pruning. The best-developed and centrally-occupied twig is chosen as leader. From the sprouts grown under it, three scaffolds are selected which will form the floors which must be subordinate to the leader, well developed and symmetrically located around the central axis, have a suitable angle of deflection (45-55º) and, if possible be proportionate. The remaining twigs are removed to the base. Selected for scaffold branches are shortened in length (60-70 cm) from the base, above the outer or lateral bud. If their length is shorter, no pruning is done. The leader cuts down in the area on the second floor, if reached such a height. When there are no suitable branches for skeletal ones or if the present ones are weak and the leader is stronger, it is shortened by 15-20 cm

above the level of the scaffolds to dominate over them.

Third dormant pruning. One or two scaffolds on the leader on 80-90 cm are selected and the first subscaffolds on the scaffolds of the first floor are determined. The leader is qoosen. Its competitors are removed. Choose a suitable branch for a scaffold, which should be well developed, grow at a wide angle from the leader and grow over one of the interstices of the floor branches. The remaining strong branches around them are removed, and the weak, growing at right angles, are left over for growing wood.

The three scaffolds of the floor are chosen to continue and their competitors are removed. On each scaffold is selected for one branch for the first subscafold. For subscafolds, strong branches are used that grow sideways and out of the scaffolds. Scaffolds are reduced to an outer bud of 70-80 cm above the selected first skeletal branches so that they remain at one level. After headed back the leader continues to dominate over the scaffolds.

The newly selected scaffold is shortened to 60-70 cm from the base to obtain the first subscaffold. In the third dormant pruning, only strong branches are removed, which are competitors of the leader, the selected scaffolds and branches, and shorten their lengths to obtain new scaffolds and subscafolds. Pruning should be limited.

Other pruning to complete the crown formation. In the fourth and at the latest the fifth year, the formation of the crown is finished, leaving the leader with 1-2 scaffolds, 20-40 cm apart, and on the scaffolds - another 1-2 scaffolds spaced apart 60-70 cm from the first, located on the other side of each scaffold. Annually, scaffold and one subscaffold are left.

With the growing wood, the competitors of scaffolds and the strong branches, growing in the crown, the damaged and broken, are removed. During the formation of the crown, neither a severe reduction nor a strong dilution should be made in order not to cause excessive growth and the fructification period to be delayed.

Another mechanism used for mechanized harvesting is a semi-flat freely formed crown (Figure 3).

First dormant pruning. The trees are cut to 130-140 cm from the soil surface. Premature twigs in the crown formation area are shortened to 15-20 buds and removed in the stem area.

Figure 3. Schematic arrangement of the scaffolds and subscaffold in semi-flat freely formed crown

Second - fifth dormant pruning. There is very little pruning, the strong and upright growing and branches in the intersection are removed.

Trimming of the crown is done in two ways - manually and mechanically.

Pruning fruiting trees. The balance between growth and fruiting is good when the average length of the trees of the fruit trees is not less than 20-25 cm, and the young - 50-60 cm.

It is achieved as 1-2 years of pruning and partial rejuvenation.

Contour pruning is done to limit crowns to space between rows and height.

It can be done manually or mechanically with RAC-6 trimming. It can be carried out simultaneously as a one-, two- or three-sided contour. It can be held consecutively for 2-3 years. Depending on the crown, the physiological state of the trees in a given plantation determines the strength and the way of pruning.

For the successful application of industrial technologies and especially for the mechanization of harvesting the crowns of the trees should not exceed 4-4.5 m high and 4.5-5 m wide. After the contour pruning the trees get the shape of a cone by providing a free stripe for passage of the servicing technique (Figure 4).

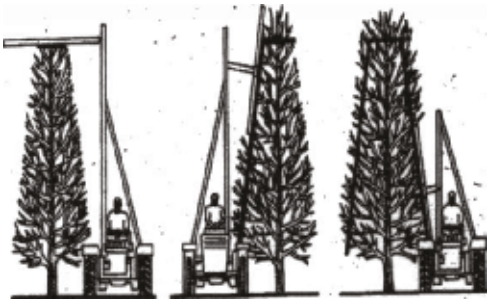

Figure 4. Contour pruning scheme

Along with the contour pruning in the fruit-bearing plantations, different sanitary, enlightening and detailed pruning can be carried out, taking into account varietal combinations and their growth and reproduction.

Formation and pruning of trees for brandy production and fruit spirit. Particularities related to this particular trend are the more freely formed crowns, by reducing cutting operations and reduced fruit requirements. After the plum tree is fully harvested, the pruning is minimized, but the fruit-bearing wood has to be periodically rejuvenated. For 4-5 years, a contour pruning is performed, which is supplemented with a low manually pruning for shaping the crown.

CONCLUSIONS

Systems for shaping and pruning plum trees in our country are differentiated according to the purpose of the fruit production, which determines the different height of the stem and the different degree of mechanization of the working processes for cultivation of the plum plantation.

For manually harvesting the fruit, the trees are formed as flat and semi-flat crowns such as oblique palmette trees or fruit hedge (free palmette), as annual and high yields are obtained.

In mechanized harvesting, trees are formed as modified central leader, semi-flat free crown and applying contour pruning that is easy to create, trees are vital and yields are very high.

For brandy production and fruit spirit - tree pruning is weaker and crowns are formed more freely to facilitate mechanized cultivation and harvesting processes.

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ASPECTS REGARDING THE QUALITY EVOLUTION OF SOME APRICOT FRUIT VARIETIES DEPENDING ON THE STORAGE CONDITIONS

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Abstract

For apricot fruits an important problem is to define the optimal picking time, the best storage variant and the variety availability for storage as to avoid having different quality levels between on–tree and in-store maturity. In response to this problem and some others that may arise, the research was performed in the orchard of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Early results have revealed that fruits from 9 apricot cultivars evaluated towards maturity in all the storage variants: NA-normal atmosphere (T=26°C), CA-cold atmosphere (T=2-3°C, relative humidity 78 to 83%) and MA-modified atmosphere (T=2-3°C, relative humidity 85 to 90%) by wrapping the storage case in plastic film. The MA storage variant may be positively considered since the total losses (both in quantity and quality) were lower than in the NA and CA variants. The best response to temporary storage was found in the late maturing varieties 'Favorit', 'Olimp' and 'Excelsior' and in the semi-early maturing variety 'Dacia', the storage period length being of 8 days in NA, 14 days in CA and 30 days in MA.

Key words*: modified atmosphere, rottenness, storage condition.*

INTRODUCTION

The present day opinion considers that the main elements defining the quality of apricots, mainly of those with double destination (fresh consumption and industrial processing) are the following: dry substance content of 18 - 20% and over 20%, titratable acidity of 1 to 2%, vitamin C of 20 - 25 mg/100 g, proteins 2 to 2.5%, pectines 0.7 to 1%, average fruit weight of 65 to 80 g and over 80 g, appealing appearance, authentic apricot fragrance (Alejandro Perez-Pastor et al., 2007).

The question that arises here is about the correlation that may be carried forth between the levels obtained by the components of fruit quality of valuable cultivar at wax ripeness and at on - tree maturity and at in-store maturity stages (Hitka, 2011).

The temporary storage of the fruit depends on temperature and relative air moisture. The damage caused by very high or low temperatures results in tissue decomposition (Bălan et al., 2008).

MATERIALS AND METHODS

A wide range of 9 varieties was analysed: 'Danubiu', 'Ovidius', 'Favorit', 'Excelsior', 'Mamaia', 'Dacia', 'Orizont', 'Olimp' and 'Harcot', which are well-known in Romania.

The fruits were harvested from the orchard of the experimental area of the Faculty of Horticulture, in Bucharest.

We recorded the fruit bearing phases, the physical and chemical features of the fruits, defined when harvested as in-completely and fully maturated and at in-store maturity, weight, weight losses and quality drops throughout storage, with special reference to the attack of *Monilinia* sp.

The fruits were stored in the facilities of the laboratory existing at the Horticultural Faculty, being conducted in three variants, and each variant was repeated three times.

NA – normal atmosphere, namely storage in shed located in the orchard in a shady place and at an ambient temperature of 26°C.

CA – cold atmosphere, 2 to 3°C temperature and 78 to 83% relative humidity.
MA – modified atmosphere achieved by wrapping the storage case in plastic film at a temperature of 2 to 3°C.

RESULTS AND DISCUSSIONS

Physicochemical features of the fruits

Harvesting at wax ripening and at full maturity

By comparing the levels of some essential components of fruit quality we noticed an evolutive change in fruit size, in soluble dry matter content, overall sugar and ascorbic acid content (Table 1 and Table 2).
Higher gains were recorded in the 'Danubiu' variety: where the increase from wax ripening to full maturity was of 5% in soluble dry matter and the 'Favorit' variety recording 10.1% increase of soluble dry matter at the beginning of ripening and a fruit size of 59 g with an increase of up to 16% of soluble dry matter and a growth of up to 68.5g of the fruit; 'Excelsior' with a content 9.4% soluble dry matter at wax ripening and 14.6% at full maturity and 'Dacia', where the levels increased by 5% for soluble dry matter and by 15 g fruit weight.

Table 1. The main physical and chemical characteristics of the fruits of some apricot varieties as determined at picking in the wax-ripe phase

Variety	Average weight (g)	Total sugar (%)	Soluble dry matter (%)	Titratable acidity (%)	Total sugar/titratable acidity ratio	Ascorbic acid (mg/100 g)
'Danubiu'	55	7.2	9.0	2.21	3.26	7.04
'Ovidius'	50	9.44	11.8	2.30	4.10	5.28
'Favorit'	59	8.08	10.1	2.04	3.96	5.28
'Excelsior'	68	7.52	9.4	1.95	3.86	5.28
'Mamaia'	65	7.84	9.8	1.48	5.30	6.16
'Dacia'	52	9.12	11.4	2.05	4.45	5.28
'Orizont'	65	10.4	13.0	1.90	5.46	5.28
'Olimp'	73	8.8	11.0	1.61	5.46	4.4
'Harcot'	74	9.44	11.8	1.82	5.19	4.52

Table 2. The levels of some fruit quality in apricot varieties at full maturity

Variety	Average weight (g)	Soluble dry matter (%)	Total sugar (%)	Titratable acidity (%)	Total sugar/titratable acidity ratio	Ascorbic acid (mg/100 g)
'Danubiu'	57.5	14.0	12.3	2.02	6.1	16.19
'Ovidius'	53.7	13.0	10.0	2.20	4.5	11.50
'Favorit'	68.5	16.0	15.3	1.91	8.0	12.60
'Excelsior	70.6	14.6	13.0	1.67	7.8	11.62
'Mamaia'	67.8	13.0	10.3	1.22	8.4	11.52
'Dacia'	67.3	16.0	13.4	1.50	8.9	14.40
'Orizont'	68.5	15.0	12.3	1.32	9.3	12.02
'Olimp'	65.7	14.0	12.6	2.12	5.9	15.40
'Harcot'	67.2	15.0	12.8	1.40	6.4	13.80

Evolution of the main physicochemical features during in-store fruit maturity

The overall sugar content recorded by us increased irrespective of the storage variant or cultivar, except for Favorit, where a slight decrease under modified atmosphere conditions was recorded.

In the cold atmosphere variant at 2 to 3°C temperature and 78 to 83% relative humidity the increase in sugar content was lower than in the normal atmosphere, still higher than in the modified atmosphere (Table 3). Difference between varieties also occurred as far as the content of sugar during storage was concerned.

Table 3. The main chemical characteristics of the fruits of some apricot varieties as determined after storage period

Variety	Storage conditions	Soluble dry matter (%)	Total sugar (%)	Titratable acidity (%)	Total sugar/titratable acidity ratio	Ascorbic acid (mg/100 g)
'Danubiu'	NA	10.0	8.0	1.6	5.0	6.06
	CA	9.5	7.6	1.7	4.5	6.26
	MA	10.0	8.0	1.9	4.2	6.45
'Ovidius'	NA	12.5	10.0	1.6	6.2	4.25
	CA	12.0	9.6	1.8	5.3	4.65
	MA	12.0	9.6	2.0	4.8	4.80
'Favorit'	NA	14.4	11.5	1.6	7.2	4.05
	CA	14.2	11.4	1.8	6.3	4.35
	MA	10.0	8.0	1.9	4.2	4.57
'Excelsior'	NA	10.5	8.4	1.5	5.6	4.15
	CA	10.0	8.0	1.6	5.0	4.45
	MA	9.5	7.6	1.7	4.5	4.70
'Mamaia'	NA	12.0	9.6	1.3	7.4	5.16
	CA	12.5	10.0	1.4	7.1	5.46
	MA	13.0	10.4	1.5	7.4	5.70
'Dacia'	NA	15.2	12.2	1.5	8.1	4.00
	CA	15.0	12.0	1.6	7.5	4.20
	MA	1.,5	10.0	1.9	5.3	4.52
'Orizont'	NA	15.9	12.7	1.4	9.1	3.92
	CA	15.5	12.4	1.6	7.7	4.11
	MA	14.0	11.2	1.8	6.2	4.40
'Olimp'	NA	8	10.2	6.7	16.9	3.80
	CA	14	14.3	6.5	20.8	3.91
	MA	30	4.1	2.3	6.4	4.00
'Harcot'	NA	8	7.6	6.9	14.5	4.92
	CA	14	15.8	6.7	22.5	5.02
	MA	30	4.2	2.4	6.6	5.15

NA = Normal atmosphere; CA = Cold atmosphere; MA = Modified atmosphere.

In a normal atmosphere the highest rises were found in the 'Favorit' variety (by 3.4%), followed by 'Dacia' (3.08%), 'Harcot' (2.76%) and 'Orizont' (2.3%). In cold atmosphere the 'Favorit' variety recorded rises of 3.32%, 'Dacia' 2.28%, 'Harcot' 2.56% and 'Orizont' 2%. In modified atmosphere, the varieties 'Dacia', 'Orizont' and 'Olimp' showed increases of only 0.8%. A different behaviour was obvious with the 'Mamaia' variety, which recorded a 2.56% increase and the 'Favorit', where the sugar content drop correlated with the flesh browning was evident.

Titratable acidity also recorded different evolutions, depending on the variety and the storage method. Its lowest value was recorded in normal atmosphere, caused by the speeding up of oxidation at 26°C.

While at harvesting at wax ripening the titratable acidity limits ranged from 1.48% ('Mamaia') to 2.3% ('Ovidius'), in the normal atmosphere the limits ranged from 1.3% ('Mamaia') and 1.6% ('Ovidius'). In the cold atmosphere the limits were from 1.4% ('Mamaia') to 1.8% ('Ovidius' and 'Favorit'); while in the modified atmosphere the limits were from 1.5% ('Mamaia') to 2% ('Ovidius'). Storing in modified atmosphere caused a higher level of organic acids following the inhibitive effect of the CO_2 content increase into the dehydrogenase activity.

A decrease in the ascorbic acid content was between the beginning of the ripening and the post maturity stage in all the investigated varieties, with different intensities in each of the three storage variant.

The normal atmosphere variant recorded an ascorbic acid content as low as 3.8 mg/100 g ('Olimp') and 6.06 mg/100 g ('Danubiu'). Higher levels were recorded in the cold atmosphere variant, of 3.91 mg/100 g ('Olimp') and 6.26 mg/100g ('Danubiu'). The highest levels were recorded in the modified atmosphere variant ('Olimp' variety featuring 4 mg/100 g) and 'Danubiu' to 6.46 mg/100 g ascorbic acid. The value of the overall sugar/titratable acidity ratio that is a milestone for assessing the gustative quality of

horticultural products, encountered changes throughout the post-harvest period, following the unequal rate of these two components.
When harvesting at wax ripeness, this ratio ranged from 3.26 (‘Danubiu’) to 5.46 (‘Orizont’ and ‘Olimp’) going up to 5 (normal atmosphere variant), 4.5 (cold atmosphere) and 4.2 (modified atmosphere) for ‘Danubiu’ and to 9.1 (normal atmosphere), 7.7 (cold atmosphere) and 6.2 (modified atmosphere) for ‘Orizont’ while for ‘Olimp’ the results were 7.5 (normal atmosphere), 7.8 (cold atmosphere) and 6.4 (modified atmosphere). A higher value of the ratio under normal atmosphere conditions and a difference between the ratio in cold atmosphere and that in modified atmosphere were highlighted because modified atmosphere slows down the metabolism of organic acids and of glucides, which results in failing to attain the characteristic gustative features.

Capacity of temporary storage of apricot varieties

The duration of apricot fruits storage picked at the wax ripening phase differed in the three variants, as it is revealed in Table 4. Thus, the optimal storage duration in normal atmosphere was of 8 days, 14 days in cold atmosphere and 30 days in modified atmosphere. Overall losses (quantitative and qualitative) in fruits stored in normal atmosphere after 8 days were high because of the high temperature and low relative humidity, ranged from 14.5% in ‘Harcot’ variety and 26.7% in ‘Ovidius’ variety.
In the case of cold atmosphere, high overall losses were also encountered, ranging from 13.2% in ‘Orizont’ and 27.9% in ’Danubiu’, that is close to previous values (normal atmosphere), attained however after a 14 days’ laps of time. The overall losses after 30 days of storage under modified atmosphere were clearly lower than those in the two preceding variants. Thus, weight losses ranged from 1.5% (Excelsior) to 5.3% (‘Danubiu’). While the qualitative ones (*Monilinia* sp. attack or inner browning) ranged from 2.7% (‘Orizont’ and 14.5% ‘Favorit’). The ‘Favorit’ variety, as already mentioned, proves improved behaviour under these conditions, since fruits do not mature and the flesh becomes brown.

Table 4. Behaviour of the apricot fruits varieties harvested at wax ripeness

Variety	Storage conditions	Storage period (days)	Weight losses (%)	Quality losses (%)	Total losses (%)
‘Danubiu’	NA	8	9.8	10.5	20.3
	CA	14	21.4	6.5	27.9
	MA	30	5.3	3.4	8.7
‘Ovidius’	NA	8	14.4	12.3	26.7
	CA	14	20.2	6.8	27.0
	MA	30	5.2	3.1	8.3
‘Favorit’	NA	8	12.1	13.5	25.6
	CA	14	20.1	7.3	27.4
	MA	30	2.6	14.5	17.1
‘Excelsior’	NA	8	14.6	10.7	25.3
	CA	14	11.1	5.8	16.9
	MA	30	1.5	2.7	4.2
‘Mamaia’	NA	8	18.0	6.2	24.2
	CA	14	1.1	5.5	16.6
	MA	30	1.6	3.4	5.0
‘Dacia’	NA	8	13.4	7.5	2.,9
	CA	14	11.9	5.9	17.8
	MA	30	4.2	2.3	6.5
‘Orizont’	NA	8	9.0	6.3	15.3
	CA	14	7.5	5.7	13.2
	MA	30	3.1	1.7	4.8
‘Olimp’	NA	8	10.2	6.7	16.9
	CA	14	14.3	6.5	20.8
	MA	30	4.1	2.3	6.4
‘Harcot’	NA	8	7.6	6.9	14.5
	CA	14	1.8	6.7	22.5
	MA	30	4.2	2.4	6.6

CONCLUSIONS

In order to obtain highly qualitative fruits as far as the organoleptic aspect is concerned, after their temporarily storage under various conditions, fruits picked at wax ripeness period must feature values of the overall sugar/titratable acidity ratio of 3.26 to 5.46.

The optimal storage period for apricot fruits picked at wax ripeness stage was of 8 days in normal atmosphere, 14 days in cold atmosphere and 30 days in modified atmosphere.

The capacity of apricot fruits which were temporarily stored varied within close limits, the best results were recorded in 'Orizont', 'Olimp', 'Dacia' and 'Harcot' varieties.

The modified atmosphere obtained inside the storage package wrapped in semipermeable plastic film keeps the overall losses at a low value throughout the storage period, if compared to the normal atmosphere and cold atmosphere variants.

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ECONOMIC EFFECT OF SUSTAINABLE APPLE PRODUCTION

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Abstract

The experiment was carried out during the period 2014-2016 at the Institute of Agriculture - Kyustendil using 'Florina' and 'Freedom' cvs. grafted on seedling rootstocks of Winter Gold Pearmain and wild apple cultivated in a high density plantation with trees of the same cultivars on clonal rootstocks MM 106 (in the row) and M 9 and Marga Hndzor (MH) (between the rows). The soil is chromic luvisols. In order to study the influence of the intercropping on the growth and production of the trees, there are 3 experiments – cultivar-rootstock trail without intercrop, cultivar-rootstock trail with intercrop in rows and inter rows spacing. The production costs required for the cultivation of one hectar apple tree orchard of the studied combinations range from 1900 euro/ha from Freedom of Winter Gold Pearmain to 282 euro /ha at Freedom on Winter Gold Pearmain with interplants on MM 106 and M9. Cost differences are the result of the number of trees per hectar, the average yields and the associated costs of collecting the additional fruit production. The average yields and the resulting gross output have a significant impact on the net profit. In 'Florina' variety, the most effective combination of wild apple rootstock with in row intercrop of MM 106, and in the inter row of MH. For Freedom, better results have been obtained using a Winter Gold Pearmain with intercrop on M9 rootstock, indicating that for the different varieties it is necessary to select suitable rootstocks.

Key words*: apple, rootstock, yield, quality, economic analysis.*

INTRODUCTION

Numerous studies in apple plantations have shown that optimal planting density is the dominant factor for yields per unit area. A decisive role in increasing the density of apple plantation has two main factors - rootstock and variety.

Trees of 'Johnagold' variety on MM 106 of the following planting distances were tested: 4 x 1, 4 x 1.5, 4 x 2, 4 x 2.5 and 4 x 3 m, with the largest cross-sectional area of the stem, the largest crown volume and the highest yield being obtained at a distance of 4 × 3 m. The results show that planting distances have a negligible effect on the quality of the fruit but are important for the coloring (Kiprijanovski et al., 2009). In an experiment in Ireland with an M9 rootstock at a planting density of 672, 961 and 1,492 trees/ha, and M27 at a density of 1,279, 1,492 and 1,957, it was found that M9 had the highest yield at the highest density (Mac an Saoir et al., 2014). In Romania, an experiment with the apple 'Florina' and 'Auriu de Bistriţa' cvs., grafted on M9 and using a different planting density (1,666,500 and 5,000 trees/hectare) formed in the 'V' system, it was found that the density of the trees and the variety influence total

yield. So, for 'Auriu de Bistrita' cv., the yield at density 2,500 exceeds control with 53% and for 5,000 with 112% and for 'Florina' cv. with 69.3 and 135.5%, respectively (Platon et al., 2014).

The use of dwarf rootstock allows intercropping of the area, early fruiting of trees, sustainable productivity, good fruit quality and high economic efficiency (Dyankov, 1995; Domozetov and Radomirska, 2009).

In the analysis of the economic performance of apple plantation grown at three planting densities, an optimal density of 2,500 trees/ha was established (Fett and Waquil, 2001).

The aim of the this study is to make a comparative analysis of variety-rootstock combinations grown as an intercrop apple plantation.

MATERIALS AND METHODS

To investigate the effect of the use of intercrops in apple plantations with different rootstocks, three planting experiments were established in the spring of 1998, with 'Florina' and 'Freedom' trees grafted on the seedlings of

Winter Gold Pearmain (WGP) and wild apple (WA) and on the vegetative rootstocks - MM106, M9 and Marga Hondzor (MH):

1. Variety-rootstock trail without intercrop - the planting distance between rows is 8 m and in the row 7 m or 179 trees/ha;
2. Variety-rootstock trail with intercrop in the row - on the seed rootstock (main trees) the planting distances are 8 x 7 m or 179 trees/ha. Trees - intercrop in the row on vegetative rootstock MM106 are planted (over one of the main trees) at 7 m (8 m x 7 m or 179 trees/ha). A total of 358 trees/ha;
3. Variety-rootstock trail with intercrop in rows and between rows - on seed rootstock (main trees) planting distances are 8 x 7 m or 179 trees/ha. Trees - intercrops in rows on vegetative rootstock MM 106 are planted (over one of the main trees) at 7 m distance (8 m x 7 m or 179 trees/ha) and between rows on vegetative rootstock M9 and MH - 2 m (8 x 2 m) or 625 trees/ha. Total of 980 trees/ha.

During the fruiting period, the main economic indicators are calculated annually: gross output, euro/ha; production costs, euro/ha; net income, euro/ha; rate of profitability, %. The necessary means of production are established on the actual costs incurred on the basis of the norms and tariffs for manual and mechanized works in the Institute of Agriculture - Kyustendil and the prices of the raw materials and supplies. The valuation of the output was made at the actual realization prices during each year.

RESULTS AND DISCUSSIONS

The values for the average stem diameter are constantly increasing for trees of all variants for both varieties. In 2014, the largest diameter of the stem was the 'Florina' tree on the WGP without intercrops and pruning - 21.60 cm and the smallest with a intercrop of MH - 11.40 cm (Table 1). In the 'Freedom' cv.with the highest thickness are the trees grafted on WGP, but with a intercrop - 17.5 cm, and the smallest at M9 - 11 cm. Trends in 'Florina' are preserved in the years 2015, 2016 and 2017, with the growth of the stem being greatest in the trees grafted on wild apple with a intercrop - 1.36 cm and smallest on those with intercrop on M9 - 0.54 cm. In 'Freedom' cv., this trend is preserved except for trees grafted on MM106. The influence of the kind of the rootstock is clearly expressed, taking into account the average values of the stem diameter of all variants. Trees of both varieties on seed rootstocks have a larger stem diameter than those on MM 106, M9 and MH. Difference was found for both types of seed rootstocks in variants with and without intercrops. The differences with Winter Gold Pearmain are statistically proven. The thicker tree stem is explained by the growth force of the seed rootstock, which induces a stronger thickening of the tree stem of the used grafts. When comparing the influence of the variety on the thickening of the stem, it can be seen that the 'Florina' trees have a thicker stems than those of 'Freedom' and the different types of rootstocks, except for MM106 and MH.

The crown height data for both varieties shows that trees on seed rootstock tended to have a higher crown height than those of the MM 106, M9 and MH clone rootstocks. Trees of 'Florina' cv. on different types of rootstocks have a larger crown volume than those of 'Freedom' (Table 2).

Average, for the period 2014-2016, tree yield for non-intercrop variants is highest in 'Florina' on a WGP rootstock that amounts to 122.77 kg/tree, followed by trees on a wild apple rootstock - 89.4 kg/tree (Table 3).

Table 3. Yield, mean weight and fruit quality

Planting combination	Variety	Rootstock	Yield per tree, kg	Average fruit weight, g	Quality, %		
					Extra	I	II
Main trees without intercrop	'Florina'	WGP	122.77	115.67	54.95	30.00	15.05
		WA	89.40	116.67	63.17	24.55	12.28
	'Freedom'	WGP	61.80	116.67	69.53	15.28	15.19
		WA	64.23	115.67	66.75	18.00	15.25
Main trees with intercrop	'Florina'	WGP	44.80	124.33	72.50	18.59	8.90
		WA	68.73	126.33	82.97	12.24	4.79
	'Freedom'	WGP	60.60	91.33	47.84	24.19	27.97
		WA	49.50	118.67	76.86	12.66	10.48
Trees - intercrops	'Florina'	MM 106	42.97	113.83	63.51	24.36	12.13
		M 9	20.17	115.00	54.67	29.83	15.50
		MH	18.40	124.00	73.12	19.76	7.12
	'Freedom	MM 106	50.55	80.67	33.02	23.42	43.56
		M 9	40,23	92,33	53,58	19,22	27,20
		MH	31,97	70,00	21,27	24,54	54,19

Table 1. Diameter of stem, cm

'Florina'	2014	2015	2016	2017	Growth, cm
WA without intercrop (st)	19.42	19.94	20.17	20.55	1.13
WGP without intercrop	21.33 ++	21.86 ++	22.13 ++	22.47 ++	1.14
WGP without pruning	21.60 ++	21.87 ++	22.29 ++	22.62 ++	1.02
WA with intercrop	18.86	19,24	19.68	20.22	1.36
WGP with intercrop	18,19 +	18.49 +	19.01	19.09 +	0.90
MM 106 – in row intercrop	14.27	14.61	14.85	15.05	0.78
M 9 – between row intercrop	12.30	12.58	12.66	12.84	0.54
MH – between row intercrop	11.40	11.60	11.76	11.98	0.58
Sd	**0.5004**	**0.5565**	**0.5853**	**0.5567**	
Gd 0.5	**1.09**	**1.213**	**1.275**	**1.213**	
f	**17.4407**	**15.1577**	**12.7847**	**14.8716**	
'Freedom'	2014	2015	2016	2017	Growth, cm
WA without intercrop (st)	16.64	16.76	17.48	18.15	1.51
WGP without intercrop	16.72	17.24	17.96	18.19	1.47
WA with intercrop	17.16	17.36	18.31	18.75	1.59
WGP with intercrop	17.52	17.88	18.35	18.75	1.23
MM 106 – in row intercrop	15.16	14.77	15.05	15.13	0.72
M 9 – between row intercrop	11.03	11.22	11.42	11.90	0.87
MH – between row intercrop	11.80	11.98	12.30	12.74	0.94
Sd	**0.8850**	**0.7926**	**0.9327**	**0.9597**	
Gd 0.5	**2**	**1.791**	**2.107**	**2.168**	
f	**0.4309**	**0.6703**	**0.3778**	**0.2420**	

Table 2. Size and volume of the crown

Variety	2014			2015			2016			2017		
	h-height, m	d-width, length, m	V - volume, m^3	h - height, m	d-width, length, m	V-volume, m^3	h-height, m	d-width, length, m	V-volume, m^3	h-height, m	d-width, length, m	V-volume, m^3
'Florina'												
WA without intercrop (st)	3.65	3.86	14.23	3.31	4.10	14.56	3.75	3.79	14.09	3.18	4.04	13.58
WGP without intercrop	3.92	4.12	17.41 ++	4.07	4.40	20.62 ++	3.92	4.23	18.35 +	3.94	4.75	23.26 +++
WGP without pruning	3.82	4.13	17.05 +	3.75	4.36	18.65 +	4.22	4.47	22.06 ++	3.56	4.40	18.03 ++
WA with intercrop	3.62	3.22	9.82 ++	3.50	3.25	9.67 +	3.88	3.34	11.32	3.20	3.31	9.17 ++
WGP with intercrop	3.38	3.13	8.66 +++	2.75	3.06	6.74 +++	3.52	3.10	8.85 +	3.09	3.26	8.59 ++
MM 106 – in row intercrop	2.95	2.79	6.01	2.61	2.69	4.94	3.08	2.74	6.05	2.86	2.86	6.12
M 9 – between row intercrop	2.29	2.32	3.22	2.35	2.30	3.25	2.66	2.22	3.43	2.28	2.27	3.07
MH – between row intercrop	2.40	2.14	2.88	2.39	2.15	2.89	2.59	2.11	3.02	2.02	2.04	2.20
Sd			**1.0266**			**1.6722**			**1.8897**			**1.2637**
Gd 0.5			**2.237**			**3.645**			**4.119**			**2.754**
f			**30.8976**			**24.9451**			**16.0754**			**48.4594**
'Freedom'												
WA without intercrop (st)	3.02	3.05	7.35	2.90	3.40	8.77	3.02	3.10	7.59	2.62	3.26	7.29
WGP without intercrop	3.28	3.02	7.83	2.75	3.18	7.28	3.35	3.05	8.15	3.12	3.38	9.51
WA with intercrop	3.35	3.19	8.92	3.10	3.28	8,73	3.68	3.04	8.90	3.08	3.49	9,82
WGP with intercrop	3.08	3.12	7.84	2.70	3.18	7.14	3.35	3.06	8.21	3.08	3.40	9.32
MM 106 – in row intercrop	3.05	2.79	6.21	2.65	2.88	5.75	3.30	2.70	6.29	3.00	3.14	7.74
M 9 – between row intercrop	2.75	2.16	3.36	2.50	2.26	3.34	2.78	1.95	2.77	2.92	2.49	4.74
MH – between row intercrop	2.59	2.27	3.49	2.46	2.20	3.12	2.76	2.10	3.18	2.72	2.46	4.31
Sd			**1.0259**			**1.2636**			**0.9618**			**1.2418**
Gd 0.5			**2.318**			**2.855**			**2.173**			**2.806**
f			**0.8864**			**1.0298**			**0.5139**			**1.4218**

The difference in tree yield at 'Florina' cv. under the influence of seed rootstocks is 33.37 kg and 'Freedom' cv. 2.43 kg. Compared to 'Florina', the 'Freedom' yields are lower with 25 kg/tree on WA rootstocks and 60.97 kg/tree on WGP. In the variants with intercrops, better results have been obtained with WA rootstock for 'Florina' - 68.73 kg/tree, and for 'Freedom' with WGP - 60.60 kg/tree.

Using vegetative rootstocks the best yielding is 'Freedom' grafted on MM 106, followed by 'Florina' on MM 106, with yield difference of 7.6 kg. The lowest average yield per tree was obtained at 'Florina' grafted on MH - 18.4 kg.

In the studied variety-rootstock combinations, the average fruit weight of both types of seed rootstocks was not significantly influenced by the variety and the rootstock and ranged of 91 - 126 g. In trees on vegetative rootstocks, it is larger for 'Florina' than for 'Freedom'.

The highest percentage of extra quality of fruit from 'Florina' on WA- 82.97 %, followed by 'Freedom' on WA - 76.86 %, and with the lowest 'Freedom' on MH - 21.27 %. On both seed rootstocks the quantity of extra-quality 'Florina' fruit is higher in the variants with intercrops. The same trend has not been established for 'Freedom' variety. At all vegetative rootstocks on 'Florina' more than 50 % of the quality fruits are obtained, while in 'Freedom' variant only on M9 rootstock.

The received gross production of variants of 'Florina' cv. has the highest value in the combination of wild apple with intercrop in the row on MM 106 and between rows on MH - 12402 euro/ha. In this indicator 'Freedom' cv. is grafted on a WGP rootstock with intercrop in row on MM106 and between row on M9 - 12065 euro/ha (Figure 1).

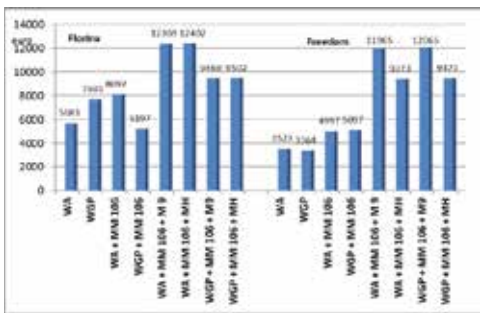

Figure 1. Gross production, euro/ha

The lowest value is the 'Freedom' cv. grafted on WGP.

The production costs required to grow one apple plantation from the studied combinations ranged from 1900 euro/ha at 'Freedom' on WGP to 2817 euro/ha at 'Freedom' on WGP with intercrops MM 106 and M9 (Figure 2).

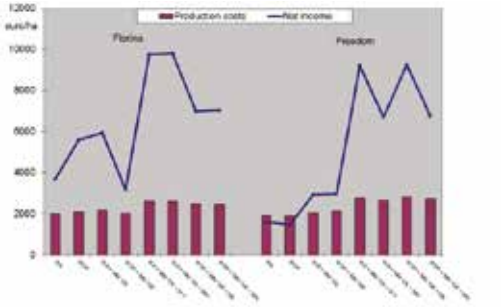

Figure 2. Production costs and net income, euro/ha

Differences in costs are a result of the number of trees per hectare, the quantity of the average yield and the associated costs of harvesting additional fruit production.

Quantity of the average yields and the gross output obtained have a significant impact on the amount of net income. In the 'Florina' cv., the most effective combination is WA rootstock with intercrop in rows on MM 106, and between rows on MH. For `Freedom`cv., better results are obtained using WGP with intercrops on M9 rootstock, indicating that it is necessary to choose suitable rootstocks for the individual varieties.

CONCLUSIONS

Trees of both varieties on seed rootstock have a larger stem diameter than those of MM 106, M9 and MH. Difference was found for both types of seed rootstocks in variants with and without intercrops. The thicker tree stem is explained by the growth force of the seed rootstocks, which induces a stronger thickening of the tree stem of the used grafts.

Trees of the 'Florina' cv.on different types of rootstocks have a larger crown volume than those of 'Freedom' cv.

The production costs required to grow one hectare of apple plantation from the combinations studied range from 1900 euro/ha

to 2817 euro/ha. Cost differences are the result of the number of trees per hectare, the quantity of the average yield and the associated costs of harvesting additional fruit production.
In 'Florina' cv., the most effective combination of a rootstock wild apple with in row intercrops on MM 106, and between rows on MH.
For 'Freedom' cv., better results are obtained using a WGP with intercrop on M9 rootstocks, indicating that it is necessary to select suitable rootstocks for the individual varieties.

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STUDIES ON THE BEHAVIOR OF SOME ELITE AND VARIETIES OF PEAR IN THE POLLINATION PROCESS

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Abstract

Fungal plant pathogens belonging to the genus Venturia cause damaging scab diseases of members of the Rosaceae. In terms of economic impact, the most important of these are Venturia inaequalis, which infects apple, and Venturia pirina, which is a pathogen of European pear. Given that Venturia fungi colonise the sub-cuticular space without penetrating plant cells, it is assumed that effectors that contribute to virulence and determination of host range will be secreted into this plant-pathogen interface. The use of resistance varieties in the pollination process is an important way to obtain varieties with genetic resistance to disease. In this paper were used as mother genitors some selections from Pyrus serotina ('9/34-94', '20/1-91' and '5/104-84') with genetic resistance to diseases and pests and as father genitors (pollen) two valuable varieties of the European assortment ('Williams', 'Beuré Bosc') and 'Cristal' cv. - Romanian pear variety registered in 2009 at Research Station for Fruit Growing Voinesti, Dambovita. The selection '9 / 34-94' performed the best results in the pollination process in crossing process with 'Williams' and 'Cristal' cvs., with 21.7%, respectively 32.11% fruit set.

Key words*: pear, resistance, pathogen, scab.*

INTRODUCTION

The *Venturia* pathogen might have originated in Asia and from there spread to Europe and recently to other apple growing countries. Hypervariability and evolution of strains that have overcome host resistance are attributed to the ability of *Venturia* to recombine its genetic material every year (Parisi L. et al., 1993).The conidia and conidiophores, together, give a characteristic velvety appearance to the young lesions of scab. The conidia of *Venturia* are capable of adhesion and germination on nonhost plants such as *Pyrus communis*, however further development to establish infections occurs only on the host plants (Chevalier M. et al., 2004). The isolates of *V. inaequalis* are hypervariable and exhibit differential pathogenicity on apple cultivars (known as differential hosts). Based upon such differences, the pathogen has been categorized into eight physiological races (MacHardy W.E., 1996; Bénaouf G. et al., 2000; Bus V.G.M. et al., 2005). However, some of the isolates of *Venturia* are capable of growing on two different differential hosts and hence it is difficult to classify them to particular race. Understanding the mechanisms of *Venturia* pathogenesis and intricacies of its interaction with apple should provide important insights for developing new strategies to combat the disease (Jha G. et al., 2009). The whole genome mutagenesis screen should be initiated to identify key virulence factors. The availability of standardized transformation methodologies in *V. inaequalis* will facilitate such efforts (Tenzer I. et al., 1997).

MATERIALS AND METHODS

Plant material evaluated included:

Pollen from pear cultivated varieties like 'Williams', 'Cristal', 'Beurré Bosc', was collected in spring 2016.

The resistance selections '9/34-94', '20/1-91', '5/104-84' were used in the controlled crossing process as mother genitors.

The scab source for inoculum was procured from the collection of the Research Station for Fruit Growing Voinesti, Romania.

These breeding progenies will be characterized after screening infections tests. Infection tests

will be performed in greenhouse conditions according to Chevalier et al. (1991) will be used for selection of resistant plants. Mixtures of isolates will be used for plantlet inoculation. Seedlings will be spraying with a conidial suspension of *Venturia inaequalis* CKE. Seedlings will be incubating for 48 hours at 18°C and 100% relative humidity. Disease symptoms will be evaluated macroscopically after 21 days of cultivation in a greenhouse. Seedlings will be dividing into 5 classes. Plants in class 0 were without symptoms of infection. Plants of class 4 had lesions with full sporulation.

RESULTS AND DISCUSSIONS

In order to plan an efficient breeding program to obtain cultivars resistant to pear scab, it is important to know the genetic control of this resistance. Although there is controversy about the genetic control of the resistance to pear scab, all authors consider that resistance could be transmitted from resistant progenitors to offspring. However, the descendants from crosses between susceptible and resistant cultivars segregated in a complex way.

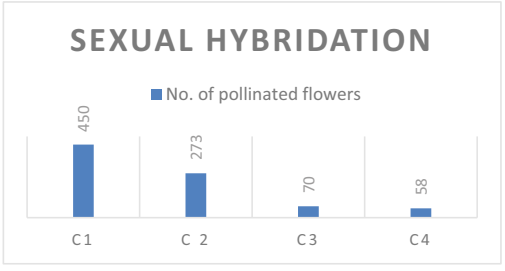

Figure 1. Number of pollinated flowers

In the Table 1 and Figure 1 we observed that the most value combination is C2 with 32.23%, percentage of fruit set, followed by the combination C1 with 21.77% percentage of fruit set:
- the combination C1 of 450 pollinated flowers, 98 fruits were bound, with a fruit set percentage of 21.77%
- the combination C2 of 274 pollinated flowers, 88 fruits were bound, with a fruit set percentage of 32.23%
- the combination C3 of 70 pollinated flowers, 5 fruits were bound, with a fruit set percentage of 7.14%
- the combination C4 of 58 pollinated flowers, 4 fruits were bound, with a fruit set percentage of 6.89%.

Table 1.The percentage of fruits set

No. crt.	Combinations	Genitors ♀ x ♂	No. of pollinated flowers	No. of fruits set	No. of harvested fruits	No. of obtained seeds
1.	C1	9/34-94 x 'Williams'	450	98	95	650
2.	C2	9/34-94 x 'Cristal'	273	88	83	535
3.	C3	20/1-91 x 'Untoasa Bosc'	70	5	5	30
4.	C4	5/104-84 x 'Untoasa Bosc'	58	4	4	32

In the Figure 2, it is noted that in the hybrid combination, C1% of the linked fruit did not produced hybrid fruit, and in the hybrid combination C2 is noted 6% of the linked fruit did not produce hybrid fruit. At C3 and C4 combinations, the linked fruits had a 100% success rate.

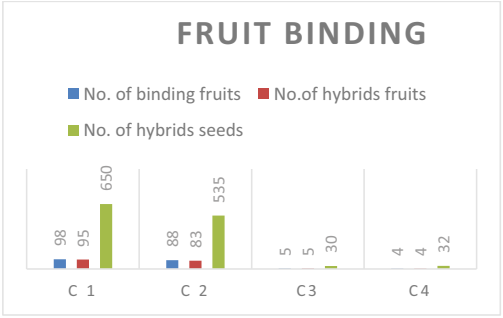

Figure 2. The fruits after pollination

From Table 2 and Figure 3 and 4 we see a success of 44% for Hybrid Combination C1, the 31% for Hybrid Combination C2, and for Hybrid C3 we have a success rate of 83% and for C4 we have a 100% success rate.
The resistant maternal progenitor (`9/34-94`, `5/104-84`, `20/1-91`) was able to transmit the scab resistance to the descendants, in agreement with previous results observed by other authors (Chevalier M., 2004).

Figure 3. Hybrids seeds

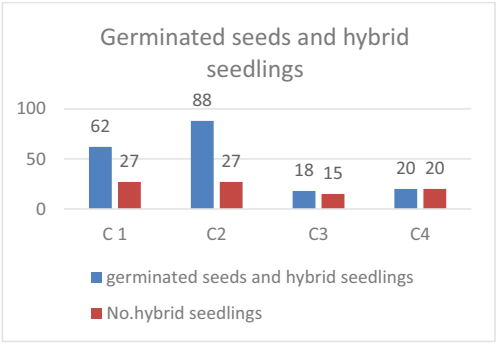

Figure 4. The percentage of germinated seeds and number of hybrid seedlings

Table 2.The percentage of germinated seeds and number of hybrid seedlings

No. crt.	Combinations	Hybrid combinations	No. of seeded seeds	No. hybrid seedlings	Percentage of rooting
1.	C1	9/34-94 x 'Williams'	62	27	44%
2.	C2	9/34-94 x 'Cristal'	88	27	31%
3.	C3	20/1-91 x 'Untoasa Bosc'	18	15	83%
4.	C4	5/104-84 x 'Untoasa Bosc'	20	20	100%

During the course of coevolution, pear has evolved mechanisms to prevent the severity of scab. The matured leaves of pear demonstrate ontogenic resistance because of which the pathogen growth is suppressed immediately after cuticle penetration and appearance of disease symptom gets delayed. The strengthened cell wall and cuticular membrane along with sub-cuticular pH of such leaves are speculated to play a role in governing such resistance. A breakdown of ontogenic resistance revealed by restored growth of the pathogen is observed in the old senescing leaves of apple. Detailed studies are needed to elucidate the functionality of such resistance and understand its breakdown mechanism.

Figure 5. Aspects of pollinations

CONCLUSIONS

It might be evident from this review that *Venturia. Sp.* is an important plant pathogen because it causes huge economic losses and also has a very interesting lifestyle. It is an appropriate time to sequence whole genome of the pathogen. The availability of genome sequence will not only stimulate research in the field of *Venturia* pear interactions and contribute to the basic understanding of this pathosystem but can also revolutionize the understanding of pathogenesis of other obligate pathogens. Understanding the mechanisms of *Venturia* pathogenesis and intricacies of its interaction with apple should provide important insights for developing new strategies to combat the disease. The whole genome mutagenesis screen should be initiated to identify key virulence factors. The availability of standardized transformation methodologies in *V. inaequalis* will facilitate such efforts.

The resistant elite progenitors (9/34-94, 5/104-84, 20/1-91) are an important step on the pear breeding program.The scab conidia could germinate on pear leaves undergoing defense responses, formation of primary hyphae is delayed and growth of subcuticular stroma is

suppressed resulting in reduced conidiation. Phenolic produced in response to *V. inaequalis* infections in pear are known to inhibit pathogen growth and are ascribed to be associated with defense mechanisms of scab resistant cultivars.

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RESEARCH ON THE ISOLATION OF GENOMIC DNA FROM OLD APPLE VARIETIES

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Abstract

The apple scab disease has probably evolved over a long time along with the apples. The disease is caused by the fungus Venturia inaequalis (Cke.) Wint, anamorph Spilocaea pomi Fr. The aim of this study were the isolation and quantification the genomic DNA on old Romanian varieties in order to select the most important to them for the Marker Assisted Selections (MAS) on apple trees. This paper presents the results of DNA isolation by exploring local populations of apple like 'Prescurate', 'Seghese', 'Vieşti' and 'Kniş' apples old varieties, that are well adapted to the conditions in Romania and that are un interesting genetic potential for resistance to scab. Only the old apple varieties were used in the study. The implementation of marker selection depends on the quality and quantity of isolated genomic DNA. The results of the quantification performed following genomic DNA isolation show a large variability in the amount of DNA in each old apple variety. The DNA concentration in apple varieties both parents and some descendants have shown that the values are higher in parents than hybrids, with the highest value for 'Turnu' variety with 179.1 ng / µl, followed by 'Calvil alb' variety with 161.1 ng / µl, then 'Varga' variety with 155.0 ng / µl.

Key words*: scab, varieties, apple, resistance.*

INTRODUCTION

Apple (*Malus x domestica* Borkh.) is one of the most cultivated fruit crops in temperate climates. The major constraint of apple cultivation is the apple scab, a fungal disease caused by *Venturia inaequalis*, which can lead to important crop losses if not properly controlled (Holb et al., 2003).

The 17 major resistance genes in apple (*Rvi1* to *Rvi17*) against *V. inaequalis* have been found (Bus et al., 2011).

Only *Rvi6* (previously *Vf* from *Malus floribunda* 821) has been extensively used for resistance breeding to date (Gessler et al., 2012).

Since the breakdown of the *Riv6* gene in the early nineties (Parisi et al., 1993), new breeding programmes have started to investigate other resistance genes for future resistance breeding (Gygax et al., 2004; Gaecia-Ruiz and Murphy, 2001).

An increase in resistance with increasing apple leaf age (ontogenic resistance) has been observed in all apple genotypes and is known to act against all known *Venturia inaequalis* strains. Up to know, no report of the breakdown of this type of resistance has been found in the literature; thus, ontogenic resistance is considered durable (MacHardy et al., 2001). Goethe (1887) and Aderhold (1900) are believed to have been the first researchers noticing age-related resistance in the *Malus-Venturia* pathosystem.

The authors observed a decrease of leaf susceptibility with increasing tissue age. Nearly three decades later, Keitt and Jones (1926) showed an increase in incubation period and a decrease of disease severity by increasing leaf age.

Following these observations, many researches have been carried out on *Malus-Venturia* interaction during leaf infection. Gessler and Stumm (1984), Li and Xu (2002), and Gusberti et al. (2012) showed that the fungus grew faster in young leaves compared to old ones.

The first unfurled and expanding leaf is considered susceptible to the apple scab disease, while the fifth leaf (starting from the top of the shoot) is considered fully resistant (MacHardy, 1996).

Disease resistance mechanisms during tissue ontogenesis have been studied in different plant pathogen systems and some factors have been

suggested to be correlated to the observed age-related resistance.

Among them, the most important appears to be chemical compounds such as salicylic acid (Kus et al., 2002; Hugot et al., 1999) and pathogenesis-related proteins (Wyatt et al., 1991), physiological barriers like the cuticle (Peries, 1962), lenticels (Kennelly et al., 2005), restricted phloem movement (Garcia-Ruiz and Murphy, 2001) or a limiting nutritional substrate for fungal infection (Juen and Hwang, 1991).

However, since a different mechanism for age-related resistance is described in each crop plant, much work remains to unveil the mechanism underlying this type of resistance in other plants. In apple, several aspects have been investigated in order to unveil the nature of ontogenic resistance.

Physiological barriers like the cuticle and papillae (Stadler, 1988), were not correlated to the age-related resistance.

The objectives of this study were the isolation and quantification the genomic DNA on old Romanian varieties in order to select the most important to them for the Marker assisted Selections on apple trees.

MATERIALS AND METHODS

Plant materials

The old Romanian apples genotypes ('Prescurate', 'Gurguiate', 'Vieşti', 'Kniş', 'Turnu', 'Moharat') were tested for their ability to support the artificial infection to scab. All this material was inoculated (by spreeding) with fiels races of *Venturia inaequalis*.

DNA isolation and SSR analysis

Genomic DNA was isolated from fresh apple leaves using the hexadecyltrimethylammonium bromide (CTAB) protocol described by. DNA concentrations were measured by a NanoDrop. Working of dilutions of genomic DNA at 100 ng/µl in TE buffer (pH 8.0) were prepared for analysis. Fresh leaf tissue (<100 mg) was sampled from inoculated and uninoculated leaf samples at 72 and 96 hpi and collected in 2 ml Eppendorf tubes (Eppendorf, Germany), previously prepared with 5 to 10, 2 mm sterile glass beads, immersed in liquid nitrogen immediately after sampling and stored at -80°C until processing. Tissues were ground twice with the FP 120 Fast-Prep machine (Bio 101 Savant Instruments Inc., Qbiogene, France) for 30 s at a speed of 5.5 m s^{-1} with an intermediate immersion in liquid nitrogen between the two grinding steps. Total RNA Isolation System (Promega Corporation, USA) and column purified following the manufacturer's instructions.

After RNA isolation and quality assessment, samples were stored at -80°C until cDNA library construction and transcriptomic assay the each population were carried out according to conditions specified in Zhebentyayeva et al., 2003.

RESULTS AND DISCUSSIONS

The results on the concentration in genomic DNA of the studied apple varieties showed that the highest concentration was recorded in 'Turnu' variety with 179.1 ng / µl, followed by the 'Calvil alb' variety with values of 161.1 ng / µl, then 'Varga' variety with 155.0 ng / µl, compared to 12.6 ng / µl 'Rosu Marin', followed by 'Sălciu' with 51.2 ng / µl (Table 1).

Table1. DNA concentration in apple varieties

No.	Cultivars	Conc DNA (ng/µl)	Rapport 260/280	Rapport 260/230	Absorbance x260
1	Prescurate	121.3	2.21	0.23	2.425
2	Turnu	179.1	2.11	0.29	3.582
3	Sălciu	51.2	2.50	0.14	1.028
4	Venchi	76.9	2.30	0.15	1.537
5	Gurguiate	114.6	1.88	0.60	2.291.
6	Iridium	55.2	2.54	0.12	1.104
7	Calvilalb	161.1	2.16	0.31	3.222
8	Vânători 48	87.2	2.23	0.17	1.744
9	Varga	155.0	2.04	0.29	3.099
10	Renet Portocaliu	143.7	1.35	0.56	2.874
11	Costat de Albeşti	46.8	2.00		0.454
12	Andrifişer	97.8	2.04	5.57	1.957
13	Roşu Marin	12.6	2.38		
14	Sângeriu	66.5	1.91	4.55	1.330
15	Roşu de Cluj	59.6	2.04	11.83	1.192

The results of the quantification performed following genomic DNA isolation show a large variability in the amount of DNA in each old apple variety (Figures 1-4).

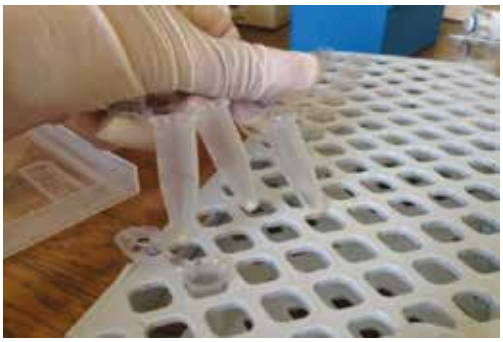

Figure 1. Apple DNA isolation

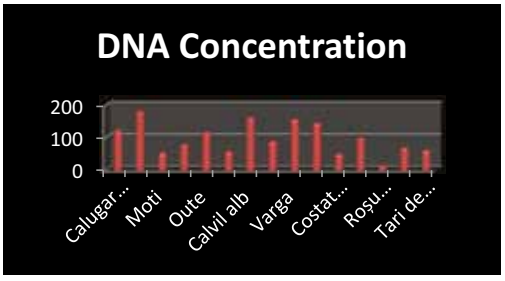

Figure 2. DNA concentration on apple cultivars

DNA purification involves the removing from the lysate all components except DNA, dividing the DNA, that is, separating the different DNA species into distinct categories. The two aspects are not necessarily constituted in separate events.
In fact, DNA purification can still begin at the stage of cell lysing.

Figure 3. Aspects of DNA isolation

Removal (after lysate formation) of all biomolecules and contaminants of DNA is accomplished by several methods: denaturation and precipitation of proteins with organic solvents such as phenol and chloroform; Following centrifugation, the precipitated proteins can be separated from the DNA, which remains in the supernatant.
The implement of resistance older apple varieties of Romanian origin could be a promising way for a viable breeding program in Romania.
First step in this work was the identification and collection (from different parts of the country) and evaluation an important number of old local varieties (Ion L. et al., 2016)

Figure 4. Apple DNA for absorbance 260

Using DNA markers of resistance to *V. inaequalis* will be based on the polymerase chain reaction (PCR).
Recovery in the Romanian breeding program the old local varieties, best suited to the climatic conditions of Romania was used like a natural source of resistance to pathogen attacks.

CONCLUSIONS

During three years of field-controlled pollination for new hybrids, other sources of resistance such as some old apple varieties, such as 'Amelie Wacsman', 'Gurguiate', 'No Name', 'Mohorât', 'Nobile de Geoagiu', 'Plocsay's favorite' etc.
The final number of signatures was relatively low.
Regarding the DNA concentration in apple varieties both parents and some descendants have shown that the values are higher in parents than hybrids, with the highest value for 'Turnu' variety with 179.1 ng / µl, followed by 'Calvil alb' variety with 161.1 ng/µl, then 'Varga' variety with 155.0 ng / µl.

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EFFECTS OF GRAFTING COMBINATIONS ON THE FRUIT QUALITY FOR THE PINOVA APPLE TREE

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Abstract

The quality of apple fruits is influenced by variety and within each variety by the rootstock and by the culture technology applied. The research presented in this paper highlighted the influence of the rootstock on the fruit quality. The experiment was conducted during 2016-2017 in the Vâlcea plant nursery, in Romania, as a comparative study for the 'Pinova' variety with several rootstocks (M9, B9, M20, Pi80, M106), including variants with grafting interstems (B9/A2, B9/M111). The size of the fruit was larger for the trees grafted on the rootstock B9 with the interstem M111, while the firmness was positively influenced by the rootstocks M9 and B9/A2. The content of soluble dry substance was favourably influenced by the rootstocks M20, B9 and Pi 80, while the titratable acidity had higher values for the fruits produced by the trees grafted on M106 and M9/M111. The total anthocyanins content was higher for the fruits obtained from the trees grafted on the rootstock B9 with the interstems M111 and A2.

Key words: *fruit weight, interstem, rootstock, soil management*.

INTRODUCTION

The apple tree is one of the most important fruit-growing specie cultivated in Romania due to its economic value and significant production. Fresh apples are highly appreciated for consumption throughout the whole year due to their healthy effects on the human body but can also be industrially processed in order to obtain various products based on pulp or juice.

The quality of fruits depends on a number of factors from which the following can be mentioned: the variety, the culture technology, the age of the trees, the pruning used (different training system) (Lord et al., 1985; Dudu et al., 2015; D' Abrosca et al., 2017).

Apple growers are constantly focused on making the apple production intensive with the aim of reducing the height of trees and the manual labour costs.

Making the production intensive is possible by grafting trees on dwarf rootstocks, particularly M9, but those trees need a supporting system.

Also, dwarf rootstocks are more sensitive to draught in comparison to standard rootstocks (Zhou et al., 2016).

In order to renounce the supporting systems and to ensure a better soil stability of trees, standard rootstocks and interstocks are used in order to decrease the vigour (Webster, 1995). The interstock influences the quality of fruits and the colour grade (Vercammen et al., 2007) and induces precocity (early fruiting) (Webster et al., 1995).

The length of the interstock influences the fruit production.

The one with a higher length leads to a higher productivity value and balances growth with fructification (Di Vaio, 2009).

The research conducted by Samad et al., (1999) with various dwarf rootstocks used did not highlight any significant differences of fruit weight in correlation with the rootstocks used.

In order to observe the way in which quality and quantity of fruits are influenced by the rootstocks and interstocks used a study was conducted using 13 grafting combinations at 'Pinova', a scab resistant apple variety, dwarf and semi-dwarf rootstocks and grafting interstocks to reduce the vigour.

MATERIALS AND METHODS

The research was conducted in the Valcea area during 2016-2017, in a 'Pinova' apple orchard established in 2015 with the planting distance

of 3.6 m x 1.25 m and a number of 2222 trees/ha.
In order to highlight the influence of the grafting interstock, two lengths were used: 30 and 40 m respectively.
The soil was maintained either worked or grassy. Researchers used 13 grafting combinations, simple or with interstock, and for each combination they chose 9 trees divided in 3 repetitions.
The following experimental variants resulted:
V1 – Pinova/M106 – control;
V2 – Pinova/M9;
V3 – Pinova/M20;
V4 – Pinova/Pi 80;
V5 – Pinova/B9;
V6 – Pinova/B9/MM111, interstock 30 cm, worked soil;
V7 – Pinova/B9/MM111, interstock 30 cm, grassy soil;
V8 – Pinova/B9/MM111, interstock 30 cm, buried;
V9 – Pinova/B9/MM111, interstock 40 cm, worked soil;
V10 – Pinova/B9/A2, interstock 30 cm, grassy soil;
V11 – Pinova/B9/A2, interstock 30 cm, worked soil;
V12 – Pinova/B9/A2, interstock 40 cm, grassy soil;
V13 - Pinova/B9/A2 interstock 40 cm, buried, grassy soil.
For combinations with interstock the influence of deep planting was tested, the interstock being planted as well.
The maintenance technology applied in the orchard was the standard one used at high-density apple culture.
At harvest, the data registered regarded the production per tree and per hectare and average samples of 15 fruits were collected from each grafting combination on which physical and chemical determinations were carried out.
The dry matter and water content of the samples were determined by oven drying for 24 hours at 105^{o}C using a UN110 Memmert oven, method used also by Delian (2011), Corollaro (2014), Muresan (2014). To determine the fruit firmness an electronic penetrometer TR was used and the results were expressed in kg/cm^2 (Saei, 2011).
Soluble solids were determined from blueberry juice (Saei, 2011; Oltenacu, 2015), with the refractive device Kruss DR301-95 (% Brix).
The titratable acidity was determined by titration with 0.1N NaOH to pH 8.1 (Saei, 2011). For titration with 0.1 N NaOH the automatic titrator TitroLine easy was used. The results were expressed in g citric acid/100g of fresh weight.
Total anthocyanins content was measured with spectrophotometrically at wavelength λ = 540 nm (Bărăscu et al., 2016), after an adapted method. The extracts were filtered under vacuum and completed up to 50 ml volume. The results were calculated using the formula: Total anthocyanins = DO_{540} x F, where DO_{540} is the absorbance and factor F = 11.16. The total anthocyanins content was expressed in mg/100 g of fresh weight.
All determinations described above were performed with Specord 210 Plus spectrophotometer. The preliminary data registered were statistically interpreted using the method of variant analysis for probability of 5%, 1% and 0.1 %.

RESULTS AND DISCUSSIONS

The average production of fruits per tree for the first 2 years of fructification (2nd and 3rd year of life) was moderate but different for the experimental variants (Table 1).
Approximately half of the variants had a relatively equal productivity.
A lower production was registered at the grafting combinations M20, MM111+B9 interstock with the length of 30 cm, buried and A2+B9 interstock of 40 cm.
The best production for 'Pinova' resulted from the combination MM111+B9 interstock of 30 cm and worked soil. Statistically, the highest production was registered at variants V6 and V7, very significant in comparison to the average one.
On the other hand, the lowest was at variants V3, V10 and V11, significantly negative.

Table 1. Fruit production and average fruit weight at the 'Pinova' apple variety

Variant	Production		Average fruit weight	
	kg/tree	t/ha	g	Std
V1	10.0^{oo}	22.22	161.47***	8.22
V2	11.9 *	26.44	137.41^{000}	7.24
V3	8.5^{ooo}	18.88	156.32***	6.53
V4	11.3 N	25.11	128.56^{000}	9.20
V5	12.0 **	26.66	151.62***	7.13
V6	12.7 ***	28.22	104.41^{000}	8.37
V7	12.3 ***	27.33	161.47***	6.57
V8	11.9 *	26.44	138.26^{000}	4.36
V9	12.1 **	26.88	194.52***	8.62
V10	9.0^{ooo}	19.99	141.91^{000}	5.34
V11	9.5^{ooo}	21.11	114.57^{000}	4.87
V12	11.2 N	24.88	157.70***	6.58
V13	11.6 N	25.77	169.04***	4.69
Average- control	11.16	24.61	147.48	4.21
LSD 5%	0.66		0.81	
LSD 1%	0.90		1.10	
LSD 0.1%	1.21		1.47	

* - significant values for the 5% probability; ** - significant values for the 1% probability; *** - significant values for the 0.1% probability.

Average fruit weight was influenced dramatically by the variants used. Thus, the biggest fruits were obtained at V9, over 194 g/ fruit, followed by V13 with 169 g /fruit and variants V1 and V7 with over 161 g/fruit. The smallest fruits were obtained at V6 with only 104 g/fruit and V11 with roughly 114g/fruit. It is worth mentioning that the first two variants with big fruits were the ones with grafting interstock on standard rootstocks. Statistically, the variants were divided in 2 groups: one under average, significantly distinctive and the other above the average, significantly positive.

The fruit diameter influenced fruit weight, even though the variation limit was small (Table 2). The correlation index between the average weight and fruit diameter was of r = -0.4881 (Figure 1). The grafting combination also influenced the fruit firmness. The strongest fruits were obtained by V2 with 10.24 kg/cm^2 followed by V11 with 9.14 kg/cm^2. Lower values were noted at V3 and V7 with 7.29 kg/cm^2 and 7.33 kg/cm^2, respectively. All other variants had intermediate values (Table 2). Statistically, values over 8.60 kg/cm^2 were significantly positive and the ones under 7.92 kg/cm^2 were significantly negative.

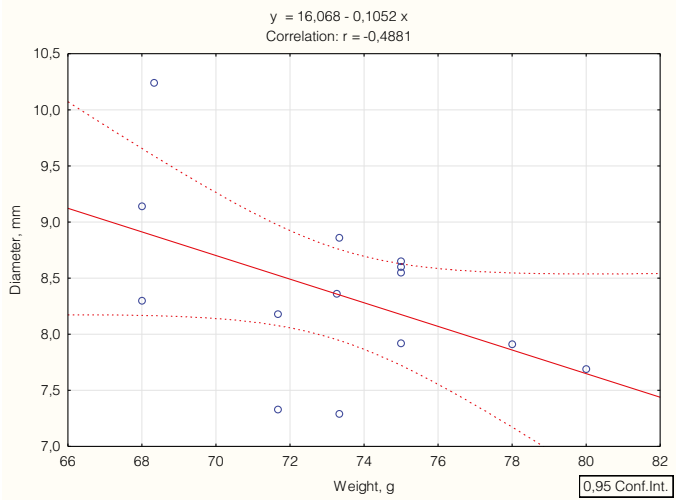

Figure 1. Correlation between fruit weight and fruit diameter

Table 2. Diameter and firmness of fruits at the 'Pinova' apple variety grafted on various rootstocks

Variant	Fruit diameter		Firmness	
	mm	Std	kg/cm^{-2}	Std
V1	75.00**	7.07	7.92^{ooo}	0.49
V2	68.33^{ooo}	5.77	10.24***	1.39
V3	73.33 N	2.89	7.29^{ooo}	0.39
V4	68.00^{ooo}	3.54	8.30 N	0.66
V5	73.33 N	5.77	8.86***	0.86
V6	75.00**	0.58	8.65***	0.44
V7	71.67 N	5.77	7.33^{ooo}	0.31
V8	71.67 N	2.89	8.18 ^{o}	1.10
V9	80.00***	0.58	7.69^{ooo}	0.43
V10	75.00**	5.00	8.60**	0.71
V11	68.00^{ooo}	3.54	9.14***	0.75
V12	75.00**	5.00	8.55**	0.75
V13	78.00 N	3.54	7.91^{ooo}	1.07
Average	73.26	4.00	8.36	0.72
LSD 5%	1.40	-	0.14	-
LSD 1%	1.90	-	0.19	-
LSD 0.1%	2.55	-	0.25	-

* - significant values for the 5% probability; ** - significant values for the 1% probability; *** - significant values for the 0.1% probability.

The water and the total dry substance content were less affected as opposed to the physical parameters of fruits (Table 3). The lowest content of water was observed at V11 of roughly 78.25%, and the highest at fruits from V13, of 81.40%. The content of total dry substance was complementary to the one of water. Statistically at V6, V9 and V11 the difference was significantly positive in comparison to the average and at V7, V8, V12 and V13 the difference was significantly negative in contrast to the average one. Glucides formulated in °Brix accumulated more at fruits from V3, reaching the maximum value (19 °Brix) and it was noted statistically as very significant. Lower values were obtained at V7 and V8, under 15.5 °Brix. The content of ash was between 0.21% at V2 and 0.44% at V11 without a visible correlation with the total dry substance and the content of glucides.

Table 3. Some biochemical parameters of fruits from the 'Pinova' variety grafted on various rootstocks

Variant	Water %	Total dry substance %	Glucides (°Brix)	Ash %
V1	80.69	19.31 N	16.6 N	0.37
V2	79.53	20.47**	17.27 N	0.21
V3	80.08	19.92 N	19***	0.27
V4	80.14	19.86 N	17.4 N	0.38
V5	80.34	19.66 N	15.8 ^{o}	0.27
V6	79.04	20.96***	16.82 N	0.24
V7	83.48	16.52^{ooo}	14.07 ^{ooo}	0.20
V8	81.33	18.67^{ooo}	15.52^{oo}	0.28
V9	78.91	21.09***	17.46 N	0.31
V10	80.21	19.79 N	16.65 N	0.26
V11	78.25	21.75***	17.5 N	0.44
V12	81.27	18.73^{ooo}	15.63 ^{o}	0.25
V13	81.40	18.60^{ooo}	15.47 N	0.28
Average- Control	80.36	19.64	16.55	0.29
LSD 5%		0.46	0.81	
LSD 1%		0.62	1.10	
LSD 0.1%		0.84	1.47	

* - significant values for the 5% probability; ** - significant values for the 1% probability; ***- significant values for the 0.1% probability.

Table 4. Titratable acidity and antocyanin content at fruits from the 'Pinova' variety grafted on various rootstocks

Variant	Titratable acidity		Anthocyanin	
	g/100 g f.w.	Std	mg/100g f.w.	Std
V1	0.379	0.001	0.763	0.037
V2	0.330	0.005	0.143	0.063
V3	0.296	0.016	0.706	0.052
V4	0.318	0.004	0.576	0.070
V5	0.348	0.012	0.773	0.060
V6	0.307	0.016	0.537	0.020
V7	0.186	0.001	0.848	0.034
V8	0.298	0.003	1.151	0.033
V9	0.369	0.004	0.603	0.870
V10	0.362	0.006	0.478	0.051
V11	0.342	0.002	0.591	0.057
V12	0.346	0.007	1.064	0.013
V13	0.270	0.005	0.673	0.085
Average - Control	0.319	0.006	0.685	0.111

Fruit acidity oscillated from simple to double, being weaker at V7, with roughly 0,186 g/ 100 g f.w. and maximum at V1, with 0.379 g/100 g f.w. All other variants registered intermediate values (Table 4). The highest quantity of anthocyanins was registered at fruits from V8 and V12 with over 1060 mg/100 g f.w and the smallest values were at fruits from V2 with only 0.143 mg/100 g f.w.

CONCLUSIONS

The present study proved how the grafting combinations influenced the size and quality of fruits of the 'Pinova' variety.

Generally, fruits of better quality were obtained at combinations were a grafting interstock was used. Interestingly, the most used rootstock, the M9, yielded well and produced firm fruits with a high content of dry substance but they were small, weakly coloured and with few minerals.

Good colours were obtained at V8 and V12 and a high content of dry substance, over 21%, was registered at V9 and V11.

The worked soil determined a better growth of fruits but it did not register other correlations for the other indicators observed. The grassy soil assured a better colouration.

The interstock of 30 cm induced a slight growth of the fruit size and a satisfactory accumulation of soluble dry substance in comparison to the interstock of 40 cm.

Deep planting of trees, including of the interstock actuated a slight growth of fruit size, a good firmness and a better colouration.

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PHYSICAL AND CHEMICAL PARAMETERS OF THE FRUIT IN FOUR *PRUNUS DOMESTICA* LOCAL POPULATIONS FROM BUZĂU COUNTY

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Abstract

The four local populations of Prunus domestica, T1, T2, T3, respectively T4, selected for observations and measurements are distinguished by a series of particular attributes of fruit: one of the four populations matures their fruits early in August while the remaining populations are ripening during September; the endocarp is not adherent to the T3 population, whereas the remainder has different degrees of adhesion; the average weight of fruit varies clearly from species to species, and the ratio between fruit weight and fruit diameter is relatively close for the 3 populations; the sugar content, with one exception, is close to the average of 15.95, and the dry matter content is also variable depending on the population.

Key words*: Prunus domestica, fruit, physical parameters, chemical parameters.*

INTRODUCTION

Among the fruit species cultivated in our country, the plum is on the first place both for the cultivated area and for the fruit yield obtained (Coman et al., 2012, http://www.fao.org/).
The pedo-climatic factors can influence the pomological, physical and biochemical proprieties of the local biotypes or genotypes of the cultivated fruit tree species (Ionica et al., 2013; Iordanescu and Costea, 2014).
It is important to have information about these particularities, useful to the breeding or growing processes, or to enlarge the assortment of local fresh, conditioning or preserved fruits (Vitanova et al., 2004; Okatan et al., 2017).
There are different researches on fruits proprieties from acquainted cultivars or local varieties, such as the nutritional characteristics of local cultivars (Rop et al., 2009), the changes that occur in their physical or chemical characteristic during the fruits ripening or keeping (Usenik et al., 2008; Miletic et al., 2012; Oltenacu and Oltenacu, 2014), the chemical compounds 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); these features, related to local environmental factors, are valuable information for fruit growers when they want to broaden their range of varieties.
By their content in antioxidant substances, along with vitamin C and minerals like calcium, potassium or magnesium (https://ndb.nal.usda.gov/), the plum fruits have a significant nutritional role in our diet.
Researches on the physical and chemical characteristics of the local varieties of plum will contribute to the desired objective, the improvement of the cultivar assortment (Botu et al., 2012).
Following this direction, our study presents the morphological and chemical characteristics of four local plum varieties from Buzău County.

MATERIALS AND METHODS

The fruits of four local populations in Pătârlagele (Buzău County) (Potor et al., 2017) were collected in full maturity in 2017.
For their characterization the following physical and chemical parameters were used: diameter, weight, respiratory intensity, the dry matter contents, titratable acidity, soluble solid and total anthocyanins content.

Figure 1. Fruit of T3 population

The four populations are distinct from the moment of fruit maturation and the degree of adherence of the mesocarp, as follows:
- the T1 population - the mesocarp partially adherent; early ripe period, in the second decade of August;
- T2 population - adherent mesocarp; the ripe period situated in the first decade of September;
- T3 population - non-adherent mesocarp (figure 1); the ripe period situated in the first decade of September, too;
- T4 population - adherent mesocarp; the latest ripe period between the 4 populations, at the end of September.

Fresh fruits were used for physical and chemical analysis.

The diameter average value was determined by measuring of 20 fruits / population with the fruit caliber; the results were expressed in mm.

The average weight was determined by weighing 30 fruits / population with the Partner-PS 600 R2 technical balance, the result being expressed in g.

Respiratory intensity, based on the CO_2 measurement of the plant material and expressed in mg CO_2 / $kg^{-1}h^{-1}$, was determined with the CO_2 analyzer. Determination of the dry matter content was achieved by weighing the fresh vegetable material and drying at 105°C with the moisture analyzer MAC 50 PARTNER, the result being expressed as a percentage.

For the total anthocyanin content, the adapted method was used after Giusti et al., 2001, respectively, the extraction in acidified methanol with 1% hydrochloric acid and spectrophotometric dosing at the wavelength of 530 nm. Results were calculated based on the formula: Total anthocyanins = DO530~~540~~ x F, where DO530~~540~~ is absorbance at wavelength λ = 530~~540~~ nm and factor F = 11.16 and expressed in mg/100g plant material (Bezdadea Cătuneanu et al., 2017).

The titratable acidity was determined according to AOAC official method 942.15 and Saad et al., 2014: 10 g of the sample were diluted with 50 ml of water and titrated with 0.1 N sodium hydroxide to pH 8.1. The formula for calculation is:

$$Titratable\ acidity = \frac{\text{V x N x C x100}}{\text{m}}$$

V = volume of titrant; N = normality of titrant; C= Citric acid equivalent; m = mass of the sample.

The analysis was performed with TitroLine easy titrator.

0.1 is the normality of NaOH (N), 0.064 is the conversion factor for citric acid, V is the volume of NaOH used (mL) and m is the mass of plum sample used (g).

The results were expressed in mg citric acid/100 g fresh sample

Total soluble solids (TSS) were determined from plum juice (Bezdadea Cătuneanu et al., 2017), with Kruss DR301-95 Digital Handheld Refractometer and the results were expressed in % Brix.

RESULTS AND DISCUSSIONS

Diameter, weight and dry matter content

Table 1. Fruits diameter, weight and dry matter content to the T1-T4 Pătârlagele local populations

Population	Diameter (mm)	Weight (g)	Dry matter content (%)
T1	28,82 ± 1.01	15,32 ± 2.05	16,99
T2	33,41 ± 2.40	18,94 ± 3.52	27,05
T3	44,29 ± 3.50	45,50 ± 7.64	18,24
T4	33,06 ± 1.48	21,14 ± 1.72	21,71

Data of Table 1 and Figure 1 show that the T3 population form the largest fruits in size and weight, but with a content in dry matter lower, 18.24%, relative to the T2 and T4 populations.

The T1 population has small fruits with a low dry matter content compared to the rest of the populations.

The ratio between dry matter content and fruit diameter is obviously higher in T1 and T3 populations, while in T2 and T4 populations the differences are smaller (Figure 2).

Comparing the data in the table 1 with those from Ionică et al., 2013, it can be noticed that 3 of the 4 populations, namely T1, T2 and T4, form smaller fruit, the average of the weight between 15.32 g and 21.14 g, are outside of the average weight range of the fruit from the mentioned paper work.

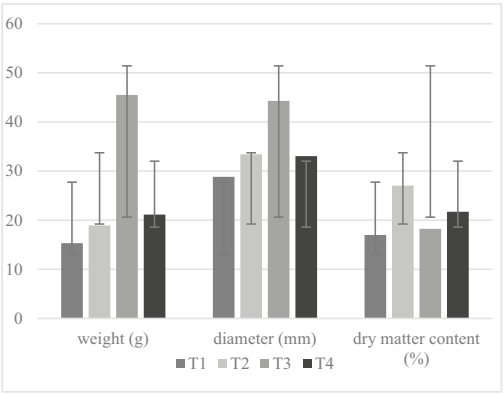

Figure 1. Physical parameters and dry matter content of fruits of the T1-T4 populations

With an average weight of 45.50 g, the fruits of the T3 population account for the highest value among the local populations of Pătârlagele; the dry matter content for T1, T3, T4, ranging from 16.99% to 21.71%, is according with the results obtained by Ionică et al.; the fruits of the T2 population, with an average of the dry matter content of 27.05%, are above the values recorded by the other populations (table 1, figure 1).

Titratable acidity, soluble solids and dry matter content

The citric acid content ranges from 0.11 mg / 100 g fresh sample to T4 and 0.81 mg / 100 g fresh sample at T1 (Table 2).

Table 2. Titratable acidity, total soluble solids and dry matter content of fruits of the T1-T4 Pătârlagele local populations

Population	Titratable acidity (mg citric acid /100 g)	Total soluble solids (% Brix)	Dry matter content (%)
T1	0.81 ± 0,089	15.78 ± 1.91	16,99
T2	0,45 ± 0,010	25.30 ± 2.76	27,05
T3	0,67 ± 0,028	15.87 ± 3.64	18,24
T4	0,11 ± 0,004	15.95 ± 1.28	21,71

The content of total soluble substances is very close to 16% in the T1, T3 and T4 populations, while T2 have the highest value of the populations studied: 25.30%.

T1 and T2 populations have the values of dry matter and total soluble solids content very close, the T3 population is showing a small difference of the two components, while to the T4 population the dry matter content is obviously higher than that in soluble solids (Figure 2).

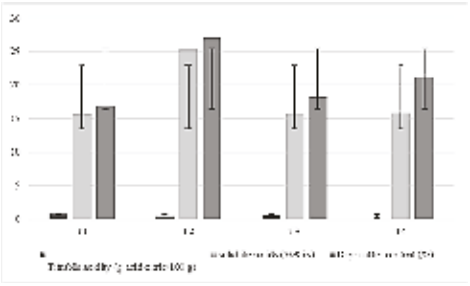

Figure 2. Acid citric, Brix and dry matter content

The higher value of the citric acid content of the T1 and T3 populations (Table 2) corresponds to a low content of total soluble solids; in the fruits of the T4 population, the citric acid content is small, similar to the content of total soluble solids, while in the fruits of the T2 population the content of total soluble solids is high and the value of the citric acid is at the medium level comparative to the rest of populations (Table 2, Figure 2).

With the exception of the T4 population where the difference between the dry matter content and the total soluble solids content is significant, to the rest of the populations the ratio between citric acid content and dry matter content is similar to the total soluble solids content (Figure 2).

Respiratory intensity and dry matter content

Table 3. Respiratory intensity and dry matter content of fruits of the T1-T4 Pătârlagele local populations

Population	Respiratory intensity (mg $CO_2/kg^{-1}h^{-1}$)	Dry matter content (%)
T1	149.60 ± 29.43	16.99
T2	64.85 ± 10.93	27.05
T3	104.66 ± 27.66	18.24
T4	61.90 ± 7.94	21.71

The respiratory intensity (Table 3) is between 61.90 mg CO_2 / $kg^{-1}h^{-1}$ (T4) and 149.60 mg CO_2 / $kg^{-1}h^{-1}$ (T1). Populations T2 and T3 have an intensity of fruit respiration approx. 2 times smaller than T4.

From Figure 3 it can be seen that the two populations with less of the respiratory intensity have the higher dry substance content.

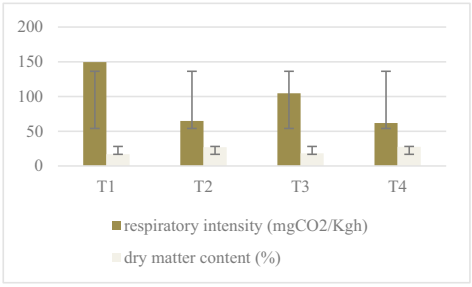

Figure 3. Respiratory intensity and dry matter content

Total anthocyanins content

With an important role in attracting consumers (Ionica et al., 2013) and in the antioxidant activity (Bezdadea Cătuneanu et al., 2017), anthocyanins are one of the significant components of plum fruits.

In the fruits of the four Pătârlagele populations the content of anthocyanins is relatively reduced, the values in ascending order from T1 to T4 range from 0.84 mg to 4.00 mg /100 g (Table 4).

Table 4. Total anthocyanins of fruits of the T1-T4 Pătârlagele local populations

Population	Total anthocyanins (mg/100 g fresh weight)
T1	0.84 ± 0.04
T2	1.45 ± 0.02
T3	1.92 ± 0.09
T4	4.00 ± 0.10

CONCLUSIONS

Three of the four populations, namely T1, T2 and T4, form small fruit; T3 population account for the highest value among the local populations of Pătârlagele.

The dry matter content for T1 – T4 populations is above 12.5%, the lower limit accepted by consumers.

The highest content of citric acid is at T1 population, and the highest content of total soluble substance is at T2 population.

High respiratory intensity is found in T1 and T3 populations.

In the fruits of the four Pătârlagele populations the content of anthocyanins is relatively reduced.

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'HARLAYNE' X 'HARCOT' – PERSPECTIVE CROSSBREED FOR COMBINING GOOD FRUIT QUALITY AND RESISTANCE TO *PLUM POX VIRUS*

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Abstract

Most apricot breeding programs aim to obtain genotypes combining good fruit qualities and resistance to Plum pox virus (PPV). Using a combination between field observations and Marker Assisted Selection improves the efficiency of the breeding process. The study was conducted at a breeding orchard in Fruit Growing Institute - Plovdiv, Bulgaria and describes progenies from the hybrid family 'Harlayne' x 'Harcot', genotyped for resistance to PPV. Fruit ripening period, fruit biometry, total soluble solids (TSS), sensory analyses, fruit ground and over color were observed. The fruit ripening period of the most of the hybrids is after Harcot, the fruits were classified from small to large size and 82% of them surpass the TSS content of the parental cultivars. According to the sensory evaluation the hybrid's fruits were highly scored (5.07-7.67). All of them were with orange ground color of varying intensity. The fruit over color was from 10 to 80 %. Two of the studied hybrids were selected and grafted on rootstock for final evaluation. A lot of the other hybrids had desirable traits and the breeding process with them will continue in F2 for improving their fruit quality.

Key words*: fruit breeding; hybrids; Plum pox virus; pomological traits; Prunus armeniaca L.*

INTRODUCTION

Most apricot breeding programs point at genotypes combining good fruit qualities and resistance to different pests. The commercial value of apricots depends entirely on the fruit quality. It is determined by the combination of physical and chemical fruit characteristics such as appearance, firmness, taste and aroma (Velisek and Cejpek, 2007). These characteristics are genetically highly variable and their phenotypic expression is influenced by the environmental conditions in the year of cultivation (Dirlewanger et al., 1999). The knowledge of the ways of inheritance of fruit quality traits would result in a higher efficiency of the breeding process and would help the choice of genitors. These traits can be improved by the breeding process and there is a great diversity of genetic resources for them (Krška et al., 2006a). Most of the fruit quality traits are under polygenic control and are quantitatively inherited (Salazar et al., 2013). The conventional breeding is successful in developing cultivars with improved fruit quality but the same cannot be said for the disease resistance. *Plum pox virus* (PPV) is the most devastating disease and a major limiting factor for the apricot cultivation. The selection of resistant genotypes requires a lot of time and significantly slows down the whole breeding process. Therefore, a number of scientists have focused their efforts on developing molecular markers associated with genes of PPV resistance (Abernathy et al., 2004). The development of Marker Assisted Selection (MAS) effectively complements plant breeding process (Singh B.D., Singh A.K., 2015) and gives us an opportunity to make it shorter by doing a proper selection at the very early stages of it. Our study describes field observations of genotyped for PPV resistance hybrid family 'Harlayne' x 'Harcot' and its aim is to investigate the way of inheritance of the fruit quality traits and evaluate the progeny.

MATERIALS AND METHODS

This research was conducted at a breeding orchard of Fruit Growing Institute - Plovdiv, Bulgaria. The hybrids were obtained by the methods of conventional fruit breeding and planted in 2011. In 2014 the progeny was genotyped for PPV resistance within the work

on project MARS (7 FP- Collaborative project nr. 613654). This hybrid family consists 153 seedlings. In the present study, the hybrids that fruited consistently over the three years (2015, 2016 and 2017) are described. An average sample of fruits was taken and biometric data was measured with Mitutoyo 500-196-30 Digimatic Absolute Caliper 150 mm.

Total soluble solid content ($Brix^0$) in juice using a handheld Sper Scientific 300019 Digital Refractometer was determined. For the descriptive characteristics of the fruits, UPOV (2007) and IBPGR (1984) descriptors were used. Sensory evaluation was done by a group of trained consumers.

For statistical data processing, Duncan's multiple range test at $P \leq 0.05$ through IBM SPSS Statistics 19 was used.

RESULTS AND DISCUSSIONS

Seventeen of the hybrids fruited in the three consistently years. Their ripening period started in the second half of June and it's duration in 2015 was 18, in 2016 - 12 and in 2017 - 20 days. In 2016 the ripening period was shorter than in the other two years probably because this trait is strongly influenced by the climatic factor (Milošević et al, 2010). During the three years, the same trend was observed - fruit ripening time is genetically variable trait and in the progeny, there are hybrids which fruits ripen earlier or later than both parental cultivars. According to Audergon et al. (2011), this is due to the genetic background of the parents which has a strong influence on the inheritance of the fruit ripening time.

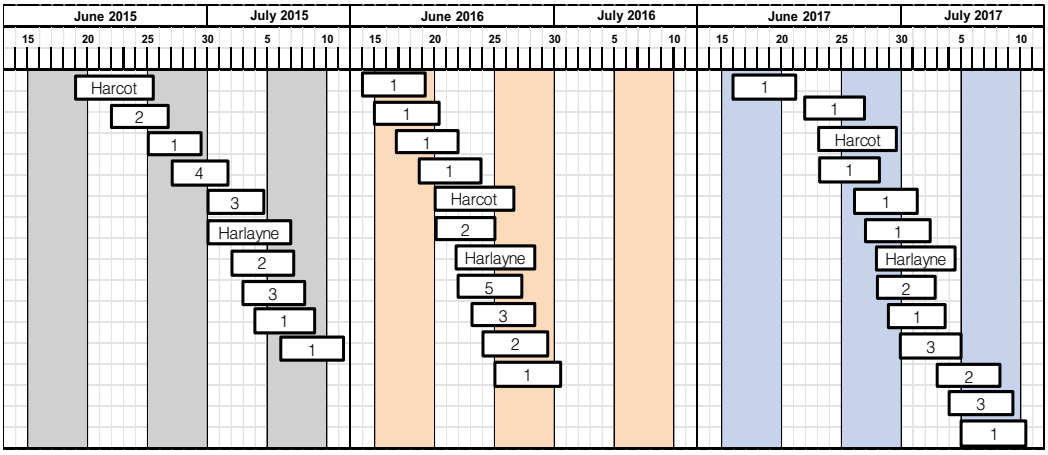

Figure1. Fruit ripening date in 2015, 2016 and 2017
Different digits show the number of hybrids ripened on that date

On figure 1 it is noticeable that most of the hybrids ripened later than 'Harcot' and 'Harlayne'. In our previous study with the crossbreed 'Modesto' x 'Harcot', the biggest group of hybrids were with intermediate ripening time (Bozhkova and Nesheva, 2016). In both cases, most of the hybrid fruits ripened later than 'Harcot' cv.

This might means that 'Modesto' and 'Harlayne' later ripening time is the dominant trait.

These results are in accordance with Nyujtó and Banai (1986) proposition for who the late ripening period is dominant. Bassi and Negri (1991) assume it is under oligogenic control and probably for that reason, there is such diversity in the hybrid family.

Table 1. Fruit size categories according to IBPGR descriptor

Fruit weight (g)	Fruit size (IBPGR)	Number of hybrids
< 20	Extremely small	0
20 - 30	Very small	0
31 - 40	Small	8
41 - 45	Small/medium	Harlayne + 3
46 - 55	Medium	3
56 - 60	Medium/large	Harcot + 2
61 - 70	Large	1
71 - 85	Very large	0
>85	Extremely large	0

Fruit weight is a trait of which depend the fruit quality and often the yield. According to the IBPGR descriptor, 47% of the fruits of the studied progeny were classified as small size (table 1). Attractive and medium-sized fruits are preferred by the producers and consumers and also are desired trait in apricot breeding (Guerriero et al.; 2005). Almost half of the studied hybrids (53 %) had small/medium to large sized fruits. Many well-known cultivars are classified as small to medium-sized fruits with an average weight up to 50-55 grams (Bozhkova and Todorova, 2012). Two of the hybrids had medium/large fruits as Harcot and one surpassed them with its large sized fruits. There is a high correlation between the main fruit physical characteristics - fruit height, fruit width, and thickness and all three of them are highly correlated with the fruit weight (Mratinic et al., 2011). Fruit biometry is important when the fruits are intended for processing, especially for their mechanical sorting (Mohsenin, 1980). All three fruit dimensions depend on the cultivar.

After Duncan's multiple range test at $P \leq 0.05$, it can be said that: according to the data for all fruit dimensions - length (FL), width (FW) and thickness (FT) the groups are overlapping and there are hybrids with intermediate phenotype and hybrids that resemble the parental cultivars (table 2).

Although the clear trend that the fruits in the progeny are getting smaller in size a few hybrids were found to surpass both parental cultivars: by FL – HH 12-42 and HH 12-26, by FW – HH 12-19 and HH 12-42, by FT – HH 12-26, HH 12-42 and HH 12-19 but the statistical difference with 'Harcot' is non-significant. The biggest fruit weight was recorded for HH 12-42 which is close to the one measured for 'Harcot'. The larger fruits had stones with higher weight. However, HH 12-42, HH 12-26, and 'Harcot' had very good stone relative share- less than 6.

Table 2. Fruit biometric analysis

Genotype	Fruit Length	Fruit Width	Fruit Thickness	Avarage Fruit Weight	Stone weight	Relative share %
HH 13-3	45.03 bcdef	41.39 abcdef	45.36 abcde	48.43 abc	3.06 abcd	6.32 abcd
HH 13-54	43.26 cdef	37.93 cdef	43.64 abcdef	41.89 bcd	3.27 abc	8.10 abc
HH 13-43	41.13 def	37.98 cdef	39.51 ef	35.53 cd	2.27 de	6.36 abcd
HH 12-63	45.74 bcde	40.97 abcdef	45.34 abcde	47.27 abcd	3.07 abcd	6.51 abcd
HH 13-14	38.58 f	37.39 def	39.72 ef	34.60 cd	2.07 e	6.04 bcd
HH 12-26	52.80 a	41.63 abcd	47.72 abc	55.87 ab	2.90 bcde	5.23 d
HH 12-67	47.49 abcd	39.60 abcdef	45.36 abcde	46.49 abcd	2.80 bcde	6.04 bcd
HH 12-42	50.65 ab	45.74 a	49.08 ab	63.09 a	3.57 ab	5.59 cd
HH 12-19	48.08 abc	44.46 ab	49.83 a	59.02 ab	3.87 a	6.56 abcd
HH 13-4	41.06 def	34.54 f	38.58 ef	32.64 cd	2.77 bcde	8.58 ab
HH 13-15	41.24 def	38.44 bcdef	40.88 def	36.45 cd	2.28 de	6.42 abcd
HH 12-62	44.56 bcdef	38.77 bcdef	42.50 bcdef	42.12 bcd	2.77 bcde	6.85 abcd
HH 12-41	43.45 cdef	36.55 def	40.31 def	36.14 cd	2.46 cde	7.30 abcd
HH 12-9	49.63 abc	38.84 bcdef	42.02 cdef	44.40 bcd	2.69 cde	6.08 bcd
HH 12-59	38.46 f	38.40 bcdef	39.60 ef	37.29 cd	2.49 cde	7.14 abcd
HH 12-60	38.80 f	34.86 f	39.49 ef	30.22 d	2.63 cde	8.82 a
HH 12-22	38.99 ef	34.96 ef	38.34 f	32.73 cd	2.53 cde	8.56 ab
Harlayne	43.57 cdef	39.72 abcdef	43.30 abcdef	43.29 bcd	2.67 cde	6.23 abcd
Harcot	49.83 abc	44.12 abc	46.58 abcd	58.44 ab	3.04 abcd	5.19 d

* Mean values followed by different letters within a column are significantly different by Duncan's multiple range test at $P \leq 0.05$.

Measuring the TSS content is a method that can quickly give us an idea of the fruit biological value. For 128 apricot cultivars cultivated in Malatya, Turkey TSS range is from 11-26.50 °Brix (Asma and Ozturk, 2005). For the cultivars 'Berge cot', 'Flavor cot', 'Lady cot', 'Tom cot', 'Perle cot', 'Jenny cot' and 'Sweet cot' TSS content ranges from 13.40 to 23.30 °Brix (Bozhkova and Nesheva, 2016). Total Soluble Solids (TSS) content is important

especially for the dried apricot production. High TSS content is associated with high amount of sugars in the apricot fruits and increases their quality and the yield of dried product (Akin et al., 2008). TSS content grows with the fruit ripening, i.e. the highest value ($Brix^0$) is found in fully ripened fruits (Xi et al., 2016). This chemical characteristic is strongly influenced by the environmental factors (Bartolini et al.; 2015).

In the present study the lowest TSS content was recorded for HH 12-42 (15.5 °Brix) and the highest - HH 12-22 (21.9 °Brix). Here the trend is reversed - TSS content increases in the progeny and 82% of the hybrids outperform both the parental cultivars (table 3).

The differences between 'Harcot' and all hybrids are non-significant. There is a statistically significant difference between the other parental cultivar 'Harlayne' and HH 12-22.

Table 3. Fruit appearance and taste qualities

Genotype	Total Soluble Solids (°Brix) (TSS)	Fruit ground color	Relative area of fruit over color (%)	Sensory score	Sensory evaluation
HH 13-3	20.07 abcd	med. orange	10-30	6.22	Good
HH 13-54	21.47 ab	med. orange	80	5.71	Good
HH 13-43	18.6 abcd	med. orange	40-60	5.90	Good
HH 12-63	18.8 abcd	light to med. orange	10	5.91	Good
HH 13-14	19.97 abcd	med. to dark orange	30-40	5.26	Good
HH 12-26	18.2 abcd	light to med. orange	50-60	7.67	Very Good
HH 12-67	19.83 abcd	light to med. orange	20-50	5.91	Good
HH 12-42	15.5 d	med. orange	40-60	6.66	Good
HH 12-19	20 abcd	med. orange	60-70	6.33	Good
HH 13-4	19.83 abcd	med. orange	40-50	5.82	Good
HH 13-15	21.00 abc	med. to dark orange	50	5.71	Good
HH 12-62	15.8 cd	med. to dark orange	30	5.53	Good
HH 12-41	16.77 abcd	med. orange	almost missing	5.11	Good
HH 12-9	18.33 abcd	light to med. orange	40-50	5.31	Good
HH 12-59	18.67 abcd	med. to dark orange	40-60	5.07	Good
HH 12-60	20.97 abc	light to med. orange	10-30	5.74	Good
HH 12-22	21.9 a	med. orange	40	5.93	Good
Harlayne	16.47 bcd	med. to dark orange	60	6.62	Good
Harcot	17.37 abcd	med. to dark orange	10	6.03	Good

Like most of the fruit characteristics, the ground color is genetically determined trait which expression is highly influenced by the environment. Consumers in our country prefer large fruits, with dark orange ground color and bright red over color (Bozhkova and Nesheva, 2016). All of the fruits of the studied hybrids were with orange ground color which shade varies from light to medium and from medium to dark orange. The red blush is the most attractive feature of apricots and the larger the area it occupies is the more seductive the fruits are. It has a great commercial impact and it is much-desired trait in the breeding programs (Mazza and Miniati, 1993). The intensity and relative area of fruit over color strongly depend on the light, radiation, irrigation and nutrition of the trees. The relative area of fruit over color for the studied hybrids ranged from 10 to 80 %. More than half of them (65%) have an over color above 40% which gives them very attractive appearance. After sensory evaluation, all the 17 hybrids were highly scored (5.07-7.67).

Their taste was evaluated as good as both parental cultivars with already proven qualities. One of the hybrids HH 12-26 is evaluated as better than the others with very good taste and

score 7.67. Usually, well-informed consumers prefer fruits with good taste and when they are valued by sensory analyzes, taste and aroma are of greater importance (Bozhkova and Nesheva, 2016).

Krška et al., (2006b) prove that Harleyne's resistance is controlled by three independent complementary dominant genes, and after 10 years of research, Polak and Kominek (2012) report it as immune to 6 strains of *Plum pox virus*. In this breeding program, this cultivar was used as a donor of resistance. Harcot is partially resistant to PPV - resistant to strain PPV - D but it is susceptible to PPV - M (Rankovic et al., 1997). Its fruits are large with excellent taste and in this breeding program and much more is used as a donor of good fruit quality (Karayiannis, 2005). After genotyping within the work on project MARS (7 FP-Collaborative project nr. 613654) 'Harlayne' was found to be resistant. Partial resistance was detected in 'Harcot'. In the progeny in 47% of the hybrids were genotyped as resistant, 33% as partially resistant and 20% - sensitive (table 4).

Table 4. Resistance to PPV virus

Genotype	PPV resistance MAS	Phenotype/PPV symptoms 2017
HH 13-3	resistant	-
HH 13-54	Missing Data	-
HH 13-43	sensitive	+
HH 12-63	sensitive	-
HH 13-14	resistant	-
HH 12-26	Missing Data	+
HH 12-67	partially resistant	-
HH 12-42	resistant	-
HH 12-19	resistant	-
HH 13-4	resistant	-
HH 13-15	partially resistant	-
HH 12-62	partially resistant	-
HH 12-41	partially resistant	-
HH 12-9	partially resistant	-
HH 12-59	sensitive	+
HH 12-60	resistant	-
HH 12-22	resistant	-
Harlayne	resistant	-
Harcot	partially resistant	-

For two of the hybrids, the data is missing. Until 2017 symptoms of PPV were observed on the stones and fruits of three of the hybrids. Two of them were genotyped as sensitive and for one of them the data is missing.

CONCLUSIONS

As a result of the hybrid analyses, for their good fruit qualities and resistance to PPV, HH 12-42 and HH 12-19 were grafted on *P.cerasifera* rootstock and continue to the next stage of the breeding process. These two hybrids were the best ones in the studied progeny. The others also have good qualities and the work with them will continue to F2 for improving their disadvantages. HH 12-26 also has good fruit qualities but on the field, it showed symptoms of PPV. The fruit weight of the resistant genotypes - HH 13-3 and HH12-22 should be improved. Because of the great heterozygosity and the big number of traits under polygenic control, picking up two hybrids out of only 17 is a considerable success for the breeding program. Usually, such a result is obtained by observing hundreds of hybrids. Very high percent of the progeny is resistant or partially resistant to the PPV. That gives us a reason to believe that the crossbreed 'Harlayne' x 'Harcot' is very perspective for the apricot breeding programs.

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EVALUATION OF INTRODUCED PLUM CULTIVARS UNDER AGROCLIMATIC CONDITIONS OF PLOVDIV REGION, BULGARIA

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Abstract

The study was carried out in the experimental plantation of the Fruit-Growing Institute - Plovdiv in the period 2014 - 2016 with six German plum cultivars: ,'Jojo', 'Topstar plus', 'Topgigant plus', 'Toptaste' and 'Tophit plus' compared to 'Stanley' as a standard. The tree volume, trunk diameter, angle of the skeletal branches, annual shoot length growth, average number of flower buds on spurs and shoots, some phenological characteristics, fruit biometrical analyses, chemical composition and the sensory characteristics were studied. The biggest tree volume and trunk diameter were calculated for the cultivars 'Toptaste' and 'Stanley'. The largest angle of skeletal branches was measured on 'Jojo'. The biggest shoot length was recorded in 'Tophit plus'. The earliest flowering time was observed on 'Jojo' and 'Toptaste', the latest one on the 'Topstar plus' and 'Tophit plus'. The ripening period of the investigated cultivars was in August and only for 'Stanley' and 'Tophit plus' in September. According to the biometric data 'Topgigant plus' and 'Tophit plus' were with the largest fruits in size. The highest sugar content and total soluble solid were found of 'Toptaste's fruits. The investigated cultivars are suitable to grow under agroclimatic conditions of Plovdiv region.

Key words*: plum cultivars, vegetative growth, phenological characteristics, fruit characteristics.*

INTRODUCTION

Plum is a traditional fruit crop in Bulgaria. 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, 2016).

The main grown cultivar is still 'Stanley', because of its good adaptability (Djuvinov V. and Vitanova I., 2002). A lot of new cultivars were registered in Europe as tolerant to Plum pox virus (Sharka disease), but few of them were accepted by the producers and spread in the orchards (Jacob, 2002; Blažek and Pištěková, 2009).

Till now only the plum cultivar 'Jojo' is known as resistant to Plum pox virus and in the past ten years was widespread in the orchards (Neumüller et al., 2010).

Unfortunately, it turned out that this cultivar is susceptible to late spring frosts, which force Bulgarian producers to look for other cultivars.

At the same time, the manipulation of tree architecture is the cornerstone of horticultural management.

According to Costes et al. (2004) an accurate knowledge of growth, branching and flowering processes within the tree canopy, i.e., tree architecture, is required to optimize the growing technologies and especially for the right choice of training and pruning methods.

As an answer to this need, some plum cultivars were introduced from Germany in the Fruit Growing Institute - Plovdiv.

In this study are presented the results of an investigation on some of those cultivars. The aim is to recommend the best one to the plum producers.

MATERIAL AND METHODS

The study was carried out in the period 2014-2016 at the Fruit Growing Institute, Plovdiv. The trees of the studied cultivars 'Jojo', 'Topstar plus', 'Topgigant plus', 'Toptaste' and 'Tophit plus' compared to 'Stanley' as a standard were planted in a collection plantation in 2011 on alluvial-meadow soil at a distance of 4×4 m and grown under non-irrigated conditions and without pruning.

Dimensions of minimum five trees were determined to be calculated the tree volume and trunk diameter. The angle between the skeletal branches and the central leader was measured

in four trees of each cultivar in three consecutive years, to trace if the angle will be changed during the period of tree growth.
One skeletal branch from four trees per cultivar was selected for measuring the average annual shoot length growth and the average number of flower buds in a spur and a shoot.
The observed phenological characteristics included flowering and fruit ripening. Biometrical, chemical and sensory analyses of the fruits were performed. Data were statistically processed by Duncan's test (Steele and Torrie, 1980).

RESULTS AND DISCUSSIONS

Data concerning the tree volume and trunk diameter are presented in Table 1. The biggest tree volume and trunk diameter were calculated for the cultivars 'Toptaste' and 'Stanley'. Their growth dynamic is the faster. The other cultivars are in the second group. Among them, the smallest tree volume was registered on 'Jojo' and 'Topgigant plus' but statistically it is not proven.
Data concerning average trunk diameter shows there are no statistically proven differences. It is known that on fruit species the larger angle of the skeletal branches is preferred (Vitanov, 1977). Out of the studied cultivars, the largest angle of the skeletal branches was recorded for the cultivars 'Jojo' and 'Topgigant plus'. Nevertheless, it was found that the wood for the 'Topgigant plus' is fragile and very often under the weight of the fruits the skeleton branches are broken.
In 'Jojo' and 'Toptaste' the larges angle was recorded for the secondary branches. The dimensions for 'Jojo' are favorable and statistically proven.

Table 1. Tree volume and trunk diameter during the period 2014-2016

Cultivar	Tree volume, m^3				Trunk diameter, cm			
	2014	2015	2016	Average	2014	2015	2016	Average
'Jojo'	1.15	1.50	1.91	1.52 b[1]	14.38	23.00	25.13	20.84 a
'Tophit plus'	1.12	2.09	2.53	1.91 b	15.33	23.33	25.33	21.33 a
'Topgigant plus'	1.20	1.61	2.10	1.64 b	17.25	25.13	27,38	23.25 a
'Topstar plus'	1.25	1.93	2.55	1.91 b	17.00	26.38	28.00	23.79 a
'Toptaste'	2.46	3.05	3.89	3.13 a	21.25	28.75	30.75	26.92 a
'Stanley'	2.77	3.02	3.82	3.20 a	22.50	28.13	30.00	26.88 a

[1]Mean values followed by different letters within a column are significantly different by Duncan's multiple range test at $P \leq 0.05$.

Table 2. Angle of the main and the secondary skeletal branches (°)

Cultivar	Branch angle º (2014 -2016)							
	2014		2015		2016		Average	
	Main branch angle	Secondary branch angle	Main branch angle	Secondary branch angle	Main branch angle	Secondary branch angle	Main branch angle	Secondary branch angle
'Jojo'	66.25	77.50	70.00	76.25	78.00	75.25	71.42 a	76.33 a
'Tophit plus'	40.00	46.25	38.75	38.75	40.25	39.50	39.67 c	41.50 cd
'Topgigant plus'	47.50	44.29	51.25	45.00	55.75	47.00	51.50 b	45.43 c
'Topstar plus'	37.50	37.14	35.00	37.50	38.00	42.00	36.83 c	38.88 d
'Toptaste'	52.50	57.50	43.75	53.75	47.25	55.50	47.83 b	55.58 b
'Stanley'	35.00	42.50	37.50	36.25	40.50	38.25	37.67 c	39.00 d

[1]Mean values followed by different letters within a column are significantly different by Duncan's multiple range test at $P \leq 0.05$.

According to Costes et al. (2004) the final size of trees is a cumulative variable resulting from the annual shoot length developed each year. In the studied cultivars, the largest annual shoot length in 2014 was established in 'Topstar plus' and 'Topgigant plus' (Figure 1). In 2015 the largest annual shoot length was reported for 'Tophit plus' and 'Toptaste' and in the third vegetation for 'Toptaste' and 'Topgigant plus'. In the same year for all investigated cultivars were obtained the highest dimensions.

That confirms the research by some authors that shoot growth is strongly influenced by climatic factors and, above all, rainfall (Seleznyova et al., 2003). The average values of the studied trait showed that 'Stanley' and 'Jojo' had the smallest total annual shoot length and 'Tophit plus' had the largest one. The mean number of flower buds in the spurs

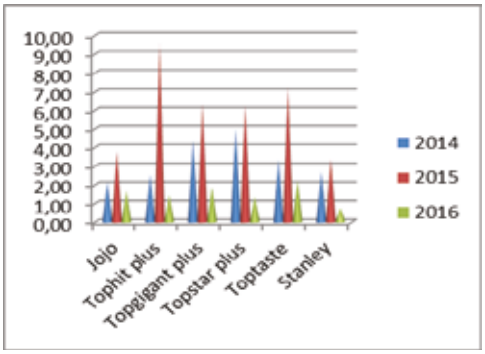

Figure 1. Annual shoot length per skeletal branch

of the studied cultivars varied from 3.26 to 3.96, but the differences between the cultivars are statistically insignificant (Table 3).

The largest number of flower buds on shoots was reported in the cultivars 'Jojo' and 'Toptaste' and it is statistically proven.

Table. 3. Number of flower buds on spurs and annual shoots in plum cultivars

Cultivar	On one spur				On 1 m of annual shoot			
	2014	2015	2016	Average	2014	2015	2016	Average
'Jojo'	3.44	3.26	3.74	3.48 a	10.35	28.13	32.45	23.64 a
'Tophit plus'	3.54	3.32	3.56	3.47 a	0.92	12.66	11.38	8.32 b
'Topgigant plus'	3.40	3.28	3.28	3.32 a	9.93	12.13	13.01	11.69 b
'Topstar plus'	3.30	3.30	3.96	3.52 a	19.88	17.98	16.88	18.25 ab
'Toptaste'	3.34	3.38	3.70	3.47 a	20.58	25.18	23.11	22.96 a
'Stanley'	3.48	3.54	3.90	3.64 a	3.86	10.45	9.40	7.90 b

Among the investigated cultivars earlier flowering time was recorded for 'Jojo', and 'Toptaste'. Two to three days later started to flower the cultivars 'Stanley', 'Topgigant plus', 'Tophit plus' and 'Topstar plus'.

Fruits of the studied cultivars ripen within a period of one month - from the beginning of August to the beginning of September (Figure 2).

Similar results about the period of ripening of those cultivars were also obtained by other authors in the Czech Republic and Poland (Blazek and Pistekova, 2009).

According to the data of the biometric analysis the fruits of 'Topgigant plus' and 'Tophit plus' were very large (Figure 3).

The fruits of the other four cultivars were medium in size and it was statistically proven (Table 4).

The stones of the cultivars 'Topgigant plus' and 'Stanley' were above 2 g in weight and in the rest varied from 1.52 to 1.87 g, but the differences between the cultivars are statistically insignificant.

The fruits of 'Topgigant plus' were large but stones were small and the relative stone to fruit ratio is low (3.17).

This data for 'Stanley' is the most unfavorable.

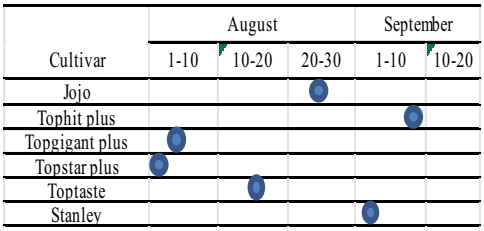

Figure 2. Fruit ripening time

Table 4. Fruit biometry (2014-2016)

Cultivar	Fruit				Stone weight (g)	Relative share (%)
	Lenght (mm)	Width (mm)	Thickness (mm)	Fruit weight (g)		
'Stanley'	50.06 ab	35.31 b	37.32 b	38.10 b	2.10 a	5.51 a
'Jojo'	47.55 ab	36.20 b	35.62 b	37.29 b	1.87 a	5.05 ab
'Toptaste'	42.67 c	36.51 b	37.61 b	35.43 b	1.66 a	4.66 abc
'Topgigant Plus'	51.11 a	42.27 a	42.78 a	53.06 a	2.10 a	3.97 bcd
'Topstar Plus'	46.15 bc	38.34 b	37.64 b	38.91 b	1.52 a	3.90 cd
'Tophit plus'	51.44 a	42.20 a	44.36 a	55.20 a	1.72 a	3.17 d

Determining the total soluble solids of the fruits is the quickest way to get information about the content of the major chemical components. The total soluble solids varied from 14.77% in 'Topgigant plus' to 25.90% in 'Toptaste' and the differences between them is significant (Table 5).

The data showed that the highest sugar content was established in the cultivar 'Toptaste'. The values for the other cultivars varied from 8.91% to 10.86% but statistically it was not proven. The acid content is low. Only in 'Topgigant plus' it was 1.75%. For all of the studed cultivars pH varied within a small range - from 2.99 in 'Topgigant plus' to 3.61 in 'Stanley'. According to the obtained data, the cultivar 'Toptaste' has the best chemical composition. The fruit quality is a complex of many different characteristics describing both external appearance and taste qualities. That is why the sensory evaluation is as important as the chemical analysis.

Table 5. Chemical composition of plum fruits (2014 - 2016)

Cultivar	Total soluble solids (°Brix)	Sugar, %			Titratable acidity, %	pH
		Total	Invert	Sucrose		
'Stanley'	18.15 b	10.77 b	6.69 bc	4.10 ab	0.82 b	3.61 a
'Jojo'	18.33 b	10.86 b	8.37 a	2.37 b	1.08 b	3.44 ab
'Toptaste'	25.90 a	13.18 a	7.79 ab	4.57 a	1.06 b	3.64 a
'Topgigant Plus'	14.77 c	8.91 b	5.70 c	3.05 ab	1.75 a	2.99 c
'Topstar Plus'	15.43 bc	9.56 b	5.37 c	4.05 ab	1.29 ab	3.07 bc
'Tophit plus'	18.13 b	9.00 b	6.43 c	2.42 b	1.03 b	3.40 ab

The results of the sensory analysis are presented in Table 6. The larger fruit size is always preferred not only for plum, but generally for all fruits. The difference in the score of this property between the studied cultivars was just 1.5 points and does not correspond exactly to the established fruit weight of the cultivars. In this case, the assessment is subjective and depends on the participants of the testing panel.

Compared to the fruit size, the score for the fruit shape varied less. Fruit colour together with fruit size contributes to fruit attractiveness. Judging by the scores given for fruit coloration, it is obvious that the dark coloured fruits are preferred (Figure 4).

Taste qualities combine the scores given for the texture, taste, aroma and sweetness. The taste is of the greatest importance for grading the cultivars in sensory evaluation.

The best taste had 'Toptaste' followed by 'Tophit plus' (Figure 5). For the other cultivars this score is not so impressive. The studied cultivars showed big variations in aroma and less in sweetness.

According to the final evaluation only for cultivars 'Toptaste' and 'Tophit plus' the sensory characteristic was excellent. Looking at the values of the chemical analysis we could not find a correlation between the chemical properties and the results of the sensory evaluation. Similar conclusions have been made in our previous studies of plum and apricot (Bozhkova, 2014; Bozhkova and Nesheva, 2016).

Table 6. Sensory evaluation of the investigated plum cultivars fruits

Cultivar	Appearance				Taste quality			Total score	Final evaluation
	Fruit size	Fruit shape	Fruit colour	Texture	Taste	Aroma	Sweetness		
'Stanley'	7.3	7.3	7.4	6.6	6.5	5.9	6.45	47.4	first class
'Jojo'	6.9	7.3	7.3	7.0	6.6	6.1	6.3	47.5	first class
'Toptaste'	6.4	7.0	6.7	7.5	8.4	7.2	7.8	51.0	excellent
'Topgigant Plus'	7.7	7.6	6.7	6.5	5.1	4.9	5.4	44.1	first class
'Topstar Plus'	7.8	7.8	7.6	6.8	5.6	5.4	6.0	47.0	first class
'Tophit plus'	8.4	8.1	7.2	7.3	7.1	6.7	6.9	51.8	excellent

Figure 3. Cultivar 'Topgigant plus'

Figure 4. Cultivar 'Jojo'

Figure 5. Cultivar 'Toptaste'

CONCLUSIONS

The biggest tree volume and trunk diameter were calculated for the cultivars 'Toptaste' and 'Stanley'. The largest angle of skeletal branches was measured on 'Jojo'.

The biggest shoot length was recorded in 'Tophit plus', but a more uniform growth rate over the years was established in 'Topgigant plus'. It was found that the wood of 'Topgigant plus' is quite fragile.

The earliest flowering time was observed on 'Jojo' and 'Toptaste', the latest one on the 'Topstar plus' and 'Tophit plus'.

The ripening period of the investigated cultivars was in August and only for 'Stanley' and 'Tophit plus' in the beginning of September.

According to the biometric data 'Topgigant plus' and 'Tophit plus' had the largest fruits in size.

The highest sugar content and total soluble solids were found in the 'Toptaste' fruits.

The investigated cultivars are suitable for growing under agroclimatic conditions of Plovdiv region.

We do not recommend to producers the cultivar 'Topgigant plus' and remind them to keep in mind that 'Jojo' is sensitive to late spring frost.

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ADVENTITIOUS SHOOT REGENERATION FROM PETIOLE EXPLANTS IN BLACK CHOKEBERRY (*ARONIA MELANOCARPA*)

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Abstract

Due to outstanding nutritional and health benefits, and also to its ornamental value, black chokeberry was gaining recently high interest from the small fruit growers in Europe. Together with vegetative propagation, in vitro micropropagation from meristems and adventitious shoots offers suitable methods for the rapid clonal propagation of new or improved cultivars, to provide sufficient quantities of planting material to the growers and to accelerate the establishment of large black chokeberry plantings. In this respect, different concentrations of N6-benzylaminopurine (BAP), dichlorophenoxyacetic acid (2,4-D) and indole butyric acid (IBA) in Murashige and Skoog (MS) and Lee-Fossard (LF) basic culture media, respectively, were assessed for their effects on adventitious shoot regeneration of the black chokeberry cultivar 'Nero'. The ability of callus formation and shoot regeneration from petiole segments was assessed using various combinations of BAP (2.5; 5.0; 10 mg L^{-1}), 2,4-D (0.25; 0.5; 1.0 mg L^{-1}), and IBA (0.25; 0.5; 1.0 mg L^{-1}). Data on callus formation and shoot regeneration were recorded after 60 days of culture. The highest percentage of black chokeberry petiole explants forming callus (100%) was found in treatments containing a combination of 2.5 mg L^{-1} of BAP, 0.25 mg L^{-1} of 2,4-D, and 0.25 mg L^{-1} of IBA in MS medium. The only growth regulators combination which resulted in 100% petiole explants forming callus on both MS and LF media was 5 mg L^{-1} of BAP, 0.5 mg L^{-1} of 2,4-D, and 0.5 mg L^{-1} of IBA. Adventitious shoot regeneration from petiole-derived callus was high in treatments with 10 mg L^{-1} and 1.0 mg L^{-1} IBA, on both MS and LF basic media. Excepting the cytokinin-auxin combination of 2.5 mg L^{-1} of BAP, 0.25 mg L^{-1} of 2,4-D and 0.25 mg L^{-1} of IBA, shoot regeneration from petioles of 'Nero' cv. was better on MS medium. However, the best adventitious regeneration and the highest number of shoots formed per explant ocurred by direct organogenesis. Thus, an average number of 4.3 shoots per petiole explant was achieved through direct organogenesis on MS medium supplemented with BAP at 5 mg L^{-1}, 0.5 mg L^{-1} of 2,4-D, and 0.5 mg L^{-1} of IBA.

Key words*: Aronia, in vitro culture, growth regulators, callus, organogenesis.*

INTRODUCTION

Aronia melanocarpa (Michx.) Elliot (black chokeberry), a native North American shrub which was naturalized and is well adapted in Europe, is an extraordinary medicine plant (McKay, 2001). 'Nero', 'Rubina', 'Viking', 'Galicjancka', 'Fertödi', 'Hugin', 'Aron' and 'Melrom' are among the most popular varieties of *A. melanocarpa* in Europe (Strigl et al., 1995; Kulling and Rawel, 2008; Walther and Schnell, 2009; Borowska and Brzoska, 2016; Șuțan et al., 2017). They differ from each other in the efficiency of juice extraction, content of total polyphenols, anthocyanins and proantho-cyanidins, total antioxidative capacity, as well as the weight and diameter of the fruit (Rop et al., 2010; Ochmian et al., 2012; Rugină et al., 2012).

Aronia melanocarpa berries constitute a very rich source of numerous substances exerting a beneficial impact on health, including mainly polyphenols (proanthocyanidins, anthocyanins, flavonoids, and phenolic acids), possessing antioxidative, anti-inflammatory, antiviral, anticancer, antiatherosclerotic, hypotensive, antiplatelet, and antidiabetic properties (Benvenuti et al., 2004; Slimestad et al., 2005; Naruszewicz et al., 2007; Jakobek et al., 2012; Bădescu et al., 2015; Borowska and Brzóska, 2016; Park et al., 2017). Now it is well known that black chokeberry possesses one of the highest antioxidant activities among fruits (Denev et al., 2012).

A. melanocarpa berries are also known to be rich of cyanidin glycosides (Wiczkowski et al., 2010). Therefore, black chokeberries has been extensively investigated for their phenolic compound content, antioxidant properties and potential positive influence on the health (Tanaka and Tanaka, 2001; Jakobek et al., 2007; Rop et al., 2010; Jakobek et al., 2012; Litwinczuk, 2013).

From the researches on the antioxidant (Kahkonen et al., 2001; Wu et al., 2004; Oszmianski and Wojdylo, 2005; Olas et al., 2008; Denev et al., 2012), anti-inflammatory (Zapolska-Downar et al., 2012), hepato-protective (Kowalczyk et al., 2003; Valcheva-Kuzmanova et al., 2004), cardioprotective (Bell and Burt, 2007; Naruszewicz et al., 2007), hypotensive and lipid lowering (Hellstrom et al., 2010; Park and Park, 2011), hypoglycaemic and antidiabetic effects (Simeonov et al., 2002; Rugină et al., 2011; Bădescu et al., 2015; Banjari et al., 2017), to those on antimutagenic (Gasiorowski et al., 1997; Duthie et al., 2007) and antitumoral effects (Malik et al., 2003; Bermudez-Soto et al., 2007; Olas et al., 2010; Sharif et al., 2013), or those on protective action against degenerative diseases, the scientific literature is rich in information highlighting their prophylactic and therapeutic properties, without suggests on any unwanted or side effect of their use (Kokotkiewicz et al., 2010).

Zielińska-Przyjemska et al. (2007) studied the *in vitro* effects of *Aronia melanocarpa* juice on oxidative metabolism and apoptosis of neutrophils from obese and non-obese individuals, and reported that *Aronia* juice exert beneficial effects in cells and may, therefore, be useful in the treatment of obesity disorders.

Bijak et al. (2011) and Sikora et al. (2012) reported the anticoagulant properties of *Aronia* extract, based on the results of their studies, which showed significant inhibition of platelet aggregation after black chokeberry extract administration.

Experimental data indicate that not only the fruit but also the leaves of *A. melanocarpa* and their products may be effective means for prevention and treatment of the effects of toxic action of some xenobiotics in humans (Borowska and Brzoska, 2016).

Recently, Park et al. (2017) reported that *A. melanocarpa* show beneficial effects against hepatic lipid accumulation along with improvements in body weight, liver functions, lipid profiles and antioxidant capacity suggesting the potential therapeutic efficacy of its juice on nonalcoholic fatty liver disease (a hepatic manifestation of metabolic syndrome). *Aronia* phenolics are considered to be also beneficial for cardiovascular health (Wu et al., 2017).

Successful plant micropropagation from *in vitro* cultured meristems has been reported for *Aronia* species, including *A. melanocarpa* (Brand and Cullina, 1990; Brand and Cullina, 1992; Petrovic and Jacimovic-Plavsic, 1992; Velchev and Mladenova, 1992; Staniene et al., 1999; Litwińczuk, 2002; Mahečić, 2009; Litwinczuk, 2013; Kwak et al., 2015; Șuțan et al., 2017) and *A. arbutifolia* (Kane et al., 1991). However, there is no relevant information on adventitious shoot regeneration using somatic tissue explants in *Aronia melanocarpa*.

Adventitious shoot organogenesis and somatic embryogenesis are the basis for implementing new genetic variability and biotechnological approaches in woody species, particularly if somatic tissues from valuable cultivars are used (Silvestri et al., 2016). Regardless of the type (direct organogenesis, indirect organogenesis or somatic embryogenesis), regeneration process is usually influenced by biotic factors including genotype, explant type, and abiotic factors such as culture media and environmental conditions. Although plant cell totipotency theoretically enables any of the cells to retain the ability to regenerate whole new plants through organogenesis or somatic embryogenesis, the regeneration capacity of plant cells usually varies between species, cultivars, and explant types (Ganeshan et al., 2002). Proper regeneration of adventitious shoots rely on composition of nutrient medium, plant grow regulators and types of explant (Popescu and Isac, 2000; Isac and Popescu, 2009).

In the present study, we investigated the ability of adventitios shoot regeneration of 'Nero', one of the most valuable black chokeberry cultivars in both central and south-eastern Europe, using petiole explants, and found that both basic culture medium and growth regulators

combination and concentration affected adventitious shoot regeneration.

MATERIALS AND METHODS

Petiole segments of about 10 mm in length, excised from *in vitro* micropropagated shoots of chokeberry cultivar 'Nero', were cultured on Murashige and Skoog (MS) (1962) and Lee and Fossard (LF) (1977) basic culture media, respectively, supplemented with N6-benzylaminopurine (BAP) in concentration of either 2.5, 5.0 or 10 mg L^{-1}, dichloro-phenoxyacetic acid (2,4-D) in concentration of either 0.25, 0.5 or 1.0 mg L^{-1}, and indole butyric acid (IBA) in concentration of either 0.25, 0.5 or 1.0 mg L^{-1} (Table 1).

For medium preparation, separate stock solutions of macronutrients and micro-nutrients were used. Iron was added to the medium as separate stock solution of ferric sodium salt EDTA (32 mg L^{-1}). BAP and IBA were disolved in 1N HCl and 1N NaOH, respectively. Dextrose was used as carbon source in the culture media (40 g L^{-1}). The pH of the culture medium was adjusted to 5.7 with 0.1N KOH before autoclaving for 20 minutes at 121°C.

The freshly prepared explants were placed aseptically onto regeneration medium in 100 ml glass jars (four petiole segments per jar), each containing 40 ml of sterile medium solidified with 0.9% plant agar (Duchefa Biochemie).

Based on our previous experience with raspberry petiole segments cultured *in vitro* (Popescu and Isac, 2000), the cut ends of chokeberry petiole segments were slightly deeped into the culture medium in order to prevent dehydration and promote the nutrients and growth regulators uptake. The explants from the same donor plantlet were randomly distributed in different jars in order to avoid the possible errors in interpretation of results due to the differences in physiological state of the source of explants. Each treatment consisted of 20 petiole segments, in five replications.

For all treatments, the petiole segments cultured *in vitro* were subjected to an initial dark treatment for one week, at 20-22°C. Subsequently, the cultures were maintained in the growth chamber at 23±1°C, under a 16 hours photoperiod of 30 lE m^{-2} s^{-1} from cool white fluorescent tubes. Transfer of the explants to fresh medium was performed every 4 weeks.

Table 1. Composition of the culture medium used for *in vitro* adventitious regeneration of shoots in 'Nero' cultivar of *A. melanocarpa* (Michx.) Elliot

Experiment	Basal medium	Growth regulators (mg L^{-1})		
		2,4-D	IBA	BAP
E1	MS	0.25	0.25	2.5
E2	MS	0.5	0.5	5.0
E3	MS	1.0	1.0	10.0
E1	LF	0.25	0.25	2.5
E2	LF	0.5	0.5	5.0
E3	LF	1.0	1.0	10.0

The observations on adventitious shoot formation by direct organogenesis or via callus were made weekly, and after two months of culture, when regeneration frequency (percentage of the petiole explants with at least one shoot) and number of shoots per explant were recorded. For all treatments were calculated both the percentage of petiole explants forming shoots and the average number of shoots per explant. Data for shoot regeneration were analyzed for significance by the standard analysis of variance (ANOVA) with mean separation by Duncan's test ($p > 0.05$).

RESULTS AND DISCUSSIONS

The *in vitro* response of black chokeberry petiole explants from cultivar 'Nero' was significantly influenced by both the culture medium and concentration of growth regulators tested. The highest percentage of petiole explants forming callus (100%) was found in

treatments containing a combination of 2.5 mg L^{-1} of BAP, 0.25 mg L^{-1} of 2,4-D, and 0.25 mg L^{-1} of IBA in Murashige-Skoog medium.

The only growth regulators combination which resulted in 100% petiole explants forming calli on both Murashige-Skoog and Lee-Fosard media was 5 mg L^{-1} of BAP, 0.5 mg L^{-1} of 2,4-D, and 0.5 mg L^{-1} of IBA.

While petiole explants cultured on MS medium formed small amounts of callus in all treatments, only a few on those cultured on LF medium were induced to form callus in treatments with IBA and 2,4-D in concentrations of 0.25 mg L^{-1} and 0.5 mg L^{-1}, respectively. Although the presence of two different auxins in the culture medium, which is not usual, would be expected to induce a higher potency of callus formation, a synergistic effect of IBA and 2,4-D was not obvious. This observation is important in the context of recent reports showing the production and accumulation of hydroxy-benzoic acids and other biologically active phenolic acids in shoot and callus cultures of *Aronia melanocarpa* (Michx.) Elliott (Szopa et al., 2013; Kwiecien et al., 2013; Szopa and Ekiert, 2014).

We did not find a strong correlation between the concentrations of the auxins in the culture medium, and type and amount of callus formed by the petiole explants. However, the green calli derived from petiole explants cultured on MS medium containing auxins in smaller concentrations showed a higher ability to form adventitios buds and regenerate shoots by indirect organogenesis (Figure 1C). Highly proliferative petiole-derived calli (Figure 1B) did not show ability to form adventitious buds.

Depending on the treatment, shoot regeneration was observed either through direct organogenesis where the regenerants emerged mostly at the cut edge of explants or wound sites (Figure 1 and Figure 2), or indirect organogenesis (via callus), where regenerants appeared on the entire surface of the petiole-derived calli (Figure 1).

Optimum combination of plant growth regulators for shoot regeneration was medium-dependent (Table 2).

Table 2. Frequency of adventitious shoot regeneration *in vitro* by either direct organogenesis or indirect organogenesis (via callus) from petiole explants of black chokeberry cultivar 'Nero'

Experimental variants	Average number of adventitious shoots regenerated per petiole explant	Average length of adventitious shoots (cm)	Average number of calli formed per petiole explant	Average number of shoots regenerated per petiole-derived callus
E1-MS	0.9±0.41 c	2.23±0.15 ab	1.1±0.20 a	0.6±0.15 b
E2-MS	4.3±0.62 a	2.80±0.48 a	0.9±0.06 ab	1.0±0.00 a
E3-MS	3.4±0.42 ab	2.03±0.60 abc	1.0±0.00 ab	0.9±0.10 a
E1-LF	1.4±0.36 c	0.97±0.19 cd	0.1±0.06 d	0.9±0.06 a
E2-LF	2.1±0.80 bc	0.92±0.12 d	0.4±0.20 cd	1.0±0.05 a
E3-LF	2.6±0.48 bc	1.56±0.21 bcd	0.6±0.17 bc	0.9±0.06 a

*Values presented are mean ± SE. Means followed by the same letter are not significantly different (Duncan test, $p>0.05$)

The best development of adventitious buds and formation of shoots was achieved on MS medium supplemented with 0.1 mg L^{-1} IBA, 0.1 mg L^{-1} 2,4-D, and 0.5 mg L^{-1} BAP. Excepting the cytokinin-auxin combination of 2.5 mg L^{-1} of BAP, 0.25 mg L^{-1} of 2,4-D and 0.25 mg L^{-1} of IBA, shoot regeneration from petioles of 'Nero' cv. was better on MS medium. However, the statistical analysis showed that there is no difference between the percentages of adventitious shoots regenerated *in vitro* by direct organogenesis from petiole explants of black chokeberry cultivar 'Nero' cultured onto LF medium, and those of adventitious shoots regenerated on MS medium (Table 2). The best adventitious regeneration and the highest number of shoots formed per explant ocurred by direct organogenesis (Figure 1 and Figure 2). Thus, an average number of 4.3 shoots per petiole explant was achieved through direct organogenesis on MS medium supplemented with BAP at 5 mgL^{-1}, 0.5 mgL^{-1} of 2,4-D, and 0.5 mg L^{-1} of IBA (Table 2).

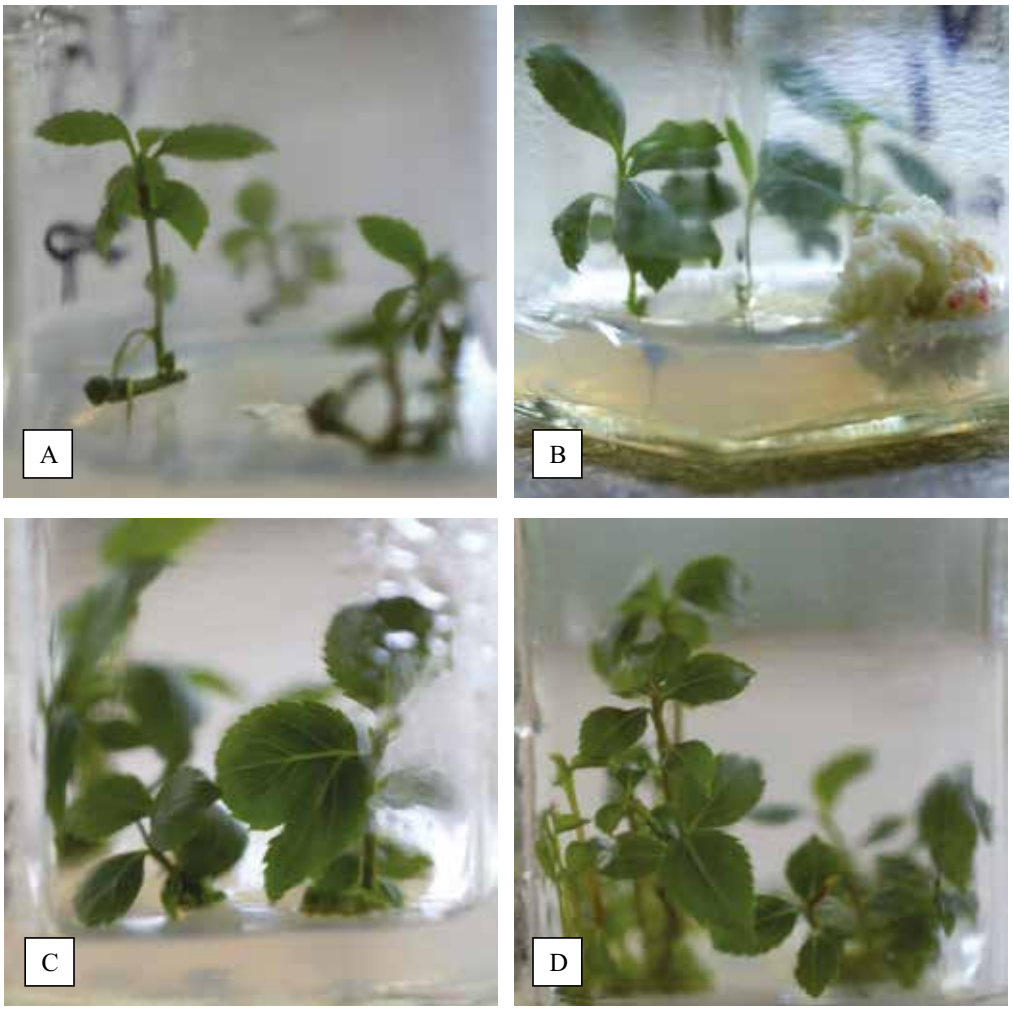

Figure 1. A-D: Adventitious shoot regeneration of black chokeberry cultivar 'Nero' through direct organogenesis from petiole segments

The petiole-derived shoots formed from adventitious buds through direct organogenesis were not uniform in appearance (Figure 2), probably due to interaction between the endogenous hormones in the plantlets used as source of explants and plant growth regulators added in the shoot induction (regeneration) medium.

Regardless of the culture medium, when petiole explants from chokeberry cultivar 'Nero' formed multiple adventitious buds (Figure 1D), only a few shoots grew and suppressed the development of the rest. Although a high frequency of shoot formation is most often desired even from the initial tissue explants, individual shoots rather than clusters are generally advantageous, because they have a higher vigor (and consequently a better ability to multiplicate), and also because the thin and crowded clusters of shoots could not be separated easily for the stage of multiplication.

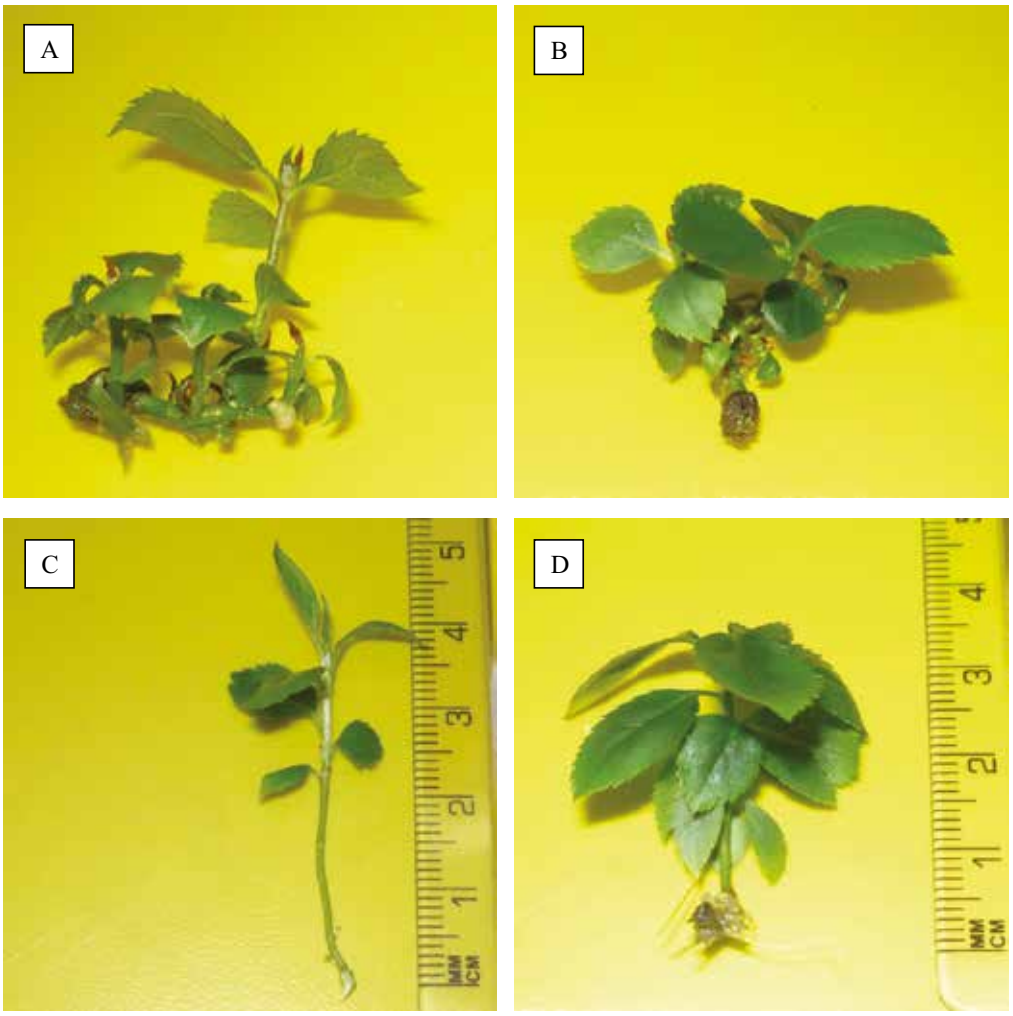

Figure 2. A-D: Length and vigour of the adventitious shoots regenerated *in vitro* by direct organogenesis from petiole explants of black chokeberry cultivar 'Nero'

As with micropropagation of *Aronia*, which is considered far more efficient than other conventional cloning methods like layering or softwood cuttings (Litwinczuk, 2013), adventitious shoot regeneration from somatic tissue explants (e.g. petiole segments) proved to be effective by direct organogenesis and even from tissue-derived calli (at a lower frequency) on Murashige and Skoog medium, and much more less effectively on Lee and Fossard medium. The effectiveness of MS medium for the *in vitro* culture of chokeberry was emphasized in many published reports (Brand and Cullina, 1990; Kane et al., 1991; Brand and Cullina, 1992; Petrovic and Jacimovic-Plavsic, 1992; Velchev and Mladenova, 1992; Staniene et al., 1999; Litwińczuk, 2002; Mahečić, 2009; Litwinczuk, 2013; Şuțan et al., 2017). Even the use of Woody Plant Medium (WPM) (Lloyd and McCown, 1980) became frequent with woody plants, including *Aronia* (Kwak et al., 2015; Chen, 2017), MS medium is a choice with reliable results, supported by many authors, such as Brand and Cullina (1990; 1992), who reported that both MS medium and WPM medium supported vigorous shoot proliferation in *Aronia arbutifolia* and *A. melanocarpa*.

Currently, experiments are underway in our laboratory to improve the conditions of *in vitro*

culture of *A. melanocarpa*, in order to achieve higher regeneration percentages per petiole explant.

CONCLUSIONS

We have undertaken the first studies to investigate the ability of adventitious shoot regeneration by either direct or indirect organogenesis from somatic tissues of *Aronia melanocarpa*.

In the current study, shoots were induced by both direct and indirect organogenesis from petiole segments of *in vitro* cultured plants of *A. melanocarpa*, cultivar 'Nero'.

Our results showed the possibility of establishing an effective *in vitro* adventitious shoot regeneration system for *Aronia melanocarpa*, which holds great promise for either micropropagation and/or genetic transformation studies in black chokeberry.

Also, the results of this investigation may be useful in optimizing shoot regeneration systems for other cultivars of *Aronia melanocarpa*, and also as an alternative allowing rapid mass propagation of elite genotypes independent from seasonal influences.

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PHYSIOLOGICAL STUDY OF VARIOUS CULTIVARS OF *PUNICA GRANATUM* (L.) WITH AN EYE TO ACCLIMATIZATION TO ROMANIA'S ENVIRONMENTAL CONDITIONS

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Abstract

The phytotherapeutic and economic importance given to Punica granatum (L.) *in the last decades indicates the rebirth of this species. In Romania, the species is barely known by farmers and hobby growers, while the pomegranate products are widely consumed. This research is supposed to ensure the physiological data that will be the basis for the development of pomegranate crop technology for eight P. granatum cultivars that were introduced for acclimatization in the experimental fields of University of Agronomic Sciences and Veterinary Medicine of Bucharest. In this study, physiological processes are described, insisting on photosynthesis, transpiration and respiration in interrelation with the chlorophyll content, water and dry matter content existing in leaves. The measurements were performed during the main growth and flowering phenophases, starting late March 2016. The results showed a connection between the photosynthesis process, leaf dry matter and leaf chlorophyll content, transpiration process and leaf water content, respiration process and the flowering phenophase (in some cultivars, not in all).*

Key words*: phenophase, photosynthesis, respiration, transpiration, chlorophyll.*

INTRODUCTION

Punica granatum (L) is a fruit-bearing shrub, belonging to the Punicaceae family, having its origins in Middle East. From its origin, considered now Iran and Afghanistan, the pomegranate spread east to India and China and west to Mediterranean countries such as Turkey, Egypt, Tunisia, Morocco, Greece, Italy and Spain. It is assumed that Spanish missionaries brought the pomegranate to the American continent in the 1500's (Hodgson, 1917; LaRue, 1980).

In Romania, the shrub can be cropped especially in the areas with a warm climate such as Dobrogea and Banat.

Pomegranate is the symbol and heraldic device of the ancient city of Granada in Spain, from which the city gets its name. The genus name, *Punica*, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate is known by the French as grenade, the Spanish as granada, and literally translates to seeded ("granatus") apple ("pomum") (Jurenka, 2008).

In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, bacterial infections or antibiotic resistance (Jurenka, 2008).

Considering lack of water resources and intensification of abiotic stresses such as drought and salinity, importance of pomegranate has increased in recent years, since this species is a tolerant fruit crop and thrives well under arid and semi-arid climatic conditions (LaRue, 1980; Jamali and Bonyanpour, 2017).

The *P. granatum* shrubs have an effective control of the plant water status by reducing transpiration due to stomatal closure and maintaining a consistent maximum stem with changes in evaporative demand (Intrigliolo et al., 2011; Parvizi et al., 2016).

This species can not be cropped only in regions with water deficit, but in seasonally wetlands as well.

The photosynthetic rate, as well as transpiration and respiration processes depend on various parameters like chlorophyll content, leaf water and dry matter content, abiotic factors (temperature, light intensity, humidity), plant age or plant phenophases (Burzo et al., 2005).

The present research, whose results are recorded in the writing, aims to thoroughly

fathom the physiological and biochemical particularities of certain *P. Granatum* cultivars of different origins, more precisely to compare certain physiological parmeteres during the species' vegetation period, informations that will allow the crop technology acquaintance for this species' acclimatization to Romania's pedo-climatic conditions.

MATERIALS AND METHODS

Plant material was represented by eight *P. granatum* cultivars*: Hicaz, Kandahar, Nikitski ranni, Echen, Mollar, Shahvar, Dolce* and *Local selection* cultivar.

The botanical characterization of the selected *P. granatum* cultivars was based on macroscopic (visual) observations made directly in the study field.

Thus, the studied plants are characterized as deciduous shrubs, with a gray-brown bark, that grow between 0.7 and 1 meter tall and have 3 to 5 branches with twigs of a length between 3 and 60 cm.

The leaves are simple, petiolated, entire, lanceolate, oposite, coriaceous and have a length of 3 to 7 cm and a width of 1 to 2 cm.

The plant assortment lies on a 60 m^2 surface and comprise a total of 13 plants of kindred age (aprox. 6 years old), originated in Europe and Middle East, planted at a 2.5 m distance between plants and 2.7 m distance between the rows (also, there is no plant support system) in the experimental field belonging to Fruit Growing Department of the Faculty of Horticulture.

The measurements were performed during the main growth and flowering phenophases, on similar leaves belonging to the middle level of the analyzed plants, starting late March 2016 (up until late June 2016), 3 times a week - for the phisyological processes - between 9:00 am and 10:00 a.m, at a light intensity with values between 1083 and 1586 $\mu mol/CO_2/m^2/s$ and an air temperature of 17.5 up to 19.7°C.

Also, the analysis of photosynthetic pigments and total water and dry matter content were completed twice a month, the determination of photosynthetic pigments was made using the leaves belonging to the upper level (young leaves) of the *P. granatum* plants taken in study.

Photosynthesis as well as transpiration and respiration process intensities were determined with the LCA-4 electronic analyser and expressed in $\mu mol/CO_2/m^2/s$ (photosynthesis and respiration) and $mmol/H_2O/m^2/s$ (transpiration).

The photosynthetic pigments content was determined using 80% acetone extraction and by colorimetry at wavelengths of 470 nm, 663 nm and 646 nm. The results were calculated using the Lichtenthaler & Wellburn's formula (1983) and the results obtained expressed in mg/100g fresh weight (FW).

The total water and dry matter content were determined through gravimetric analysis. And expressed in percentages.

RESULTS AND DISCUSSIONS

The intensity of the Photosynthesis process in *P. granatum* leaves during the Growth Phenophase

The maximum photosynthetic rate is recorded, for most species, before growth cessation of the leaves, when they reached 37 - 90% of their foliar surface (Burzo et al., 2004).

From Table 1 data analysis, it is observed that the photosynthesis process recorded similar values at *Echen* and *Mollar* cultivars (8.26 $\mu mol/CO_2/m^2/s$). They carried out the photosynthesis process with the highest intensities, 1.30 times higher than *Hicaz* and *Dolce*, 1.23 times higher than *Kandahar*, 1.94 times higher than *Shahvar* and 1.51 times more intense than *Local selection* cultivar.

Table 1. The intensity of the Photosynthesis process in *P. granatum* leaves during the Growth Phenophase

Cultivar	Light intensity ($\mu mol/m^2/s$)	Temperature (°C)	Photosynthesis intensity ($\mu mol/CO_2/m^2/s$)
Hicaz	1245	18.0	6.32
Kandahar	1189	18.1	6.68
Nnikitski ranni	1204	17.7	8.01
Echen	1153	17.5	8.26
Mollar	1083	18.6	8.26
Shahvar	1073	19.0	4.25
Dolce	1126	17.7	6.32
Local selection	1119	17.8	5.45

Nikitski ranni also recorded a high photosynthetic rate of 8.01 $\mu mol/CO_2/m^2/s$.

The *Shahvar* cultivar, during the growth period, carried out the photosynthesis process with the lowest intensity, compared to the other studied cultivars, namely 4.25 μmol/CO_2/m^2/s.
Of note were *Hicaz*, *Dolce* and *Kandahar* cultivars, whose photosynthesis processes were carried out with related intensities, the values being between 6.32 and 6.68 μmol/CO_2/m^2/s.
Of the obtained data, it was found that the photosynthesis process was influenced by the type of cultivar taken in study, representing an internal factor that the specialty literature signals in modifying dynamics of the photosynthesis process (Burzo et al. 2005; Dobrescu, 2007; Bădulescu, 2016).

The intensity of the Photosynthesis process in *P. granatum* leaves during the Flowering Phenophase

During the appearance of the first flowers at *Hicaz*, *Kandahar*, *Nikitski ranni* and *Local Selection* cultivar, the photosynthesis process was carried out with a high intensity in all studied cultivars.
The highest photosynthesis process intensity was determined at *Hicaz*, its value being 11.58 μmol/CO_2/m^2/s, followed by the *Echen*, 10.33 μmol/CO_2/m^2/s and *Nikitski ranni*, 9.71 μmol/CO_2/m^2/s (Table 2).

Table 2. The intensity of the Photosynthesis process in *P. granatum* leaves during the Flowering Phenophase

Cultivar	Light intensity (μmol/m^2/s)	Temperature (°C)	Photosynthesis intensity (μmol/CO_2/m^2/s)
Hicaz	1402	18.8	11.58
Kandahar	1514	19.7	8.37
Nnikitski ranni	1415	18.2	9.71
Echen	1425	19.5	10.33
Mollar	1505	18.5	9.03
Shahvar	1498	18.2	5.19
Dolce	1567	19.1	7.86
Local selection	1586	18.5	9.16

The lowest intensity was recorded at *Shahvar* cultivar (5.19 μmol/CO_2/m^2/s).
There was recorded an increase of the photosynthesis process during flowering phenophase, compared to growth phenophase, thus: at the flowering phenophase moment, the *Hicaz* cultivar intensified its photosynthesis process 1.83 times, *Kandahar* 1.25 times, *Nikitski ranni* 1.21 times, *Echen* 1.25 times, *Mollar* 1.09 times, *Shahvar* 1.22 times, *Dolce* 1.24 times and *Local selection* cultivar 1.44 times.
The *Hicaz* cultivar (with the highest photosynthetic rate) carried out the photosynthesis process with an intensity of 1.38 times higher than *Kandahar*, 1.19 times higher than *Nikitski ranni*, 1.12 times higher than *Echen*, 1.28 times higher than *Mollar*, 2.23 times higher than *Shahvar*, 1.46 times higher than *Dolce* and 1.29 times higher than *Local Selection*.

Total leaf Chlorophyll content of *P. granatum* during the Growth Phenophase

Following the chlorophyll pigment analysis, the highest content in total leaf chlorophyll was found at *Nikitski ranni* cultivar, 38.78 mg/100g FW, followed by *Echen* cultivar, 28.14 mg/100g FW (Table 3).

Table 3. Total leaf Chlorophyll content of *P. granatum* during the Growth Phenophase

Cultivar	Total Chlorophyll (mg/100g FW)
Hicaz	23.29
Kandahar	26.41
Nnikitski ranni	38.78
Echen	28.14
Mollar	22.80
Shahvar	24.44
Dolce	22.09
Local selection	26.38

Regarding these 2 cultivars, there was a relevancy between the total leaf chlorophyll content and the intensity of the photosynthesis process. Therefore, the photosynthesis processes of these cultivars showed the highest intensity, given the highest content of chlorophyll (Tables 3, 1).
As for *Hicaz*, *Kandahar*, *Mollar*, *Shahvar*, *Dolce* and the *Local Selection* cultivars, there was no interdependence between the total leaf chlorophyll content and the intensity of the photosynthesis processes.

Total leaf Chlorophyll content of *P. granatum* during the Flowering Phenophase

During the flowering phenophase, it was noted a different accumulation of chlorophyll pigments of the analyzed cultivars. The highest

values were shown at *Kandahar* (which flourished), *Nikitski ranni* (also flourished) and *Shahvar*, and the lowest were recorded at *Echen* (Table 4).

Table 4. Total leaf Chlorophyll content of *P. granatum* during the Flowering Phenophase

Cultivar	Total Chlorophyll (mg/100g)
Hicaz	27.33
Kandahar	30.89
Nnikitski ranni	32.27
Echen	22.90
Mollar	28.89
Shahvar	32.63
Dolce	28.15
Local selection	26.39

The *Hicaz*, *Dolce* and *Mollar* cultivars recorded a higher chlorophyll content during flowering phenophase, compared to growth phenophase, with the remark that *Dolce* and *Mollar* reported a similar increase rate of chlorophyll content. These cultivars showed a connection between the chlorophyll content and the intensity of the photosynthesis process. At the *Dolce* cultivar, the photosynthesis process intensified with the same rate that the chlorophyll content increased (1.25 times) (Tables 4, 2).

It is worth mentioning that during the flowering phenophase, the chlorophyll content of *Local selection* cultivar did not change compared to the growth phenophase and the photosynthesis process was not influenced by this biochemical indicator. Therefore, these two physiological parameters were not influenced by the phenophase at this cultivar.

The intensity of the Transpiration process in *P. granatum* leaves during the Growth Phenophase

Data analysis revealed an intense transpiration at *Echen* cultivar (4.82 mmol/H_2O/m^2/s), followed by *Shahvar* (4.58 mmol/H_2O/m^2/s), and *Mollar* (4.13 mmol/H_2O/m^2/s) (Table 5).

Referring to *Echen* cultivar, the intensity of transpiration process was: 1.41 times lower at *Hicaz*, 1.96 times lower at *Kandahar*, 2.49 times lower at *Nikitski ranni*, 1.16 times lower at *Mollar*, 1.05 times lower at *Shahvar*, 2.12 times lower at *Dolce*, and 1.32 times lower at *Local selection* cultivar.

The lowest transpiration intensity was recorded at *Nikitski ranni*, of 1.93 mmol/H_2O/m^2/s .

Analyzing the amount of water present in leaves and the intensity of transpiration process, it was observed that the *Echen* cultivar correlates the considerable quantity of water present in leaves with the high intensity of transpiration process (Table 5, 7).

The studied cultivars carried out the transpiration process with different values, albeit they showed a similar water content in their leaves. This occurrence is explained by the absorption of a distinct amount of light energy caused by the difference in leaf size of each analyzed cultivar (the light intensity directly influences the transpiration process through its caloric effect) (Burzo and Dobrescu, 2011; Dobrescu, 2007).

Table 5. The intensity of Transpiration process in *P. granatum* leaves during the Growth Phenophase

Cultivar	Light intensity (µmol/m^2/s)	Temperature (°C)	Transpiration intensity (mmol/H_2O/m^2/s)
Hicaz	1245	18.0	3.30
Kandahar	1189	18.1	2.45
Nnikitski ranni	1204	17.7	1.93
Echen	1153	17.5	4.82
Mollar	1083	18.6	4.13
Shahvar	1073	19.0	4.58
Dolce	1126	17.7	2.27
Local selection	1119	17.8	3.64

The intensity of the Transpiration process in *P. granatum* leaves during the Flowering Phenophase

During the flowering phenophase, according to specialty literature data, plant water requirements are higher and stimulate water absorption at the root level, which enhances the transpiration process (Burzo, 2016; Burzo et al., 2005; Dobrescu, 2007).

The transpiration process was carried out with a distinct intensity from one cultivar to another: the highest transpiration intensities, with values between 5.15 and 5.56 mmol/H_2O/m^2/s were recorded at *Shahvar* and *Mollar* cultivars, followed by *Echen* and *Local Selection* cultivars of 4.34 and 4.90 mmol/H_2O/m^2/s.

The lowest values were registered at *Nikitski ranni* and *Hicaz* of 3.50 and 3.60 mmol/H_2O/m^2/s (Table 6).

Table 6. The intensity of Transpiration process in *P. granatum* leaves during the Flowering Phenophase

Cultivar	Light intensity ($\mu mol/m^2/s$)	Temperature (°C)	Transpiration intensity ($mmol/H_2O/m^2/s$)
Hicaz	1402	18.8	3.60
Kandahar	1514	19.7	4.12
Nnikitski ranni	1415	18.2	3.50
Echen	1425	19.5	4.34
Mollar	1505	18.5	5.56
Shahvar	1498	18.2	5.15
Dolce	1567	19.1	3.92
Local selection	1586	18.5	4.90

The transpiration process was strongly influenced in its development by the phenophase, stating that in all cultivars the transpiration intensity increased during the flowering phenophase, compared to growth phenophase, for example 1.12 times, respectively 1.34 times at *Shahvar* and *Mollar* cultivars.
High transpiration values during the flowering phenophase can be explained due to both high light intensity and high temperature, external factors that directly influence this process.

Total leaf water and dry matter content of *P. granatum* during the Growth Phenophase

During growth phenophase, the highest water content was determined at *Echen* cultivar, of 68.18%, which was 1.05 times higher than the other studied cultivars (Table 7).

Table 7. Total leaf water and dry matter content of *P. granatum* during the Growth Phenophase

Cultivar	Water content (%)	Dry matter content (%)
Hicaz	64.88	35.12
Kandahar	65.62	34.38
Nnikitski ranni	64.40	35.60
Echen	68.18	31.82
Mollar	65.89	34.11
Shahvar	65.71	34.29
Dolce	62.87	37.13
Local selection	64.55	35.45

At *Echen*, the leaf water content had positively influenced the process of photosynthesis which recorded the highest intensity (it is known that the water represents a key matter for the photosynthesis process) (Tables 7, 1).
Also, at *Echen* cultivar, it was recorded a high transpiration intensity, this process being conditional on the leaf water content (Tables 7, 5).
The *Dolce* cultivar showed the lowest leaf water content, of 62.87%, compared to the other analyzed cultivars. It was noted that, for this cultivar, the low leaf watter content diminished the intensity of photosynthesis and transpiration processes (Tables 7, 1, 5).
The rest of the studied *P. granatum* cultivars showed an approximately equal leaf water content, with values between 64.8% and 65.8%.
The highest leaf dry matter content was registered at *Dolce* cultivar, of 37.13%, followed by *Nikitski ranni* and "*Local Selection*".
It was found that the *Echen* cultivar, whose leaves had the lowest dry matter content, of 31.82%, can not be explained because the intensity of the photosynthesis process was higher (it is known that photosynthesis process leads to the accumulation of leaf dry matter) (Tables 7, 1).
Data obtained showed that the *Kandahar*, *Mollar* and *Shahvar* cultivars had a similar leaf dry matter content, therefore this indicator does not allow the correlation with the photosynthesis process, process that presented fluctuations of values for each individual studied cultivar (Tables 7, 1). It is considered that part of leaf dry matter resulted from photosynthesis was translocated to growing vegetative organs.
The accumulation of dry matter in plant organs is mostly the result of the photosynthesis efficiency. Also, the amount of dry matter in *P. granatum* leaves varies, depending on the cultivar type, cultivar age, or the leaves position on the branches.

Total leaf water and dry matter content of *P. granatum* during the Flowering Phenophase

During the flowering phenophase, the leaf water content of *P. granatum* cultivars showed a decreasement of approximately 1.04 times at all studied cultivars compared to the growth phenophase (Table 8).
Also, it was found that the leaf water content did not influence the transpiration process which registered different intensities from one cultivar to another (Tables 8, 6).

Table 8. Total leaf water and dry matter content of *P. granatum* during the Flowering Phenophase

Cultivar	Water content (%)	Dry matter content (%)
Hicaz	62.59	37.41
Kandahar	64.26	35.74
Nnikitski ranni	58.11	41.89
Echen	61.15	38.85
Mollar	63.27	36.73
Shahvar	62.53	37.47
Dolce	61.60	38.40
Local selection	63.18	36.82

At *Hicaz*, *Kandahar* and *Local Selection*, the leaf water content had values between 62.59% and 64.26%, lower than the ones registered during the growth phenophase. These values are in interrelation with the high intensities of the transpiration process (some of the water quantity present in leaves was eliminated) (Table 8, 6).

The lowest leaf water content was determined at *Nikitski ranni*, of 58.11%.

During the flowering phenophase, the highest leaf dry matter content was recorded at *Echen* cultivar, which was 1.22 times higher compared to growth phenophase. Also its photosynthesis process intensified 1.25 times (probably, the biosynthesized organic substance following the photosynthesis process was present in the dry matter content) (Tables 8, 2).

Withal, the leaf dry matter content of *Hicaz, Kandahar, Nikitski ranni, Mollar, Shahvar, Dolce, Local selection* registered an increase-ment, compared to the growth phenophase, explained by a high photosynthesis process intensity (Tables 8, 2). Yet these cultivars' leaf dry matter content was 1.15 times lower that *Echen*'s.

Therefore, this biochemical indicator can characterize and differentiate the analyzed cultivars, influencing their metabolism and, the degree of acclimatization.

The intensity of the Respiration process in *P. granatum* leaves during the Growth Phenophase

During the growth phenophase, the respiration process was carried out with different intensity values at the analyzed cultivars: between -2.46 (*Mollar*) and -3.36 μmol/CO_2/m^2/s (*Shahvar*) (Table 9).

Table 9. The intensity of Respiration process in *P. granatum* leaves during the Growth Phenophase

Cultivar	Light intensity (μmol/m^2/s)	Temperature (°C)	Respiration intensity (μmol/CO_2/m^2/s)
Hicaz	1245	18.0	- 2.77
Kandahar	1189	18.1	- 2.94
Nnikitski ranni	1204	17.7	- 3.18
Echen	1153	17.5	- 2.59
Mollar	1083	18.6	- 2.46
Shahvar	1073	19.0	- 3.36
Dolce	1126	17.7	- 3.13
Local selection	1119	17.8	- 3.06

The lowest respiration intensity was registered in *Mollar* cultivar leaves, that also had the slightest biometry (based on macroscopic observations).

The *Shahvar* cultivar had the highest respiration process intensity.

The biochemical energy requirement for biosynthesis of organic substances involved in the growing process made the *Shahvar* cultivar have the largest biometry (based on microscopic observations).

The intensity of the Respiration process in *P. granatum* leaves during the Flowering Phenophase

During the flowering phenophase there was a difference in the floral evocation of the analyzed cultivars, as follows: *Hicaz*, *Kandahar*, *Nikitski ranni* and *Local selection* cultivar were characterized by the appearance of the first flowers while the other cultivars were not.

It was revealed that the respiration process had higher intensities in flowery cultivars compared to the ones whose floral evocation did not happen (Table 10).

Analyzing the cultivars in terms of metabolism, namely the photosynthesis and respiration values, representing the anabolic side, respectively the catabolic side, it was found that the early flowery cultivars had a more intense metabolism compared to those of which the flowering process did not manifest during the determination.

Table 10. The intensity of Respiration process in *P. granatum* leaves during the Flowering Phenophase

Cultivar	Light intensity ($\mu mol/m^2/s$)	Temperature (°C)	Respiration intensity ($\mu mol/CO_2/m^2/s$)
Hicaz	1402	18.8	- 3.75
Kandahar	1514	19.7	- 4.85
Nnikitski ranni	1415	18.2	- 4.50
Echen	1425	19.5	- 3.55
Mollar	1505	18.5	- 2.88
Shahvar	1498	18.2	- 3.56
Dolce	1567	19.1	- 3.61
Local selection	1586	18.5	- 4.04

CONCLUSIONS

The *Hicaz*, *Nikitski ranni*, *Echen*, *Mollar* cultivars are the first ones that entered the vegetative period, justifying a more intense metabolic reactions during the research.

Only *Hicaz*, *Nikitski ranni*, *Kandahar* and *Local Selection* cultivars registered the floral evocation during the research, marking the beginning of acclimatization process

Regarding *P. granatum* species, the physiological processes and biochemical parameters are directly influenced by the type of cultivar and by the phenophase.

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EFFECT OF DIFFERENT AMINO ACID FOLIAR FERTILIZERS ON YIELD AND FRUIT QUALITY OF 'REDIX' APPLE CULTIVAR

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Abstract

In fruit orchards the foliar application of nutrients is very important especially in certain periods when fruit trees required important quantity of different compounds. The present study was aimed to determine the effect of foliar application of amino acids on yield and fruit quality of 'Redix' apple cultivar. The field experiments were carried in two succesive seasons of 2016 and 2017, in a superintensive apple experimental orchard of USAMV Bucharest. The fertilizers utilized in this experiment were Terra sorb complex, Terra sorb foliar, Naturamin, Hit AA in different concentration. Fruit quality: average fruit size, weight, firmness, shape index (length/diameter) and also fruit chemical features: dry matter, total soluble solids content of fruits and fruit acidity were recorded. Results indicates that all the applied treatments were very effective in increase yield and fruit quality of 'Redix' than control unfertilized.

Key words*: firmness, weight, acidity, foliar application.*

INTRODUCTION

The production of apple using sustainable and environmentally friendly agricultural practices plays an essential role in determining their market value and nutritional benefits.
In recent years, different strategies have been applied to enhance the quality and productivity of apple without compromising quality standards (Tanou et al., 2017; Sala et al., 2014; Jivan and Sala, 2013; Amiri et al., 2012).
One of these is using fertilizers which can be applied through the plant root suystem or through leaves and each of these treatments has its advantages and disavantages (Murtic et al., 2017; Dudu et al., 2015; Amiri et al., 2012; Jafapour and Poursakhi, 2011).
Foliar fertilization has advantages of low application rates, uniform distribution of fertilizer materials, easiest method of application and quick responses to applied nutrients (Abd El-Gleel Mosa et al., 2015).
Amino acids act as buffers for maintain favorable pH value within the plant cell and also have a chelating effect on micro nutrients when applied together making absorption and transportation of micro nutrients easier inside the plant (Abo-Elmagd et al., 2015).
Today, foliar spraying of aminoacids in most of the fruit orchards in the world has become usual and its positive effects on fruit growth and quality characteristics are evident (Murtic et al., 2017; Arabloo et al., 2017; Molaie et al., 2013; Fayek et al., 2011; Morales-Payan and Stall, 2003; Koksal et al., 1999).
The objectiv of this study were to test several amino acids formulations by applied them during growth and development of fruit and determining the impact on quality and yield of the 'Redix' apple cultivar.

MATERIALS AND METHODS

The experiment was carried out at the USAMV Bucharest experimental orchard during the period 2016-2017 in an superintensive orchard planted in 2012 with 'Redix' apple cultivar grafted on the M9 rootstock, at planting distance of 3.5 x 1 m.
In both years of investigation the following foliar fertilization treatments were applied to the plots:

Vo - Control (water only);
V1 - Terra sorb complex applied to leaves as a 0.2% solution;
V2 - Terra sorb foliar applied to leaves as a 0.2% solution;
V3 - Naturamin applied to leaves as a 0.2% solution;
V4 - Hit AA applied to leaves as a 0.2% solution.

First treatment was applied at petal drop and next treatments were done every two weeks. Each year, the chemical composition of the fruits was analysed with specific methods in three replications. Yield per tree (kg) 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 traits.

Dry matter of fruits was determined with a gravimetric method through drying an aliquot ~5 g of fruit tissue at 105°C to constant weight.

Titratable acidity (TA) was determined by titration of an extract of fruit homogenate with 0.1 N NaOH to the end point of pH 8.1 and expressed as malic acid percent.

Soluble solids (SS) expressed as °Brix were measured in juice pressed from the whole fruit sample using a digital refractometer (model PR-101, ATAGO, Tokyo, Japan) at 21°C.

Fruit firmness (kg/cm^2) was measured using a penetrometer (FT-327) with a 11-mm diameter probe from three different areas (top, middle and bottom) of the whole fruit.

RESULTS AND DISCUSSIONS

According to our results the foliar application of amino acids improved both quality and yield of 'Redix' apple fruit.

The obtained results showed that the foliar application of amino acids gave great increases in the yield, fruit firmness, average fruit weight, soluble solids content, and decreased the fruit acidity.

It can be noticed that foliar fertilizers application does not significantly influence fruit diameter and fruit length in both seasons as compared to the control.

The fruit weight of apple increased signify-cantly from 173.26 g in the control variant to 188.80g in Terra sorb foliar variant (V2) and 190.86 g in Naturamin variant (V3) and also 225 g in Hit AA (V4) in 2016 year (Table 1).

The similar results were obtained by Arabloo et al., 2017 who have found a significant increase in fruit weight for 'Golden Delicious' and 'Granny Smith' cultivars fertilized with amino acids.

The average fruit weight was higher in 2017 compared to 2016.

The all foliar application also resulted in a significant increase in yield of apple fruit as compared to the control. Kamiab et al. (2015) reported that foliar spraying of amino acids increased the quantitative and qualitative characteristics of pistachio.

Table 1. Influence of foliar fertilizer on fruit size, fruit weight and fruit yield of 'Redix' cultivar during 2016-2017

Treatment	Fruit weight (g)		Fruit diameter (mm)		Fruit length (mm)		Fruit yield/tree (kg)		Yield (t/ha)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
V0 - water	173.26	183.06	77.00	70.60	9.03	8.71	10.57	12.27	30.20	35.06
V1 - Terra sorb complex	175.21	186.75	75.00	71.05	10.51	9.52	20.32	18.30	58.05	52.28
V2 - Terra sorb foliar	188.80	198.28	77.50	73.70	10.33	9.82	24.36	16.66	69.60	47.60
V3 - Naturamin	190.86	192.21	79.00	74.55	9.13	10.20	20.07	21.33	57.34	60.94
V4 - Hit AA	225.12	197.99	81.20	71.80	9.06	10.68	21.84	30.69	62.40	87.68

Data presented in table 2 show that in all foliar treatments (V1-V4), dry matter content was higher in comparison to control variant (V0) both in 2016 as weel as in 2017. The highest dry matter content in apple fruit was determined in variant V4 (16.35 g%) followed by the variant V3 (16.32 g%) and V2 (16.07 g%) compared to the control variant V0 (14.91 g%). The values of this compound are slightly low in 2017 compared with 2016.

Table 2. Influence of foliar fertilizer on fruit quality parameters of 'Redix' cultivar in 2016-2017

Treatment	Dry matter (g%)		Firmness kg/cm^2		Soluble solids °Brix		Titratable acidity (malic acid %)		SS/TA ratio	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
V0-water	14.91	14.65	9.03	8.71	10.62	10.2	0.96	0.85	11.06	12
V1 - Terra sorb complex	15.60	14.98	10.51	9.52	12.04	11.03	0.85	0.82	14.16	13.45
V2 - Terra sorb foliar	16.07	15.08	10.33	9.82	11.10	10.65	0.91	0.84	12.20	12.68
V3 - Naturamin	16.32	15.11	9.13	10.20	12.78	11.76	0.88	0.80	14.52	14.70
V4 - Hit AA	16.35	15.68	9.06	10.68	13.80	12.20	0.81	0.79	17.04	15.44

Foliar application of V1 and V2 foliar fertilizers resulted in a significant increase in apple fruit firmness. Firmness of the fruit to which the Naturamin (9.13 kg/cm^2) and Hit AA (9.06 kg/cm^2) fertilizers has been applied was very close to the value of the control (9.03 kg/cm^2) in 2016 year. Similar results for fruit firmness were obtained by Arabloo et al., 2017 on 'Golden Delicious' and 'Granny Smith' apple cultivars. Milosevic and Milosevic, 2015 reported that spraying with foliar fertilizer increased fruit firmness of 'Idared' apple cultivar .

The TA content of apple fruit was not significantly affected by the application of foliar fertilizers. However application of Naturamin and Hit AA fertilizers decreased titratable acidity percent.

From the results obtained, the all foliar application of amino acids improved TSS and TSS/ acid ratio and decreased acidity percentage in the fruits as compared to the control in the two seasons studied. The results of our experiment confirm data obtained by Abo-Elmagd et al., 2015, that found a positive effect of amino acids on the soluble solids content of apple fruit. However results obtained by Malik and Singh, 2006 showed that total soluble solids content of fruit was reduced by the aqueous solutions of amino acids when sprayed on mango, cv. 'Kensington pride" like foliar fertilizers. Also positive impact of amino acids foliar spray on fruit quality was also supported by other authors (Fayek et al., 2011; Khan et al., 2012).

CONCLUSIONS

The foliar application of amino acids had a positive effect to improve the yield and fruit quality of 'Redix' apple trees.

Application of Naturamin and Hit AA fertilizers had the highest positive effect to improve the percentages of yield and average fruit weight. Also, it increased dry matter content of apple in both seasons, as compared to the control treatment.

All fertilizers used decreased the percentage of acidity, but differences were not significant among treatments for each year studied.

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RESEARCHES ON GENETIC RESISTANCE TO APRICOT VARIETY 'BERGERON' TO FROST FROM WINTER

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Abstract

The research was conducted in a orchard of apricot aged 7 years, located at 50 km away from Bucharest. Trees planted at a distance of 4x4m were studied during the vegetation season paying particular attention to resistance to low temperature. In the winter of 2013, temperatures in winter decreased to -20 degree C and produced losses of flower buds. In the spring of 2014 the 'Bergeron' variety bloomed well, 100% of flower buds being resistant to negative temperatures from winter. In 2015 was a warm winter but the temperature decreased in January to -29 degree C and the trees suffered a thermal shock. Following, resistance to frost decreased and the percentage of flower buds affected was 98.2%. Production losses were very high and vegetative buds were not affected by frost and the trees were properly vegetated grew normally. In January of 2016 were recorded temperatures of -25 degrees C associated with very cold wind, the flower buds were affected in 100%. In 2017 'Bergeron' variety was not affected.

Key words*: flower buds, temperatures, thermal shock.*

INTRODUCTION

The apricot is a species appreciated for fruit quality and is grown successfully in areas with high temperatures (Cociu V. et al., 2000; Sestraş R. et al., 2004). In Romania, grows in the S part of the country and in the Plain of V. In Romania, the apricot, do not make the fruits every year because of the low temperatures that affect the flowers (Branişte N. et al., 2007). The 'Bergeron' is a new variety introduced in crop and has not been studied for resistance to low winter temperatures and temperatures during the flowering period (Ghena N., Branişte N., 2003).

We made a trial with 'Bergeron' variety to study resistance to winter cold, fructification and fruit quality.

As a result of the researches we were able to establish the resistance limit at low temperatures and I observed that the 'Bergeron' variety behaves differently from one year to another depending on the evolution of the temperatures. In cold winters associated with wind, production losses are very high, up to 100% (Păun C., 2017).

Researchers are concerned about obtaining apricot varieties that start late in vegetative and resist winter frost.

MATERIALS AND METHODS

Research and observations were made in an orchard set up in 2010, located at a distance of 50 km N, compared to Bucharest. The studies began in 2013, when the orchard was in the 3rd year of vegetation, and the trees began to fruit and continued until 2017. The biological material studied was the 'Bergeron' apricot variety produced in Romania, grafted on the Myrobolan 29 C rootstock. The trees were planted at a distance of 4 m between the rows and 4 m between the plants per row and were led as a vessel. The fructification cuts were made in August, after harvesting the fruit. Soil has been kept clean by weeds by manual and mechanical work. Were studied 50 trees in 5 rehearsals. To record the temperatures during winter and during the vegetation period we used our own thermometers and meteorological data from the nearby weather station. To determine the percentage of buds affected by low winter temperatures, we made observations in the laboratory where we analyzed at microscope the fruit buds and vegetative buds and in the spring we made observations on the field. Observations were made in November (before frost), in February, at the beginning of the vegetative period (March) and during the

vegetative period. After the flowering we determined the percentage of flowers formed to determine the influence of winter cold on fruit buds. During the vegetation period, we made observations on the percentage of fruit formed, the length of the shoots, the production of the fruit obtained and the qualities of the fruits. The shoots were measured at the end of July, after stopped of growth. The fruits were weighed in the laboratory and analyzed for determinate the average fruit weight, colour, pulp weight and kernel weight.

RESULTS AND DISCUSSIONS

In the 3rd year of vegetation, the 'Bergeron' variety went through the winter without losing the fruit buds. This year, the first fruit production was obtained.

Research into the resistance of fruit buds to winter cold began in the winter of 2014 when the trees were 4 years old.

The year 2014 was a normal year from the thermal point of view and the trees resisted very well during the winter.

In spring, the trees flourished normally without the loss of fruit buds.

Fruit buds resisted 100% and fruit production was normal (Table 1).

Table 1. Behavior of the 'Bergeron' variety in the winter 2014

Variety	Rehearsal	Nr. buds analyzed	% viable fruit buds	% affected buds
'Bergeron'	R1	380	100	0
	R2	300	100	0
	R3	420	100	0
	R4	200	100	0
	R5	470	100	0
Average		354	100	0

Table 2. Behavior of the 'Bergeron' variety in the winter 2015

Variety	Rehearsal	Nr. buds analyzed	% viable fruit buds	% affected buds
'Bergeron'	R1	500	1.4	98.6
	R2	347	2.7	97.3
	R3	289	1.6	98.4
	R4	320	1.5	98.5
	R5	415	1.8	98.2
Average		374,2	1.8	98.2

Table 3. Behavior of the 'Bergeron' variety in the winter 2016

Variety	Rehearsal	Nr. buds analyzed	% viable fruit buds	% affected buds
'Bergeron'	R1	620	0	100
	R2	514	0	100
	R3	387	0	100
	R4	427	0	100
	R5	489	0	100
Average		487.8	0	100

Table 4. Behavior of the 'Bergeron' variety in the winter 2017

Variety	Rehearsal	Nr. buds analyzed	% viable fruit buds	% affected buds
'Bergeron'	R1	382	98.7	0.7
	R2	417	98.4	0.6
	R3	354	99.7	0.3
	R4	472	99.4	0.6
	R5	395	99.3	0.7
Average		404	99.1	0.5

Table 5. Analysis of the influence of low temperatures on the percentage of fruit formed

Variety	Rehearsal	2014 % fruit formed	2015 % fruit formed	2017 % fruit formed
'Bergeron'	R1	31	58	27
	R2	28	60	31
	R3	38	62	25
	R4	37	58	28
	R5	35	55	24
Average		33.8	58.6	27

In 2014 and 2017, winter temperatures decreased to -25 ° C, but the temperature drop was gradual, and the buds were resistance and was achieved a normal production. In 2015 the heat shock destroyed the buds, and in 2016 the very cold wind from the winter period amplified the cold and destroyed all the fruit buds (Table 2 and Table 3). Observations regarding the genetic resistance of flowers at low temperatures during bloom showed that the 'Bergeron' variety very well tolerates low temperatures. In years with high production, when winter frost did not destroy fruit buds, the flowers were not affected by low temperatures during the flowering period. In 2014 and 2017 during the bloom when the flowers were opened, during the night were temperatures of -3 ... -4 degree C, and the apricot flowers were

not affected (Table 4). The results regarding the influence of low temperatures on the percentage of fruits formed are presented in table 5.

During the vegetation period observations were made on the length of the shoots, number of shoots/tree, number of vegetative buds/shoots and number of fruits buds/shoot (Table 6).

The fruits were analyzed morphologically and was determined the production on the tree (Table 7). At the end of the growing season (July), the shoots were measured in length and the number of vegetative and fruit buds was determined on each shoots for estimate the production of the following year.

The correlation between fruit weight and pulp weight is presented in figure 1, and the correlation between fruit weight and pulp weight is presented in figure 2.

Table 6. Observations on the growth of shoots

Variety	Year	The length of shoots (cm)	Nr. of shoots/tree	Nr. of vegetative buds/shoots	Nr. of fruit buds /shoot
'Bergeron'	2014	62	120	58	121
	2015	58	134	49	102
	2016	50	119	47	94
	2017	45	143	38	84
Average		43	102.4	38.4	80.2

Table 7. Observations on characteristics fruits and productivity

Variety	Year	Average fruit weight (g)	Weight of pulp (g)	Weight of kernel (g)	Fruit production (kg/tree)
'Bergeron'	2014	86	80.2	5.8	14
	2015	90	84.3	5.7	2
	2016	0	0	0	0
	2017	92	85.9	6.1	21
Average		89.3	83.4	5.8	12.3

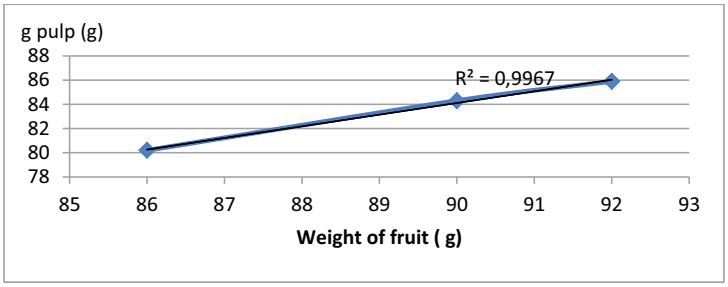

Figure 1. The correlation between fruit weight and pulp weight

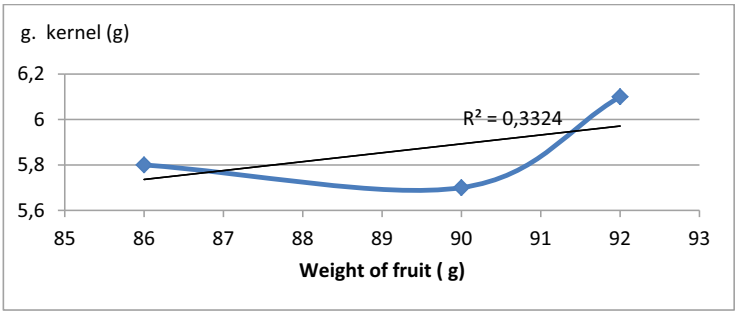

Figure 2. The correlation between fruit weight and pulp weight

CONCLUSIONS

'Bergeron' variety is a relatively resistant variety to low winter temperatures and resistance depends on how low the temperature is.

For protect the 'Bergeron' variety to low temperature from winter, the orchard must be established in areas where the wind does not blow.

Under thermal shock, fruit buds can be affected to 98.2%, and cold wind causes 100% fruit bud losses.

If the fruit buds are not affected during the winter, the 'Bergeron' variety fructify normally, being resistant to low temperatures during the flowering period

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STUDY OF THE CYTOLOGICAL CHARACTERISTICS AND GERMINATION POTENTIAL OF SOME PEACH CULTIVARS FROM RSFG CONSTANŢA

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Abstract

The paper shows the results of the genetic potential of some peach cultivars (Prunus persica L.) used as genitors in the breeding process. A detailed morphological and structural description of pollen grains of four peach cultivars 'Raluca', 'Florin', 'Filip' and 'Monica' was done. The pollen was collected from peach flowers in April from the demostrative plots at Research Station for Fruit Growing Constanta (RSFG Constanta). The viability and pollinated tube cultures were made in order to determine the fertility and germination capacity of the analyzed biological material. The one with the highest viability of the pollen grains was recorded at 'Raluca'. 'Florin' cv. has been shown the highest germination capacity on a 15% sucrose nutrient medium after a 2 hour sprouting time (89.6%), followed by a 5% sucrose nutrient medium with after 2 hours of germination time (88.9%).

Key words: *Prunus persica L., pollen, sucrose.*

INTRODUCTION

The peach is one of the most appreciated species that is very very well adapted to the southeastern part of the country.
The studied peach cultivars were created to RSFG Constanta and belong to the fresh consumption type.
Pollen sterility is a character that is finding in few cultivars of peach, being a recessive and monogamy character (Scott and Weinberger, quoteds by Cociu V., 1981).
Reached by stigma, due to wind, insects, birds, water and humans, the pollen grains are soaked with water from the stigmatic liquid, it is swells and germinates: the exine cracks and intine exits out like a pollinic tube through the germination pore.
The pollen is retained by the stigma and absorbs the water from the adhesive mixture that is produced by it; it is moisturizes and then germinates.
In general, pollen moisturizing lasts from few seconds to few minutes and germination begins after 45-210 minutes, both phenomena depending on the species (Andrei, 1978; Şerbănescu-Jitariu and Toma, 1980).

MATERIALS AND METHODS

In order to analyze **the viability of the pollen**, were used substances that bind H_2-catalysed dehydrazes resulting a new colored substance (*formazam*) that distinguished the viable pollen from the unviable one. The mature anthers strike on a lamella in a drop of 0.5% carmin acetic solution; 3 - 5 preparations are microscopic analysis, respectively 5 microscopic fields of each preparation; microscopic fields include red pollen granules because viable granules are colored in red while unviable pollen retains its original yellow colored and there are wrinkled (Andrei and Paraschivoiu, 2003; Doroftei et al., 2008). The statistical interpretation was based on the number of germinated and non-germinated pollen grains from a total of 1000 pollen grains from the analyzed microscopic fields in 3 repetitions, in order to determine the germination capacity of pollen grains.
For microscopic analysis, observations were made and images were taken on a FLUO 2 research microscope provided with a BEL PHOTONICS DV-1300 video camera.
Determination of pollen germination capacity was achieved by collecting pollen of

peach cultivars at the time of full flowering: put 5%, 10% and 15% sucrose solutions on one blade, sprinkle mature pollen on each blade and analyzed at a microscope at 30 minute intervals (up to two hours); the coloring with a 0.5% carmin acetic solution for 2-3 minutes, then covering with the lamella. Three to five preparations are analyzed for each experimental variant, 5 microscopic fields of each preparation are numbered, and germinated and non-germinated pollen granules are included in the microscopic fields (Andrei and Paraschivoiu, 2003; Doroftei et al., 2008).

In order to determine the germination capacity of pollen grains the statistical interpretation was based on the number of germinated and non-germinated pollen grains from a total of 1000 pollen grains from the analyzed microscopic fields in 3 repetitions. For microscopic analysis, observations were made and images were taken on a FLUO 2 research microscope provided with a BEL PHOTONICS DV-1300 video camera.

RESULTS AND DISCUSSIONS

Morphological an structural characteristics of pollen grains to peaches cultivars studied

The cross sections in the antennas at different stages point to the existence of a homogeneous mass of meristematic cells (at the beginning); differentiation of the array of hypodermic cell strings (at a more advanced stage); repeated division of the anther's cells and differentiation of the primary parietal layer as well as the primary sporogen layer.

The parietal layers that appear a little bit later are: epidermis, endothelium, several medium layers and the inner most layer called wallpaper, whose physiological importance is special because it serves to nourish spores.

The sporogenic tissue has as orgin primary sporogenic cells. Some of the sporogen cells degenerated frequently and are absorbed by the rest of the cells. The resulted microspors from the microsporus parent cell divisions are disposed in tetrades that can be of different types.

The distinct elements of the pollen grains are the polar axis, the equatorial axis, and the poles. Pollen granules analyzed have different shapes: spherical, oval or triangular, with average dimensions ranging from 19-30 µm (Table 1).

The pollen membrane has a complex composition that differs from the outside consisting of ectexin and endexin.

Exina is covered with tubers or beaks of different shapes and sizes, with positive elements prevailing, the surface of which is praying, with ornamental elements larger than one micrometer, elongated and distributed irregularly.

In the field of microscopy they appear as elongated, irregularly spaced ridges (Table 1, Figure 1).

Apertures or germinating apertures include colps and pores. Colps have different shapes and can form with the granule (with regular margins) or later than the granule (with irregular margins).

On the analyzed microscopic preparations we usually observed 3 colts, but their number can be between 2 and 4.

The pores are cone-shaped acamerate. The main pollen morphological types observed at the microscope were: bicolpat, polarized or tricolpic pollen, tricolor (Table 1).

Table 1. The characteristics of the analized pollen from four peach cv.

Cultivars	Average size	Shape	Elements of the exine	Apertures
'Raluca'	28 µm	triangular, spheroidal	rugulată	tricolpat triporat
'Florin'	30 µm	triangular,	rugulată	tricolpat triporat
'Filip'	26 µm	triangular, spheroidal	rugulată	tricolpat triporat monocolpat monoporat
'Monica'	23 µm	triangular, oval	rugulată	tricolpat triporat bicolpat biporat

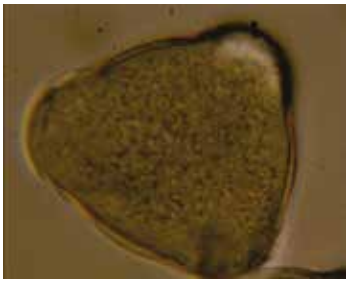

Figure 1. Morphological aspect of pollen grains in *Prunus persica* L. 'Monica' (1000x). There are exine, intine, 3 germinating pores and the surface of the exine

The viability of the pollen it can be seen as one of the most fertile peach from the cultivars analyzed, with the highest viability is 'Raluca' and low fertile with minimal viability 'Florin' (Table 2).

Table 2. The viability of the pollen for the analized cultivars

Cultivars	The total number of the analized pollen grains	The number of unviable pollen grains	The number of viable pollen grains	Viability %
'Raluca'	1000	125	875	87.5
'Florin'	1000	258	742	74.2
'Filip'	1000	189	811	81.1
'Monica'	1000	176	824	82.4

Germination capacity The comparative analysis of the four peach cultivars showed that the highest germination capacity was observed to the 'Florin' cultivar on a 15% sucrose nutrient medium after a 2 hours germination time (89.6%) (Figure 2), followed by 5% sucrose nutrient medium after 2 hours of germination time (88.9%). This cultivar proved to be fertile, although viability was the smallest of the four analyzed varieties (74.2%) (Table 3).

At 'Raluca' cultivars, the highest germination capacity was observed on a 5% sucrose nutrient medium after a 2 hours (85.9%) germination time (Figure 3) and the lowest on a 10% sucrose nutrient medium after a germination time of 30 minutes (22%) (Table 3).

At the 'Filip' cultivars, the highest germination capacity was observed on a 5% sucrose nutrient medium after a 2 hours germination time (72.4%) (Figure 4) and the lowest on a 15% sucrose nutrient medium after a germination time of 30 minutes (42%).

'Monica's' cultivar highest germination capacity was observed on a 10% sucrose nutrient medium after a 2 hours germination time (61.2%), and the lowest on a 5% sucrose nutrient medium after a germination time of 30 minutes (24.3%) (Figure 5).

Table 3. The germination capacity of analyzed peach cultivars

Cultivars	Treatment	Total number of pollen grains	Germinated pollen grains	Non-germinated pollen grains	Germination capacity %
'Raluca'	Distillated water (Control)	1000	0	1000	0
	Sucrose 5%, 30 minutes	1000	556	444	55.6
	Sucrose 5%, 1 hour	1000	668	332	66.8
	Sucrose 5%, 2 hours	1000	859	111	85.9
	Sucrose 10%, 30 minutes	1000	220	780	22.0
	Sucrose 10% 1 hour	1000	276	724	27.6
	Sucrose 10% 2 hours	1000	283	717	28.3
	Sucrose 15%, 30 minutes	1000	478	522	47.8
	Sucrose 15%, 1 hour	1000	544	456	54.4
	Sucrose 15%, 2 hours	1000	604	396	60.4
'Florin'	Distillated water (Control)	1000	0	1000	0
	Sucrose 5%, 30 minutes	1000	678	322	67.8
	Sucrose 5%, 1 hour	1000	790	210	79.0
	Sucrose 5%, 2 hours	1000	889	111	88.9
	Sucrose 10%, 30 minutes	1000	584	416	58.4
	Sucrose 10% 1 hour	1000	667	333	66.7
	Sucrose 10% 2 hours	1000	834	166	83.4
	Sucrose 15%, 30 minutes	1000	632	368	63.2
	Sucrose 15%, 1 hour	1000	821	179	82.1
	Sucrose 15%, 2 hours	1000	896	104	89.6
'Filip'	Distillated water (Control)	1000	0	1000	0
	Sucrose 5%, 30 minutes	1000	482	518	48.2
	Sucrose 5%, 1 hour	1000	663	327	66.3
	Sucrose 5%, 2 hours	1000	724	276	72.4
	Sucrose 10%, 30 minutes	1000	355	645	35.5
	Sucrose 10% 1 hour	1000	507	493	50.7
	Sucrose 10% 2 hours	1000	654	346	65,4
	Sucrose 15%, 30 minutes	1000	420	580	42.0
	Sucrose 15%, 1 hour	1000	506	494	50.6
	Sucrose 15%, 2 hours	1000	591	409	59.1
'Monica'	Distillated water (Control)	1000	0	1000	0
	Sucrose 5%, 30 minutes	1000	243	757	24.3

Cultivars	Treatment	Total number of pollen grains	Germinated pollen grains	Non-germinated pollen grains	Germination capacity %
	Sucrose 5%, 1 hour	1000	305	695	30.5
	Sucrose 5%, 2 hours	1000	347	653	34.7
	Sucrose 10%, 30 minutes	1000	462	538	46.2
	Sucrose 10% 1 hour	1000	506	494	50.6
	Sucrose 10% 2 hours	1000	612	388	61.2
	Sucrose 15%, 30 minutes	1000	269	731	26.9
	Sucrose 15%, 1 hour	1000	318	682	31.8
	Sucrose 15%, 2 hours	1000	364	636	36.4

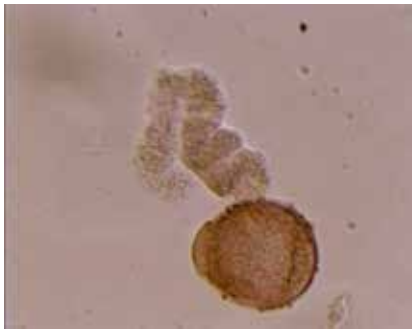

Figure 2. Pollen grains of *Prunus persica* 'Florin' on sucrose 15%, 2 hours (400x)

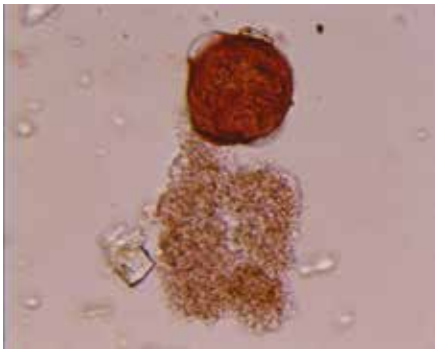

Figure 3. Pollen grains of *Prunus persica* 'Raluca' on sucrose 5%, 2 hours (400x)

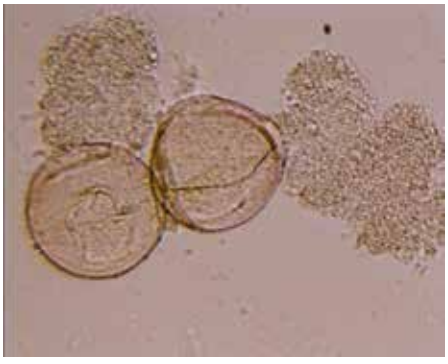

Figure 4. Pollen grains of *Prunus persica* 'Filip' on sucrose 5%, 2 hours (400x)

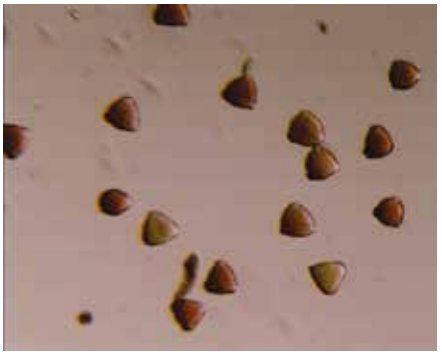

Figure 5. Pollen grains of *Prunus persica* 'Filip' on sucrose 5%, 30 minutes (100x)

CONCLUSIONS

The analyzed pollen granules have different shapes: spherical, oval or triangular, with average dimensions ranging from 19-30 μm.
Exina is covered with tubers or beaks of different shapes and sizes with positive elements predominantly, the surface is rugulate, with ornamental elements larger than one micrometer, elongated and distributed irregularly. Three colps and three pores were usually observed on the analyzed microscopic preparations, but their number may range from 2 to 4. The pores are conical.
From all peach varieties analyzed the one with the highest viability is 'Raluca' and the least viable is 'Florin'.
The comparative analysis of the five peach and nectarine varieties showed that the highest germination capacity was observed in the 'Florin' variety on a 15% sucrose nutrient medium after a 2 hours sprouting time (89.6%) followed by the medium 5% sucrose nutrition after 2 hours of germination time (88.9%).
The comparative analysis of the four peach cultivars showed that the highest germination capacity was observed in the 'Florin' cv. on a 15% sucrose nutrient medium after a 2 hours sprouting time (89.6%) followed by the 5% sucrose nutrition medium after 2 hours of germination time (88.9%).

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BEHAVIOR OF SOME APRICOT CULTIVARS GRAFTED ON NEW VEGETATIVE ROOTSTOCKS

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Abstract

In the Dobrogea area an important component in obtaining orchards with notable economic performances is the finding of the best variety-rootstock combinations. The different physical and chemical structure and composition of soil in Dobrogea obliges to find scientific solutions that will come to the future farmers. At RSFG Constanta in the period 2013-2018, the compatibility and development of a large apricot species grafted on different rootstocks originating in the Mediterranean area was assessed. Thus, the 'Amiral' apricot variety was grafted on the following rootstocks: Adesoto, Myrobolan 29 C, Weiwa, Ishtara. As a result of the measurements and the analyzed data, it was noticed that the Ishtara rootstock shows an increased compatibility and, once again, imparts a medium vigour, which means that by introducing the multiplication of this rootstock we have high-density orchards, so the fruit growing in the Dobrogea area a model to be followed for other apricot-favorable areas.

Key words*: Prunus armeniaca (L.), compatibility, orchard systems.*

INTRODUCTION

The used rootstocks for apricot are originated from six species of *Prunus* genus: *P. armeniaca* L., *P. cerasifera* Ehrh., *P. domestica* L., *P. insititia* Jussl., *P. persica* Stock., *P. amygdalus* L. (Cociu et al., 1993). All over the world the apricot rootstocks are used according to the pedoclimatic conditions, affinity, cropping systems, etc. Among the most frequently used are small apricot, franc apricot, Manicot (GF 1236), cherry plum, myrobolan plum, franc plum (Bălan et al., 2008).

MATERIALS AND METHODS

The study was carried out in apricot demonstrative lots at Research Station for Fruit Growing Constanta, located in south-eastern Romania, near the Black Sea.

The site is located at 44°10' Northern latitude and 28°29' Eastern longitude, and 70 m above sea level. Climate is continental with warm and droughty summers, frequent dry winds all the year round and temperate winter generally without snow.

The mean annual temperature is 12.0°C and the total active temperature is 3988°C, out of which 3170°C during the growing season; the annual precipitation amount is 400 mm, out of which during the growing season (April 1 to September 30), 240.7 mm. The lowest winter temperatures below -20°C are not very often: 1 out of 10-15 years and so are the spring frosts susceptible to cause apricot yield damage.

The climatic water deficit reaches as much as 400 mm/year, so irrigation application is needed for apricot. The zonal soil type is a calcaro-calcic chernozem formed on loess, with loam texture and a proper capacity of water preserving, holding and circulation. The humus content ranges between 2.5 and 4%; pH of the soil is poor alkaline (7.0-8.1).

Observations and determinations were observed in Field I and II within the RSFG fruit tree nursery. In the third year (2017) fifty fruit trees of 'Amiral' cv. grafted on each mentioned vegetative rootstocks were planted in an experimental field. The planting scheme was chosen 4/4 m, the orientation of the rows was from North to South and the shape of the crown was improved vase.

RESULTS AND DISCUSSIONS

All four rootstocks come from the Mediterranean, are used in countries like Italy, France, Spain, Turkey.

Ishtara - is a French rootstock that has medium-to-small vigour, it ensures precocity and good fruit quality; it does not ensure proper tree anchoring and has no affinity with all apricot cultivars.

Weiwa - *Prunus domestica* rootstock obtained from the cultivar 'Wangenheims'. This roostock is very resistant to "apricot and plum decline" and is tolerant to phytoplasma disease. Trees grafted onto Weiwa rootstock have 30% less vigour compared to Myrobolan 29 C (http://www.vitroplant.it/weiwa/?lang=en).

It has great affinity with all apricots and plums. Weiwa has a very positive impact on both cropping efficiency and fruit size. The rootstock is not suckering.

Adesoto - Selection of Pollizo de Murcia obtained in Spain. Interesting for the positive grafting affinity and adaptability to very calcareous and dry soils. Tolerates to *Armillaria* root-sucking.

Myrobolan 29 C - Clone selected from a progeny of *Prunus cerasifera* in California. Rootstock for plums and most apricot cultivars. Suitable for all soil types, adapts well to dry soils and heavy soils with low permeability.

Moderately resistant to *Agrobacterium tumefacens, Verticillium* and *Leptonecrosis*, susceptible to *Pseudomonas syringae* and resistant to root-knot nematodes (*Meloidogyne* spp).

Resistant to calcareous soils (8-9% active lime). Suitable for replanting. All plum cultivars shows perfect grafting compatibility and high yield efficiency. With plum cultivars, this rootstock reduces vigour by 15-20% compared to 'Myrobalan B'. Many apricot cultivars rootstocks have been breeded and are spread out without knocking their behavoir in fruit tree nursery (Field I and Filed II) regarding the grafting compatibility, scion growing and the productivity.

The percentage of grafting was 62% at Adesoto up to 98% at Ishtara (Table 1).

Regarding the trunk diameter, the lowest value was recorded at Ishtara (11.85 mm) and the highest value (18.57) was found at Myrobolan 29 C cultivar (Table 2).

In Field II the height of the trees recorded was 146 cm at Ishtara and 180 cm at Myrobolan 29 C.

The number of anticipated shoots varies from 3 to the Weiwa cultivar up to 7 for the Ishtara cultivar. The number of fruit buds ranges from 4 to Adesoto and 9 up to Ishtara.

Table 1. Number of trapped eyes and percentage of grafting, RSFG Constanta

No.	APRICOT				
	Rootstocks	Variety	The number of grafted seedlings (August 2015)	Nr. eyes caught in the first inventory (April 2016)	Percentage of grafting
1.	ADESOTO	'Amiral'	50	31	62
2.	ISHTARA		50	49	98
3.	MYROBOLAN 29 C		50	48	96
4.	WEIWA		50	42	84

*The used grafting height was 5 cm above the soil.

Table 2. Behavior of some apricot rootstocks in field II of RSFG nursery Constanta, 2016

No.	Rootstocks	Cultivar	Ø (mm)	H (cm)	Number of anticipations (pieces)	Number of fruit buds (pieces)
1	ADESOTO	Amiral	16.02	163	4	4
2	ISHTARA		11.85	146	7	9
3	MYROBOLAN 29 C		18.57	180	5	5
4	WEIWA		17.56	171	3	6
Average			16.00	165	4.75	6.00

Figure 1. Diameter of trunck in second year at Myrobolan 29 C

Figure 2. Tree height in second year at Adesoto rootstock

Figure 3. Adesoto rootstock at planting in nursery

Figure 4. Ishtara rootstock at the planting time

CONCLUSIONS

Grafting percentage was 98% for Ishtara variety.

The Ishtara cultivar shown the lowest value for the thickness of the trunk.

The number of anticipated shoots is higher for the Ishtara cultivars.

The number of fruit buds is higher for the Ishtara cultivars.

The lowest height compared to the other rootstocks was registered with the Ishtara variety.

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PRODUCTIVITY OF GOOSEBERRY VARIETIES IN THE REPUBLIC OF MOLDOVA CONDITIONS

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Abstract

In the paper are reflected the results of the scientific researches carried out in Republic of Moldova regarding the determination of the phonological phases of the cultivation of gooseberry, the productivity and the exploitation period of the intensive plantation established in 2004 with the 'Colobok', 'Captivator', 'Grushenka', 'Sadko', 'Smena', 'Severnîi capitan' varieties during the years 2015-2017 on irrigated land. Among the studied gooseberry varieties with few thorns, resistant to mildew, low temperatures and drought, showed great fruits are: 'Smena' (3.5 g), Colobok (3.4 g) and with small fruits: 'Severnîi capitan' (1.7 g) and 'Grushenka' (1.9 g). The average weight of the gooseberry fruits through the studied varieties ranged between 1.4 g and 3.3 g. The average harvest of the varieties studied grew between 6.2-18.1 t/ha.

Key words: *gooseberry, fruits weight, phenophases, variety, yield.*

INTRODUCTION

Variety occupies a central place in increasing productivity, being an independent and absolutely determined factor. On the basis of a more productive variety, without additional costs, it is possible to increase the crop yield 1.5 times and more. An intensive variety of gooseberry must combine a whole range of qualities such as: the adaptation reaction to the conditions of the growing medium, in case it does not adapt well, the accumulated energy is spent on adaptation and not on the formation of the crop, the poor resistance to diseases and pests can destroy more than 1/3 of the crop, early entry bearing, self-fertility, which guarantees harvest in unfavorable conditions for flowering, fruit quality, cold resistance, drought and the final result - the crop (Sergheeva, 1985).

In order to optimize the fuller realization of the productive potential, it is necessary to know the tolerance potential of the variety for cultivation in different ecological areas, the conditions of which correspond better to the requirements of the plantation. Although the level of resistance, which is specific to each species, variety, or even plant, is a hereditary, genetically controlled character, under optimal conditions it is latent, unrealized, and only occurs under extreme stress conditions (Ştefârţă, Alunici et al., 2001).

Gooseberry productivity vary strongly depending on climatic conditions specific to the year, characteristic features of varieties and may decrease from 12 t/ha to over 1 t/ha **(**Olhina, Riabuschina,1987).

Gooseberry harvest depends on the genetic potential of the variety, the climatic conditions established during the growing season of each year and their pollination by the bees. The productivity of gooseberry plants is influenced directly by the variety (Kawecki, 1989).

Form the productivity point of view, the studied gooseberry varieties were divided in four categories: low productivity ('Grushenka' - 1.2 t/ha), medium productivity ('Zenit' - 3.5 t/ha, 'Someş' - 3.3 t/ha, 'Orlionok', 'Finik' - 2.8 t/ha), high productivity 5.5 - 4.2 t/ha - 'Scedrâi', 'Ruskii', 'Smena', and very high productivity: 'Severnîi capitan' - 14.6 t/ha (Sava, 2010).

The varieties with vigorous growth as 'Captivator', 'Severnîi capitan', 'Colobok', under irrigation conditions, in intensive plantations (1.5 x 1.0 m) can give a high average yields ranging from 13.8 to 19.5 t/ha (Sava, 2012).

MATERIALS AND METHODS

The investigations were carried out during the years 2015-2017 according to the methods established studying shrubs and influenced the

cultivation conditions in determining the duration of the phenological phases of development of the gooseberry plants, the fruit mass and the productivity of the intensive plantation, established in the year 2004 with a planting distance of 1.5 x 1.0 m, on irrigated land, with varieties which have a few thorns and are resistant to mildew: 'Smena', 'Sadko', 'Colobok', 'Captivator', 'Severnîi capitan', 'Grushenka'.

RESULTS AND DISCUSSIONS

Climate change and its influence on the cultivation conditions of shrub species becomes more evident. The climatic conditions during the 2017 vegetation period were manifested by lower temperatures than in the previous years, starting in the third decade of April, which has led to a delay in going through the phenological phases of the development of gooseberry plants, and the rains from the end of May to the beginning of June and the low temperatures at night delayed the growth of plants.
The observations made during the evolution of the phenological phases of gooseberry during the vegetation period, 2015-2017, were introduced in Table 1.

Table 1. The duration of the phenological phases of gooseberries plants

Name of phenophases, duration between them (days)	Years			Ave rage
	2015	2016	2017	
Budding date	16.03	06.03	08.03	
Duration between phases (days)	34	34	32	33
Blooming	19.04	03.04	09.04	
Duration between phases (days)	59	72	73	68
Ripening	17.06	19.06	21.06	

As can be seen from Table 1 data, the duration between budding and blooming phases in 2015 reached 34 days. In 2016, the vegetation period began earlier, similar to the year 2002, when positive temperatures were established at the end of February and the budding took place on 06.03, the period until the flowering lasted 34 days and the flowering started on 03.04.2016. At the end of March there were frosts higher than -5 degrees C, which affected the first young leaf of goosebrry, a phenomenon that has not happened in the past. In 2017, the vegetation period began earlier, similar to 2016, and the budding took place on 08.03., the period until the flowering lasted 32 days and the flowering began on 09.04.2017. At the end of April (19-22) frosts took place and wet snow fell with a layer of 50 cm, which affected the harvest, the plants being with formed small fruits.

Figure 1. Climatic conditions during the vegetation period, 19-22.04.2017

Figure 2. Growing of gooseberry fruits

The productivity of gooseberry varieties studied per hectare depends on the climatic conditions of the year, the characteristics of each variety, the capacities to adapt to the new cultivation conditions, crop maintenance, the age of plantation etc.
The study of the gooseberry varieties introduced during 2007-2017 allowed their appreciation by mass of the fruit and the production obtained, and the results are included in Table 2.

Table 2. Production and average mass of gooseberries fruits depending on variety and year conditions

Variety	2015		2016		2017		Average	
	fruit mass, g	yield, t/ha	fruit mass, g	yield t/ha	fruit mass, g	yield, t/ha	fruit mass, g	yield, t/ha
Colobok	2.5	8.9	1.9	6.0	3.4	12.7	2.6	9.2
'Captivator'	2.2	19.2	2,9	18.0	2.1	17.1	2.4	18.1
'Sadko'	3.0	6.7	2,7	6.7	3.1	6.7	2.9	6.7
'Severnîi capitan'	1.4	15.9	1,7	22.0	1.7	15.1	1.6	17.7
'Smena'	3.0	4.7	3,4	6.7	3.5	7.3	3.3	6.2
'Grushenka'	1.1	14.0	-	-	1.7	9.8	1.4	11.9
Average	1.1-3.0	4.7-19.2	1.7-3.4	6.0-22.0	1.7-3.5	6.7-17.1	1.4-3.3	6.2-18.1

As shown in Table 2, the average weight of the fruits of gooseberry varieties ranged from 1.4 to 3.3 g values. The largest fruits were obtained in 2017, the maximum mass ranged from 1.7 g to 3.5 g.

The varieties with large fruits are: 'Smena' (3.5 g), 'Colobok' (3.4 g), 'Sadko' (3.1 g). The varieties with small fruits are: 'Severnîi capitan' (1.7 g) and 'Grushenka' (1.9 g). The average of fruit weight ranged from 1.4 g (variety 'Grushenka') to 3.3 g (variety 'Smena').

The average fruit harvest obtained for the gooseberry varieties studied ranged between 6.2 (variety 'Smena') and 18.1 t/ha (variety 'Captivator'). The maximum yield of 22.0 t/ha of gooseberry fruits was obtained in the 2016 year for the variety 'Severnîi capitan', and in 2015 year - 19.2 t/ha for the 'Captivator' variety.

Fig. 3. Gooseberry varieties with high profuctivity - 'Severnîi capitan'

Fig. 4. Gooseberry varieties with high productivity - 'Captivator'

CONCLUSIONS

On the basis of the research carried out on the gooseberry varieties introduced to the pedoclimatic conditions of the Republic of Moldova, it was established that:

The phenological phases of the development of the gooseberry plants are influenced by the climatic conditions, and on average between the stages of budding and blossoming there is a period of 33 days, and between the flowering and the maturing of the fruit the interval is 68 days. The average weight of gooseberry fruits varied between 1.4 g in the 'Grushenka' variety and 3.3 g in the 'Smena' variety.

The average fruit harvest obtained in the gooseberry varieties ranged between 6.2 ('Smena' variety) and 18.1 t/ha ('Captivator' variety).

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STUDIES ON *IN VITRO* FERTILIZATION BIOLOGY OF *MESPILUS GERMANICA* L. CV. 'İSTANBUL'

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Abstract

This study was aim to investigate the fertilization biology of 'İstanbul' medlar variety. For this purpose, firstly pollen production capacity and morphological homogeneity were assessed (hemocytometer slide). The pollen viability was tested with TTC and IKI staining methods. Pollen germination experiments were carried out in different medium with different sucrose concentration (agar in plate method) during 2 h, 6 h, 12 h and 24 h period. The number of anthers in a flower 36.5, pollen number in an anther 160.55 and pollen number in a flower 12753.8 were found. Morphological homogeneity was high with 98%. Pollen viability rates varied between 94% (TTC) and 96% (IKI). Contents of medium V: 20% sucrose 1% agar-agar+5 ppm boric acid and 24 hours incubation duration were stated as optimum pollen germination rate and tube growth.

Key words*: Medlar, pollen germination, morphological homogeneity, pollen tube growth, TTC.*

INTRODUCTION

Medlar (*Mespilus germanica* L.) has been cultivated for over thousands of years in temperate zones of Anatolia. The medlar, called as 'Muşmula', 'Beşbıyık' or 'Döngel' (in Turkish), is botanically classified as a pome and produces edible fruits (Atay, 2013). *Mespilus germanica* L. belongs to Rosaceae family and it grows mainly in frost-free areas, and on rocks and poor soils (Haciseferoğullari et al., 2005). The flowers are hermaphrodite, five-piece, white-pink color, and each bud has one flower. Flowers generally open in May-June. In Turkey, they are abundant particularly in north and west-Anatolia and Marmara regions. Medlar is one of the latest maturating fruits and the ripening occurs in late October before frosts in Turkey. The fruits are used as a nutrition component by the local population and are prepared by the local people as marmalade or pickle (Ercisli et al., 2012).

Fertilization success in plants is the result of processes that take place during the progamic phase (Thompson, 2004; Güçlü and Koyuncu, 2017). Pollen germination and pollen tube growth are important components of fertilization success in fruit trees (Janick and Moore, 1996; Tosun and Koyuncu, 2007). The first condition of formation of seed and fruit is developing healthy male and female organs of the flower and cells, except for an apparent partenocarpy of some cultivars. Pollen performance, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed-producing plants. Pollen performance is clearly affected by the genotype of the pollen (Acar and Kakani, 2010; Hedly et al., 2004). There have been some studies about 'İstanbul' medlar, but apparently almost no work has been done on fertilization. The aim of this study is to examine the pollen performance of the 'İstanbul' medlar variety and determine the optimum pollen germination medium protocol.

MATERIALS AND METHODS

In the study, pollens taken from 'İstanbul' medlar trees in the orchard of Eğirdir Fruit Research Institute were used. Pollens were obtained from flowers at balloon stage. The flowers were transferred to the laboratory immediately. Anthers were removed and placed into the dark-colored bottle to promote dehiscence at room temperature. For the pollen

performance, pollen production capacity, morphological homogeneity, pollen viability rates were investigated. In addition, optimum germination medium and incubation period were determined. The pollen production capacity and morphological homogeneity percentages of pollens were assessed with the hemocytometer (Marienfeld, Germany) slide (Eti, 1990). Imperfectly shaped pollen grains were considered as aborted pollen. The final percentage of morphological homogeneity (MH) was defined as:

$$MH = \frac{Ns - Na}{A} x100$$

Ns: Number of shaped pollen per area;
Na: Number of aborted pollen per area;
A: Total number of pollen area.

In the stain tests, pollen viability was estimated by using TTC (2, 3, 5-triphenyl tetrazolium chloride) and FDA (fluorescein di acetat) stains. Pollens were scattered onto TTC and FDA solutions, and stained pollens were counted after 2 hours and 15 minutes, respectively. To determine the pollen viability, pollens of each cultivar (of four different areas) were observed onto two slides under a light microscope (×100 magnification). The stained pollen was considered as viable in these tests.

Four concentrations of sucrose were tested in the germination medium for the *in vitro* germination test.

I. 5% sucrose + 1% Agar-Agar + 5 ppm H_3BO_3 (Boric Acid)
II. 10% sucrose + 1% Agar-Agar + 5 ppm H_3BO_3
III. 15% sucrose + 1% Agar-Agar + 5 ppm H_3BO_3
IV. 20% sucrose + 1% Agar-Agar + 5 ppm H_3BO_3

The 'agar in plate' method was used to establish pollen germination and pollen tube growth (Koyuncu and Tosun, 2009). Pollen tube long at least as its diameter was considered to be 'germinated'. The percentage of pollen germination was determined after 2 h, 12 h and 24 h incubation period at 21°C. An ocular micrometer was used to measure pollen tube length, under a light microscope, at a magnification. Four Petri dishes were used for germination and pollen tube growth experiments. For each assay, 2 mL of medium was placed into Petri dishes. Counts were made from 4 different microscope fields (100-150 pollen grains per field for each Petri dishes) (Hedly et al., 2004; Koyuncu, 2006). Statistical analysis was conducted using Duncan's multiple range test within the general linear model procedure of SPSS 16.0.

RESULTS AND DISCUSSIONS

Pollen production capacity and pollen viability

Pollen production amount and viability results were shown in Table 1. Pollen morphological homogeneity (98%) and pollen viability (96%) were found very high IKI viability test (96%) was higher than TTC (94%) test. Similarly, researcher reported that pollen viability in the IKI test was generally higher (84.83-100%) and stable than TTC test (11.78-91.66%) (Çavusoğlu and Sülüsoğlu, 2013). Koyuncu (2006) studied strawberry pollens using TTC and reported that pollen viability ratios reached 82% (Allstar and Elvira) and 86.5% (Chandler). Koyuncu and Tosun (2009) used TTC, FDA and IKI stain tests for the same sweet cherry cultivars. They reported that the pollen viability differed according to stain methods and cultivars.

Table 1. Pollen production capacity, morphological homogeneity and pollen viability tests

Pollen production capacity and morphological homogeneity				
Cultivar	N	M	PN	MH
İstanbul	16 ?	160 ??	98565 84	98%

Pollen viability tests		
	TTC	IKI
İstanbul	94 %	96%

N: Numbers of anthers in a flower; M: Mean pollen number in an anther; PN: Pollen number in a flower; MH: Morphological homogeneity.

Pollen germination tests

For the optimum germination medium different sucrose concentrations were tried.

A linear equation best described the relationship between the germination percentage and different concentration of sucrose (r 0.966). In Medium I, sucrose concentration was the lowest, pollen germination has not started in 2 hours whereas the others even low gave germination at 2

hours with increasing sucrose concentration. As seen Figure 1, pollen germination rates increased with increasing sucrose concentration. The highest ratio of pollen germination was obtained from Medium IV (20% sucrose + 1% Agar-Agar + 5 ppm H_3BO_3). In terms of mean pollen germination rate, it followed by Medium III (16.82%) and Medium II (7.25) (Table 2). This suggests that the increased concentration of sucrose increases the germination rate. Çavuşoğlu and Sülüsoğlu (2013) reported that pollen germination varied between 16.4%-66.67% for all germination media. Another study which was conducted in Arabidopsis emphasized sugar-dependent multilayer regulation of Arabidopsis pollen germination is supported, which makes this approach a valuable experimental system for future studies addressing sugar sensing and signaling (Hirsche et al., 2017). Güçlü and Koyuncu (2017) found the suitable germination medium 0.5% agar + 15% sucrose + 5 ppm H_3BO_3 (Boric acid) for apple's pollen.

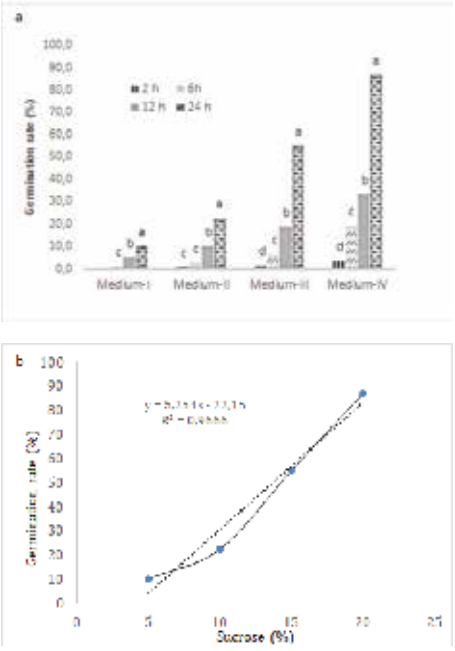

Figure 1. *In vitro* pollen germination percentages of medlar at 2 h, 6 h,12 h and 24 hours ($p<0.05$) at different sucrose concentration (a); Regression curve between germination rate and sucrose concentration (b)

When different incubation periods are compared in the same germination medium, the difference between the values is statistically significant ($p<0.05$). Pollen germination raised as much as 3 fold between 6 h and 12 hours reached own maximum percentage at 24 hours (10.1; 22.3; 54.9; 86.8, respectively) for all mediums. Results of incubation duration experiments were similar to the findings of Yıldız and Yılmaz (2002), who reported that the germination pollen of strawberry cultivar 'Tufts' began within 1 h at 24°C. Tosun and Koyuncu (2007) also reported that the germination rates increased with incubation period in cherries.

Table 2. *In vitro* pollen germination percentages of medlar in different sucrose concentrations and incubation periods

	Incubation Period				
Germination Medium	2h	6h	12h	24h	Mean
Medium I	0.5c*	1.1c	5.2d	10.1d	1.57d
Medium II	0.8c	2.7c	9.9c	22.3c	7.25c
Medium III	1.5b	5.1b	18.7b	54.9b	16.82b
Medium IV	3.3a	19.9a	33.5a	86.8a	43.52a

*Values within a column followed by different letters are significantly different ($p<0.05$).

When the data were evaluated, in a medium with different sucrose concentrations, a linear increase was observed between the rate of germination and the duration of incubation (Figure 2).

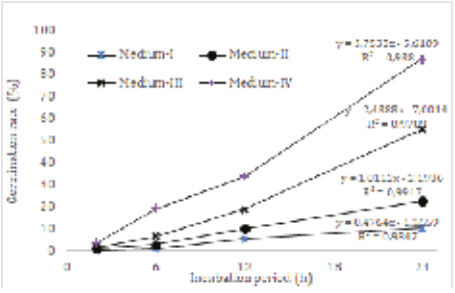

Figure 2. Linear regression curves between germination rate and incubation period

The *in vitro* elongation of pollen tubes was affected by different sucrose concentration (Table 3). The effect of different concentrations of sucrose on the pollen tube elongation was statistically significant ($p<0.05$). As well as the pollen germination rate, pollen tube growth

increased during the incubation period and measured at 24 hours later. As the sucrose concentration increased, the length of the pollen tube increased, reaching the highest value in Medium IV (164.2 µm). The shortest pollen tubes were measured at Medium I, the lowest sucrose concentration (16.8 µm).

Table 3. Pollen tube growth of medlar in different sucrose concentrations (µm) 24 hours later

Germination medium	Pollen tube lenght (µm)
Medium I	16.8d*
Medium II	35.9c
Medium III	110.7b
Medium IV	164.2a

*Values within same column followed by different letters are significantly different ($p<0.05$).

Koyuncu and Güçlü (2009) reported that the *in vitro* pollen germination and tube growth were clearly affected by incubation period. Sharafi (2011) found the *in vitro* medium containing 17% sucrose, 10 ppm acid boric and 1.2% agar. Cultured pollens were incubated in dark condition at 25°C for 24 h optimum medium for pollen germination and tube growth.

CONCLUSIONS

The amount of pollen production and the morphological homogeneity level were determined for 'İstanbul' medlar variety.
Pollen viability found high (98% for IKI).
Pollen germination rates increased by increasing with incubation period.
Medium IV (20% sucrose + 1% Agar-Agar + 5 ppm H_3BO_3) and 24 hours incubation period was found optimum pollen germination and pollen tube growth condition. Fertilization biology studies should be continued at *in vivo* conditons. We hope these results will be usefull for researchers and growers for future breeding studies.

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RELATIONSHIP BETWEEN VEGETATIVE GROWTH AND NUT CHARACTERISTICS IN ALTERNATE BEARING PISTACHIO (*PISTACIA VERA*) CULTIVARS EXPOSED TO DROUGHT

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Abstract

Among abiotic stress factors, drought stress is of the most influential factors limiting plant growth and impairing plant metabolism. In addition to the abiotic stress factors, alternate bearing is a main physiological problem for horticultural plants including olive, pistachio, hazelnut and apple. In this context, a 3-year field study was designed for three pistachio cultivars, namely 'Uzun', 'Siirt' and 'Ohaidi'. The cultivars were exposed to irrigated and non-irrigated conditions. After experimental period, yield, shoot length and nut characteristics were measured. According to years, shoot length, 100 nut weight, nut internal weight, nut length, nut width and nut thickness decreased but blank nut ratio increased in productive year as a yield. Regarding with varieties, there was no significant difference yield, blank nut, and split nuts among cultivars but shoot length, nut width and thickness were higher in 'Ohaidi' cultivar. Lowest 100 nut weight and nut internal weight were determined in 'Uzun' cultivar and highest nut length was observed in 'Siirt' cultivar. Irrigation only affected the shoot length from given properties. As a result, yield of the tree affected vegetative development and nut characteristics. Along with this study, finally vegetative growth and fruit characteristics were correlated.

Key words*: alternate bearing, pistachio cultivars, Pistacia vera, vegetative growth, nut characteristics.*

INTRODUCTION

Turkey has an important place in the world with its unique ecological conditions and is the gene center of pistachio and pistachio production, spreading in the south-eastern parts of Turkey. The irregular fruiting behaviour (alternate bearing) and its effects in yield reduction in pistachio are of the major problems. Alternate bearing is related to many factors mainly classified as intrinsic factors such as endogenous hormones, nutrition, carbohydrate accumulation allocation between reproductive and vegetative organs of the plant, rootstocks, cultivars, genetic factors (Nzima et al., 1997; Spann et al., 2008; Kumar et al., 2016; Goldschmidt and Golomb, 1982; Rosecrance et al., 1998; Durand et al., 2013; Kafkas et al., 2006) and extrinsic such as environmental, climatic and soil properties, climatic changes in rainfall and temperature, compensate chilling and total heat demand of trees, inadequate pollination, traditional cultural practices, fertilization, harvesting, prolonged period of water stress etc. (Elloumi et al., 2013; Acar and Eti, 2007; Khoyerdi et al., 2016; Marcinska et al., 2013; Dag et al., 2009; Kallsen, 2017). Disturbance in adequate and balanced nutrition of the plant lead to low productivity and quality. The alternate bearing is the result of the disruption deviating from optimal nutrition caused by domination of fruit in relation to nutrient use competition between fruit, buds and leaves (Crane and Nelson, 1971). Pistachio is mainly grown under rain fed conditions in the Mediterranean basin and considered as drought and saline-resistant crop (Behboudian et al., 1986; Rieger, 1995). Irrigation is of the essential factor for plant yield. In pistachio, irrigation improves nut quality and reduces the alternate bearing pattern (Kanber et al., 1993).

Plenty of flower buds are formed every year in pistachio that makes it different from other

species showing alternate bearing. However, the flower buds fall off during the summer months.

The alternate bearing in pistachio is the result of flower buds falling off, not concerned with flower bud formation (Crane, 1971).

Of the abiotic stress factors, drought is considered as the most hazardous factors on sustainable agriculture worldwide (Din et al., 2011; Anjum et al., 2012; Lefèvre et al., 2012).

Drought is assumed to be the consequences of soil and/or atmospheric water deficit, causing substantial eco-physiological constraints to plant survival and to crop productivity and quality (Boyer, 1982).

Drought associated studies are great concern of the plant scientists for developing, improving or exploring the drought-tolerant plant species (Hamrouni et al., 2001; Al-Barrak, 2006; Bettaieb et al., 2009; Bybordi, 2010).

In the current study, it was aimed to reveal the relationship between yield and vegetative growth and nut characteristics in pistachio cultivars bearing alternatively in response to the irrigated and non-irrigated conditions.

MATERIALS AND METHODS

Experimental Material

The research was carried on 25 years old cultivars 'Siirt', 'Uzun' and 'Ohaidi' grafted onto a *Pistacia vera* rootstocks which were cultivated in 7 x 2 m intervals at Pistachio Research Institute.

The experiments were designed under irrigated and non-irrigated conditions according to the randomized plot design with four replicates.

Yield, vegetative growth and nut characteristics

Yield (kg/tree) was obtained by dividing the total harvested product per tree for each variety.

Shoot length (SL) was found by measuring the shoots at the end of vegetation. Counts and measurements of the fruits in the clusters were made for the fruit characteristics. Nut length (NL), nut width (NW), nut thickness (NT), nut weight (100-NW), internal nut weight (INW), shelling percentage (SP) and blank nut (BN) are also evaluated for the fruit characteristics.

Statistical analysis:

SPSS statistical program was used to determine statistical significance levels by employing the independent one-way ANOVA followed by Duncan multiple range test and the differences between individual averages were considered to be statistically important at $p< 0.05$. The results were expressed as mean.

RESULTS AND DISCUSSIONS

The yield, vegetative growth and nut characteristics values of 'Uzun', 'Siirt' and 'Ohaidi' pistachio varieties were measured for three years on irrigated and non-irrigated conditions and the inter-parameter relationships were determined by statistically evaluating the changes between parameters according to years, irrigation and varieties. The obtained values and changes are shown in Table 1. Changes in yield, growth and nut characteristics' parameters according to irrigation, cultivars and years are represented in Table 2. Accordingly, irrigation increased shoot length. Of the varieties, 'Ohaidi' with more shoot, 'Siirt' and 'Ohaidi' with more 100-nut weight and internal nut weight, 'Siirt' with broader nut and 'Ohaidi' with thicker nuts were of the results of the present study.

When differences between the years were investigated, the yields of the following years were about the same, while the first year received more yield than the other years. During the year with fewer yields, higher shoot length and lower blank nut, 100-nut weight, internal nut weight, split nut, nut length, width and thickness were obtained.

Changes and correlations between the yield, shoot growth and nut characteristics under irrigated and non-irrigated conditions for 'Uzun' cultivar are shown Table 3 and Figure 1. In terms of correlation coefficient (r) values there were negative correlation between yield and blank nut under non-irrigated (-0.811) condition, yield and 100-nut weight under irrigated (-0.844), yield and shelling percentages under irrigated (-0.888) condition, yield and nut width under non-irrigated (-0.807) condition, yield and nut thickness under irrigated (-0.974) and non-irrigated (-0.979)

Table 1: Growth and nut characteristics' parameters under irrigated and non-irrigated conditions for pistachio cultivars

		'UZUN'			'SIIRT'			'OHAIDI'		
		1.Year	2.Year	3.Year	1.Year	2.Year	3.Year	1.Year	2.Year	3.Year
Yield (kg/tree)	IR	4.6	2.9	3.3	5.6	3.7	3.8	4.5	3.1	4.8
	N-IR	6.4	3.2	5.1	7.7	3.7	3.6	6.4	2.8	4.3
	AVE	5.49	3.05	4.20	6.65	3.68	3.71	5.44	2.93	4.58
Shoot lenght (cm)	IR	12.8	13.0	21.5	16.6	18.6	22.5	25.0	25.9	32.0
	N-IR	13.0	14.3	19.8	13.8	13.1	21.2	21.5	24.1	27.9
	AVE	12.90	13.65	20.65	15.20	15.85	21.85	23.25	25.00	29.95
Blank Nut (%)	IR	17.7	15.7	9.2	22.9	9.7	8.0	18.1	10.9	7.1
	N-IR	14.0	22.2	11.7	25.4	17.8	11.2	40.1	14.6	9.7
	AVE	15.85	18.95	10.45	24.15	13.75	9.60	29.10	12.75	8.40
Nut weight (g/100 nut)	IR	168.5	181.2	187.7	187.0	249.7	252.3	198.4	244.6	249.9
	N-IR	165.1	183.4	185.5	179.2	245.3	246.0	220.6	243.9	246.9
	AVE	166.8	182.3	186.6	183.1	247.5	249.2	209.5	244.3	248.4
Internal nut weight (g/100 nut)	IR	59.0	56.0	61.6	69.7	92.3	94.4	81.0	91.1	95.2
	N-IR	61.4	56.9	59.6	74.4	88.8	89.7	90.6	93.5	93.2
	AVE	60.20	56.45	60.60	72.05	90.55	92.05	85.80	92.30	94.20
Shelling (%)	IR	30.0	51.5	59.0	36.0	68.5	80.0	26.0	76.5	77.0
	N-IR	57.5	56.0	61.5	32.5	65.0	75.0	30.0	43.5	74.0
	AVE	43.75	53.75	60.25	34.25	66.75	77.50	28.00	60.00	75.50
Nut lenght (mm)	IR	25.0	25.5	26.1	24.5	27.0	27.4	23.0	23.8	23.0
	N-IR	24.5	25.0	26.1	26.0	26.5	27.4	23.0	23.8	22.5
	AVE	24.75	25.25	26.10	25.25	26.75	27.40	23.00	23.80	22.75
Nut width (mm)	IR	13.5	13.5	14.0	14.2	15.1	15.0	14.5	15.0	15.5
	N-IR	13.0	13.5	13.5	14.2	15.1	14.5	15.0	14.5	15.0
	AVE	13.25	13.50	13.75	14.20	15.10	14.75	14.75	14.75	15.25
Nut thickness (mm)	IR	12.1	13.0	13.0	13.0	14.3	14.2	15.3	15.0	15.0
	N-IR	12.1	13.0	12.5	13.5	14.3	14.2	15.3	14.5	14.5
	AVE	12.10	13.00	12.75	13.25	14.30	14.20	15.30	14.75	14.75

Table 2: Changes in yield, growth and nut characteristics' parameters according to irrigation, cultivars and years

		Yield kg/tree	Shoot length (cm)	Blank Nut (%)	Nut weight (g/100 nut)	Internal nut weight (g/100 nut)	Shelling (%)	Nut length (mm)	Nut width (mm)	Nut thickness (mm)
IRRIGATION	IR	4	20.8 a	13.2	213.3	78.2	56.1	24.8	14.4	13.6
	N-IR	4.8	18.7 b	18.6	212.9	78.0	55.0	24.6	14.2	13.6
CULTIVARS	'UZUN'	4.2	15.6 b	15.1	178.6 b	59.2 c	52.6	25.2 b	13.5 b	12.4 c
	'SIIRT'	4.7	17.6 b	15.8	226.6 a	84.9 b	59.5	26.2 a	14.4 a	13.8 b
	'OHAIDI'	4.3	26.0 a	16.8	234.0 a	90.8 a	54.5	22.8 c	14.9 a	14.7 a
YEARS	1.YEAR	5.9 a	17.1 b	23.0 a	186.5 b	72.7 b	35.3 c	24.2 b	14.0 b	13.3 b
	2.YEAR	3.2 b	18.1 b	15.1 b	224.7 a	79.9 a	60.2 b	25.0 a	14.3 ab	13.8 a
	3.YEAR	4.2 b	24.1 a	9.6 b	228.0 a	82.3 a	71.1 a	24.9 a	14.6 a	13.8 a

condition, positive correlation between yield and internal nut weight under irrigated (0.999) condition. There was no particular correlation between yield and nut length.

Table 3. Correlation coefficient (*r*) values of the growth and developmental parameters under irrigated and non-irrigated conditions for 'Uzun' cultivar

		Y	SH	BN	100-NW	INW	SP	FL	FW
SL	IR	-0.312							
	N-IR	-0.073							
	AVE	-0.136							
BN	IR	0.500	-0.979						
	N-IR	**-0.811**	-0.525						
	AVE	-0.315	**-0.898**						
100-NW	IR	**-0.844**	0.773	-0.887					
	N-IR	**-0.748**	0.716	0.218					
	AVE	**-0.775**	**0.731**	-0.355					
INW	IR	0.565	0.834	-0.703	0.294				
	N-IR	**0.999**	-0.066	-0.815	-0.744				
	AVE	**0.791**	0.500	**-0.830**	-0.226				
SP	IR	**-0.888**	0.714	-0.843	0.996	0.209			
	N-IR	0.366	0.901	-0.842	0.343	0.373			
	AVE	-0.639	**0.849**	**-0.828**	**0.981**	-0.034			
NL	IR	-0.695	0.900	-0.970	0.972	0.510	0.948		
	N-IR	-0.201	0.992	-0.411	0.800	-0.194	0.838		
	AVE	-0.411	**0.959**	**0.736**	**0.894**	0.234	**0.964**		
NW	IR	-0.292	1.000	-0.974	0.760	0.845	0.700	0.891	
	N-IR	**-0.807**	0.648	0.309	0.996	-0.803	0.254	0.741	
	AVE	-0.541	**0.907**	-0.628	**0.951**	0.087	**0.993**	**0.989**	
NT	IR	**-0.974**	0.517	-0.682	0.943	-0.041	0.968	0.839	0.500
	N-IR	**-0.999**	0.117	0.784	0.777	-0.999	-0.325	0.244	0.832
	AVE	**-0.979**	0.333	0.117	**0.887**	-0.651	**0.781**	**0.989**	0.700

Considering average value there were no correlation between yield and shoot length, blank nut, shelling percentage, nut length and nut width but there were correlation 100-nut weight (-0.775), internal nut weight (0.791) and nut thickness (-0.979).

As regards to correlations between each other except yield, correlations were found between shoot length and blank nut (-0.898), 100-nut weight (0.731), shelling (0.849), nut length 0.959) and nut width (0.907), between blank nut and internal nut weight (-0.830), shelling (-0.828) and nut length (0.736), between 100-nut weight and shelling (0.981), nut length (0.894), width (0.951) and thickness (0.887), between shelling percentage and nut length (0.964), width (0.993) and thickness (0.781), between nut length and nut width (0.989) and thickness (0.989), between nut width and nut thickness (0.700).

Changes and correlations between the yield, shoot growth and nut characteristics under irrigated and non-irrigated conditions for "'Siirt'" cultivar are shown Table 4 and Figure 2. Effects of yield on vegetative growth and nut characteristics were more significant in 'Siirt' cultivar than the 'Uzun' and 'Ohaidi' cultivars.

Table 4. Correlation coefficient (*r*) values of the growth and developmental parameters under irrigated and non-irrigated conditions for 'Siirt' cultivar

		Y	SH	BN	100-NW	INW	SP	FL	FW
SL	IR	**-0.729**							
	N-IR	-0.450							
	AVE	-0.568							
BN	IR	**0.989**	-0.824						
	N-IR	**0.895**	-0.801						
	AVE	**0.958**	**-0.779**						
100-NW	IR	**-0.997**	0.782	-0.998					
	N-IR	**-0.999**	0.439	-0.890					
	AVE	**-0.999**	0.593	**-0.967**					
INW	IR	**-0.992**	0.808	-1.000	0.999				
	N-IR	**-0.999**	0.478	-0.909	0.999				
	AVE	**-0.997**	0.629	**-0.977**	**0.999**				
SP	IR	**-0.955**	0.899	-0.989	0.976	0.984			
	N-IR	**-0.979**	0.623	-0.967	0.976	0.985			
	AVE	**-0.969**	**0.754**	**-0.999**	**0.976**	**0.985**			
NL	IR	**-0.985**	0.837	-0.999	0.996	0.999	0.992		
	N-IR	**-0.786**	0,906	-0.979	0.779	0.805	0.896		
	AVE	**-0.953**	**0.790**	**-0.999**	**0.962**	**0.973**	**0.998**		
NW	IR	**-0.999**	0.690	-0.979	0.991	0.984	0.937	0.974	
	N-IR	**-0.742**	-0.265	-0.365	0.750	0.721	0.589	0.169	
	AVE	**-0.926**	0.215	**-0.780**	**0.914**	**0.895**	**0.804**	**0.768**	
NT	IR	**-1.000**	0.713	-0.985	0.995	0.989	0.948	0.981	0.999
	N-IR	**-0.991**	0.325	-0.827	0.992	0.986	0.942	0.695	0.826
	AVE	**-0.997**	0.502	**-0.933**	**0.994**	**0.988**	**0.947**	**0.927**	**0.952**

According to correlation coefficient (r) values there were negative correlations between yield and shoot length under irrigated (-0.729), yield and 100-nut weight under irrigated (-0.997) and

non-irrigated (-0.999) condition, yield and internal nut weight under irrigated (-0.992) and non-irrigated (-0.999) condition, yield and shelling percentages under irrigated (-0.955) and non-irrigated (-0.979) condition, yield and nut length under irrigated (-0.985) and non-irrigated (-0.786) condition, yield and nut width under irrigated (-0.999) and non-irrigated (-0.742) condition, yield and nut thickness under irrigated (-1.000) and non-irrigated (-0.991) condition, positive correlation between yield and blank nut weight under irrigated (0.989) and non-irrigated (-0.895) condition. There was no particular correlation between yield and nut length.

Considering average value there were correlations between yield and blank nut (0.958), 100-nut weight (-0.999), internal nut weight (-0.997), shelling percentage (-0.969), nut length (-0.953), width (-0.926), and thickness (-0.997). As regards to correlations between each others except yield, correlations were found between shoot length and blank nut (-0.779), shelling (0.754) and nut length (0.790), between blank nut and 100-nut weight (-0.967), internal nut weight (-0.977), shelling (-0.999), nut length (-0.999), width (-0.780) and thickness (-0.993), between 100-nut weight and internal nut weight (0.999), shelling (0.976), nut length (0.962), width (0.914) and thickness (0.994), between internal nut weight and shelling (0.985), nut length (0.973), width (0.895) and thickness (0.988), between shelling percentage and nut length (0.998), width (0.804) and thickness (0.947), between nut length and nut width (0.768) and thickness (0.927), between nut width and nut thickness (0.952).

Changes and correlations between the yield, shoot growth and nut characteristics under irrigated and non-irrigated conditions for 'Ohaidi' cultivar are shown Table 5 and Figure 3. According to correlation coefficient (r) values there were negative correlations between yield and 100-nut weight under non-irrigated (-0.862) condition, yield and internal nut weight under non-irrigated (-0.949) condition, yield and nut length under irrigated (-0.986) condition, positive correlation between yield and blank nut weight under non-irrigated (0.837) condition, yield and nut width under non-irrigated (0.814) condition, yield and nut thickness under non-irrigated (0.910) condition. Considering average value there were correlations between yield and 100-nut weight (-0.696), nut length (-0.814) and thickness (0.763).

Table 5. Correlation coefficient (*r*) values of the growth and developmental parameters under irrigated and non-irrigated conditions for 'Ohaidi' cultivar

		Y	SH	BN	100-NW	INW	SP	FL	FW
SL	IR	0.541							
	N-IR	-0.490							
	AVE	-0.075							
BN	IR	0.011	-0.835						
	N-IR	**0.837**	-0.887						
	AVE	0.618	**-0.830**						
100-NW	IR	-0.261	0.671	-0.968					
	N-IR	**-0.862**	0.864	-0.999					
	AVE	**-0.696**	**0.768**	**-0.995**					
INW	IR	-0.073	0.799	-0.998	0.982				
	N-IR	**-0.945**	0.748	-0.970	0.980				
	AVE	-0.605	**0.839**	**-1.000**	**0.993**				
SP	IR	-0.342	0.606	-0.943	0.996	0.962			
	N-IR	-0.389	0.994	-0.830	0.803	0.669			
	AVE	-0.516	**0.893**	**-0.992**	**0.974**	**0.994**			
NL	IR	**-0.986**	-0.394	-0.176	0.417	0.237	0.493		
	N-IR	-0.531	-0.479	0.018	0.028	0.225	-0.573		
	AVE	**-0.840**	-0.479	-0.093	0.195	0.076	-0.032		
NW	IR	0.165	0.919	-0.984	0.909	0.972	0.870	0.001	
	N-IR	**0.814**	0.108	0.364	-0.407	-0.579	0.218	-0.924	
	AVE	0.179	**0.968**	**-0.663**	0.582	**0.675**	**0.751**	**-0.684**	
NT	IR	0.350	-0.599	0.940	-0.996	-0.960	-1.000	-0.500	-0.866
	N-IR	**0.910**	-0.807	0.989	-0.995	-0.996	-0.736	-0.132	0.500
	AVE	**0.763**	**-0.702**	**0.980**	**-0.995**	**-0.976**	**-0.947**	-0.289	-0.500

shelling (0.994), nut width (0.675) and thickness (-0.976), between shelling percentage and nut width (0.751) and thickness (-0.947), between nut length and nut width (-0.684).

The reasons underlying alternate bearing are classified as (environmental, climatic and soil properties) and intrinsic factors (nutritional status, endogenous balance of hormone and interactions between organs). Extrinsic factors affect bearing alternatively to yield because of climatic changes in rainfall and temperature (Elloumi et al., 2013). Intrinsic factors are related to endogenous hormones, nutrition and carbohydrate accumulation allocation between reproductive and vegetative organs of the plant (Nzima et al., 1997; Spann et al., 2008).

The results indicated that yield affected the vegetative growth and nut characteristics. In the year of low yield, shoot length was increased accordingly blank nut, 100-nut and internal nut weight, shelling percentage, nut length, width and thickness decreased. Irrigation increased shoot length.

Drought conditions decrease rate of cell division and expansion, stem elongation, plant water and nutrient uptake and water use efficiency (Li et al., 2009), shoot and root dry weight, leaf relative water content, total chlorophyll, carotenoids and increased oxidative stress products, some osmoregulators and antioxidant agents (Khoyerdi et al., 2016; Marcinska et al., 2013; Shamshiri and Fattahi, 2014; Spann et al., 2009).

Because of competition between developing fruit and vegetative growth, heavy fruit load uses the plants of nutrients and carbohydrates which are required for vegatative growth (Goldschmidt, 1999; Stevenson et al., 2000). A heavy fruit loaded branch will have little vegetative growth and little fruit on olive trees (Lavee, 2007).

Competition for energy resources between the vegetative shoot meristem and fruit reduce vegetative growth of the tree, so it designates the reproductive status of the tree the following year (Samach and Smith, 2013). For this reason in alternate bearing varieties cultural practices such as pruning, girdling, applications of plant growth regulators and flower and fruit thinning are to maintain a balance of vegetative and reproductive shoots (Pellerin et al., 2011; Dag et al., 2009).

In pistachio nut, the nut splitting is a genetic characteristic and nut splitting ratio is affected by rootstock, cultivar, plant nutrition, alternate bearing, climatic conditions, cultural management and pollen source (Takeda, 1979; Crane et al., 1982).

Percentage of split, non-split and blank nuts varied by shoot type, clusters harvested from long-shoots have higher total yield and split nut compared to short-shoot clusters because of the locally higher carbohydrate supply from the greater leaf area of the long-shoots (Kumar et al., 2016). Because of photosynthesis and the transportation of in other photo-assimilate (Marschner, 1995) to the developing nuts, 100-nut weight, split nuts, and internal nut weight increased.

Nutrient status of trees and fertilization has effects on nut yield and quality in pistachio. Nitrogen, phosphorus, potassium and foliar boron application influenced vegetative growth measurements, the flowering and fruit set, reduced buds abscission, blank pistachios and in turn improved nut quality characteristics including, nut length, width and thickness (Kumar et al., 2016).

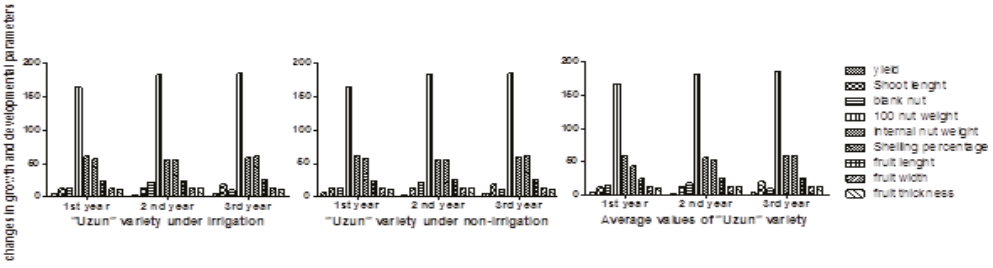

Figure 1. Changes in growth and nut characteristics under irrigated and non-irrigated conditions for 'Uzun' cultivar

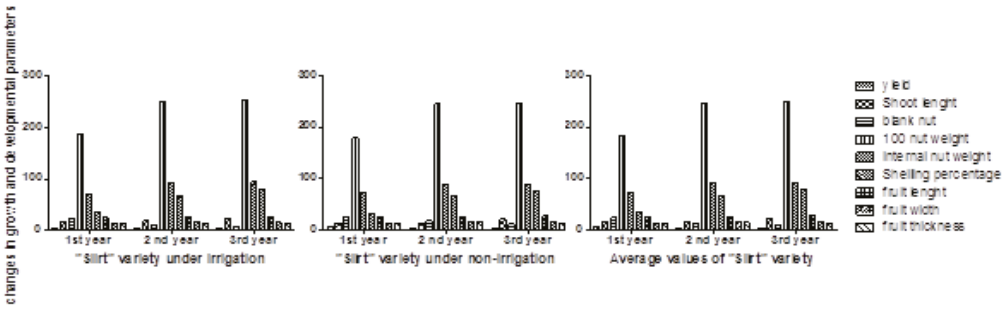

Figure 2. Changes in growth and nut characteristics under irrigated and non-irrigated conditions for 'Siirt' cultivar

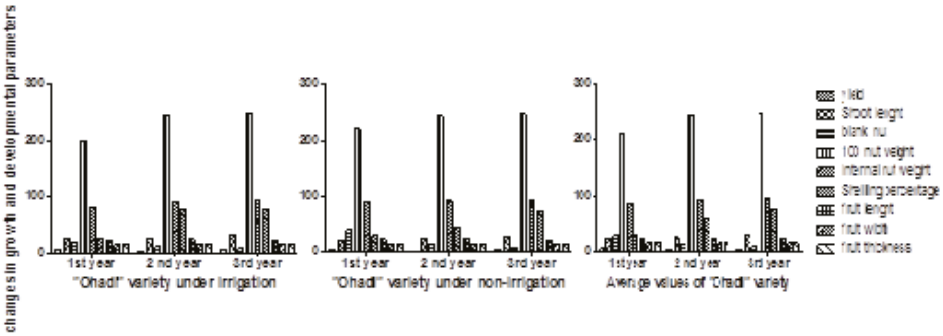

Figure 3. Changes in growth and nut characteristics under irrigated and non-irrigated conditions for 'Ohaidi' cultivar

Application of potasium improved nut quality in pistachio with an increased percentage of split nuts and 100-nut weight and reduced blank nut percentage (Zheng et al., 2001; Mimoun et al., 2004).

CONCLUSIONS

Alternate bearing and drought conditions are of the significant economic problems for fruit numbers and subsequently nut industries worldwide.

Hence, monitoring the changes in alternate bearing pistachio cultivars under drought conditions is of the essential steps for understanding alternate bearing mechanisms for the plant.

During the year with fewer yields, higher shoot length and lower blank nut, 100-nut weight, internal nut weight, split nut, nut length, width and thickness were obtained.

Effects of yield on vegetative growth and nut characteristics are more significant in 'Siirt' cultivar than the 'Uzun' and 'Ohaidi' cultivars.

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EVALUATION OF POMOLOGICAL PROPERTIES OF SOME ORANGE VARIETIES FROM WEST MEDITERRANEAN TURKEY

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Abstract

Citrus trees can be productively grown in Mediterranean region of Turkey. In this study, six orange varieties (Belladonna, Biondo, Biondo Riccio, Calabrese, Mediterranean Sweet, Parson Brown), grafted C. aurantium L. var. 'Yerli', were re-evaluated under Antalya ecological conditions. During the 2 consecutive trial years, fruit dimensions (weight, height, width), rind thickness, seed number, total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, fruit juice content, pH, vitamin C content were analyzed. There were statistically differences according to some pomological properties of six varieties. Mediterranean Sweet has the biggest fruits (187.54 g). The highest TSS ratio (11.0%) and vitamin C content (61.20 mg/100ml) was in Parson Brown orange. Biondo fruits have the lowest rind thickness.

Key words*: Citrus, C. sinensis, fruit quality.*

INTRODUCTION

Mediterranean basin has suitable ecological conditions to enable the edible production of citrus fruits. Citrus species are the most important fruit groups for Turkey in view of production and export quantities. Orange (*Citrus sinensis*) is the first species among *Citrus* with high level of production. Tropical and semitropical citrus fruits are more concentrated in subtropical regions (Davies and Albrigo, 2005). Located in the Mediterranean basin, Turkey has quite favorable ecological conditions for production of quality edible citrus (Tuzcu, 1998). China, Brazil and India are the top countries in world citrus production (FAO, 2017). Turkey is the 9th country with 4,293,007 tons among the major citrus producers in 2016 (TUIK, 2017). The citrus fruits are generally the first in the total export of fresh fruits and vegetables in Turkey (AKİB, 2017).

Although Turkey is no homeland of citrus, a lot of varieties have been brought from different countries has led to the emergence of an important gene source. The ecological conditions of the Mediterranean and Aegean regions in Turkey allow for citrus cultivation successfully. In his way, Turkey has been the potential to competing with other Mediterranean countries in terms of quality (Tuzcu, 1998). It is important to evaluate well-adapted, productive and having good fruit quality varieties for optimal growing (Yılmaz et al., 2013).

The fruit quality of the citrus species is affected by cultivars or types, rootstocks, ecology and cultural practices (Ozcan and Ulubelde, 1984; Economides and Gregoriou, 1993; Castle 1995; Tuzcu et al., 1999). Similarly Hodgson (1967) emphasized that fruit quality is influenced by three main factors as climate, rootstock and nutrition. Moreover, climate is the most important parameter affecting fruit quality and size.

In this study, the pomological characteristics of some sweet orange varieties have been evaluated in the Antalya region, which has an important potential for citrus growing.

MATERIALS AND METHODS

The present research was conducted on citrus germplasm collection of Bati Akdeniz Agricultural Research Institute (BATEM) in Antalya (Turkey) during 2015 and 2016. Six orange varieties (Belladonna, Biondo, Biondo Riccio, Calabrese, Mediterranean Sweet and Parson Brown), budded on the sour orange 'Yerli' (*Citrus aurantium* L. var. 'Yerli') in

1938, were used as plant material. The experimental plot located at 36° 52' 29.9'' N and 30° 43' 28.5'' E latitude, the altitude is about 37 m. Sweet orange orchard had a typical Mediterranean climate, and soil had alkaline reaction, calcareous, unsalted, and rich in phosphorus.

The fruits were randomly harvested from all sides of different 4 trees for each variety in middle-January on both trial years. 20 healthy fruits were used for pomological analyzes. The fruit quality parameters of orange varieties were assessed according to (Ozsan and Bahçecioglu, 1970).

Different physical and chemical parameters i.e. fruit weight, fruit height, fruit diameter, rind thickness, segment number, seed number, juice content, total soluble solids (TSS), total acidity (TA) and ascorbic acid were evaluated. Fruit dimensions and peel thickness were measured with a digital caliper (0.01 mm sensitivity). The fruits, which had almost same color and dimensions, were weighed by 0.01 g sensitivity balance (Shimadzu) and their juice extracted. The juice retrieved was weighed and calculated the fruit content percentage. Segment number and seed number were counted. Total soluble solid (TSS in ^{o}Brix) was measured using hand refractometer (N.O.W Tokyo 0~32). Titratable acidity (TS) was determined with AOAC method (NaOH 0.1 N to pH 8.1) using a titrator and expressed as grams of anhydrous citric acid/100 ml fruit juice. Maturity index (TSS/TA) was calculated. pH of fruit juice was measured by digital pH meter (WTW Ino Lab). The ascorbic acid content was determined as reported earlier by using method of Horwitz (1975) and expressed mg/100 ml.)

The experimental design was performed according to a randomized design with 4 replications, each replication consisting of one tree. Data analysis was done by analysis of SAS statistical software and means were separated by using LSD test at ($P \leq 0.05$). Average data of last 2 years were presented in this present study.

RESULTS AND DISCUSSIONS

It has been observed that the sweet orange varieties, budded on *C. aurantium* L., are able to maintain their commercial significance in terms of both yield and quality even in advanced ages under appropriate cultural conditions (data were not shown here).

Results of average of some pomological characteristics of six orange varieties for last two consecutive years are shown in Table 1 and Table 2.

Fruit quality is an occurrence that expresses many different parametrizes. Each fruit has specific fruit quality factors although there are similarities among others. Janick and Moore (1975) set these factors as: fresh size, shape, colour, peel, juice soluble solid, solid: acid ratio, flavor, ease of peeling, seed content and juice content.

It is well known fruit size is very important especially consuming as fresh fruit. The variance analysis showed that fruit weight of orange varieties was significant ($P \leq 0.05$). The highest fruit weight was the obtained from Mediterranean Sweet (187.54 g) while the lowest one was Biondo Riccio (151.71 g). Tuzcu (1990) reported these values of different orange varieties grown in Turkey as; 191.16 g (Yafa), 160.81 g (Valencia), 160.99 g (Hamlin), 198.91 g (Finike Yerli), 162.36 g (Alanya Dilimli), 165.97 g (Kozan Yerli), 164.06 g (Dörtyol Yerli). The weight of different Valencia clones in Antalya changed between to 228.71 g (VAA 75) and 214.82 g (VAA 59) (Tuncay, 2005). Fruit weight was determined by Altan (1995) as 205 g (Hamlin), 164 g (Magnum Bonum), 148 g (Dörtyol Yerli), 196 g (Kozan Yerli) and 157 g (Valencia) in Çukurova Region. Khan (2015) stated that fruit weight was between 189.75 g (Pineapple) to 140.50 g (Robel) in Pakistan. Even if fruit weights of our results are different, they are included in the boundary of others. The current differences can be attributed to differences of tree age and ecology.

The highest average fruit height for last two years was taken from Calabrese (70.99 mm) and Mediterranean Sweet (70.02 mm). Statistically significant differences were obtained between orange varieties expressed in Table 1. Similarly, fruit diameter of trial oranges has significant difference ($P \leq 0.05$) according to variance analysis.

As seen in Table 1, Mediterranean Sweet had the widest fruits diameter with 71.81 mm, while Biondo Riccio had the small one with

67.09 mm. Tuncay (2005) stated that the fruit length/width of Valencia and Yafa clones grown in Antalya were the 77.14 mm (VAA 72)/ 76.13 (VAA 70) mm and 91.21 mm/ (YAA 46) 80.92 mm (YAA 45).

When compared to the rind thickness of the fruits there was no significant differences, even though the thin-rind fruit obtained from cv. Biondo (3.66 mm), Mediterranean Sweet (4.07 mm) had the thicker-rind. All these reports indicated that fruit dimensions and rind thickness can vary to ecology.

Tuzcu et al. (1999) have stated that segment number is an important genetic factor and also it does not affected to rootstocks, climatic conditions and etc. This notification is confirm to our results, which there was no significant difference of segment number among trial varieties (Table 1).

Fruit quality and its appearance are very important from a consumer's viewpoint. Especially seedless fruit are desired quality characteristics for orange citrus. In this study, Calabrese, Biondo, Biondo Riccio and Mediterranean Sweet oranges were defined as commercial seedless varieties in Antalya (south Turkey).

Table 1. Main fruit characteristics of orange varieties (mean of two years)

Variety	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Rind thickness (mm)	Segment number	Seed number
Belladonna	175.18±21.77 ab	66.38±1.50b	70.99±1.97a	3.80±0.32	9.37±0.44 c	6.84±1.56 cd
Biondo	162.36±15.55 bc	65.67±1.10 bc	67.68±2.84b	3.66±0.35	9.45±0.24 c	4.55±1.56 ab
Biondo Riccio	151.71±30.64 c	63.54±4.81 c	67.09±4.14b	3.92±0.13	9.36±0.36 c	5.55±1.06 bc
Calabrese	173.43±14.89 ac	70.99±1.64a	69.53±2.62ab	3.97±0.22	9.87±0.27 b	3.07±0.96 a
Mediterranean Sweet	187.54±10.32 a	70.02±2.50 a	71.81±1.37a	4.07±0.17	10.33±0.33 a	5.16±0.84 bc
Parson Brown	160.24±15.38 bc	63.07±1.03 c	68.99±2.23 ab	3.97±0.24	9.91±0.36 b	8.09±3.17 d
LSD (0.05)	22.006	2.8231	3.0545	NS	0.3876	1.964

*Mean separation within columns by LSD multiple range test P≤0.05.

Fruit quality, is eventually a matter of consumer preference, can measure as physical traits and chemical composition. In current study, some chemical properties are evaluated and shown in Table 2. Juice content changed between 59.78% (Calabrese) 56.91% (Mediterranean Sweet). There was no significant difference among orange cultivars. Juice content was expressed as different ratio in a lot of research established different ecology (Tuzcu, 1990; Tuzcu et al., 1993; Yılmaz et al., 2013). Total soluble solid (TSS), juice acidity (TA) and TTS/TA are abundant quality parameters in citrus.

Table 2. Main fruit quality properties of orange varieties (mean of two years)

Variety	Fruit juice content (%)	Total Soluble solids (%)	Acidity (%)	Soluble solids/ Acidity	pH	Ascorbic acid mg/100ml
Belladonna	56.94±2.09	8.33±0.57 bc	1.44±0.12	5.90±0.95	3.38±0.43	54.86±2.04bc
Biondo	59.70±2.37	9.30±0.95 b	1.70±0.31	5.81±1.65	3.31±0.37	49.60±1.76de
Biondo Riccio	57.22±3.04	8.85±1.20 bc	1.71±0.41	5.72±2.05	3.27±0.33	57.10±2.58b
Calabrese	59.78±3.62	7.48±1.44 c	1.51±0.11	5.04±1.25	3.07±0.21	46.71±1.40e
Mediterranean Sweet	56.91±2.33	8.83±1.82 bc	1.49±0.23	6.68±2.51	3.08±0.13	52.44±6.21cd
Parson Brown	56.82±1.15	11.00±1.94a	1.58±0.20	7.26±2.11	3.32±0.51	61.20±2.86a
LSD (0.05)	NS	1.6049	NS	NS	NS	3.6987

*Mean separation within columns by LSD multiple range test P≤0.05.

Parson Brown had the highest with 11% TTS but Calabrese had the lowest (7.48%), and difference among orange varieties was significant (P≤0.05). Our TTS results are almost similar to reports of Khan (2015) and Tuzcu (1990). TA, TTS/TA and pH values of trial varieties were no significant. Fruit quality is foremost an inherent scion cultivar trait. It can be modified but not radically changed without genetic manipulation (Castle, 1995). Among the secondary factors, climate is widely recognised as a major fruit quality factor

judging by the prominence of climatic adaptation on plant breeders' lists of objectives (Hodgson, 1967; Janick and Moore 1975). In citrus, rootstocks have many scion interactive effects; the principal internal factors are juice content and colour, and soluble solids, acid concentrations and their ratio (Wutscher, 1988).

Citrus fruits have been consumed for good sources of antioxidant. Stuetz et al. (2010) stated that they were acknowledged as a good source of ascorbic acid and carotenoids. There were statistically significant differences among cultivars in terms of ascorbic acid ($P \leq 0.05$) (Table 2). Parson Brown had the highest ascorbic acid (61.20 mg/100 ml) content. The lowest was determined in the Calabrese variety (46.71 mg/100 ml). Ascorbic acid value of orange was found between 36.90 mg/100 ml (cv. 'Lsen Asfour', in Tunus) by Tounsi et al., (2011) and 61.7 mg/100 ml (cv. 'Hamlin', in Çukurova) by Altan (1995). Gülbahar et al. (2009). Research as indicated that ascorbic acid contents of fruits are affected by genetic, climatic factors, soil structure repining level and also quantity and quality of sunlight. This statement confirms the differences in the results of obtained from different researches on ascorbic acid content. On the other hand, among the secondary factors, climate is widely recognised as a major fruit quality factor judging by the prominence of climatic adaptation on lists plant breeders (Hodgson, 1967).

CONCLUSIONS

The present study demonstrated differences in fruit quality parameters in some orange varieties grown in Antalya (south-west Turkey) conditions. Mediterranean Sweet orange variety had high performance of almost all quality parameters, especially fruit weight. The Calabrese, Biondo, Biondo Riccio and Mediterranean Sweet varieties were as relatively commercial seedless. These results considered to be a valuable reference for forthcoming studies on pomological and biochemical characteristics of oranges to decide the most favorable one for commercial production. In addition, it has been observed that the sweet orange varieties, budded on *C. aurantium* L, are able to maintain their commercial significance in terms of both yield and quality even in advanced ages under appropriate cultural conditions.

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POLLEN PERFORMANCES OF NATURALLY GROWN BLACKBERRIES IN ISPARTA - TURKEY

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Abstract

Fertilization biology is one of the most important criteria used to identify as candidate genotype and variety. In this research pollens of 6 wild-grown blackberry shrubs, preselected from Isparta - Turkey, were used. In order to determine the pollen performance which is the basis of the studies on fertilization biology, the amount of pollen production, pollen viability degree and the morphological homogeneity rate were determined. In addition, temperature experiments (15^{0}C, 20^{0}C and 25^{0}C) were performed to determine the optimum pollen germination condition. However, the effects of some growth regulators and mineral substances (GA_3, KNO_3, BA) on pollen germination and tube growth were investigated. N6 had the highest values in terms of anther number (71.2) average number of pollens per anther (13151.7), number of pollen per flower (414245.7), and morphological homogeneity (95.8%). 20^{0}C was found optimum temperature for pollen germination and tube growth. Gibberellic acid and Potassium nitrate were determined as promoter while benzyl adenine had inhibitory effect on pollen germination and tube growth. As a result of fertilization biology studies N6 can be thought promising, terms of pollen performance.

Key words*: Rubus canescens, pollen tube growth, TTC, KNO_3.*

INTRODUCTION

Turkey is located in the region of the intersection of three phytogeography regions. While Turkey flora has approximately 11,466 plant taxon, whole Europe continent has approximately 12,000 plant taxon. *Rosaceae* family has some are thorny and trailing that are rarely grassy, bush and tree exist. This family is represented by 115 genus and approximately 3200 species in the world. *Rosaceae* family members are represented by 35 genus and 318 species in Turkey. *Rubus* taxon is an important genus of *Rosaceae* family.

It has about 16 species in Turkey (Güner, 2012). *Rubus canescens* DC. var. *canescens* is low, usually trailing shrub. The red purplish body is angular and often prickly. Thorns are short, flat and sickle-shaped. Leaves are ternate or sometimes pedate with 3 or 5 leaflets, discolorous; leaflets tomentellous, dull green or greyish green, canescent-tomentose with stellate hairs below, bidentate, lateral ones subsessile, terminal one petiolulate, cuneate-obovate to rhombic; stipules linear. Flowering shoots are erect, 15-40 cm, angled, pubescent to shortly tomentose, armed like the turions. Sepals are ovate-oblong, acute, pubescent tomentose, reflexed after flowering. Petals are white (drying pale yellowish), obovate-oblong (rarely broader), 5-8 (-10) mm. Fruits are about 1 cm in size and red purple color. Drupelets are black, small and numerous, glabrous (Davis, 1972).

It is light-half-shadow plant. It is resistant to cold and hot. It likes moisture but can also grow in dry places. It is used of forceps, diuretic and diabetes in folk medicine. The liquid obtained from the fruits is used as a mouthwash against inflammations of the tonsils (Durmuşkahya, 2006).

Fertilization success in plants is the result of processes that take place during the progamic phase (Thompson, 2004; Güçlü and Koyuncu, 2017). Pollen germination and pollen tube growth are important components of fertilization success in fruit trees (Janıck and Moore, 1996; Tosun and Koyuncu, 2007).

The first condition of formation of seed and fruit is developing healthy male and female organs of the flower and cells, except for an apparent partenocarpy of some cultivars.

Pollen performance, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed-producing plants.
Pollen performance is clearly affected by the genotype of the pollen (Acar and Kakani, 2010; Hedly et al., 2004).
Pollen-pistil interactions and environmental factors also affect pollen performance (Dafni and Firmage, 2000). Temperature is one of the most important environmental factors for pollen germination, fruit set and seed set. Temperature has been shown to affect the chemical composition of pollen, pollen viability, pollen tube growth as well (Johanson and Stephanson, 1998).
Pollen germination and pollen tube growth are important research materials for morphological, physiological, biotechnological, ecological, evolutional, biochemical, systematic and molecular studies. Additionally testing pollen performance could be helpful for a fruit cultivation of genetic progeny for breeding purpose, and especially for selecting which cultivars should be used by researchers and growers.
For this purpose we tried to observe the pollen performances of 6 wild-grown blackberries.

MATERIALS AND METHODS

Pollens were collected from flowers of 6 wild-grown blackberry shrubs (N1, N2, N3, N4, N5, N6) at balloon stage.
The 50 flowers at balloon stage were picked up. The flowers were transferred to the laboratory immediately. Anthers were removed and placed into the dark-colored bottle to promote dehiscence at room temperature. For the pollen performance, pollen production capacity, morphological homogeneity, pollen viability rates were investigated.
In addition, optimum germination temperature and optimum germination medium consist of some growth regulators and mineral substances were determined. The pollen production capacity and morphological homogeneity percentages of pollens were assessed with the hemocytometer (Marienfeld, Germany) slide (Eti, 1990). Imperfectly shaped pollen grains were considered as aborted pollen.
The final percentage of morphological homogeneity (MH) was defined as:

$$MH = \frac{Ns - Na}{A} x100$$

Ns: Number of shaped pollen per area;
Na: Number of aborted pollen per area;
A: Total number of pollen area.

Pollen viability was determined by using TTC (2, 3, 5-triphenyl tetrazolium chloride) staining test. A few drops of 1% TTC (0.2 g. TTC and 12 g. sucrose were dissolved in 20 ml distilled water) were dropped by Pasteur pipettes on microscope slides and pollen were shaked with a slim brush (each brush used only one plant type) covered with a coverslip of used four microscope slides with three replication were counted after 2 hours.
To determine the pollen viability, pollens of each cultivar (of four different areas) were observed onto two slides under a light microscope (×100 magnification). The stained pollen was considered as 'viable' in the test. The 'agar in plate' method was used to establish pollen germination and pollen tube growth (Koyuncu and Tosun, 2009).
Pollen tube long at least as its diameter was considered to be 'germinated'. For temperature experiments; pollen grains were sowed in the medium containing 15% sucrose+0.5% agar-agar+5 ppm (H_3BO_3) boric acid at 15^0C, 20^0C and 25^0C in the dark.
For the growth regulators and mineral substances experiments GA_3, KNO_3 and BA were added at the determined doses as a preliminary experiment (50 ppm GA_3, 50 ppm KNO_3, and 50 ppm BA) (Tosun and Koyuncu, 2007b).
An ocular micrometer was used to measure pollen tube length, under a light microscope, at a 40x magnification. Four Petri dishes were used for germination and pollen tube growth experiments. For each assay, 2 mL of medium was placed into Petri dishes. Counts were made from 4 different microscope fields (100-150 pollen grains per field for each Petri) (Hedly et al., 2004; Koyuncu, 2006).
Statistical analysis was conducted using Duncan's multiple range test within the general linear model procedure of SPSS 16.0.

RESULTS AND DISCUSSIONS

Pollen production capacity and pollen viability

Pollen production amount and viability results were shown in Table 1. The difference in the amount of pollen production of genotypes was statistically significant ($p<0.05$). The highest results were taken from N6, in terms of numbers of anthers in a flower (71.2), mean pollen number in an anther (13,151.74) and pollen number in a flower (414,245.07). However, the difference between pollen viability and morphological homogeneity were not statistically significant ($p<0.05$). The pollen viability rate ranged from 79.88% (N2) to 83.22% (N6). Morphological homogeneity rates were found upper than 92%. Türemiş and Derin (1999), reported that pollen viability levels varied from 79.75% to 91.94% some blackberry cultivars. Koyuncu (2006) studied strawberry pollens using TTC viability test and reported that pollen viability ratios reached to 82% for cvs. 'Allstar' and 'Elvira' and 86.5% for cv. 'Chandler'. Koyuncu and Tosun (2009) used TTC, FDA and IKI stain tests for the sweet cherry cultivars. They reported that the pollen viability differed according to stain methods and cultivars. The viability and morphological homogeneity related to pollen quality. They are the most important properties in fruit trees. These properties are useful for plant breeders, geneticists and growers. However, an easy method for determining pollen viability is required to increase the efficiency of the breeding program and the selection of a suitable pollinizer while the orchard is being established (Ercisli, 2007).

Table 1. Pollen production capacity, morphological homogeneity and pollen viability test

	n	m	pn	pv (%)	MH (%)
N1	67.4b*	12919.14b	391742.11b	83.10x	95.12x
N2	65.5b	12431.10b	371130.07b	79.88	93.5
N3	66.3b	12782.19b	383435.14b	81.16	92.2
N4	66.9b	12830.71b	392424.16b	80.23	92.9
N5	65.8b	12754.09b	374126.09b	81.63	94.0
N6	71.2a	13151.74a	414245.07a	83.22	95.8

n: numbers of anthers in a flower; m: mean pollen number in an anther, pn: pollen number in a flower; pv: pollen viability, MH : morphological homogeneity.

*Values within a column followed by different letters are significantly different ($p<0.05$).[x]The difference between the values is not statistically significant. SUB TABEL 1!

Pollen germination and pollen tube growth tests

Three constant temperature regimes (15^0C, 20^0C and 25^0C) were evaluated on the pollen germination and expressed as the percentage of germinated pollen (Table 2). As seen as Table 2, the effects of different constant temperature on pollen germination were statistically significant ($p<0.05$). N6 is the best genotype with highest value (52.25) in terms of pollen germination rate, at all temperature. This is followed by N1 (32.40) and N5 (23.39), respectively. The lowest pollen germination rate was obtained from N3 (7.29). 20^0C was determined as the optimum germination temperature for all genotypes. When the temperature rose up from 15^0C to 20^0C, the pollen germination rate also increased dramatically, but when it reached 25^0C, germination began to fall down. This shows us of high temperatures had the negative effect on the pollen germination.

Table 2. *In vitro* pollen germination (%) of blackberries pollen at different temperatures after 24 hours incubation, an a medium containing 15% sucrose + 0.5% agar-agar + 5 ppm (H_3BO_3)

	Incubation temperature (°C)			
	15	20	25	Mean
N1	9	48.21	40	**32.40b**[x]
N2	8.56	12.9	12	**11.15d**
N3	2.96	10.5	8.41	**7.29d**
N4	5.78	13.24	10.67	**9.90d**
N5	7.25	33.59	29.34	**23.39c**
N6	16.64	75.87	64.23	**52.25a**
Mean	**8.37c**[y]	**32.39a**	**27.44b**	

[x]Values within a column followed by different letters are significantly different ($p<0.05$).
[y]Values within same row followed by different letters are significantly different ($p<0.05$).

The *in vitr*o elongation of pollen tubes was affected by incubation temperature (Table 3). Responses of tested cultivars to different temperatures were statistically significant ($p<0.05$). As seen as Table 3, the longest pollen tubes for all varieties were measured at 20^0C when the shortest ones at 15^0C. The most augmentation pollen tube elongation was obtained from N6 at 15 to 20^0C (95.88 µm-154.36 µm). Pollen tube length of N6 was above the average (Figure 1).

Table 3. The effect of incubation temperature on pollen tube growth (μm) after 24 hours

	Incubation temperature (°C)			
	15 °C	20 °C	25 °C	Mean
N1	67.86	89.74	82.51	**80.04d**[x]
N2	91.26	101.36	95.9	**96.17b**
N3	72.25	98.25	84.63	**85.04d**
N4	84.26	97.65	92.54	**91.48c**
N5	92.33	106.67	99.51	**99.50b**
N6	95.88	154.36	148.69	**132.98a**
Mean	**83.97c**[y]	**108.01a**	**100.63b**	

[x]Values within a column followed by different letters are significantly different (p<0.05).
[y]Values within same row followed by different letters are significantly different (p<0.05).

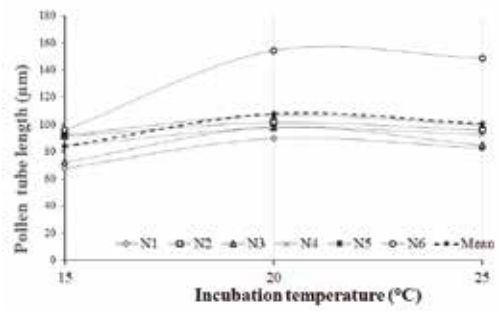

Figure 1. Pollen tube elongation at different incubation temperature

Temperature is among the most important environmental factors affecting plant reproductive processes such as pollen germination pollen tube growth and fruit set (Kakani et al., 2005). Temperature ranges and optimum temperature values for pollen germination and tube growth were studied for different fruit species, including strawberry (Koyuncu, 2006), pears (Vasilakasis and Porlingis, 1985), cherries (Tosun and Koyuncu, 2007a), pistachio species (Acar and Kakani, 2010), pomegranate (Gökbayrak and Engin, 2018) The optimum temperature required for pollen germination and tube growth was about 20^0C for apricot, cherry and sour cherry (Austin et al., 1998; Koyuncu and Tosun, 2009). These results parallel to our findings. Another study which was conducted at apricots as our study, pollen germination and tube growth above 25°C reduced (Egea et al., 1992). Low or high temperatures have negative effects on pollen germination and tube growth. The optimum temperature for pollen germination varies among species and cultivars of the same species (Mert, 2009; Kuroki et al., 2017).

The effects of chemicals on pollen germination and tube growth were statistically different, ($p<0.05$). Potassium nitrate and gibberellic acid were determined as promoter while benzyl adenine effects as inhibitory pollen germination and tube growth (Tables 4, 5). Pollen germination rate decreased to 24.82 in BA supplemented medium and increased to 30.72% in GA_3 added medium by 41.16%. The study which was conducted at blackberries it is reported that the effects of minerals on pollen germination were found to vary according to cultivars and doses (Türemiş and Derin, 1999). Kumar et al. (2016) found GA_3 as a promoter for ornamental tropical tree species pollens.

Table 4. The effect of different plant growth regulator on pollen germination (%)

	Control	**BA**	**KNO_3**	**GA_3**	Mean
N1	48.21	36.2	52.7	55.3	**48.10b**[x]
N2	12.9	8.2	14.71	19.34	**13.78d**
N3	10.5	7.7	18.6	24.5	**15.32d**
N4	13.24	10.2	18.22	26.4	**17.01d**
N5	33.59	27.7	39.79	45.61	**36.67c**
N6	65.87	58.9	68.71	75.8	**67.32a**
Mean	**30.72c**[y]	**24.82d**	**35.46b**	**41.16a**	

Table 5. The effect of different plant regulators on pollen tube length (μm) after 24 hours

	Control	**BA**	**KNO3**	**GA3**	Mean
N1	98.22	94.65	114.26	128.11	**108.81c**[x]
N2	103.3	82.33	109.55	117.09	**103.06c**
N3	94.68	103.26	113.6	120.39	**107.98c**
N4	145.63	127.58	139.41	154.69	**141.82b**
N5	130.65	107.89	156.86	165.99	**140.34b**
N6	151.36	144.85	161.48	174.25	**157.98a**
Mean	**120.64c**[y]	**110.09d**	**132.52b**	**143.42a**	

[x]Values within a column followed by different letters are significantly different (p<0.05).
[y]Values within same row followed by different letters are significantly different (p<0.05).

Pollen germination regulated by water, amino acids, sugars, calcium and growth regulators such as gibberellins, auxins and kinetin. Gibberellins have been traced in developing pollen grain after anthesis (Singh et al., 2002). Our results have been supported these findings. Pollen performance criteria (pollen vitality,

germination and pollen tube growth rate) are critical for discharging male gametes in the embryo sac and are a prerequisite for fertilization and fruit set. *In vitro* germination studies are powerful tools for genetic, physiological, biochemical and cytochemical studies for a wide range of plant species belonging to different families (Radıčević et al., 2013). These studies are also a good predictor of *in vivo* pollen behavior but only for autotrophic phase of pollen growth where the initial steps of pollen germination and pollen tube growth are independent of style nutrients, sugars and plant growth regulators. They help with selections for breeding programmers, *in vitro* assessments can also help to predict possible problems of sterility of that particular genotype in commercial orchards (Fotirić Akšić et al., 2017)

CONCLUSIONS

Pollen performances of wild-grown black-berries were determined. N6 can be thought promising type in for pollen performances. 20^0C was found optimum incubation temperature for pollen germination and pollen tube growth.

Potassium nitrate and gibberellic acid were determined as promoter while benzyl adenine effects as inhibitory pollen germination and tube growth

Fertilization biology studies should be continued at *in vivo* conditions.

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EFFECT OF POSTHARVEST OXALIC ACID TREATMENT ON COLD STORAGE OF APRICOT CV. 'APRIKOZ'

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Abstract

In this study, effects of postharvest oxalic acid (OA) treatments on storage life and quality of apricot cv. 'Aprikoz' were investigated. Fruit were harvested at optimum stage (firm ripe stage) and transported to the postharvest physiology laboratory, immediately. Apricots were immersed in different doses (0, 1, 2, 4 and 8 mM) of OA solution+ Tween 20 for 10 minutes. After treatments, fruit were held at room conditions for drying during 30 minutes. Dried fruit were placed in modified atmosphere packages (MAP) and stored for 40 days at 0°C and 90±5% relative humidity. The weight loss, total soluble solids content, titratable acidity, fruit flesh firmness, fruit skin color, respiration rate and gas composition of MAP were determined at the beginning of the storage and 10-day intervals during the cold storage period. As a result, all doses of OA gave better results than control group in terms of some quality parameters. Especially, 1 mM dose of OA was the most effective treatment for decreasing weight loss and maintaining fruits firmness. The results suggest that OA has the potential to extend the storage life of apricot by delaying quality loss.

Key words*: apricot, oxalic acid, modified atmosphere, cold storage.*

INTRODUCTION

Apricots are one of the most popular fruit in both domestic and international markets owing to its delicious taste and aroma (Özdoğru et al., 2015). Apricots have sufficient amounts of sucrose, glucose, fructose and high antioxidant components (lycopene, β-carotene, vitamins A and E) and minerals (K, P, and Mg). Consumption of apricots plays an important role in preventing diseases and maintaining healthy life (Muradoğlu et al., 2011). Orchard conditions, developmental stage at harvest, and other postharvest factors such as chilling injury, high temperature and mechanical damage influence fruit quality. Fruit quality in apricot is an important factor that affects consumers' perception. Apricots as climacteric stone fruit have a limited postharvest life (Ezzat et al., 2017). The main factor limiting the postharvest life of apricot fruit are very rapid maturation process due to the high rate of respiration after harvest (Abd El Wahab, 2015; Jing et al., 2018). Oxalic acid (OA), as a final metabolite product in plants (Martinez-Espla et al., 2014), plays an important role in physiological functions such as regulating stress responses, resistance against disease (Wu et al., 2011). In addition, postharvest treatments with OA were effective in delaying some processes of climacteric fruit through an inhibition of ethylene biosynthesis (Martinez-Espla et al., 2014). Another beneficial effect of pre-storage OA applications is reduced chilling injury in pomegranate and mango in connection with an enhance in antioxidant capacity (Li et al., 2014). In previous studies, it was reported that both pre- and postharvest OA treatments extended storage life, and maintained fruit quality during the storage period. Zheng et al. (2007a; 2007b) stated that OA treatments in mango and peach fruit were effective in extending the postharvest life. Razavi and Hajilou (2016) treated sweet cherries with OA and stored them for 20 days under cold temperature, and reported that OA delayed the postharvest ripening. In literature, it can be found some researches related to OA but there are not enough studies about the effect of OA on the postharvest life and quality of apricot

during cold storage. The aim of this study was to investigate the effect of postharvest OA treatment on cold storage of apricot cv. 'Aprikoz'.

MATERIALS AND METHODS

Harvest and postharvest treatments

The fruit of apricot cv. 'Aprikoz' were harvested at commercial harvest stage (yellowish-green and firm ripe), and transported to the postharvest laboratory, immediately by a frigofric car. Fruit were selected for uniformity (color, size and shape) and any blemished/diseased fruit were discarded. Selected apricots were randomly divided into five groups. First group (control) apricots were dipped into distilled water + 0.01% Tween-20 (a surfactant) for 10 min. The other four group apricots were dipped into in different doses (1, 2, 4 and 8 mM) of OA solution + 0.01% Tween 20 for 10 minutes. After dipping treatments, fruit were held at room conditions for drying during 30 minutes. Dried fruit were placed in modified atmosphere packages (MAP) (25 µm low-density polyethylene) and stored for 40 days at 0°C and 90±5% relative humidity. All analyses were performed at harvest date and 10 days intervals during cold storage.

Chemical and physical analysis

Weight loss of apricots was measured over 15 fruit in each replicate and expressed as the percentage of loss of weight with respect to the initial weight. Weight loss was determined by the formula; Weight loss = [(First weight - Last weight)/First weight] × 100. Fruit flesh firmness was measured over 15 fruit in each replicate. Fruit flesh firmness (a small slice of fruit skin was removed from each side of a fruit) was determined using a digital texture machine and measured via compression using a 50 N load cell and a stainless steel, 5.1 mm diameter. The results were expressed as Newton (N). Total soluble solid (TSS) content was measured using a digital refractometer (Atago Pocket PAL-1) and expressed as percentage. Titratable acidity (TA) was determined by a digital pH meter (Hanna Instruments HI 9231) and titrimeter (Digitrat, Isolab), and expressed as percentage. Fruit skin color was determined using a Minolta CR-300 colorimeter over 15 fruit in each replicate. The values were expressed by the CIE L^* (brightness-darkness), a^* ($+a^*$: red, $-a^*$: green) and b^* ($+b^*$: yellow, $-b^*$: blue) system. Respiration rate and ethylene production were measured in 600-700 g of fruit samples for each replicate. Fruit were weighed and placed in 2 L airtight jars for 2 h at 20ºC. Then gas sample was taken from jars and injected into gas chromatographs. Results were expressed as µL/kg.h for ethylene production and mL CO_2/kg h for respiration rate. Gas concentration (O_2 and CO_2) in the packages was measured by Gaspace 2 (Gas Headspace analyzer, Systech Instruments) and expressed as percentage. External appearance was rated on a hedonic scale of 1-9 (1-3: unmarketable, 5: marketable, 7: good, 9: very good), taste was rated on a hedonic scale of 1-5 (1: very bad, 2: bad, 3: medium, 4: good, 5: very good) and internal browning was rated on a hedonic scale of 0-4 (0: healthy, 1: 1-10%, 2: 11-33%, 3: 33-66%, 4: 66-100%). The experiment was set up according to the factorial randomized design with 3 replications (40 fruit per replication). Data were subjected to analysis of variance (ANOVA, JMP7), means were separated by means of Tukey test ($P<0.05$).

RESULTS AND DISCUSSIONS

Weight loss. Weight losses of apricots treated with different doses of OA, during the cold storage, was given in the Table 1. Weight loss, one of the most important factors limiting the storage life of the products, has increased continuously during storage. But this increase was found to be lower in OA treatments (except for 4 mM) than in control groups. The weight loss of the apricots treated with dose of 1 mM OA (1.36 %) was significantly delayed compared to control and other doses at the end of cold storage (Table 1.) Fruit lost their weight mainly due to respiration and transpiration through skin and various metabolic activities. The positive effects of OA on weight loss of fruit might be due to slowed metabolic process and decreased respiratory rate (Razzaq et al., 2015). According to some researches which are parallel to our results, OA-treated fruit exhibited reduced weight loss compared with control (Sayyari et al., 2010; Razzaq et al.,

2015). Sayyari et al., 2010 reported that pre-storage OA treatments reduced the weight loss in pomegranate fruit stored at 2°C for 84 days. Also, Razzaq et al. (2015) reported that OA treatments reduced weight loss in mango fruit.

Table 1. Weight loss (%) of 'Aprikoz' apricots treated with OA during MAP storage at 0°C

Treatment	10 d	20 d	30 d	40 d	Means
Control	0.55	1.34	1.94	2.58	1.59 ab*
1 mM	0.56	1.17	1.53	2.23	1.36 c
2 mM	0.64	1.43	1.55	2.53	1.53 ab
4 mM	0.60	1.50	1.93	2.60	1.65 a
8 mM	0.51	1.34	1.57	2.45	1.46 bc
Means	0.57 d	1.35 c	1.70 b	2.47 a	

*Means followed by the same letter in the same row and column are not statistically significant (P<0.05); d: days.

Fruit flesh firmness. The OA treatments had positive effects on fruit firmness. The flesh firmness in treated fruit was maintained compared with the control, thus fruit softening rate was delayed by OA during storage. The highest firmness value (34.43 N) was determined in 1 mM OA treatment, whereas the lowest (30.71 N) was from control fruit (Table 2). There are similar results obtained by other researchers. Studies on peach (Razavi and Hajilou, 2016; Zheng et al., 2007b), mango (Zheng et al., 2007a) and plum (Wu et al., 2011) showed that OA treatments maintained flesh firmness, delayed softening and extended the postharvest life of fruit. Correspondingly Wu et al. (2011) stated that the application of OA delayed softening of plum fruit. They suggested that the inhibition of softening was associated with decreased polygalacturonase (PG) and pectin methyl esterase (PME) activities; that is, the retardation of pectin solubilization/degradation (Razavi and Hajilou, 2016).

Total soluble solid (TSS) content and titratable acidity (TA). The effects of OA treatments on TSS content and TA were statistically significant (P<0.05). The TSS and TA decreased in all treatments with increasing storage period. The TSS of fruit treated with 1.0 and 8.0 mM doses were significantly higher than those of control. The TSS contents varied between 10.50% and 9.57% at the end of 40 days (Table 3). The TA contents of fruit gradually decreased over the storage period regardless of treatments. The highest TA value was obtained from 1 mM dose of OA varying in the range of 1.10-0.92 throughout storage period. Zheng et al. (2007a) reported that OA treatments in mango, a climacteric fruit, increased TSS while TA was decreased. Adverse results of Zheng et al. (2007a; 2007b) related to TSS may be attributed to different species and storage condition.

Table 2. Firmness (N) of 'Aprikoz' apricots treated with OA during MAP storage at 0°C

Treatment	0 d	10 d	20 d	30 d	40 d	Means
Control	36.10	31.46	29.64	29.20	27.13	30.71 c*
1 mM	36.10	35.87	34.23	33.89	32.04	34.43 a
2 mM	36.10	35.00	33.65	32.55	30.31	33.52 ab
4 mM	36.10	33.24	32.30	29.55	28.96	32.03 bc
8 mM	36.10	31.96	31.71	29.34	29.30	31.68 c
Means	36.10 a	33.51 b	32.30 bc	30.91 cd	29.55 d	

*Means followed by the same letter in the same row and column are not statistically significant (P<0.05); d: days.

Table 3. TSS and TA of 'Aprikoz' apricots treated with OA during MAP storage at 0°C

	Treatment	0 d	10 d	20 d	30 d	40 d	Means
TSS	Control	11.53	11.63	11.00	10.80	9.97	10.99 bc*
	1 mM	11.53	11.70	11.47	11.20	10.50	11.28 a
	2 mM	11.53	10.93	10.87	10.63	10.47	10.89 bc
	4 mM	11.53	11.00	10.97	10.70	9.57	10.79 c
	8 mM	11.53	11.90	11.43	10.57	10.27	11.14 ab
	Means	11.53a	11.39ab	11.19b	10.78c	10.15d	
TA	Control	1.10	1.02	1.00	0.89	0.87	0.98 ab*
	1 mM	1.10	1.07	1.02	0.93	0.92	1.01 a
	2 mM	1.10	1.04	0.96	0.92	0.86	0.98 ab
	4 mM	1.10	0.95	0.94	0.90	0.81	0.94 b
	8 mM	1.10	0.94	0.91	0.90	0.77	0.92 b
	Means	1.10 a	1.00 a	0.97 a	0.91 b	0.85 b	

*Means followed by the same letter in the same row and column are not statistically significant (P<0.05); d: days.

Ethylene production and respiration rate. In the present study, the effect of the treatments on ethylene production (µL/kg h) and respiration rate (mL CO_2/kg h) was significant ($P<0.05$). The highest ethylene production (0.58 µL/kg h) was determined from untreated fruit, whereas the lowest ethylene production (0.46 µL/kg h) was detected in 8 mM OA-treated fruit. The OA treatments influenced the ethylene production of apricots depending on dose levels. The average ethylene production of fruit decreased with increasing doses of OA showing its obvious effect on ethylene biosynthesis (Table 4). Respiration rate is an important factor in maintaining quality during cold storage and shelf life of fruit. For this reason, it is important to reduce the respiration rate of the apricots during storage. It can be seen from data that the respiration rates of OA treated fruit are suppressed better than control group. The lowest respiration rate (44.64 mL CO_2/kg h) was detected in 4 mM OA-treated fruit. Similarly in a previous study, the decreased ethylene production by OA contributed to the delaying of ripening of plum fruit, and reported that reduced ethylene production in OA-treated fruit might be ascribed to the reduced 1-aminocyclopropane-1-carboxylic acid synthase (ACS) activity (Wu et al., 2011). In addition, it was reported that OA treatments reduced respiration rate of peach fruit, and inhibited the ethylene production rates in mango and plum (Huang et al., 2013).

Fruit skin color. The results of the effects of OA treatments on the change of fruit skin color are given in Table 5. The effects of storage period and treatments on L^*, a^* and b^* value of fruit skin were statistically significant, ($P<0.05$). L^* value, which shows fruit brightness of fruit skin, decreased during the storage period. The highest L^* value was 72.93 with OA 8 mM treatment. The a^* and b^* values of the fruit generally showed an increase during storage. All treatments showed similar characteristics of the a^* value when compared to the control group. The highest a^* (-6.80) and lowest b^* (44.68) values were obtained from 4 mM OA treated fruit. The skin color of apricots is one of the symbols of fruit senescence. In general, the color of the skin changes from yellow-green (at harvest) to orange-yellow (at the end of storage). OA-treated fruit turned to orange-yellow slowly compared to control fruit during storage. Turning color to dark-orange has been associated with ripening of apricot. OA delayed the ripening process of climacteric fruit such as mango (Zheng et al., 2007a) and peach (Zheng et al., 2007b), due to the inhibition of ethylene production.

Sensory analyses. Storage period and treatments affected significantly the external appearance, taste scores and internal browning of apricots during storage ($p<0.05$).

OA treated apricots preserved their external appearance and taste values better than control fruit (Table 6).

The average highest external appearance (8.79) and taste (4.83) scores were obtained from 1 mM OA treatment during storage.

Table 4. Ethylene production (µL/kg h) and respiration rate (mL CO_2/kg h) of 'Aprikoz' apricots treated with OA during MAP storage at 0°C

	Treatment	0 d	10 d	20 d	30 d	40 d	Means
Ethylene Production	Control	0.54	0.54	0.63	0.73	0.48	0.58 a*
	1 mM	0.36	0.56	0.52	0.67	0.57	0.54 ab
	2 mM	0.37	0.42	0.42	0.80	0.65	0.53 ab
	4 mM	0.40	0.50	0.40	0.50	0.67	0.50 ab
	8 mM	0.43	0.58	0.42	0.45	0.45	0.46 b
	Means	0.42 c	0.52 abc	0.48 bc	0.63 a	0.56 ab	
Respiration Rate	Control	95.97	67.46	48.90	32.77	34.74	55.97 a*
	1 mM	75.52	42.99	48.01	41.14	34.79	48.49 bc
	2 mM	86.28	59.50	50.39	36.55	33.80	53.31 ab
	4 mM	80.01	35.38	33.33	43.25	31.25	44.64 c
	8 mM	84.71	52.83	35.70	39.77	34.35	49.47 abc
	Means	51.63 a	43.27 b	38.70 c	33.79 cd	34.50 d	

*Means followed by the same letter in the same row and column are not statistically significant ($P<0.05$); d: days.

Table 5. Change color (L^*, a^*, b^*) of 'Aprikoz' apricots treated with OA during MAP storage at 0°C

	Treatment	0 d	10 d	20 d	30 d	40 d	Means
L^*	Control	74.07	72.32	73.65	71.90	70.81	72.55 ab*
	1 mM	74.07	73.47	72.75	71.54	70.51	72.47ab
	2 mM	74.07	73.14	73.35	72.26	68.24	72.21 b
	4 mM	74.07	72.65	73.30	71.84	68.13	71.99 b
	8 mM	74.07	74.28	74.22	73.43	68.65	72.93a
	Means	74.07 a	73.17 c	73.45 b	72.19 c	69.26 d	
a^*	Control	-11.79	-9.75	-4.32	-6.02	-4.85	-7.34ab*
	1 mM	-11.79	-8.37	-4.56	-5.60	-7.69	-7.60b
	2 mM	-11.79	-7.53	-5.10	-4.95	-6.51	-7.17ab
	4 mM	-11.79	-7.11	-5.04	-4.54	-5.53	-6.80a
	8 mM	-11.79	-6.87	-5.39	-7.10	-6.41	-7.51ab
	Means	-11.78 d	-7.92 c	-4.88 a	-5.63 ab	-6.19 b	
b^*	Control	43.99	45.18	47.64	46.84	45.16	45.76a*
	1 mM	43.99	47.47	45.71	46.89	44.46	45.70a
	2 mM	43.99	45.20	46.96	45.53	43.00	44.93b
	4 mM	43.99	43.85	45.38	46.94	43.27	44.68b
	8 mM	43.99	47.01	47.34	46.19	43.89	45.68a
	Means	43.99 c	45.74 b	46.60 a	46.47 a	43.95 c	

*Means followed by the same letter in the same row and column are not statistically significant (P<0.05); d: days.

Table 6. The external appearance, taste and internal browning scores of fruit during MAP storage at 0°C

	Treatment	0 d	10 d	20 d	30 d	40 d	Means
External Appearance	Control	9.00	9.00	9.00	7.45	8.33	8.12 d*
	1 mM	9.00	9.00	9.00	8.60	8.05	8.79 a
	2 mM	9.00	9.00	9.00	8.50	7.96	8.71 b
	4 mM	9.00	9.00	9.00	8.20	7.80	8.63 c
	8 mM	9.00	9.00	9.00	8.14	6.15	8.59 c
	Means	9.00 a	9.00 a	9.00 a	8.18 b	7.66 c	
Taste	Control	5.00	5.00	5.00	4.20	3.20	4.42 c*
	1 mM	5.00	5.00	5.00	4.86	4.30	4.83 a
	2 mM	5.00	5.00	5.00	4.25	4.00	4.65 b
	4 mM	5.00	5.00	5.00	4.10	4.00	4.62 b
	8 mM	5.00	5.00	4.70	4.05	3.80	4.57 b
	Means	5.00 a	5.00 a	4.94 a	4.29 b	3.86 c	
Internal browning	Control	0.00	0.00	0.60	1.50	1.90	0.84 a*
	1 mM	0.00	0.00	0.00	1.10	1.40	0.50 c
	2 mM	0.00	0.00	0.00	1.20	1.60	0.56 bc
	4 mM	0.00	0.00	0.00	1.40	1.80	0.64 bc
	8 mM	0.00	0.00	0.00	1.60	1.70	0.66 c
	Means	0.00 c	0.00 c	0.12 c	1.36 b	1.68 a	

*Means followed by the same letter in the same row and column are not statistically significant (P<0.05); d: days.
External appearance: 1-3: unmarketable, 5: marketable, 7: good, 9: very good; Taste: 1: very bad, 2: bad, 3: medium, 4: good, 5: very good; Internal browning: 0: healthy, 1: 1-10%, 2: 11-33%, 3: 33-66%, 4: 66-100%.

Control fruit gave the lowest external appearance (8.12) and taste (4.42) scores. Internal browning of fruit increased compared to the initial values at the end of cold storage regardless of treatment, but the lowest value was determined in 1 mM OA treatment. The OA treatments limited internal browning incidence of apricots (Table 6). It can be said that internal browning is the important and limiting factor for marketable quality of apricots (Koyuncu et al., 2010).

Gas composition. The CO_2 concentration in the packages increased, while a decreasing was found in O_2 concentration compared to initial levels during storage period (Table 7). The effects of storage period and treatments on O_2 and CO_2 values in MAP were significant (P<0.05). The highest average O_2 (15.06%) and

the lowest CO_2 (5.65%) concentrations were measured in packages of 1 mM OA treatment. This means that the 1 mM dose of OA suppressed respiration rate of apricots better than the other treatments. Generally, OA treatments decreased respiration rate of apricots compared to control group according to gas compositions in MAP. Our findings related to respiration rate (Table 4), which indicates suppressing effect of OA on respiration, support present results.

Table 7. The O_2 (%) and CO_2 (%) composition of MAP during storage at 0°C

Treatment		10 d	20 d	30 d	40 d	Means
O_2	Control	15.55	14.55	14.15	11.60	13.96ab*
	1 mM	15.85	15.90	15.25	13.23	15.06 a
	2 mM	15.95	14.35	14.11	12.96	14.34 ab
	4 mM	15.80	15.45	13.90	13.63	14.70 ab
	8 mM	15.85	13.15	12.65	10.90	13.14 b
Means		15.80a	14.68ab	14.01bc	12.46c	
CO_2	Control	5.88	6.30	7.10	8.65	6.98 a*
	1 mM	4.15	4.90	6.30	7.25	5.65 b
	2 mM	5.70	5.95	7.40	8.20	6.81 a
	4 mM	5.85	6.15	7.30	7.90	6.80 a
	8 mM	4.82	6.15	7.30	8.35	6.66 a
Means		5.28 c	5.89 c	7.08 b	8.07a	

*Means followed by the same letter in the same row and column are not statistically significant (P<0.05); d: days.

CONCLUSIONS

In conclusion, all doses of OA gave better results than control group in terms of some quality parameters. Especially, 1 mM dose of OA was the most effective treatment for decreasing weight loss and maintaining fruit flesh firmness, TA and sensory quality. The results suggest that OA has the potential to extend the storage life of apricot by delaying quality loss. Based on our results, it can be indicated that OA could maintain fruit firmness and delayed quality loss of apricot by suppression of ethylene production and respiration rate.

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EFFECTS OF PRE-HARVEST RETAIN TREATMENTS WITH MAP ON COLD STORAGE QUALITY OF SWEET CHERRY CV. '0900 ZIRAAT'

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Abstract

Effect of pre-harvest treatments of ReTain [active ingredient: Aminoethoxyvinlglycine (AVG) 15 %] on cold storage quality of sweet cherry cv. '0900 Ziraat' grafted on Gisela 5 (Prunus cerasus × Prunus canescens) rootstock was investigated. For this purpose ReTain within concentrations of 50, 100, and 150 mg/L was applied as a spray 25 days before harvest. Fruits were harvested at the optimum harvest date and stored in modified atmosphere packages (MAP) at 0°C temperature and 90±5% relative humidity conditions for 6 weeks. Weight loss, fruit skin colour, fruit firmness, total soluble solid and titratable acidity content were determined during the storage period. In addition, sensory analysis was performed. Weight loss increased in all treatments during storage. Titratable acidity of fruit decreased with increasing storage period, but the highest average acidity was determined in fruit treated with 100 mg/L ReTain. The treatment of 50 mg/L of ReTain maintained fruit firmness better than those of others. These results indicated that pre-harvest ReTain treatment can be a tool for maintaining some quality attributes of sweet cherry during cold storage.

Key words: *'0900 Ziraat', sweet cherry, MAP, ReTain, cold storage.*

INTRODUCTION

Sweet cherry is one of the most important crops of Turkey, constituting approximately 20-25% of world production with the production capacity of 599,650 tons (2016 year) (FAO, 2018). In addition to production, sweet cherry, cv. '0900 Ziraat', is an important species for export of Turkey (Eştürk et al., 2012). But fruit losses after harvest are still high depending on the species, harvest methods, transporting and marketing conditions and length of storage. Sweet cherry decays rapidly after harvest, so the consumption period is short and limited. The main causes of fruit deterioration are water loss, stem browning, softening, colour changes and pitting (Bernalte et al., 2003). Water loss and firmness are important quality attributes of cherries, and are directly related to storability (Martinez-Romero et al., 2006). Increase in these postharvest losses can cause great economic losses (Esti et al., 2002).

Many pre- and post-harvest technologies have been used to delay these losses, but the use of chemicals has been limited in some countries. So, alternative technologies for preservation are needed, which have to be considered as healthy and environmentally friendly (Serrano et al., 2005). Among these technologies, the use of modified atmosphere packaging (MAP) has been reported to be effective in cherry storage (Spotts et al., 2002; Tian et al., 2004; Kupferman et al., 2005). The MAP is used for keeping the postharvest quality of fruit by lowering the respiration rate of fruit (decreasing O_2 and increasing CO_2) (Kader et al., 1989). MAP extends the postharvest life of cherry and decreases decay resulting from softening, and maintain green stem colour (Kupferman et al., 2005). As in other fruit, plant growth regulators are used to increase quality and to extend post-harvest life of sweet cherries (Onursal et al., 2013). ReTain is, a commercial product that includes 15% AVG, a plant growth regulator, and effects harvesting criteria (Clayton et al., 2000). Aminoethoxyvinylglycine (AVG) is a natural compound produced in plant tissues (Rath et al., 2006). Researchers have shown that pre-harvest AVG treatment has maintained skin colour and firmness of some fruits (Jobling et al., 2003; McGlasson et al., 2005; Çetinbaş et

al., 2012). This research was carried out to determine the effects of pre-harvest ReTain (AVG) treatment on quality of '0900 Ziraat' sweet cherry during cold storage.

MATERIALS AND METHODS

Plant material and retain treatment

The study was conducted at Fruit Research Institute, Isparta-Turkey. The uniform trees, cv. '0900 Ziraat' sweet cherry, on Gisela-5 (*Prunus cerasus* × *Prunus canescens*) rootstock, were used. Standard cultural practices including, thinning and pesticide sprays were provided to the trees.

For pre-harvest treatment, 50, 100 and 150 mg/L Retain and Tween-20 (0.01%) (as a surfactant) were sprayed on sweet cherry trees as well as distilled water with Tween-20 (pre-harvest control group) 25 days before commercial harvest (when fruits turned to straw-color). Cherries were harvested at commercial harvest maturity from an orchard and transported to the laboratory immediately. Harvested cherries were dipped in cool water (0-2°C) for 8-10 minutes for pre-cooling. After pre-cooling, cherries were placed on the MAP (Xtend) and stored at 0°C and 90±5% relative humidity for 6 weeks.

Chemical and physical analysis

Weight loss of cherries was measured over 5 kg fruits in each replicate and expressed as the percentage of loss of weight with respect to the initial weight. Weight loss was determined by the formula; [(First weight - Last weight) / First weight] × 100.

Fruit skin color was determined using a colorimeter (CR400, Minolta Co., Japan) over 15 fruits in each replicate. Minolta color measurement apparatus was calibrated according to the standard white calibration plate (Y: 92.3, x: 0.3136 and y: 0.3194). The values were expressed by the CIEL* (brightness-darkness), a* (+ a*: red, ** - a*: green) and b* (+ b*: yellow, - b*: blue).

Fruit firmness was determined using a texture analyzer (Guss FTA Type GS14, Strand, South Africa). It was defined as the maximum load required to penetrate the probe (5 mm diameter) into the fruit flesh (6 mm). The results were expressed in Newton (N).

Total soluble solids (TSS) content was measured using a digital refractometer (HI 96801, Hanna, UK) and expressed as a percentage (%). Titratable acidity (TA) was determined by an automatic titrator (Mettler Toledo T50, Switzerland) and expressed as grams of malic acid equivalent per 100 g fresh weight.

Overall acceptability was rated on a hedonic scale of 0-2 (0: good commercial quality, 1: some damage but still commercially salable, 2: not commercially salable), described by Feng et al. (2004). Taste was rated on a scale of 1-5 (1: very bad, 2: bad, 3: medium, 4: good, 5: very good), described by Erbaş and Koyuncu (2016).

The experiment was set up according to the factorial randomized design with 3 replications (5 kg fruit per replication). Data were subjected to analysis of variance (ANOVA, JMP7), means were separated by means of LSD test ($P<0.05, 0.01, 0.001$).

RESULTS AND DISCUSSIONS

Weight loss which is the most important factor limiting the storage period, increased during storage in all treatments, especially in control treatment (Table 1). In this study, the weight loss of cherries was low levels because of the water vapour permeability properties of the MAP. The effects of storage periods on weight loss were statistically significant but there was no significant effect of treatments ($P<0.01$) (Table 4). At the end of storage, the weight loss of cherries was between 2.36% (control) and 2.07% (100 mg/L) (Table 1). The results found in this research agree with those of previous studies (Üstünel et al., 2008; Machado et al., 2010).

Table 1. Effect of pre-harvest ReTain treatment on weight loss of '0900 Ziraat' cherries stored at MAP

	1 w	2 w	3 w	4 w	5 w	6 w	Means
Control	1.69	2.04	2.05	2.19	2.78	3.38	2.36^{ns}
50 mg/L	1.10	1.66	1.87	2.64	2.92	2.97	2.19
100 mg/L	1.57	1.61	2.22	2.27	2.34	2.42	2.07
150 mg/L	1.64	2.01	2.03	2.21	2.81	3.18	2.31
Means	1.50C*	1.83C	2.04BC	2.33ABC	2.71AB	2.99 A	

*Means followed by different letters with in the same row is significantly different at $P<0.01$; ns: nonsignificant; w: weeks.

Softening of cherries reduces both market value and consumer acceptability. Changes in fruit firmness during cold storage are presented in

Table 2. The effect of storage time and treatments on the fruit firmness were statistically significant (P<0.01) (Table 4). Fruit firmness decreased with increasing storage periods but generally, fruit softening were minimized by ReTain treatment regardless of doses. At the end of the storage, 100 mg/L doses of ReTain was the best treatment for maintaining of firmness compared to other treatments. The positive effects of ReTain treatment combined with the MAP on fruit firmness were recorded in this study. In previous studies, pre- and post-harvest ReTain treatments maintained fruit firmness better than control groups during the storage (Drake et. al., 2006; Kharoshaki et. al., 2008; Lara, 2013). These results can be explained by delaying water loss and maintaining pectin level in the cell wall of fruit related to ReTain.

A large part of the TSS is composed of sugars, and change of TSS during the storage is due to changes in fruit carbohydrate structure. The water loss of fruit affects fruit TSS content, and generally it increase (Kader, 1989). But in this study, the weight loss of cherries was very low (Table 1) so the increasing of TSS was low, too. The TSS of fruit increased at the end of 42 days compared to initial values, with fluctuation during storage (Table 3). No significant interaction existed between treatments and storage periods, but differences between storage periods and treatments for TSS values were significant (Table 4). The lowest change in TSS value according to initial values was obtained from dose of 150 mg/L (Table 3). Similar observations were recorded by Remon et al. (2000) and Onursal et al. (2013) for cherries. The main factor of taste formation in fruit and vegetables are TA (Karaçalı, 2009). Storage periods and treatments significantly affected TA contents of cherries (Table 4). During the 6-weeks cold storage, the lowest average (0.65%) TA value was obtained from 50 mg/L dose of ReTain, and the highest (0.68%) in 100 mg/L dose of ReTain. 150 mg/L dose of ReTain treatment was the best treatment for maintaining the TA. During storage, TA values were continuously decreased (Table 3). Our results are in agreement with Khorshidi et al. (2011) who reported that sweet cherries, a nonclimacteric fruit, use sugars and acids for respiration, so TA can decrease depending on storage period.

The colour of sweet cherries is probably the main quality attribute considered by consumers. Changes in fruit skin color during cold storage are given in Figure 1. L^* values, which shows fruit brightness-darkness, decreased throughout storage. Fruit in control group lost their brightness more than ReTain treatments. While the highest mean L^* value was obtained from the dose of 100 mg/L (31.55), the greatest decrease (27.09) occurred in control group at the end of the storage. Similar trend was also observed for a^* and b^* values of skin colour. The a^* ($+a^*$: redness, $-a^*$: greenness) and b* ($+b^*$: yellowness, $-b^*$: blueness) values steadily decreased during the storage. However, the amount of decrease in ReTain-treated cherries less than control treatment (Figure 1). ReTain treatments have been reported to be beneficial in maintaining fruit colour (Onursal et al., 2013).

The results of the sensory analyses are presented in Figure 2. Overall acceptability and taste decreased during storage. Storage period and treatments affected significantly the external appearance and taste scores of cherries during storage (p<0.01) (Table 4). ReTain-treated cherries (especially 100 mg/L dose) preserved their taste scores better than control fruit. The better taste was obtained from 100 mg/L dose during 5 weeks, while control fruit had a bad taste after 3 weeks of storage. Similar results were reported by Drake et al. (2006) and Olmstead et al. (2012).

Table 2. Effect of pre-harvest ReTain treatment on fruit firmness of '0900 Ziraat' cherries stored at MAP

	0 w	1 w	2 w	3 w	4 w	5 w	6 w	Means
Control	2.65	2.55	2.47	2.45	2.39	2.17	1.68	2.34b*
50 mg/L	2.65	2.60	2.59	2.57	2.49	2.48	2.52	2.56a
100 mg/L	2.70	2.34	2.71	2.71	2.75	2.67	2.37	2.61a
150 mg/L	2.52	2.43	2.39	2.40	2.35	2.30	1.99	2.34b
Means	2.64A*	2.48AB	2.54A	2.53A	2.50AB	2.41BC	2.14C	

*Means followed by different letters with in the same row and column are significantly different at P<0.01; w: weeks.

Table 3. Effect of pre-harvest ReTain treatment on total soluble solid content and titratable acidity of '0900 Ziraat' cherries stored at MAP

		0 w	1 w	2 w	3 w	4 w	5 w	6 w	Means
TSS	Control	15.81	16.07	16.37	17.00	16.50	16.07	15.93	16.25 b*
	50 mg/L	15.43	15.80	16.67	16.77	17.97	16.98	15.70	16.34 a
	100 mg/L	16.83	17.40	17.83	17.87	18.07	17.97	17.43	17.63 a
	150 mg/L	15.60	16.37	16.80	17.43	17.23	16.63	15.63	16.53 b
	Means	15.92AB*	16.41BC	16.92B	16.25C	17.27BC	16.91A	16.17BC	
TA	Control	0.79	0.72	0.68	0.63	0.63	0.60	0.57	0.66 bc
	50 mg/L	0.76	0.72	0.65	0.64	0.60	0.59	0.58	0.65 c
	100 mg/L	0.80	0.75	0.69	0.68	0.65	0.61	0.60	0.68 a
	150 mg/L	0.78	0.74	0.67	0.65	0.64	0.61	0.61	0.67 ab
	Means	0.78A*	0.73B	0.67C	0.65C	0.63D	0.61D	0.59D	

*Means followed by different letters with in the same row and column are significantly different at P<0.01; w: weeks.

Table 4. Anova for dependent variables for treatments, storage period and their interactions for cherries

	Weight loss	Firmness	Overall acceptability	Taste	L*	a*	b*	TSS	TA
SP	**	**	**	**	**	**	**	**	**
T	ns	**	**	**	**	**	**	**	**
SP × T	ns	ns	ns	ns	**	**	**	ns	ns

SP: Storage period; T: Treatments; ns: represents non-significance at P< 0.05; **Represents significance at the 0.01; TA: Titratable acidity; TSS: Total soluble solid.

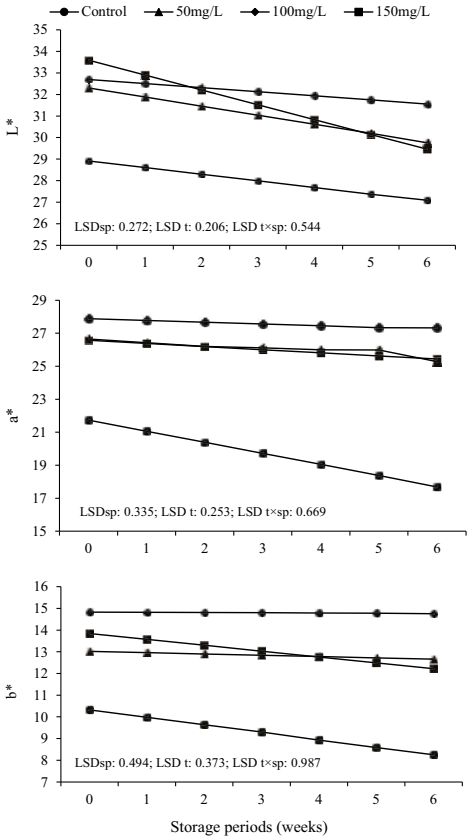

Figure 1. Effect of pre-harvest ReTain treatment on fruit skin color (L*, a* and b*) of '0900 Ziraat' cherries stored at MAP (SP: Storage period; T: Treatments)

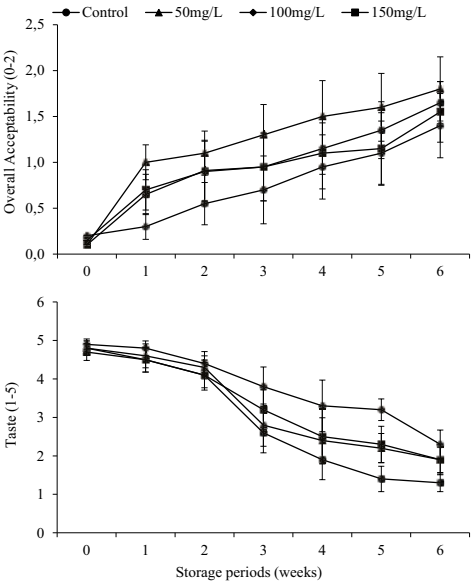

Figure 2. Effect of pre-harvest ReTain treatment on sensory analysis of '0900 Ziraat' cherries stored at MAP Vertical bars represent the standard error of the mean (n=3). Overall acceptability: 0: good commercial quality, 1: some damage but still commercially salable, 2: not commercially salable; Taste scores: 1: very poor, 2: poor, 3: mild, 4: good, 5: excellent

CONCLUSIONS

At the end of the 6 weeks of storage, AVG treatment was more effective for maintaining

postharvest quality of cherries compared to control treatment. Treated cherries with AVG delayed colour changes, softening and loss of acidity. In addition, AVG treatment was effective in the maintaining sensory quality during the storage. Especially 100 mg/L AVG as pre-harvest treatment was the most effective dose and it can be used to maintain sweet cherry postharvest quality during storage of 35 days at 0°C.

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AN INVESTIGATION ON EFFECTS OF DRY AND WET CLIMATE CONDITIONS ON PISTACHIO (*PISTACIA VERA*) YIELD IN MIDDLE EUPHRATES BASIN, SOUTHEAST OF TURKEY

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Abstract

Extreme conditions in the climate play an important role on the plant growth and yield of crops. Meteorological drought is one of these extreme climate conditions. In this study, 16 days photosynthetic activity of pistachios was investigated by integrating both field research and modern techniques (remote sensing and statistical methods). Furthermore, the effects of climatic factors on the yield of pistachio were analysed. Along with the present study, the relationships between pistachio and dry and wet climatic conditions were presented. According to the results of the drought analysis of both Birecik (a district in the south-eastern parts of Turkey) and Gaziantep (a province in the south-eastern parts of Turkey) stations, pistachio is significantly affected by extreme climatic conditions. The photosynthetic activity of pistachio increased during the periods of wet climatic conditions.

Key words*: climate, drought, photosynthetic activity, Pistacia vera.*

INTRODUCTION

Turkey's southern line constitutes one of the sensitive areas where short or long-term climate change in the world can be experienced. Southeast Anatolia is particularly vulnerable to climate change (Cosun and Karabulut, 2009). One of the most important consequences of climate change is the increase in extreme climatic conditions. The arid period, in which the humid period with extreme precipitation and the amount of rainfall and humidity are well below the average, is the extreme climate. Depending on the climate change, there is an increase in the frequency of extreme climatic conditions in the area where Gaziantep, Şanlıurfa and Adıyaman province are located.

Depending on this increase, significant changes occur in the phenology and yield of agricultural products. Also, many previous studies report that changes in climate varieties can lead to phenological changes in agricultural products and changes in phenological cycles will have significant effects on agricultural production (Box et al., 1989; Alexandrov and Hoogenboomb, 2000; Chmielewski and Rötzer, 2001; Ichii et al., 2002; IPCC, 2007; Sanchísa and Feijoo Bello, 2009; Cheng and Wu, 2011).

The study of the impact of climate change on the phenology of agricultural products has a major precaution in terms of food supply and socio-economic in the world with a population of over 7 billion. One of the most worried issues for the future is the danger of agricultural production due to extreme climatic conditions and the resulting scarcity. The answer to the question of how global climate change will impact on agricultural production is among the most important concerns of concern all over the world (Tubiello and Fischer, 2007).

Pistacia vera (pistachio) belonging to Anacardiaceae is of the characterized crop for the South-eastern parts of Turkey. Southeastern Anatolia Region of Turkey is the most pistachio producing region. This region is followed by the Mediterranean and Aegean

regions (Külekçi and Aksoy, 2011; Ertürk et al., 2015). More than 80% of the pistachio-planted areas in the Southeast are in Gaziantep and Şanlıurfa. Gaziantep and Sanliurfa also covers a portion of 78% of the pistachio field in Turkey. Therefore, any possible incident regarding drought that may emerge in Sanliurfa and Gaziantep affect significantly the production of pistachios in Turkey. In Turkey, pistachio plantings of that period it was first built in 1960 production was 34 tons (Gul and Akpinar, 2006) in a humid climate today late in the period, this figure rises to 150 tons. In the years when the climate is arid (for example in 2014), pistachio production has been determined as 80 tons. Therefore, it is necessary to extensively analyze the effect of climate conditions on the production of pistachio nuts. The results of the drought analysis applied to the climate data obtained from the General Directorate of Meteorology (MGM) show that both in Sanliurfa and in Gaziantep, the years of 2012 and 2013 are humid. On the other hand, in 2014, it is observed that dryness occurs in these two stations where pistachio production is highest. Significant changes in Turkey's pistachio yield during dry and wet climate periods are great concern.

In this context, the current study was designed to investigate the possible influence of dry and wet climate conditions on the pistachio nut yield from two stations, namely Gaziantep and Sanliurfa, which are considered as main pistachio suppliers for Turkey.

MATERIALS AND METHODS

For the current study, three provinces in the south-eastern parts of Turkey (Gaziantep, Sanlıurfa and Adıyaman) were selected to due to their potential pistachio production (Figure 1). In this study, during the dry and wet climatic periods, the yield and phenology of pistachios were monitored. In this context, Moderate Resolution Imaging Spectroradiometer (MODIS) and National Oceanic and Atmospheric Administration (NOAA) Advanced Very High Resolution Radiometer (AVHRR) satellite data were used. The meteorological satellites carrying the NOAA-AVHRR sensor with spatial resolution of 1 km are scanning the globe twice a day. The first of these takes place at around 10 pm local time and the other at 2.30 pm. All these collected data are collected via various earth stations (Karabulut, 2006).

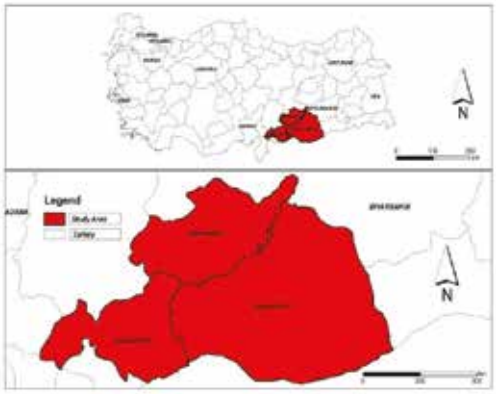

Figure 1. Location map of study area

MODIS NDVI images with a spatial resolution of 250 m are published in 16 day composites for drawing twice a day. Radiometric resolution of the images taken twice a day for 16 days is published by compositing the images that are free from brdf and cloud. The MODIS NDVI images, consisting of 4800 rows and 4800 columns, are capable of analyzing the variation in vegetation activity over a wide area (Çelik and Sonmez, 2013).

Another plant index model that has recently been used frequently is Enhanced Vegetation Index (EVI). EVI images are not affected much by aerosol and cloudiness. The EVI images provided by MODIS to its users are calculated by the following formula (Huete et al., 2002; Galvão et al., 2011; Zhang et al., 2014):

$$EVI = G \times (\text{Near Infrared-Red}) / (\text{Near Infrared} + C1 \times \text{Red-C2} \times \text{Blue} + L)$$

The gain factor used in the formula is 2.5, L = 1, C1 = 6, C2 = 7.5. With this formula values appear in the band interval ranging from -1 to +1. EVI displays, which are not much affected by atmospheric conditions, do not show too much deviation during the year as a trend.

In order to determine the periods when the climate was arid and humid, Standardized Rainfall Index (SPI) developed by Mckee et al. (1993) was used and SPI was calculated by the

following formula;

$$SPI = \frac{X_i - X_i^{ort}}{\sigma}$$

According to the results of the formula, when the index falls below zero, it is considered as the beginning of the month, and when the index is positive, the month is considered as the end of the drought.

RESULTS AND DISCUSSIONS

Monitoring of agricultural areas in short periods is of great importance in terms of revealing the effects of environmental phenomena (such as climatic and biotic factors). In this regard, remote sensing technology has many advantages over conventional methods since traditional methods are inadequate in detecting short-term changes in large agricultural areas. In this study, 16 days photosynthetic activity of pistachios was investigated by integrating both field research and modern techniques (remote sensing and statistical methods). Furthermore, the effects of climatic factors on the yield of pistachio were analysed. Along with the present study, the relationships between pistachio and wet and dry climatic conditions were presented. It has been observed that extreme rainfall conditions have caused considerable changes on the pistachio phenology and physiology. The area harvested, yield and production of pistachio between 2000-2016 years in Turkey are represented Table 1 and Figure 2 (FAOSTAT, 2016). Pistachio yield and production values fluctuate between years. Fluctuation or irregular bearing are considered as consequences of endogenous and exogenous factors. Endogenous factors are defined as plant own characteristics including cultivar, agricultural practises, genetic and physiology of plant (Nzima et al., 1997; Spann et al., 2008). Exogenous or environmental factors such as climatic variation of temperature and precipitation, wet and dry climate condition are significant predictor on yield (Elloumi et al., 2013).

Middle Euphrates region provides a significant portion of which Turkey pistachio production. Turkey meets 20-25% of the world pistachio production. Yield and yield per tree are low in Turkey. Kumar et al. (2016) reported that the low yield of pistachio could be consequences of periodicity, inadequate pollination, fertilization, water stressful conditions and traditional cultural practices.

Table 1. Area harvested, yield and production of pistachio between 2000-20016 in Turkey

Years	Area harvested (ha)	Yield (t/ha)	Production (t)
2000	36349	2.0633	75000
2001	36999	0.8108	30000
2002	37428	0.9351	35000
2003	37570	2.3955	90000
2004	37572	0.7985	30000
2005	40000	1.5000	60000
2006	40000	2.7500	110000
2007	40661	1.8056	73416
2008	40954	2.9329	120113
2009	43063	1.8994	81795
2010	42310	3.0253	128000
2011	44097	2.5399	112000
2012	53071	2.8264	150000
2013	54451	1.6272	88600
2014	56186	1.4238	80000
2015	57996	2.4829	144000
2016	60814	2.7954	170000

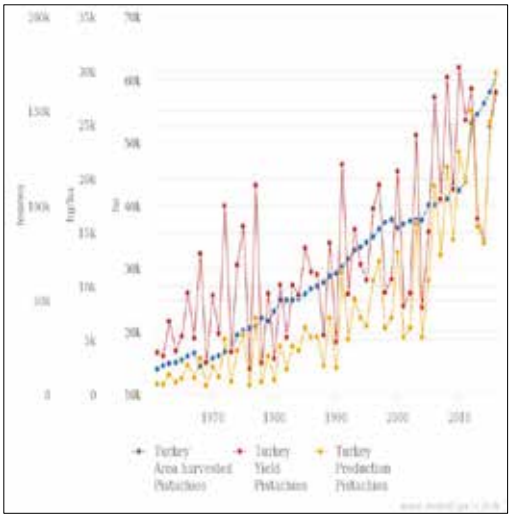

Figure 2. Changes Area harvested, Yield and Production of pistachio between 2000-20016 in Turkey

Variation in climatic changes is of the predictive factors on pistachio yield in Turkey. In the study area, there has been extreme dry period (severe dry, very severe drought, extraordinary dry) 5 times in last 54 years. In

contrast to this, it is observed 8 times extreme humid period (very humid, extremely humid, extraordinary humid). The year 2014 is the most arid period of the last 54 years in the study area. 2012 is one of the five most humid periods of the last 54 years (Figures 3, 4).

Pistachio nut yield changes depending on dry and wet periods in Gaziantep affect the average yield of Turkey. Accordingly, the year 2014 is an extraordinary dry period in the meteorological station of Gaziantep, Şanlıurfa and Adıyaman. Turkey's average yield was 1.4238 t/ha of pistachios for 2014. In contrast, the average value of Turkey was found to be 2.8264 t/ha for 2012 when is excessive wet period.

Pistachio trees require very hot climates in summers and cold climates in winters. In this context, pistachios are resistant to extreme hot conditions, while photosynthetic activity declines during the dry periods. It is observed that the photosynthetic activity of pistachio is particularly low in the growing season during the dry season of 2014. In contrast, the Enhanced Vegetation Index (EVI) values are above normal in the corresponding humid period of 2012 (Figure 5).

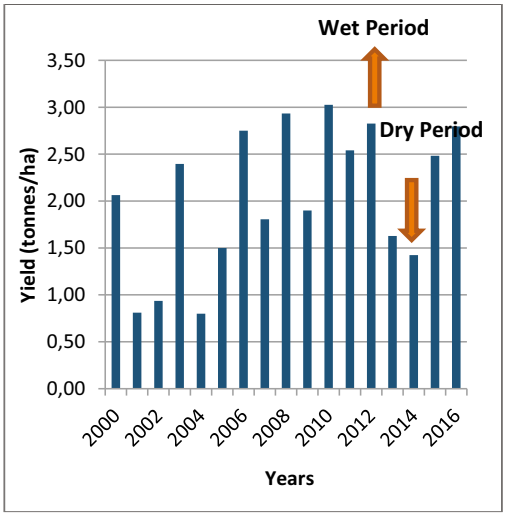

Figure 3. Yield of pistachios in wet and dry periods

As reported in many and various studies, any shift in metabolic activities of plants which might be consequences of plant-own structure or external-induced perturbations result changes in biochemical and physiological aspects as a consequence of molecular level changes. As a response to drought conditions, Khoyerdi et al. (2016) reported the shifts in value of vegetative growth, RWC, TChl and carotenoids, TSP and an increase in MDA and H_2O_2 etc. while reduction rate concerned with cell expansion and division rate were reported by Li et al. (2009).

Based on the literature, we cannot deduce or simplify the factors responsible for irregular bearing, which is of the complex phenomenon of the plant kingdom (Spann et al., 2009).

CONCLUSIONS

Extreme conditions in the climate play an important role on the crop production. Meteorological drought is one of these extreme climate conditions. Depending on global climate changes, plant growth and physiology are influenced and causes many problem in agriculture. According to the results of the drought analysis of Gaziantep, Sanliurfa and Adiyaman (in the south-eastern parts of Turkey) stations, pistachio is significantly affected by extreme climatic conditions. The photosynthetic activity of pistachio trees grown in Gaziantep, Sanliurfa and Adiyaman increased during the periods of wet climatic conditions. Especially at the beginning of 2010, pistachio plant exhibited highest photosynthetic activity and the activity was considered to be associated with wet climatic conditions in both areas at the end of 2009. The present results indicate that the pistachio does not react immediately to wet climatic conditions, suggesting that pistachio respond or develop any mechanisms to this climatic stimulant 2 and 3 months after the humid climatic conditions.

It has been observed that pistachio was affected by extreme climatic conditions and extreme rainfall conditions have caused considerable changes on the pistachio phenology and physiology. As a result it was observed that the photosynthetic activity of pistachio increased during the periods of wet climatic conditions.

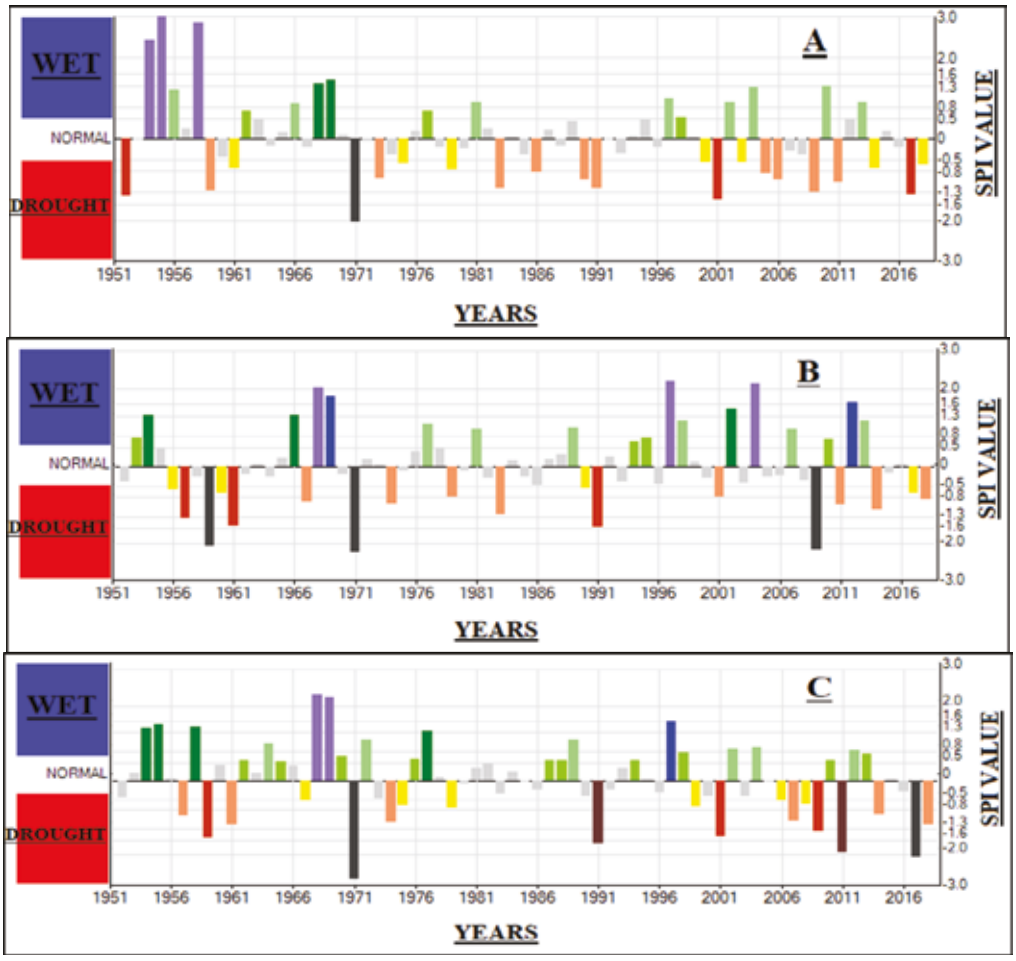

Figure 4. Drought analysis results of Standardized Precipitation Index (SPI) of the Adıyaman (A), Gaziantep (B) and Sanliurfa (C) (1952-2018) Source: General Directorate of Meteorology (MGM)

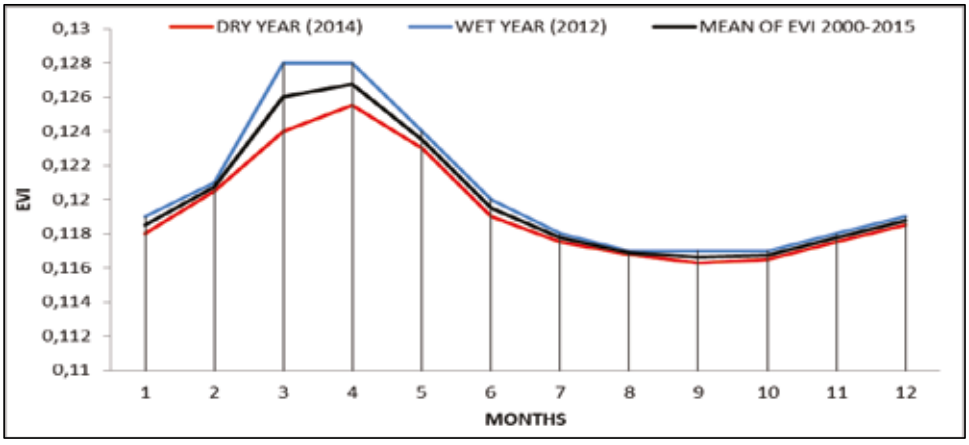

Figure 5. Photosynthetic activity in wet and dry periods according to EVI analysis results

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LOW COST AND LABORATORY SCALE NIR SPECTROSCOPY FOR QUALITY EVALUATION OF FRUITS AND VEGETABLES

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Abstract

NIR spectroscopy has proved to be one of the efficient and easy tools to monitor the quality of agricultural products. NIR spectrometers are versatile devices to monitor the ripeness or quality parameters of the fruits. We demonstrate a low-cost spectrometer design that is produced with off the shelf components. In this work, the development, characterization and validation of a prototype is discussed. The proposed device has a dedicated user interface on the PC to plot and analyze spectral data. The performance of the proposed spectrometer is comparable to existing laboratory scale spectrometers in terms of stability and resolution. The spectral resolution and response range of the proposed spectrometer are 20 nm and 640-1050nm, respectively. Proposed device consists of MEMS based Hamamatsu spectrometer sensor (C11708MA), microcontroller (Arduino) and IR light source. Roles of the Arduino are generating essential control signals and sampling output of the C11708MA. These spectral response data have a huge advantage in generating data sets that may be useful in building machine learning based models.

Key words*: fruit and vegetable quality spectrometer, near infrared spectroscopy, non-destructive detection.*

INTRODUCTION

Spectroscopy is gaining importance for fruit firmness detection, pharmaceutical and environmental monitoring. Near infrared (NIR) is a fast and non-destructive method that can be applicable to any biological or non-biological materials. NIR technique has been widely used to measure internal qualities of various fruits such as tomato, apple, and kiwifruit (Huang, 2018; Ye et al., 2017; Li et al., 2017). Spectroscopy technique can be utilized in detecting plant diseases (Khaled et al., 2018). Besides, multiple spectrometer modules can be installed into packaging or processing lines for measuring quality characteristics of the goods. However, most spectrometer equipment which is utilized in the industry or laboratory is expensive and bulky. Although, there has been some work on portable and low cost spectroscopy devices, there is a need for several improvements in terms of quality and repeatability of data (Das et al., 2016). Also, excessive price of these devices makes them less accessible for many researchers. This work aims to demonstrate a low-cost spectrometer design that is produced with off the shelf components.

An NIR spectrometer consists of a light source (tungsten, halogen or LED), sample presentation accessory, detector, and optical components, such as lenses. NIR radiation covers the range of the electromagnetic spectrum between 780 and 2500 nm. NIR spectrometer is a device that irradiates the sample with NIR radiation and measures the reflected or transmitted radiation.

There are three different measurement setups for obtaining near infrared spectra. These are reflectance, transmittance and interactance modes. In reflectance mode light source and sensor are mounted under a specific angle. In transmittance mode the light source is positioned opposite to the sensor. In interactance mode the light source and detector are positioned parallel to each other.

Micro Electro Mechanical Systems (MEMS) offers inherently device miniaturization and wide applications in sensors and actuators Thanks to MEMS technology spectrometer components can be easily produced in one package. Proposed spectrometer utilized with

MEMS based Hamamatsu spectrometer sensor (C11708MA) (Hamamatsu, 2018). C11708MA spectrometer sensor allows designing low price and high-performance spectrometer design. Longitudinal section view of the C11708MA is given is Figure 1.

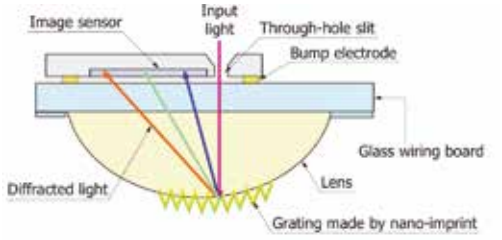

Figure 1. Longitudinal section view of the C11708MA (Hamamatsu, 2018)

C11708MA is equipped with 256-pixel CMOS linear image sensor, micro optical slit (75 x 750 µm), a grating that is formed on a convex lens. These features have made the C11708MA very compact and versatile.

In this work, we demonstrate a low-cost spectrometer design that is produced with off the shelf components.

The development, characterization and validation of a prototype are discussed. The proposed device has a dedicated user interface on the PC to plot and analyse spectral data.

The performance of the proposed spectrometer is comparable to existing laboratory scale spectrometers in terms of stability and resolution. Technical specifications of the C11708MA are given in Table 1.

Table 1. Technical specifications of the C11708MA

Dimensions	27.6 x 16.8 x 13 mm
Weight	9g
Spectral range	640-1050 nm
Supply Voltage	5 V
Power Consumption	30 mW
Number of pixels	256

The digital resolution and response range of the proposed spectrometer are 2 nm/pixel and 640-1050 nm, respectively. The designed device and supplied software is user friendly so that anybody with basic knowledge of spectroscopy can easily use it. Spectrometer device communicate with computer via USB cable.

MATERIALS AND METHODS

In this section, the essential components of the proposed spectrometer are described in detail. The block diagram of the spectrometer system designed in this study is given in Figure 2.

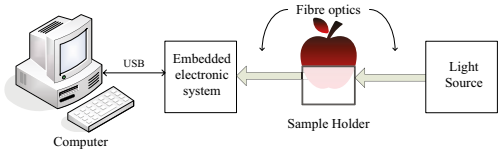

Figure 2. System containing computer, embedded system and light source

Proposed device consists of three parts: computer, embedded electronic system and light source. Light source holds the NIR lamp and fibre optic cable in an appropriate geometry. In our work, we prefer tungsten lamp as the source of the NIR light. Higher light intensity levels can be obtained by using tungsten lamp. Power consumption of tungsten lamp is somewhat high respect to the LED and laser sources. In future works for the mobile and battery-based operation system can easily utilize with LED light source.

Embedded electronic system is responsible for the generating control signal of the MEMS spectrometer chip.

The block diagram of the embedded electronic system is given in Figure 3.

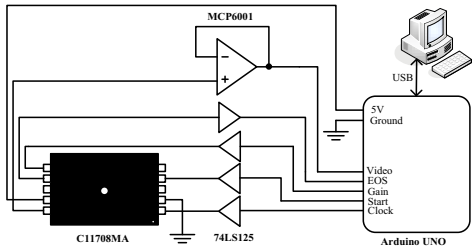

Figure 3. Block diagram of the embedded electronic system

Arduino UNO generates clock pulse for C11708MA which was set to 1 kHz in the proposed design. Arduino sends a start pulse to C11708MA to trigger the pixel read-out process and the interval between two start pulses is accepted as the integration time of the sensor. When Arduino receives a capture command from MATLAB, a start pulse was

triggered which initiated the charge integration of each pixel. The C11708MA provided an End of Scan (EOS) pin which was used to terminate the read out process. As declared above, main purpose of the embedded electronic system is sampling and sending the spectrum data to the computer. Embedded electronic system is controlled by the computer software for coherent detection of spectrum. Embedded electronic system utilized with low cost Arduino microcontroller board. Roles of the Arduino are generating essential control signals and sampling output of the C11708MA. Arduino UNO contains built in 10-bit precision Analog to Digital converter (ADC). Resolution of this ADC is suitable for NIR spectrometer application. C11708MA contains 5 digital I/O interface pins for logical control and one analog output for spectral response data. To avoid excessive current sink from analog output port buffered with OPAMP (MCP6001) buffer amplifier.

The embedded software based upon the open-source Arduino IDE platform. The Arduino firmware was developed with the Arduino IDE and provides an easy to navigate hierarchical menu system for selection of device functions. The computer software was developed in the MATLAB environment. The computer software stores and displays received spectrum information.

As decelerated above, C11708MA contains 256 pixels to detect specific wavelengths. These pixels are numbered and these numbers have to be transformed into the corresponding wavelength. This can be done by a multiorder polynomial as given in Eq. (1).

$$\lambda(nm) = A_0 + B_1 x + B_2 x^2 + B_3 x^3 + B_4 x^4 + B_5 x^5 \quad (1)$$

where, x is the pixel number and A_0, B_1, B_2, B_3, B_4 and B_5 are calibration coefficients whose values are supplied by the sensor producer. This approximation polynomial gives a precision wavelength vector. Computer software generates a wavelength vector by using Eq. (1). Computer software also detects the peak and Full Width at Half Maximum (FWHM) values of the spectrum. Peak and FWHM values are important for evaluating the properties of the sample.

Off the shelf fibre optic cable has been used for the assessment of light levels inside the fruit sample which has been illuminated with NIR light source. The probe has minimal effect on the light being measured and enables the direct measurement of light levels at any location in the fruit.

The spectrometer, light source and sample holder design was wholly completed in Sketchup (Sketchup, 2018), a solid modelling software designed by Google. The design of the case body is shown schematically in Figure 4. The case was printed with polylactic acid (PLA) media.

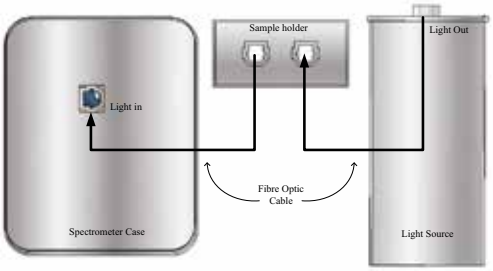

Figure 4. 3D design of the spectrometer

RESULTS AND DISCUSSIONS

The spectra of apple and carrot fruit samples are shown in Figures 5, 6, respectively. The similar spectral pattern was also observed in the spectra of apple fruit measured in the interactance mode.

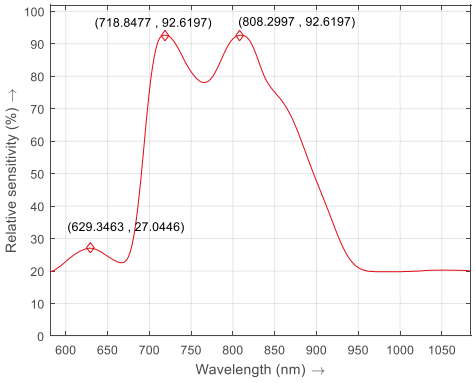

Figure 5. Transmittance spectrum of apple

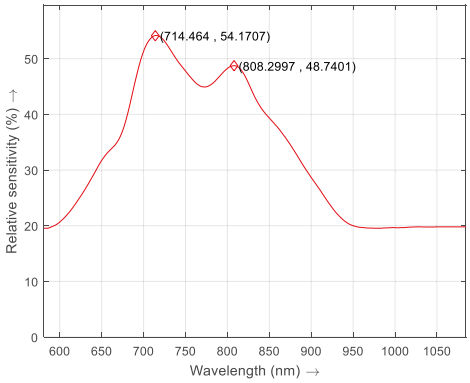

Figure 6. Transmittance spectrum of carrot

CONCLUSIONS

In conclusion, the designed MEMS based NIR spectrometer is a portable and low-cost device. In the Results and Discussions section it was shown that proposed device works with sensitivity and resolution acceptable for many tasks both in education and research. Future work is necessary for this design to realize the full potential of the tool for fruit internal properties detection applications. As discussed above main role of this device is obtaining set of spectral response data for various types of fruits. These spectral response data may be useful in building machine learning based models. In addition, work is needed to make the NIR spectrometer portable, such as the incorporating batteries.

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INCREASING THE ECONOMIC PERFORMANCE BY PROMOTING HIGH-DENSITY APPLE ORCHARDS IN THE DÂMBOVIŢA FRUIT BASIN

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Abstract

The researches carried out at the Research and Development Station for Fruit Growing Voineşti in the period 2015 - 2017, had as objective the evidence of modern plantations established after 2007 by the producers of SC Mere de Voineşti SRL; Owner Dan Ionescu, and I.I. Luminita Marin from Malu cu Flori. The tree culture in high density system extends mainly to apple trees in the Dâmboviţa fruit-tree basin, the interest of fruit growers is evident in the promotion of productive varieties, well adapted to the pedoclimatic conditions of the area, grafted on M.9 rootstock planted at a distance of 3,5 x 1 m, with a density of 2,857 trees/ha. The biggest yield belonging to SC Mere de Voineşti SRL were harvested from following varieties: 'Golden delicious' (43.2 t/ha), 'Braeburn' (35.4 t/ha) and 'Gala' (35.2 t/ha), trees that are 9-11 years old, in comparison with 'Gala' apple varieties (36.2 t/ha) and 'Idared' (33.8 t/ha) of 7-8 years old. In the apple tree orchard for 3 years, in the 'Golden delicious' and 'Ionaprince' varieties, production of 40.2 t/ha and 38.6 t/ha was achieved in 2017. At the Owner Dan Ionescu, with 8 years old trees, the most productive varieties were: 'Golden delicious' (38.9 t/ha), 'Idared' (34.7 t/ha) and 'Gala' (32.7 t/ha), and the producer I.I. Luminita Marin , located in Malu cu Flori, with trees in the 4th year after planting, recorded 34.3 t/ha in the 'Red Gala' variety, 46.8 t/ha in 'Golden delicious' and 42.8 t/ha to 'Pinova'. The high density apple system is recommended for expansion in the well-established fruit-growing areas of our country, including the Dâmboviţa fruit basin, due to the high yield and efficiency, the way of periodic and rapid replacement of the assortments, demanded more and more by consumers.

Key words*: high apple density system, apple assortment, yield, crop systems.*

INTRODUCTION

Fruit growing remains one of the field of horticulture of great interest to growers in the traditional fruit-growing areas in Romania. Both from an economic and social point of view, the cultivation of trees and in particular the apple trees provides permanent activity and manages to capitalize inappropriate areas for other agricultural crops.

The extension of modern apple culture systems with higher precocity and short-term exploitation is a way of periodic and rapid replacement of the assortments (Petre Gh., 2006). In this regard, introduction of modern techniques and novelties in the production adapt better the apple to the high quality European standards (Comanescu D., 2015; Petre Gh. et al., 2005).

Apples are nowadays cultivated in high density system and faster expand in Romanian fruit growing areas, the results achieved confirm its full economic efficiency. The generalization of the high-density apple system (Petre Gh. et al., 2009), represents the upgrade of the modernization of the fruit growing sector in our country, including the Dâmboviţa basin.

MATERIALS AND METHODS

By implementing the fruit tree thematic subprogram of PNDR in the period 2015 - 2020, the aim is to increase the technical and economic competitiveness in fruit growing sector, by promoting technologies adapted to the pedoclimatic conditions in Romania, with the goal to set up new apple plantations, including in the Dâmboviţa fruit basin.

In order to emphesize the interest of the Dâmboviţa fruit growers in the promotion of modern culture systems, during the period 2015-2017 some high-density apple plantations, established after 2007, by some apple producers from Dâmboviţa fruit basin

were highlighted: SC Mere de Voineşti SRL; Owner Dan Ionescu; I.I. Luminita Marin from Malu cu Flori.
Data on the planted area, varieties and rootstocks used, planting distances, soil maintenance, crown shape, phytosanitary treatments scheme, fertilization etc. were recorded. Also, the production potential of the apple trees was monitored, especially since the newly established plantations used trees from Italy or Netherland, with varieties different from the ones regulary cultivated in Romania.

RESULTS AND DISCUSSIONS

The high density apple system offers the possibility of easy change the assortment (due to the short period of cultivation), pedestrian labour in the orchard, increased performance in terms of yield and production quality.
In recent years, in the Dâmboviţa fruit tree basin, especially apple tends to generalize the high density culture system, the interest of the fruit growers is evident through the establishment of new modern plantations, using European varieties, proved to be well adapted to the pedoclimatic of the county.

Evidence of high-density apple systems in the Dâmboviţa fruit basin

The significant successes achieved in the world fruit tree, but especially in the European one in recent years, as well as the valuable experience gained, led some of the farmers in the Dâmboviţa fruit basin to show interest in expanding the apple-tree system, replacing the old plantations with over aged trees characterized by a low production potential.
The tendency to expand the high-density apple orchards to some of the Dâmboviţa fruit growers is outlined in Table 1 by planted areas, the age of trees and the density of planting.

Table 1. The examples of high density apple orchards in the Dâmboviţa County

No	Owner	Area planted (ha)	Year of planting	Trees age (years)	Plantng distances (m)	Trees/ha
1	SC Mere de Voineşti SRL	4	2007	11	3.5 x 1	2,857
		4	2009	9	3.5 x 1	2,857
		2	2010	8	3.5 x 1	2,857
		7	2011	7	3.5 x 1	2,857
		6	2015	3	3.5 x 1	2,857
2	Dan Ionescu	5	2010	8	3.5 x 1	2,857
3	I.I. Luminita Marin - Malu cu Flori	1.50	2014	4	3.5 x 1	2,857

From the data presented in the above table, SC Mere de Voineşti SRL has an area of 23 ha, cultivated with apple in a high density system, with trees aged from 3 to 11 years, planted at a distance of 3.5 x 1 m, of 2,857 trees/ha. At the same planting density (2,857 trees/ha), but with the age of trees of 4-8 years old, there are apple orchards belonging to Dan Ionescu farmer, with a surface of 5 ha and I.I. Luminita Marin - Malu cu Flori with a surface of 1.5 ha.
The 23 ha, cultivated with apple at SC Mere de Voineşti SRL, are equipped with a 3-wire trellising system, the first one placed at 60 cm from the ground, on which the drip irrigation system of 2.2 l/h is tied up.
The irrigation system can be used for fertilizers. The apple assortment consists of varieties 'Braeburn', 'Gala', 'Ionaprince', 'Granny Smith', 'Golden delicious', 'Stark' group of different types grafted on M9 rootstock. The fruit trees were purchased from Italy.
The area of 5 ha cultivated with apple by Dan Ionescu, the assortment consists of the apple varieties 'Golden delicious', 'Idared', 'Granny Smith', 'Braeburn', 'Gala' grafted on the M9 rootstock. The Dutch system is present with a wood tutor for each individual tree.
The irrigation system is layered on the ground and provide a flow rate of 2 l/h.
At the I.I. Luminita Marin from Malu cu Flori, the assortment consists of 'Pinova', 'Red Gala' and 'Golden delicious' varieties grafted on M9. The support system has 2 wires, the first wire is sitated at 60 cm from the ground level, on which the drip irrigation pipes are installed and work with a flow rate of 2 l/h. Each tree is supported by a bamboo stick.

Apples cultivated on these plots, are susceptible to diseases.

Therefore, to ensure a proper phytosanitary state, a number of 16-18 phytosanitary treatments have been applied annually.

In the overproduction years, the chemical thinning of the fruit, followed by a manual thin, was applied to obtain fruits with valuable appearance and price on the market.

In all of these apple orchards, the trees are trained as Spindle bush and the soil is maintained between the rows covered with grass and clean within the row using different herbicides.

Evaluation of production potential in apple-tree orchards in the Dâmboviţa fruit basin

In 2017, due to the low temperatures below the freezing threshold recorded in the area, the yields were substantially reduced depending on the variety and age of the trees.

Analyzing the productions obtained by SC Mere de Voineşti SRL during the 3 years of study (2015-2017), it is obvious that the most productive apple varieties in the 9-11 years old trees were 'Golden delicious' with 43.2 t/ha, followed by the 'Braeburn' apple variety with 35.4 t/ha and 'Gala' with 35.2 t/ha (Table 2).

Table 2. The apple yield obtained at the varieties cultivated in the high density system between 2015-2017 by SC Mere de Voineşti SRL

Apple trees age (years)	Trees/ ha	Variety/M9	Yield (t/ha), year			Average (t/ha)
			2015	2016	2017	
9 - 11	2,857	'Gala'	42.0	47.1	16.5	35.2
		'Golden delicious'	36.0	75.1	18.6	43.2
		'Braeburn'	48.0	50.0	8.3	35.4
		'Stark delicious'	20.0	45.1	15.0	26.7
		'Granny Smith'	21.0	41.7	12.0	24.9
7 - 8	2,857	'Gala'	36.8	53.1	18.6	36.2
		'Stark delicious'	20.6	40.3	28.6	29.8
		'Ionaprince'	20.4	45.1	15.2	26.9
		'Idared'	35.6	47.1	18.6	33.8
3	2,857	'Golden delicious'	18.2	14.6	40.2	24.3
		'Stark delicious'	15.4	12.8	28.3	18.8
		'Ionaprince'	12.6	15.7	38.6	22.3

In the 7-8 years old trees, the highest yields, averaging 3 years of production, were recorded by 'Gala' variety with 36.2 t/ha, followed by the 'Idared' variety with 33.8 t/ha. In the apple orchard with 3 years old trees, in 2017, the 'Golden delicious' variety, recorded a production of 40.2 t/ha and 38.6 t/ha for the 'Ionaprince' variety. The production was considered as normal because the flower buds in the frozen moment was in the balloon stage and were not affected.

Dan Ionescu, in 2017, in 8-year-old trees, registered a drastically reduced production compared to the previous year due to the frost phenomena. The smallest production was recorded in the 'Braeburn' variety by 8 t/ha (considered less adapted to the climatic conditions in the Voineşti area). The highest yields, were obtained by 'Golden Delicious' with 31.3 t/ha, followed by 'Idared', with 20.6 t/ha (Table 3).

Table 3. The apple yield obtained at the vaieties cultivated in the high density system between 2015-2017 by Dan Ionescu and I.I. Luminita Marin - Malu cu Flori

Apple trees age (years)	Trees/ ha	Variety/M9	Yield (t/ha), year			Average (t/ha)
			2015	2016	2017	
8	2,857	'Golden delicious'	25.0	60.3	31.3	38.9
		'Idared'	18.3	65.1	20.6	34.7
		'Granny Smith'	18.2	30.3	15.5	21.3
		'Braeburn'	24.3	59.4	8.0	30.6
		'Gala'	24.8	58.8	14.6	32.7
4	2,857	'Pinova'	30.0	35.7	42.8	36.2
		'Red Gala'	30.0	25.1	34.3	29.8
		'Golden delicious'	29.8	30.0	46.8	35.5

Analyzing the production recorded over the 3 years of study, the highest yields were recorded in the 'Golden delicious' apple variety with 38.9 t/ha, 'Idared' by 34.7 t/ha and 'Gala' with 32.7 t/ha.
Luminita Marin, located in Malu cu Flori, harvested from the 4th year trees, in 2017 34.3 t/ha to 46.8 t/ha, depending on variety, as follows: 'Red Gala' - 34.3 t/ha, 'Golden delicious' 46.8 t/ha and 'Pinova' 42.8 t/ha. Analyzing the productions recorded during the 3 years of study, 'Pinova' was the most productive apple variety with 36.2 t/ha close to next one 'Golden delicious' with 35.5 t/ha.

The efficiency of the high-density apple system compared to other cultivation systems

The intensification of the fruit growing sector in our country must accept as an important objective the introduction of new intensive crop cultivation systems adapted to the new socio - economic conditions and to the continuously developing technical and material basis.
In connection with the introduction of new crop systems, it should be noted that the conventional or classic culture system has a number of limits beyond which it is not overpassed, regardless of the applied technologies.
The production of fruit is delayed in the classical orchards, from 8-10 years onwards after planting. Some high-volume main works, such as pruning and harvesting fruit, require excessive workload due to the high tree height, 7-10 m, which forces growers to use large and inconvenient stairs. The mechanized execution of phytosanitary treatments, soil mainenance is hampered by the globular form of the crown or is realised with low efficiency and uneconomically.
Intensive crops and, above all, high-density plants offer greater flexibility in changing fruit varieties due to the lower economic exploitation period of these orchards (22 to 30 years in intensive orchards, 15-16 years to high density ones).
The yields of the high density production of apple during the exploitation period are superior, reflected by the productive potential differences compared to the intensive and classic culture systems (Figure 1).

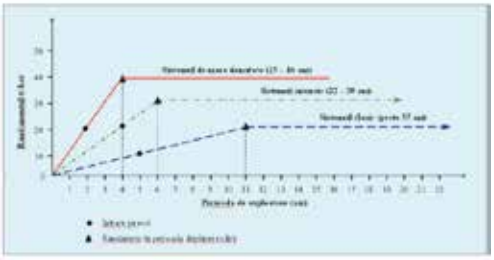

Figure 1. The efficiency and the exploitation period of apple orchards in different cultural systems

The differences between the apple culture systems are quite obvious in terms of fruit input of the trees, the economic growth and the level of production during the full production period, as well as the upper limit of the exploitation period.
What really differentiates apple cultivation systems is the precocity. The classic system start be economically after 10-12 years from planting, to the intensive system after 6-7 years, and to the high density after 3-4 years.
If we take into consideration the first 10 years since planting, it is clear that the yields produced significantly differences in the apple culture systems. If the high-density and even intensive system yields/ha is high as a result of the rapid entry of the fruit trees and the achievement of a large productive volume of the crown through the planting density itself, the classic system should expect much more for both the bearing of fruit trees and the formation of the skeleton branches for yield bearing.
The high density apple system is recommended for extension in the well-established fruit-growing areas of our country, including the Dâmboviţa fruit basin, due to the high yield and efficiency, the way of periodic and rapid replacement of the assortments, but also the obtaining of bigger apples volumes demanded more and more by consumers.

CONCLUSIONS

The high density system expands mainly to apple trees in the Dâmboviţa fruit basin, the interest of fruit growers is evident in the promotion of productive varieties well adapted to the pedoclimatic conditions of the area, grafted on the M9 rootstock planted at a

distance of 3.5 x 1 m, with planting density of 2,857 trees/ha.

Although the production was diminished by the low temperatures recorded during the flowering period in 2017, the highest average production for three years period belonging to SC Mere de Voineşti was recorded in 9-11 years old apple trees by the varieties: 'Golden delicious', 'Braeburn' and 'Gala' and in 7-8 years old apple trees, the varieties 'Gala' and 'Idared'.

In the apple tree orchard for 3 years, the 'Golden delicious' and 'Ionaprince' varieties produced 40.2 t/ha and 38.6 t/ha in 2017.

In Dan Ionescu orchard, the 8 years old trees performed better in varieties such as: 'Golden delicious', 'Idared' and 'Gala'.

Luminita Marin from Malu cu Flori, highlight the 4th year trees, that produced from 34.3 t/ha to 46.8 t/ha It was remarked 'Red Gala', 'Golden delicious' and 'Pinova'.

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TWENTY YEARS OF EXPERIENCE IN INTENSIVE PLUM PRODUCTION ON *PRUNUS CERASIFERA* EHRH. ROOTSTOCK

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Abstract

As a result of two decades long research this paper provides basic parameters and characteristics of training system plum spindle on Myrobalan (Prunus cerasifera Ehrh.) seedling rootstock. Utilization of Myrobalan rootstock induces intensive plum growth in initial years of production. This growth can only be controlled by application of adequate and precise pomotechnics and induction of early fruiting as a result of applied treatments. Obtained results indicate that training principles for plum spindle system are similar for most analyzed cultivars. On the other hand, cultivar specifics concerning biological predispositions for this training system may greatly influence success of production. Certain cultivars ('Čačanska Lepotica' and 'Stanley') can serve as model plants for correct and successful high intensity plum production in spindle system. All factors should be taken into consideration, when defining training systems adequate for plum (Prunus domestica L.) on Myrobalan rootstock, so the optimal combination could be defined depending on growing conditions and level of application of agrotechnical measures. Level of applied measures in orchard needs to be one of the most important elements of financial feasibility of plum production in spindle system. Planting density depending on the cultivar and growing conditions is between 1000 and 2000 trees per hectare. Increase in number of individual spindles per hectare is possible by modification of planting system ("V" spindle planting) or modification of training system (multi-leader spindle systems), which implies different approach to production.

Key words*: canopy management practices, cultivar, density.*

PLUM PRODUCTION ON MYROBALAN SEEDLING ROOTSTOCK

The introduction of dwarfing rootstocks in fruit production has led to its intensification, primarily through changes in orchard systems and increases in planting density and yield per unit area (Hrotko et al., 1998; Achim et al., 2017). Apart from significant initial investments, vegetative rootstocks of weak vigor require optimum growing conditions and proper cultural and tree management practices to manifest their positive characteristics. Impressive results in improving orchard systems for apple and pear have given rise to intensive research in dwarfing rootstock selection for stone fruits as well. However, intensive systems of production on dwarfing rootstocks have not been as successful for stone fruits as for pome fruits (Paunović et al., 2011). This can be explained by the fact that dwarfing rootstocks modify scion growth and development through physiological growth suppression and initiation of greater generative bud differentiation. In pome fruits, this results in enhanced fruiting potential, and the fact that pome fruit generative buds contain vegetative cones means that an increase in the total number of generative buds per tree does not induce a decrease in leaf surface area per fruit i.e. there is no decrease in the total number of leaves required for successful fruit development in trees bearing a high crop load (Lučić et al., 1996). On the other hand, in stone fruits, growth suppression and increased generative bud differentiation lead to a decrease in vegetative buds per tree and, hence, leaf biomass per fruit. There is the impression that intensive production systems for stone fruits can be successfully established on semi-dwarfing rootstocks using specific canopy management practices that enable fruiting each year. Regardless of the development of plum vegetative rootstocks, the Myrobalan *Prunus cerasifera* Ehrh. seedling is still a predominant rootstock in the Bosnia and Herzegovina and

Serbia, and some other Balkan countries as well (Botu and Botu, 2017). Less favorable soil conditions for plums grown in this area, partial use of cultural and tree management practices in orchards, higher costs of vegetative rootstocks and the resulting nursery trees, as well as the marked presence of the traditional approach to plum production are just some of the reasons for the current state. Plum is grown on Myrobalan rootstock mostly in extensive or semi-intensive orchards, with an average planting density of 400 to 800 trees per hectare (Micic et al., 2005; Botu and Botu, 2017), and using only minimum tree management practices (pruning during dormancy). Nevertheless, experience indicates that the intensification of plum production on Myrobalan rootstock is possible and justified, if all necessary tree management practices are used, especially in the initial years of production (Cvetkovic et al., 2017b). Considering the high fruiting potential of currently grown plum cultivars, the specific manner of formation and character of fruiting wood, as well as the pronounced tendency for decreased generative bud differentiation in certain cultivars (regardless of rootstock type), strong vegetative growth of the Myrobalan seedling rootstock can have a significant effect on the regular formation and renewal of fruiting wood. This paper presents 20 years of experience in intensive plum production on Myrobalan rootstock, specific characteristics of the "plum spindle" training system, as well as the biological predisposition of certain cultivars to this training system.

CHARACTERISTICS OF MODERN "PLUM SPINDLE" TRAINING SYSTEMS

It is possible to train plum trees to a modified spindle system, slightly higher (3.2-3.5 m) than the standard spindle, with a trunk height of up to 0.5 m, and with more pronounced main lateral branches at the base of the tree. The branches should be distributed irregularly, spirally along the central leader, taking care that their length is shortened towards the top so that the tree takes the final form of a "conical frustum" (Mićić et al., 2005). During the first 2 or 3 years after planting, trees are trained according to the spindle training principles. During the period, it is necessary to use all tree management treatments which, apart from having a role in tree architecture, ensure the fastest possible formation of fruiting wood and fruit-bearing branches, as the best procedure for initial vigor suppression. Successful plum production is only possible if intensive summer pruning operations are used (during the growing season), which is not the case in conventional training systems. To this end, all pruning treatments should be used at optimum dates. Notching is applied during dormancy or immediately before the growing season (Lučić et al.,1988; Glisic, 2012; Cvetković et al., 2017a). Plum has a biological predisposition to creating "floors" i.e. branches growing in several blocks along the central leader; therefore, notching provides great results in balanced targeted positioning of the shoots along the central leader. Effects of notching are not related to its timing, and the cultivar-dependent response to growing point initiation ranges from 95.77-98.11% (Cvetković et al., 2017a). The formation of a crotch angle of approximately 90° relative to the central leader provides the best predisposition to the creation of quality growth of main lateral branches and fruit-bearing branches. In some cultivars ('Čačanska Lepotica'), this procedure is sufficient for the formation of main lateral branches properly positioned relative to the central leader; however, this is not the case for most cultivars. Spreading the branches and maintaining their position at an initial crotch angle of approximately 90° is usually performed with different types of wires and twines (Mićić et al., 2005; Cvetković et al., 2017b), keeping in mind the timing. Maintaining crotch angles by shoot twisting must be applied successively during the growing season before the newly formed shoots are lignified at the base. Twisting is uniquely and highly effectively used in plum (Mićić et al., 2005; Glisic, 2012; Cvetković et al., 2017b). Pruning during dormancy is applied in accordance with standard principles and cultivar specificity. Succession of old lateral branches is usually done by "stub cuts" and successive use of undercutting to initiate the growth of lateral shoots which will take the role of main lateral branches. Training plum trees to a spindle system ensures satisfactory yields

(25-40 t/h), high quality fruit and ease of access for fruit harvest, thus increasing the number of extra quality fruits in the total production structure.

CULTIVAR SPECIFICITY AND SUITABILITY FOR THE "PLUM SPINDLE" TRAINING SYSTEM

The biological specificity regarding the growth and development of individual cultivars, as well as their response to tree management treatments are of special importance for defining a proper approach to training certain cultivars to the spindle system. Morphological characteristics of growth and development largely affect the formation of the "plum spindle" training system and high-density planting. Tree architecture, type of growth of fruit-bearing branches, intensity of formation and character of fruiting wood, dominant type of fruit-bearing branches, fruit positioning on the tree, degree of fruit-bearing branch shedding and moving further from the central leader after fruiting and the specific activation of new points of growth for the scheduled succession of main lateral branches are just some of the cultivar-specific morphological characteristics of growth and development that greatly affect the spindle training approach taken for individual cultivars. Certain biological characteristics of cultivars (Table 1) can pose significant difficulty to the approach taken to the formation and maintenance of the training system, which has to be taken into consideration while designing the training system. Experience indicates that the above listed cultivars can be conditionally classified in three groups (Table 2), based on their biological predisposition to spindle training as well as on treatment intensity for the satisfactory formation and maintenance of the training system. 'Čačanska Lepotica' and 'Stanley' are in the first group as model cultivars for intensive production; their characteristics are suitable for spindle system training. All treatments are highly effective and successful, even though they are somewhat more extensive and demanding in 'Stanley'. A more complex approach is necessary for 'Čačanska Najbolja', 'Čačanska Rana' and 'Katinka'. The greatest difficulty in the production of 'Čačanska Najbolja' and 'Čačanska Rana' is strong initial vigor and self-sterility issues which very often additionally contribute to strong vigor. 'Katinka' requires more precise pruning combined with some other measures to achieve the satisfactory fruit size.

Table 1. Biological specificity of growth and development that impede spindle training system

Cultivar	Specificity
'Čačanska Najbolja'	vigorous self-sterile late first bearing very intensive shoot growth in initial years of production insufficient number of strong shoots adequate for main lateral branches non-uniform lateral branching of the central leader acute crotch angles of the lateral branches relative to the central leader lack of response to treatments inducing the succession of main lateral branches
'Elena'	vigorous very intensive shoot growth in initial years of production long shoots extremely acute crotch angles of lateral branches relative to the central leader fast shoot lignification at the base highly intensive transfer of growth points to top parts of the tree unfavorable response to the succession of main lateral branches
'Čačanska Rana'	self-sterile occurrence of alternate bearing late first bearing shoots have specific growth dynamics insufficient number of strong shoots adequate for main lateral branches relatively unfavorable response to the succession of main lateral branches
'Stanley'	intensive shoot growth in initial years of production too high percentage of short fruit-bearing branches in the total fruit-bearing branch structure
'Katinka'	alternate and partially alternate bearing tendency to form thin long fruit-bearing branches dominant short fruit-bearing branches on main lateral branches affinity towards shedding of the base of main lateral branches transfer of growth points to top parts of the tree
'Čačanska Rodna'	alternate bearing intensive growth of new shoots – longer treatment period long elastic shoots inappropriate for treatments during the growing season relatively unfavorable branching angles relative to the central leader extreme shedding of main lateral branches after fruiting intensive transfer of growth points to the periphery of the tree
'Čačanska Lepotica'	lack of formation of long main lateral branches

'Čačanska Rodna' and 'Elena' belong to a separate group as they are highly demanding as regards training to the spindle system and require high-intensity treatment regardless of orchard age.

Table 2. Cultivar predisposition to spindle training and treatment intensity for proper training (more pluses indicate better predisposition i.e. greater treatment intensity)

Cultivar	Predisposition	Treatment intensity
'Čačanska Najbolja'	++	+++
'Elena'	+	+++
'Čačanska Rana'	++	++
'Stanley'	+++	+
'Katinka'	++	++
'Čačanska Rodna'	+	+++
'Čačanska Lepotica'	+++	+

PLANTING DENSITY

Planting density is dependent on the expression of cultivar vigor in combination with myrobalan rootstock, as well as on cultivar specificity regarding tree architecture (Table 3).

Table 3. Planting density

Cultivar	Spacing between rows (m)	Spacing within the row (m)	Number of plants/ha
'Čačanska Najbolja'	4.2 – 4.6	2.0 – 2.4	900 – 1190
'Elena'	4.2 – 4.6	2.0 – 2.2	980 – 1190
'Čačanska Rana'	4.0 – 4.4	1.8 – 2.0	1130 – 1380
'Stanley'	3.8 – 4.2	1.6 – 1.8	1320 – 1640
'Katinka'	3.8 – 4.0	1.6 – 1.8	1380 – 1640
'Čačanska Rodna'	3.8 – 4.2	1.4 – 1.6	1480 – 1870
'Čačanska Lepotica'	3.6 – 4.0	1.4 – 1.6	1560 – 1980

The highest spacing is required for cultivars with strong vigor, which require more complex tree management treatments. Experience shows that 'Stanley' can be grown successfully at a narrower spacing within the row (1.5 m), which is also true for 'Čačanska Lepotica' (1.2-1.4 m). The intensification of plum production using the spindle training system is additionally possible through the "V" planting system (Mićić et al., 2005), which allows an increase in planting density by additional 25-30%, thus increasing crop yield (Mitrovic et al., 2005). On the other hand, the need to use complex trellises in this training system makes investment more expensive. At the same time, difficulty in pruning areas of the tree facing inwards of the row and the frequent inability to use adequate disease control imply the necessity to move the intensification of plum training systems on Myrobalan rootstock in some other direction.

CONCLUSIONS

Myrobalan (*Prunus cerasifera* Ehrh.) seedling is the dominant rootstock for plum in the Western Balkans. Even though it induces stronger vigor, with adequate and timely use of all necessary tree management treatments, it is possible to control growth and establish an appropriate balance of vegetative and generative growth in initial years.

Plums can be successfully grown in a modified spindle system, with a planting density of 900 to 2000 trees per hectare, which is largely affected by cultivar-specific growth and development.

Cultivars demonstrate significant differences in their response to intensive tree management treatments, which should be considered when designing an orchard.

Tree management treatments during the growing season are essential for plums to be trained to the spindle system.

Treatments during dormancy should be corrective and less severe so as to prevent additional vigor.

Plum production using this system involves more manual labor in the total cost structure, but it ensures higher yields and optimal quality of the fruit.

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SENSORIAL EVALUATION OF 26 HIGHBUSH BLUEBERRY VARIETIES IN ROMANIA

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Abstract

Among many blueberry growers and general consumers perception, often are cross-cutting issues. The quality of the fruits depends on many factors such as variety, area were fruits are produced (environmental conditions), ripening and harvest time, producing technology etc. Of course many other influencing factors could intervene during the picking time till consumption of fruits and affects the fruit appearance, firmness, colour or other internal or external parameters. Besides all these reasons, the final consumer has particular demands and preferences which stays at the base of their choices. In 2017, a tasting session with 26 blueberry varieties cultivated in Romania were evaluated by different categories of consumers. Cultivar tested were: 'Coville', 'Chandler', 'Azur', 'Darrow', 'Blueray', 'Legacy', 'Simultan', 'Brigitta', 'Augusta', 'Spartan', 'Vital', 'Pemberton', 'Delicia', 'Reka', 'Toro', 'Berkeley', 'Denise blue', 'Duke', 'Safi'r, 'Lax', 'Hannah's choice', 'Bluecrop', 'Patriot', 'Nelson', 'Bluetta', 'Pink lemonade'. All these varieties were evaluated against fruit size, skin colour, firmness, juiciness, taste and flavor. 'Coville' and 'Chandler' performed better as general total score. 'Chandler' and the Romanian variety 'Augusta' remarked by large fruits and were appreciated accordingly. 'Coville' and 'Blueray' showed good appearance of the skin colour since the most firm fruits were noticed at 'Legacy'. Most tasty and aromatic fruits were scored for 'Coville'.

Key words*: Vaccinium corymbosum L., organoleptic assessment, consumer preferences.*

INTRODUCTION

As many other fruits, blueberries are subject of organoleptic analysis (Polashock J. et al.). General consumers perception is of great interest when it comes to retailers and influences the fresh fruit market. But, even with the latest perfomant lab equipments (Saftner R. et al., 2008) the consumers preference is stil a cross-cuting issue. It was demonstrated that major traits of the varieties that influence the positive perception of fruits quality are the taste, fruit size, color and flavor (Gilbert J.L. et al., 2015).

Indeed, the quality of the fruits depends on many factors such as area were fruits are produced (environmental conditions), ripening and harvest time, producing technology etc. Many other influencing factors could intervene during the picking time till consumption of fruits and affects the fruit appearance, firmness, colour or other internal or external parameters. Besides all these reasons, the final consumer has particular demands and preferences which stays at the base of their choices (Yue C. and Wang J., 2017).

Evaluation of blueberries traits is a continuously process required by the breeders, producers and consumers. Many studies were conducted in this regard (Itle R.A. and NeSmith D.S., 2017) trying to emphasize which is the best solution and what varieties are most suitable for a specific regions, countries or consumer preference.

Therefore, the main objective of the present study was to investigate the general consumers preference in Romania for the fresh blueberry fruits since the last 10-15 years, the blueberry consumption increase worldwide very fast.

A subsequent goal was also to decelate the consumers preference regarding each criteria of assessment. This could be also relevant for the breeders when they decide to release new varieties on the market.

MATERIALS AND METHODS

In July 17, 2017, a tasting session with different 26 blueberry varieties cultivated in Romania (pot crop system), Dambovita county were evaluated by different categories of consumers: students, professors, researchers,

farmers and regular people. Also it was a balanced distribution between young, mid age and elder people as long as male/female participation.

The blueberry varieties tested were: 'Coville', 'Chandler', 'Azur', 'Darrow', 'Blueray', 'Legacy', 'Simultan', 'Brigitta', 'Augusta', 'Spartan', 'Vital', 'Pemberton', 'Delicia', 'Reka', 'Toro', 'Berkeley', 'Denise blue', 'Duke', 'Safir', 'Lax', 'Hannah's choice', 'Bluecrop', 'Patriot', 'Nelson', 'Bluetta', 'Pink lemonade'. Seven of them are Romanian varieties and the other 19, international blueberry varieties (Figure 1).

Figure 1. The 26 blueberry varieties in the scene of reviewers (17.07.2017)

All these varieties were evaluated against six criteria: fruit size (1…5), skin color (1…5), firmness (1…3), juiciness (1…3), taste (1…7) and flavor (1…5). From the total of 28 points, 10 points have been allocated to the fruit appearance (fruit size plus skin color), 6 points to consistence/texture (firmness and juiciness) and 12 points to taste (taste and flavour). The varieties were reviewed by 25 assessors delivering at the end the sheets with the scores for each analysed item.

RESULTS AND DISCUSSIONS

In the first step, all the scores were summed and the varieties ranked from the highest value to the lowest one.

The overall chart highlight 'Coville' variety that gathered 23.18 points out of maximum 28 points (Figure 2) followed closer by 'Chandler' with 22.43 total points.

Both varieties showed very large fruits and confirms the appreciation of general consumers for the higher size of the fruits.

Nevertheless, the appearance of this particular two varieties was enhanced by the silver pruin of the skin and the freshness look.

At the bottom of the list, was sitated a novel blueberry variety 'Pink lemonade' with pinkish and aromatic fruits that did not comply with the consumers expectation.

'Bluetta' as a early variety was overripened at the time of consumption and therefore has registered less points for most of the criteria.

It is remarkable the presence of two Romanian blueberry varieties ('Azur' and 'Simultan') in the top 10 most appreciated ones by evaluators.

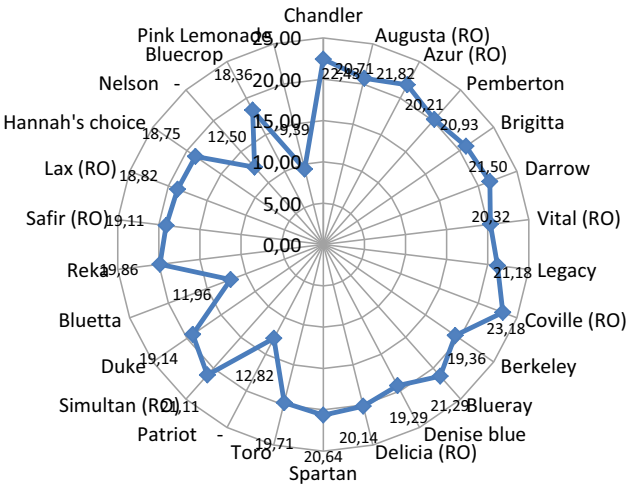

Figure 2. The overall total scores of the tested blueberry varieties

In relation with the fruit size, blueberry varieties ranged between 1.86 at the 'Pink lemonade' and 4.68 points at 'Chandler' out of the maximum 5 points foreseen in this criteria. For only 0.04 points less, the Romanian variety 'Augusta' followed the first variety in the class (Figure 3). Together with 'Azur' situated in the third position indicate that national varieties also could be reliable on the international competitive market.

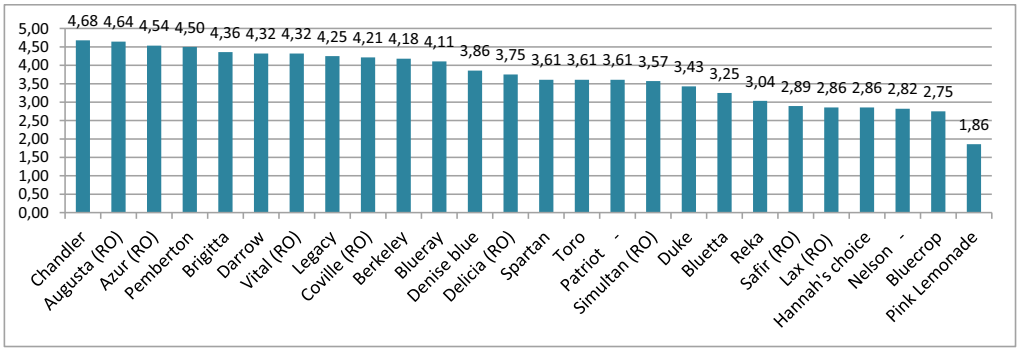

Figure 3. The ranking list of the blueberry varieties according to the fruit size

In the overall appearance perception, the color of the fruits skin is very significant. Thus, a proeminent uniform blue of the blueberries skin is an advantage for the varieties like 'Coville', 'Blueray' or 'Safir' (Figure 4).

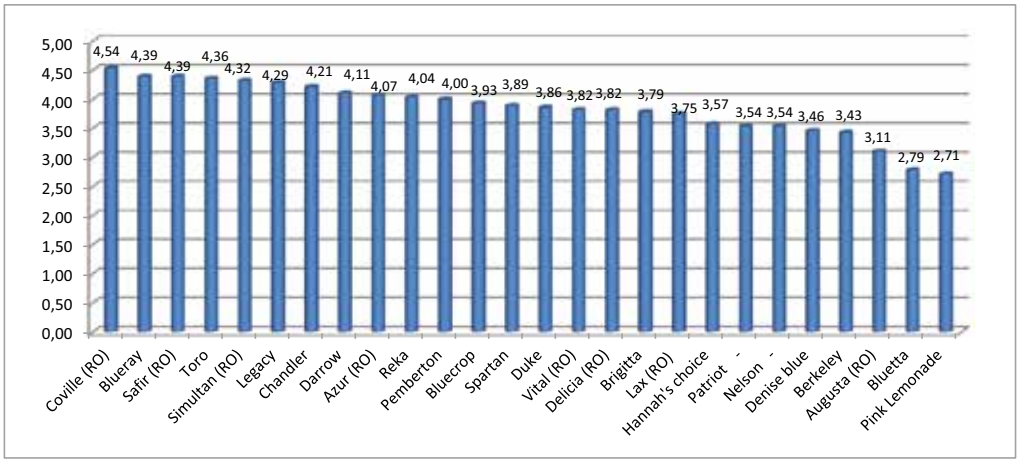

Figure 4. The arch of the blueberry varieties ranked by skin color total scores

The fruits firmness is nowadays, beside the shelf life of the product, more and more desired in the fresh consumption. In this respect we assist at a different rank of the blueberry varieties when it comes to a crispy pulp. In the top of the list, 'Legacy' confirms the texture preference of the evaluators and the literature too (Strik B.C. et al., 2017). 'Reka' and the Romanian variety 'Delicia' took an upper position in the ranking list, next by 'Chandler' and 'Duke', varieties that are well known for their firm fruits (Figure 5).

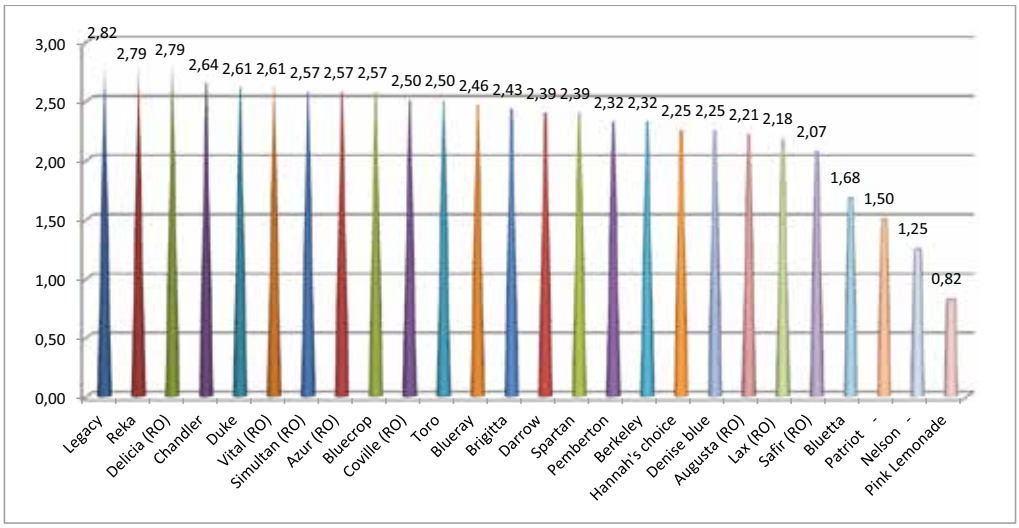

Figure 5. The variability of pulp firmness for the 26 blueberry varieties

The consistence of the fruits is direct influenced by the variety, harvest time, post-harvest storage conditions and many other factors that intervene in the process. Therefore, the values registered by the varieties tested in the same day are in a flesh juiciness stage according to their postharvest evolution. Even so, it were remarked old varieties like 'Darrow' or 'Brigitta' next by four Romanian blueberry varieties: 'Vital', 'Lax', 'Simultan' and 'Azur'. Except few varieties like 'Toro', 'Nelson', 'Patriot', 'Bluetta' and 'Pink lemonade', al the other varieties did not vary too much in the scores given by the evaluators (Figure 6).

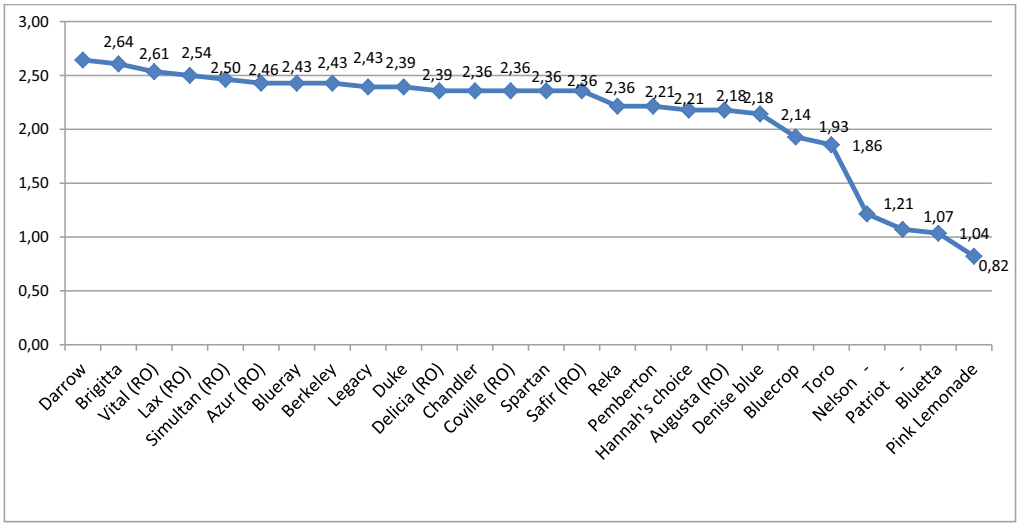

Figure 6. The juiciness spectrum of the 26 blueberry varieties

Taste is imediately after appearance the most important trait of the fruit assesed by the consumer.

It is definetely one of the criteria and reason for the consumer to return and taste again. It represents the path to remember the name of the variety when the client wants to buy again. In this regard, a special attention was payed by the breeders trying to match the consumer preference. The results obtained in our tasting session restore the performance of an old blueberry variety, famous for taste and medium

to large fruits, namely 'Coville'. It convinced in a significant and evident way by gathering no less than 5.36 points (Figure 7) out of the maximum 7 followed at some distance by 'Augusta' and 'Azur' with the same score (4.68 points). Both are Romanian varieties less popular that other foreign varieties. 'Augusta' performed better due to its special and particular taste and flavor.

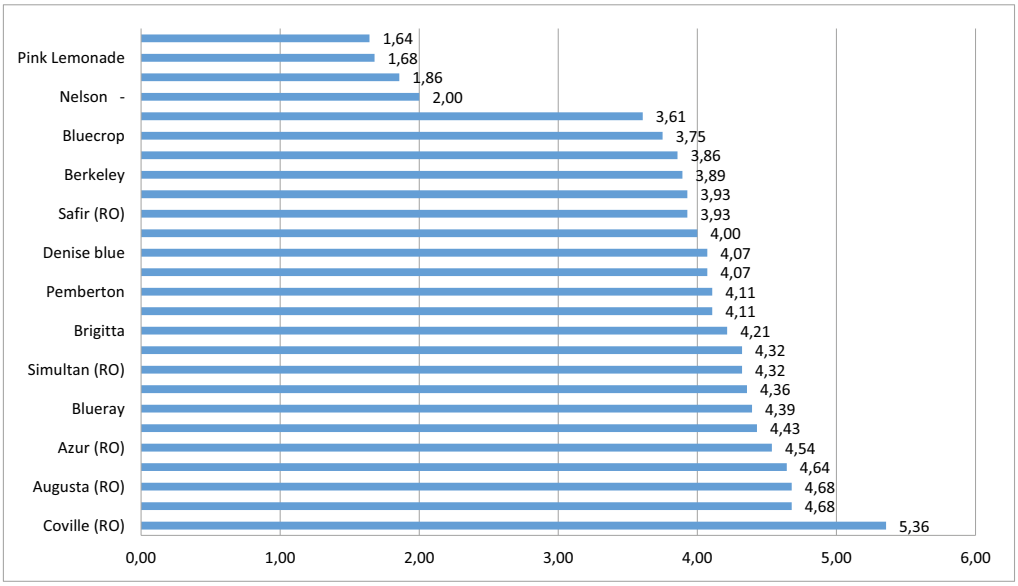

Figure 7. The ranking of all 26 bluebery varieties according to the consumers taste preference

The flavor of the fruits (Figure 8) completes the taste and inprint the consumer memory. As many regular and unadvised consumer, the taste results are similar with the flavor one. As it was expected, the first two varieties 'Coville' and 'Augusta' were assigned as most flavored varieties recording the highest scores.

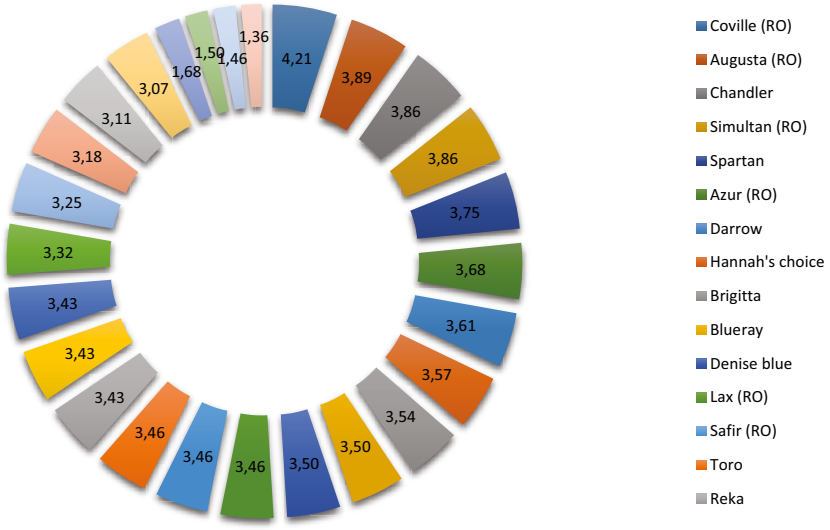

Figure 8. Consumers preference range for the blueberry flavor assesed in the tasting session

It is again remarkable the fact that 'Coville' detached in a significant way from the second position also for this item analysed. Another Romanian blueberry variety ('Simultan') remains in the top preference of the consumers for the flavor, considered one of this variety major advantage.

CONCLUSIONS

'Coville' and 'Chandler' varieties performed better as overall total score and taste in the consumers preference range.
'Chandler' and the Romanian variety 'Augusta' were remarked for their large fruits.
'Coville' and 'Blueray' showed good appearance of the skin color.
The firmest fruits were noticed at 'Legacy' and 'Reka'.
Most tasty and aromatic fruits were ones from 'Coville' and again 'Augusta'.
High aromatic fruits were positive evaluated at 'Coville', 'Augusta' and 'Chandler'.
Evaluation of the blueberry varieties from the consumers preference point of view has to be understood as a dinamic process and must be periodically repeated.

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EVALUATION OF THE CONSUMER PREFERENCE FOR SWEET CHERRY FRUITS AT THE "SWEET CHERRY FEST" IN ISTRIȚA - BUZĂU

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Abstract

Since 2013, the Univeristy of Agronomic Sciences and Veterinary Medicine of Bucharest yearly organizes at the Istrița Nursery and Didactic-Experimental Farm the "Sweet Cherry Fest" in the first decade of June. The event is part of the "Agriculture for Life, Life for Agriculture" International Conference programme and consist in a large sweet cherry exhibition and tasting of fresh sweet cherry fruits gathered from own trial orchards or from comercial sweet cherry plantations within the country. Depending on the environmental conditions and weather conditions of each year, the samples of cherries for tasting session varies a lot but comprise as many as possible varieties, most of them from early to medium harvest time. In 2017, the tasting panel evaluated 24 samples coming from 17 varieties. These were as follows: 'Early red', 'Burlat', 'Celeste', 'Kordia', 'Firm red', 'Grace Star', 'New Star', 'Early Bigi', 'Giant red', 'Summit', 'Ferrovia', 'Ulster', 'Van', 'Rubin', 'Katalin', 'Skeena' and 'Lapins'. Best score was recorded by 'Early red' followed by 'New Star' and 'Giant red'. The fruit size and appearance of the 'Early red' and 'Giant red' varieties were rewarded by tasting panel with high appreciation scores. The taste was also remarked by the consumers for 'Early red' and also the aroma for 'New Star'.

Key words*: variety, organoleptic assesment, taste, fruit appearance, consumer preference.*

INTRODUCTION

Since 2013, the Univeristy of Agronomic Sciences and Veterinary Medicine of Bucharest yearly organizes at the Istrita Nursery and Didactic-Experimental Farm the "Sweet Cherry Fest" in the first decade of June.
The event is part of the "Agriculture for Life, Life for Agriculture" International Conference programme and consist in a large sweet cherry exhibition and tasting of fresh sweet cherry fruits gathered from own trial orchards or from commercial sweet cherry plantations within the country.
Depending on the environmental conditions and weather conditions of each year, the samples of cherries for tasting session varies a lot but comprise as many as possible varieties, most of them from early to medium harvest time.
Besides many researches conducted in this field (Amidei R. et al., 2017; Ross C.F. et al., 2010; Chauvin M.A. et al, 2009), the sensorial analyses taken by panelists versus common consumers support retailers, farmers or breeders to figure out the key drivers for sweet cherry market need (Revell J., 2008).
Different compounds of the fruits are identified (Legua P. et al., 2017) and associated with the consumers preference (Vavoura M.V. et al., 2015; Serradilla M.J. et al., 2012).
But characterization of sweet cherry cultivars only by lab instruments is unpaired without tasting evaluation session.

MATERIALS AND METHODS

The exhibition and tasting session within the 4th edition of the "Sweet Cherry Fest" took place in Istrita Nursery and Farm in 10th of June, 2017.
The tasting panel consist of 44 persons which assessed 24 samples represented by 17 varieties (Figure 1). The gender balance was good, in the session participating 25 male and 19 female above 18 years.

Sweet cherry varieties introduced in the contest were as follows: 'Early red', 'Burlat', 'Celeste', 'Kordia', 'Firm red', 'Grace Star', 'New Star', 'Early Bigi', 'Giant red', 'Summit', 'Ferrovia', 'Ulster', 'Van', 'Rubin', 'Katalin', 'Skeena' and 'Lapins'.

Figure 1. Participants during the tasting session - 10.06.2017 at Istrita, Buzau

The samples provenience were mainly from the University of Agronomic Sciences and Veterinary Medicine Bucharest.
The host - Istrita Nursery and Fruit growing Farm provided nine varieties and the Faculty of Horticulture Bucharest came with 10 sweet cherry varieties from the didactic and experimental field situated in the Bucharest campus.
Two private companies: "Livada fermecata" Agroclaas SRL (Haret, Vrancea) and Multifruct SRL (Chiselet, Calarasi) introduced in the tasting session another five varieties.
From the Research and Development Fruit Growing Station Iasi, the varieties and hybrids were presented only in the exhibition.
The samples were encoded and each participant move from one variety to another marking in the tasting sheet their appreciation (Figure 2).
In total, eight items were analyzed for the sweet cherry assessment.
The fruit size, appearance, skin color, firmness, juiciness, juice color, taste and flavor.
The external traits gather 10 points, the internal characters 9 points and the taste 7 points maximum plus 4 points from the flavor.
Total score is 30 points.


Sărbătoarea Cireșului la Istrița

Ceа de a 4-a ediție - 10 iunie 2017

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Figure 2. Tasting sheet used by the participants in the sweet cherry quality assessment

RESULTS AND DISCUSSIONS

All the samples accepted in the contest were scored by the evaluators and centralized after collecting the sheets.
Summarizing the scores from each item we obtained the total scores that ranked better the 'Early red' variety from the Istrita Nursery and Farm (27.25 points out of max 30 points) (Figure 3).
The second best variety was 'New Star' from the Istrita Nursery and Farm with 26.44 points followed by 'Giant red' from the same institution (25.51 p).

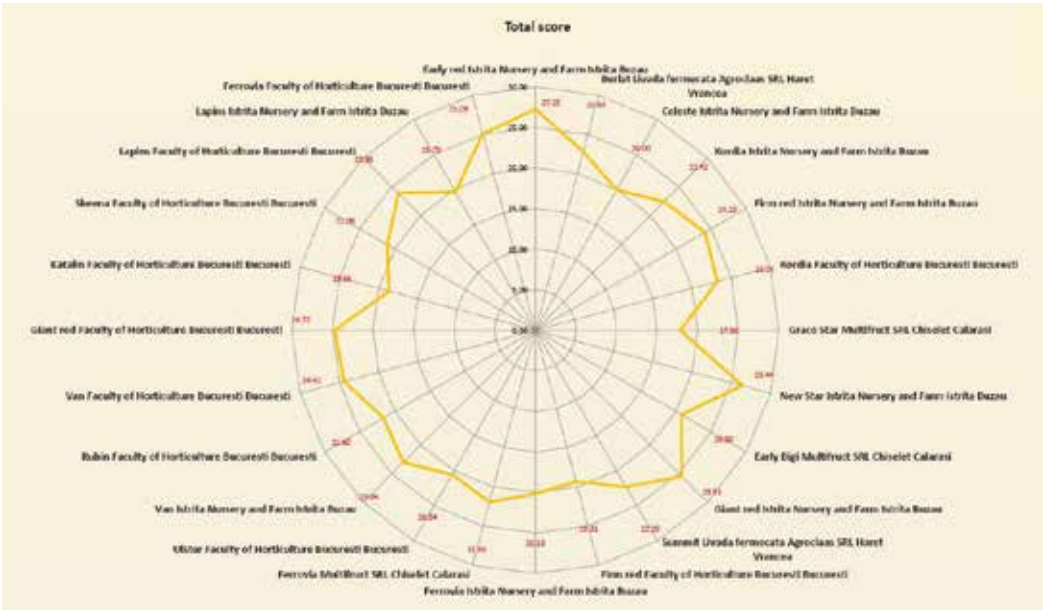

Figure 3. The distribution of total scores among all sweet cherry samples participating in the tasting evaluation session (2017)

Considering the size of the fruit, the highest scores were registered by 'Early red' (Figure 4), 'Firm red' and 'Giant red' from the Istrita Farm and Nursery Station. Bigger fruits were also remarked at 'Burlat' from "Livada fermecata" and 'Grace star' from Multifruct SRL.

Figure 4. The appearance and fruit size of 'Early red' variety

In terms of appearance of the fruits, the most appreciated variety was 'Burlat' from the "Livada fermecata", Haret, Vrancea which gain an average of 2.94 points out of maximum 3.0 points. In the second position was notced 'Early red' from the Istrita Farm and Nursery and in the third level 'Celeste' from the same institution.

The highest appreciation for the skin color was highlighted by the same 'Early red' from Istrita followed closely by 'Burlat' from "Livada fermecata" (Figure 4).

Surprisingly, 'Ferrovia' from the Multifruct SRL impress the tasters with a very firm fruits (2.81 p out of 3.0 p). The second place was ocupied by 'Firm red' from Istrita, the name of the variety is in this way reconfirmed.

'Celeste' from Istrita was the variety with higher content of juice in the perception of assessors together with 'Early Bigi' from Multifruct SRL.

The color of juice emphasized 'Burlat' from "Livada fermecata" and 'Early red' from Istrita followed by 'Kordia' and 'Celeste' from Istrita too.

The most important feature of the sweet cherry fruit is the taste. As we expected, 'Early red' from Istrita was very much appreciated, with an average score of 5.98 p out of 7.0 p. 'Burlat' even that is an old variety earn the respect

of the evaluators which scored with 5.42 p. (Figure 5).

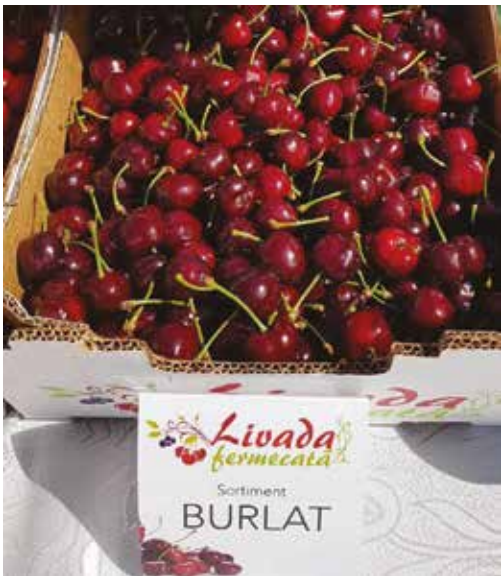

Figure 5. Variety 'Burlat' from "Livada fermecata", Vrancea

'Kordia' and 'Celeste' from Istrita also were remarked from this point of view. The most disliked varieties were 'Lapins', 'Katalin' and 'Ferrovia'.

A strong correlation between taste and aroma was found, most of the evaluators ranking higher the same varieties of sweet cherry. Therefore, 'Early red' and 'Burlat' plus 'Kordia' and 'Celeste' were considered the most aromatic fruits between the samples.

CONCLUSIONS

Best total score was recorded by 'Early Red' from Istrita Nursery and Farm followed by 'New Star' and 'Giant red' from the same institution.

The biggest fruit size was remarked at 'Early Red', followed by 'Firm red' and 'Giant red' varieties from Istrita Nursery and Farm.

In terms of fruit appearance and skin color, 'Burlat' from "Livada fermecata" and 'Early red' from Istrita were rewarded by the evaluators with higher scores.

The firmest pulp had 'Ferrovia' from Multifruct SRL and 'Firm red' from Istrita.

The taste and aroma of 'Early red', 'Burlat' and 'Kordia' was remarked and highly appreciated by the consumers.

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THE BEHAVIOR OF GALA, JONAGOLD, GOLDEN DELICIOUS AND GRANNY SMITH APPLE VARIETIES IN ORGANIC FARMING SYSTEM

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Abstract

In the present study, the behaviour of the 'Gala', 'Jonagold', 'Golden delicious' and 'Granny Smith' apple varieties, grown in an organic orchard in Arad County, Romania is presented. The intensive orchard was planted in 2010, on 3.275 ha with apple trees, grafted on M9 rootstock, using 2.6 m x 0.8 m planting distances and 3.5 m high concrete poles with 5 lines of wires, as trellis system. The total yield and productivity of the apple varieties are presented and compared with the initial data estimated in the business plan of the structural funds' project. 2017 was the first year in which the total harvested production surpassed the estimation from the business plan, seven years after planting, but the overall real total production was still 68.1 tones, lower than previsioned. Fruit quality was reduced due to scab attack symptoms, sunburns, cracking and low calibre. Even so, by using intelligent marketing strategies as "buy locally" and "pick it yourself" besides the organic distribution chains, the economic losses were diminished. Considering our results, we do not recommend the cultivation of high disease sensitive apple varieties in organic orchards in areas with high infection pressure and without an efficient phytosanitary protection. The cultivation of scab resistant varieties and a proper orchard management could be the solution for the development of the organic apple production.

Key words: *apple production, high density, productivity, fruit quality.*

INTRODUCTION

As eating healthy and life quality becomes more and more important, the market shifts its focus and the business owners reorient themselves towards the organic products and services that satisfy the clients needs. EU makes serious efforts to fulfill their citizen expectations and promote and support the organic farming developpment. The organic farming in Romania is still at its beginning, the dynamic of organic production indicators showing different fluctuations, except the organic fruit and vineyard areas, where the trend was constantly ascending (MADR, 2018). This ascending situation was also sustained by the EU structural funds given for agriculture and rural development. Although the total organic area cultivated in Romania decreased with 21.5% in the period 2012-2016 (EUROSTAT, 2017), the organic orchard and vineyard area tripled in the same period (Burghelea et al., 2016). Romania makes serious efforts to support and promote organic farming, including advantages offered by different EU structural funds measures for agriculture for those activating in the organic sector. For the period 2007-2013 the procentage of the European Union subsidy received by Romania was 52.02% in average (Marinas et Prioteasa, 2016), altghough some afirm. For the Measure 1.2.1 (Agriculture), in October 2016, in Romania, out of 8738 projects proposals, 3849 were aproved and 2789 finalised, with a total of 662,274,906 euro payments.

Apple is the most important fruit species in Romania, our country having the second highest area cultivated with the apple in Europe (after Poland), representing a share of 10.2% of the total EU-28 production area and only a share of 3.6% of the total EU-28 harvested production (EUROSTAT, 2016).

Organic fruit growing has increasingly gain interest both from Romanian and European farmers or investors, althgough uncertified organic food (as farmers' market, self-production etc.) is considered by consumers to

be more “organic” than certified organic food (Petrescu et al., 2016).
The company Fruit4you, Belgian investors, which owns the 3,275 ha of organic apples in Horia village, Vladimirescu commune, Arad county, received more than 350,000 euro for the implementation of their organic apple orchard.
In this paper, we present the economic results of the organic apple orchard in Arad County, in terms of production, productivity, income in the 2011-2017 period and we compare these results with the previsions made in the structural funds business plan.

MATERIALS AND METHODS

The data used for this study were gathered from the apple orchard belonging to company Fruit4you, lat: 46.1986, long: 21.4285 (Figure 1). As the investors were from Belgium, the same technology as in Belgium was used for the apples plantation. The soil was prepared in the spring and the planting was done with a planting machine.

Figure 1. The Fruit4you orchard

The trees were imported from a Belgian nursery, transported in a temperature-controlled cargo and kept at 25-28°C (the normal temperature environment), after arrival, with the roots in water for 24 hours to get hydrated. The planting was done in June-July 2011, because of the delays in trees delivery. The late planting period caused a premature period of dormancy, which made the young trees apparently looking as they were died in the autumn. The delay in plants delivery also forced the investor to build a buffer for the irrigation system that collects the water from the well and reduce the water temperature difference before the irrigation. The planting machine, with an potential efficiency of 4,800 trees/day manage to plant the orchard (3,275 ha) in four days. On the 90 m x 360 m plot 3 years old plants of ‘Gala’, ‘Jonagold’ (‘Red Prince’) ‘Golden delicious’ and ‘Granny Smith’ varieties, grafted on M9 rootstock, were planted.
The investors preferred to plant at 2.6 m x 0.8 m, for a „fruit wall shape” high density orchard, with 4,000 trees/ha, having 3.5 m high concrete poles and 5 lines of wires. The poles were distributed every 10 m, for 360 m, with a space in the middle of the orchard, where the tractor can make the “U” turn. The varieties were planted by groups of four rows, starting and the scheme repeated for 27 lines. The inter rows were mowed mechanically and the row was cleaned by hand.
Fertirrigation was insured by drip irrigation lines placed directly at the ground level, working on 4 sectors, every day, with 2 hours of watering per sector.
The hail and pest protection was insured by a hail protection net that covers the top and the four laterals of the orchard (Figure 2).

Figure 2. The trellis and the hailnet system

No tree pruning was done in the first year. In the second year the trellis system was put in place, the first three wires, followed by the next fourth and fiftth wires in the third year.
The canopy forming prunings were performed after the Belgian method, during the flowering time, which also implies slow shoot growths of around 30 cm/year.
As the orchard is included in the organic farming system, the insects were controled with pheromone disruptors and the hail nets, that were kept closed from May 1st to September

30.800 units/ha of Isomate CLR mating disruptors (active substance codlemone), were used each year, being placed at height of the 3rd wire (~ at 1.8 m), one every 3 trees, one the edges and one every 5 or 8 trees in the centre of the orchard. Every year the dispensers were put in place at the beginning of May (Bujdei et al., 2016). The insects attack was estimated under 1%, in the flowering period only, when the net is still closed, for a better pollination.
After the net was closed, no insect issues were observed in all 6 years of cultivation. Mineral oil was sprayed in spring 2012 and treatments with cooper (Bouille Bordelaise WDG) were applied during the seasons.
The fruits were picked at maturity and kept in cold storage, at 4°C.

RESULTS AND DISCUSSIONS

Our paper presents the real experience of one applicant to 1.2.1 Rural Development Measure, focusing on the real challenges that one investor met within an organic apple orchard.

Yield, productivity and economic efficiency

The average fruit production/ha was estimated at 18.58 t/ha in the business plan and the realized production was of 15.91 t/ha, with 14.35% less (Table 1). The differences were much higher in the first five years, only 50% of what was estimated was realised until 2016. The year 2017 was a very good year for apple growing in the region with an average annual production of 48.55 t/ha, which represented a record.

Table 1. The estimated and the realized apple production in the 2011-2017 period

Year	Estimated yield (t)	Real yield (t)	Difference (t)	Difference %
2011		3.03		
2012	30.00		-14,10	-47.00
2013	55.00	23.40	-31.60	-57.45
2014	70.00	27.30	-42.70	-61.00
2015	70.00	42.60	-27.40	-39.14
2016	70.00	41.40	-28.60	-40.86
2017	70.00	159.00	89.00	127.14
Total yield	365.00	312.63	-52.37	-14.35
Total annual yield	60.83	52.11	-8.73	-14.35
Annual yield/ha	18.58	15.91	-2.67	-14.35

A similar high yield was reported by Sumedrea et al. in 2016, a production of 44.40 t/ha for 'Golden delicious' Clone B. As in the period 2012-2015, the productions were lower than expected, the producer tried in one year, on one row, a production without thinning.
The result was promising, with 35% higher production on that row, but in the following year no production was obtained, as no flowering buds were differentiate.

Table 2. The estimated and realized productions of each apple variety in the 2011-2017 period

Year	Golden (t)	Gala (t)	Jonagold (t)	Granny Smith (t)	Total yield (t)
2011	1.00	1.00	1.00	0.30	3.30
2012	4.50	5.40	5.10	0.90	15.90
2013	3.00	9.00	11.40	-	23.40
2014	8.40	8.40	10.50	-	27.30
2015	13.50	16.50	12.60	-	42.60
2016	15.00	17.40	9.00	-	41.40
2017	48.60	53.00	57.40	-	159.00
Total yield/ variety	94.00	110.70	107.00		312.90
Average yield /ha	14.35	16.90	16.34		

'Gala' variety was the most productive one, with a productivity of 16.90 t/ha, calculated for the 2011-2017 period (Table 2).
The 'Granny Smith' variety was replaced after the first 2 years, due to its scab sensitivity and low production. Because of scab attack, the general aspect of the trees and apples was unsatisfactory, which forced the producer to sell the 'Granny Smith' apple at a reduced price (50% lower than expected). Although this variety should produce big apples, no fruit was bigger than 65^{+} mm (Figure 3).

Figure 3. Small fruits produced by 'Granny Smith'

'Jonagold' had a lower productivity when compared with 'Gala', but the apples were in majority of 1st category (80+ calibre) (Figure 4). 'Golden delicious' had in average 65% scab free apples, with fruits varying in size between 65 and 80+mm. 'Gala' was more affected by scab, with around 45% scab free apples and the calibre between 60 and 70 mm.

Figure 4. The calibre measurments for 'Jonagold' apples

Regarding the income, in the period 2012-2016 all our estimations were unrealistic, as the real income between 39 and 61% of the estimated one (Table 3).

Table 3. The estimated and actual orchard incomes in the 2011-2017 period

Year/ to	Estimated income (euro)	Real income (euro)	Difference (euro)	Difference (%)
2011				
2012	17,523	4,437	-13,086	-74.68
2013	32,126	5,986	-26,140	-81.37
2014	40,887	13,029	-27,858	-68.13
2015	40,887	17,427	-23,460	-57.38
2016	40,887	14,113	-26,774	-65.48
2017	40,887	42,400	+1,513	+3.70

The average price of apples was 2.5 lei/kg (55 euro cent/kg) and the producer was able to maintain this price only because he has built through the structural funds a cold storage, that allowed him to keep and sell the fruits later than all the other producers in the area and also to deliver in high quantities at once, loading a full truck. Still, the price was not as expected.
Another issue was the lower production obtained in comparison with the estimated one. Beside the stress, this fact leads to the loss of important contracts and direct income losses.

Crop issues

The most frequent crop issues that the producer faced were sunburns, cracking and scab (figures 5, 6 and 7) and the most susceptible variety was 'Gala'.

Figure 5. Sun burns and cracking on 'Gala' variety

Figure 6. Scab symptoms on 'Gala' variety leaves

Regarding scab, more than 45% of the 'Gala', more than 35% of 'Golden' and more then 15% of 'Jonagold' fruits had the disease marks, a fact wich led to a loss due to the lower selling price.

Selling strategies

The producer mainly used two selling strategies: "buy locally" and "pick it yourself".

Figure 7. The "buy locally" and "pick it yourself" announcements on Fruit4you Facebook page

The main retailer client was Real Hyper Market Arad, but a part of the fruits also were bought by the Belgian fruits and vegetables dealer, Fresh Fruit Service BvB.

CONCLUSIONS

The estimations in the business plan when the producer applied to structural funds were more optimistic than the results obtained in reality, especially regarding the income and this had a negative impact on the business.

The highly scab sensitive varieties: 'Gala', 'Jonagold' ('Red Prince') 'Golden delicious' and 'Granny Smith' faced serious problems under a high infection pressure and the lack of good protection products. Cooper sulphate couldn't offer an effective protection against scab and the percentage of affected fruits was extremely high, from 15-45%. 'Granny Smith' was eliminated for this reason, after two years of cropping. Insects attack on fruits was kept less than 1% by using winter oil treatment, matting disruption and net closed orchard. The level of fruit production and its poor quality affected seriously the orchard incomes, the results being with 57.38-81.37% lower than the estimated ones. Only in 2017, the income overpassed the estimation, due to an exceptional production.

Taking in consideration our experience, we recommend to other farmers:

- to set up of the irrigation drip line at 60-80 cm height instead of laying directly on the soil - no rats eating the tube and no need to massive replacement due to destruction during weeding;
- to use higher planting distances between rows, to at least 3.2 m;
- to use mechanical equipments for weed control on the fruit tree row;
- to associate in growers groups, in order to fill the eventual gaps that some may have in some years, reduce the cost of cold storage and not at last, to be able to negotiate with the buyer for fair selling prices.

The general conclusion is to avoid the cultivation of scab sensitive varieties in organic apple orchards and to replace them with scab resistant ones. A proper orchard management is needed to control the phytosanitary issues, to ensure a proper fertilization and a rational tree growth and fruit bearing.

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SOME RESULTS ON NUTRITIONAL PROPERTIES OF ORGANIC ROSE PETALS AND RELATED PROCESSED PRODUCTS

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Abstract

The actual paper presents the first results on the nutraceutical substances found in the rose petals of different varieties, grown organically in different experimental variants. The petals were collected from each variant and analysed in fresh and dehydrated status. Rose petal jams produced with different ingredients (ginger, lemon, seabuckthorn) were also analysed. Total carotenoids content in fresh petals for all 'Crown Princess Margareta' variants was influenced by the mulch and ameliorative variants. Dried petals preserved the carotenoids content while in the rose petal jam was very low. Total anthocyanin content in fresh petals of 'Falstaff' variants were in average at 33.06 mg/100 g. Wool was beneficial, the maximum values on anthocyanin being registered in these variants. Water content was similar in all variants with an average of 84.14%. Total soluble solid (TSS) content varied with the mulch variants (wood chips and wool) and also with the plant ameliorative species. The maximum value obtained was 11.10%. Both rose varieties analyzed have close TSS values.

Key words*: organic horticulture, carotenoids, anthocyanins, rose petal jam*

INTRODUCTION

From ancient times, roses have been used in medical treatments and as food ingredients. Still now, rose petals and fruits are used in different combinations in pharmaceutical and food products, being highly appreciated for their bioactive substances: etheric oils, tannins, carotenoids, anthocyanin, organic acids, vitamins, minerals etc. (Vasilca Mozăceni, 2002; Milică et al., 2010; Lambraki, 2001.)
Rose petals, like the rose fruits, are an import source of antioxidants (Barros et al., 2011).
The actual paper presents the first results on the nutraceutical substances found in the rose petals of different varieties, grown organically in different experimental variants.

MATERIALS AND METHODS

The organic culture was established in 2015 at the Didactic Experimental Field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, using three climbing David Austin rose varieties: 'Falstaff', 'Brother Cadfael' and 'Crown Princess Margareta'.
'Falstaff', created in 1999, is a large and full petalled flowers with a dark crimson colouring. The growth is strong and can reach 2.0 - 2.5 m high as a climber.
'Brother Cadfael', created in 1986, has a large, globular with a clear pink flowers. It has a particulary strong and rich old rose fragance and can reach 2.5 - 3.0 m high.
'Crown Princess Margareta', created in 1999, has a strong and fruity fragance like a Tea Rose type. It grows to 2.5 - 3.0 m high, usually bearing clusters of large apricot colour flowers. (Austin, 2012; Wagner, 2010).
Before and after establishing the organic rose culture, three ameliorative species: *Sinapis alba* L.*, Phacelia tanacetifolia* L. and *Tagetes patula* L. were use to increase the soil biologic activity and for soil disinfection and disinsection.
All the three ameliorative species were sown in the spring, by combining the three species in 7 variants: V1 *Sinapis*, V2 *Sinapis* + *Phacelia*, V3 *Phacelia*, V4 *Sinapis* + *Tagetes*, V5 *Sinapis* + *Tagetes* + *Phacelia*, V6 *Tagetes* + *Phacelia*, V7 *Tagetes* and a control parcel V8, was kept as black field, without sowing.
They were trimmed and incorporated into the soil after the flowering period.

Starting with 2016, on the rose rows, the soil was mulched with saw dust and wool for each initial variant (Vn): Vn.1. wood chips and Vn.2. wool, while the control Vn.3., was represented by unmulched soil. Both mulched rows had the same 1 m width with the specific material.

For each experimental variant, the same technology was applied using manure fertilizing at planting and other organic products in the following years. Plant protection was ensured with copper, sodium bicarbonate, sulfur, pepper, garlic and propolis tincture. Different biostimulators and caw milk were used to increase the plant immunity system.

The petals were collected from each variant begining with May, in the morning (08.00 - 10.00), being collected initially in plastic bags and transported in cold rooms.

A part of the rose petals were dehydrated with an Escalibur dehidrator, 3 hours at 40°C. The dehydrated petals were kept in paper bags at room temperature.

From fresh petals were produced more variants of rose petal jam (D1 - 'Crown Princess Margareta', D2 - 'Falstaff', D3 - 'Brother Cadfaell') with different ingredients: lemon (Dn.1), ginger (Dn.2), seabuckthorn (Dn.3).

The receipe for every variant consisted in a base formula (sugar siroup: 1.5 kg sugar at 1.0 l water with 0,5 kg fresh rose petals) with ingredients added in the final stage (0.3 ml lemon juice, 1.2 ml seabuckturn or 50 g. ginger)

This study presents the first results on some nutraceutical substances found in rose petals and rose jams, as total carotenoids and total anthocyanins, influenced by the three varieties and the applied organic growing technologies. The content in total soluble solids and dry matter is also reported.

Total soluble solids were determined from rose petal juice (Yoon, 2005; Saei, 2011; Mureşan, 2014; Oltenacu, 2015; Bezdadea Cătuneanu et al., 2017), with refractive device Kruss DR301-95 (% Brix).

Dry matter and water content of the samples were determined by oven drying for 24 hours at 105^{o}C using a UN110 Memmert oven, method used also by Moura (2005), Skupień (2006), Delian (2011), Corollaro (2014), Mureşan (2014), Ticha (2015), Bezdadea Cătuneanu et al. (2017).

Total anthocyanins content was studied to 'Falstaff' variety and was measured with Specord 210 Plus spectrophotometer at λ = 540 nm (Bărăscu et al., 2016; Bezdadea Cătuneanu et al., 2017), after an adapted method. The extracts were filtered under vacuum and completed up to 50 ml volume. The results were calculated using the formula: Total anthocyanins = DO_{540} x F, where DO_{540} is absorbance at wavelength λ = 540 nm and factor F = 11.16. The total anthocyanins content was expressed in mg/100 g in fresh weight.

Total carotenoids were studied to 'Crown Princess Margareta' variety. The identification and quantification of total carotenoids content was made after an adapted method after Lichtenthaler and Wellburn (1983) and Arnon (1949).

The analyses were conducted in the Research Center for Studies of Food and Agricultural Products Quality, University of Agronomic Studies and Veterinary Medicine of Bucharest.

RESULTS AND DISCUSSIONS

For the '***Crown Princess Margareta'*** variety were analysed total soluble solids, dry matter and total carotenoids content in fresh petals, dry petals and jam comparing the variants of ameliorative species and mulch used (Table 1).

Table 1. Influence of variants on evolution of total soluble solids and dry matter on fresh petals of 'Crown Princess Margareta' variety

Variant	Dry matter content (D.M. %)	Water content (U) (%)	Total Soluble Solids (TSS) (% Brix)
V1.1	19.33	80.67	8.90
V2.1	16.38	83.62	8.50
V3.1	15.06	84.94	9.80
V4.1	14.97	85.04	7.80
V5.1	15.60	84.40	7.60
V6.1	17.02	82.98	9.70
V7.1	14.66	85.34	8.10
V8.1	15.09	84.91	7.90
Average Vn.1	***16.01***	***83.99***	***8.54***
V1.2	15.51	84.49	8.00

Variant	Dry matter content (D.M. %)	Water content (U) (%)	Total Soluble Solids (TSS) (% Brix)
V2.2	17.16	82.84	10.30
V3.2	15.16	84.84	8.20
V4.2	17.43	82.57	8.50
V5.2	16.47	83.53	7.80
V6.2	17.50	82.50	8.70
V7.2	15.83	84.17	8.10
V8.2	17.98	82.03	9.80
Average Vn.2	***16.63***	***83.37***	***8.68***
V1.3	15.75	84.25	8.00
V2.3	15.24	84.76	7.80
V3.3	16.70	83.30	9.60
V4.3	13.47	86.54	10.20
V5.3	15.98	84.02	9.70
V6.3	17.82	82.18	7.80
V7.3	18.63	81.37	7.60
V8.3	14.88	85.12	9.00
Average Vn.3.	***16.06***	***83.94***	***8.71***
Average Vn.n.	**16.23**	**83.77**	**8.64**

Water content was similar in all variants, with an average of 83.77%. In the wood chip mulch variants the minimum was in V1.1 (80.67%) and the maximum in V6.1 (85.34%) and V4.1 (85.04%). In the wool mulch variants the minimum was in V8.2 (82.03%) and the maximum in V3.2 (84.84%). In the control mulch variants the minimum was in V7.3 variant (81.37%) and the maximum in V4.3 variant (86.54%).

V6 variant registered the lowest value of water content in average (82.56%) and V4 variant the maximum value (84.71%).

Total soluble solids for all the 'Crown Princess Margareta' variants were in average at 8.64 (% Brix).

In the wood chips mulch variants the minimum were in V5.1 (7.60%), V4.1 (7.80%) and V8.1 (7.90%); the maximum values were in V3.1 (9.80%) and V6.1 (9.70%).

In the wool mulch variants the minimum were in V5.2 variant (7.80%) and the maximum in V2.2 (10.30%) and V8.2 (9.80%).

In the control mulch variants the minimum were in V7.3 (7.60%), V2.3 (7.80%) and V6.3 (7.80%); maximum were in V4.3 (10.20%), V5.3 (9.70%) and V3.3 (9.60%).

V7 variant registered the lowest value of total soluble solids in average (7.93%) and V3 variant the maximum values of TSS (9.20%).

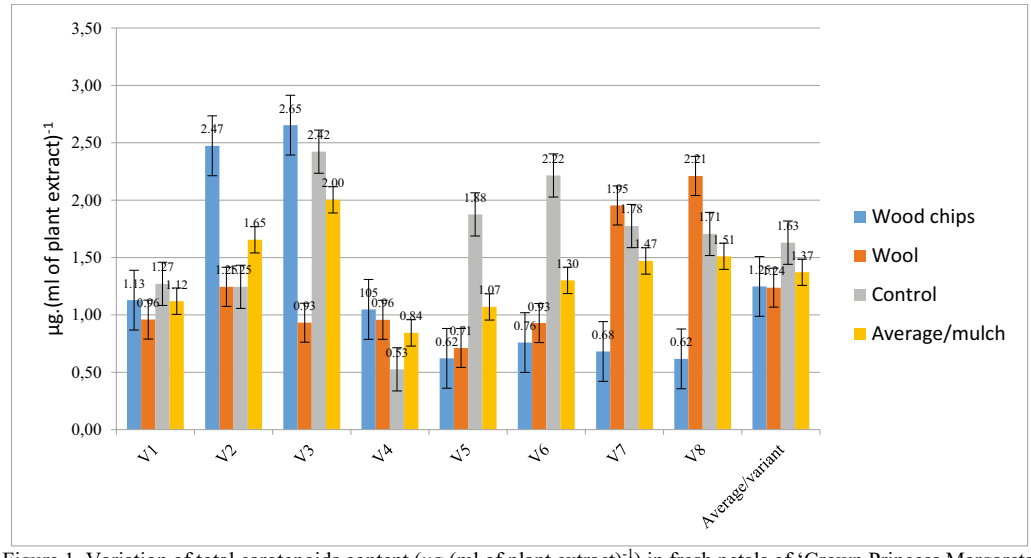

Figure 1. Variation of total carotenoids content (µg (ml of plant extract)$^{-1}$) in fresh petals of 'Crown Princess Margareta' variety, according to mulch and ameliorative species variants

Total carotenoids content in fresh petals for all 'Crown Princess Margareta' variants were in average at 1.37 (µg.(ml of plant extract)$^{-1}$).
In the wood chips mulch variants the minimum were in V5.1 (0.62), V7.1 (0.68), V8.1 (0.62); the maximum values were in V3.1 (2.65) and V2.1 (2.47).
In the wool mulch variants the minimum were in V5.2 variant (0.71%) and the maximum in V8.2 (2.21%).
In the control mulch variants the minimum were in V4.3 (0.53) and the maximum were in V3.3 (2.42%).
V4 variant registered the lowest value of total carotenoids content in average (0.84) and V3 variant the maximum values (2.00) (Figure 1).

Table 2. Total soluble solids content, dry matter and total carotenoids content on dehydrated rose petals and rose petal jam from 'Crown Princess Margareta' variety

Variant	Dry matter content (D.M. %)	Water content (U) (%)	Total Soluble Solids (TSS) (% Brix)	Total carotenoids (µg.(ml of plant extract)$^{-1}$)
U	91.90	8.10		2.07±0.170
D1	20.00	80.00	68.50	0.13±0.082
D4	26.00	74.00	61.30	0.05±0.055
D7	20.00	80.00	68.10	0.09±0.008
Average D.n	***22.00***	***78.00***	***65.97***	***0.09±0.063***

In the dry petals of 'Crown Princess Margareta' variety, total carotenoids were similar to the average of fresh petals (2.07±0.170) (Table 2).
In all rose petal jams from this rose variety, total carotenoids were significant lower than in fresh or dry petals.

For the 'Falstaff' variety total soluble solids, dry matter and total anthocyanin content in fresh petals, dry petals and jam were analysed comparing the variants of ameliorative species and mulch used (Tables 3, 4).
Water content was similar in all variants, with an average of 84.51%. In the wood chip mulch variants the minimum was in V7.1 (76.85%) and the maximum in V8.1 (86.69%). In the wool mulch variants the minimum was in V4.2 (83.30%) and the maximum in V7.2 (86.60%). In the control mulch variants the minimum was in V5.3 (84.22%) and the maximum in V2.3 (86.81%).

Table 3. Influence of variants on evolution of total soluble solids and dry matter on fresh petals of 'Falstaff' variety

Variant	Dry matter content (D.M. %)	Water content (U) (%)	Total Soluble Solids (TSS) (% Brix)
V1.1	16.06	83.94	7.20
V2.1	13.68	86.32	8.00
V3.1	14.63	85.37	8.30
V4.1	19.87	80.13	8.30
V5.1	15.20	84.80	10.30
V6.1	18.59	81.41	9.90
V7.1	23.15	76.85	10.00
V8.1	13.31	86.69	8.70
Average Vn.1	***16.81***	***83.19***	***8.84***
V1.2	15.09	84.91	8.40
V2.2	16.35	83.65	8.00
V3.2	15.91	84.09	10.10
V4.2	16.70	83.30	7.40
V5.2	15.25	84.75	8.80
V6.2	14.88	85.12	11.10
V7.2	13.40	86.60	8.50
V8.2	14.33	85.67	7.10
Average Vn.2	***15.24***	***84.76***	***8.68***
V1.3	14.70	85.31	7.30
V2.3	13.19	86.81	6.70
V3.3	14.34	85.66	7.90
V4.3	14.38	85.62	8.50
V5.3	15.78	84.22	7.70
V6.3	13.96	86.04	7.80
V7.3	13.86	86.14	6.90
V8.3	15.09	84.91	7.80
Average Vn.3	***14.41***	***85.59***	***7.58***
Average Vn	**15.49**	**84.51**	**8.36**

V4 variant registered the lowest value of water content in average (83.02%) and V8 variant the maximum value (85.76%).
Total soluble solids for all the 'Falstaff' variants were in average at 8.36 (% Brix). In the wood chips mulch variants the minimum was in V1.1 (7.20%) and the maximum was in V5.1 (10.30%) and V7.1 (10.00%). In the wool mulch variants the minimum was in V8.2 variant (7.10%) and the maximum in V6.2 (11.10%).

In the control mulch variants the minimum were in V2.3 (6.70%), V7.3 (6.90%) and maximum was in V4.3 (8.50%).

V2 variant registered the lowest value of total soluble solids in average (7.57%) and V6 variant the maximum values of TSS (9.60%).

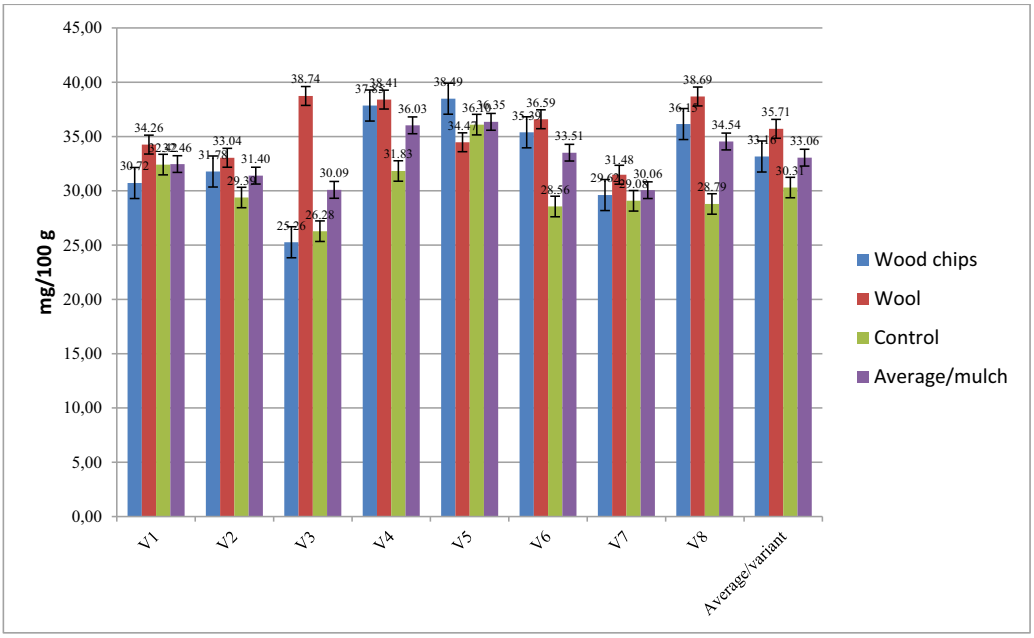

Figure 3. Variation of total anthocyanin content (mg/100 g) of 'Falstaff' variety, according to mulch and ameliorative species variants

Total anthocyanin for all the 'Falstaff' variants were in average at 33.06 mg/100 g.

In the wood chips mulch variants the minimum was in V3.1 (15.26) and maximum was in V5.1 (38.49).

In the wool mulch variants the minimum was in V7.2 variant (31.48%) and the maximum in V3.2 (38.74), V8.2 (38.69) and V4.2 (38.41).

In the control mulch variants the minimum was in V3.3 (26.28) and maximum was in V5.3 (36.10).

V7 and V3 variants registered the lowest values of total anthocyanin in average (30.06 respectively 30.09); V5 and V4 variants registered the maximum values of total anthocyanin in average (36.35 respectively 36.03). In the dry petals of Falstaff variety, total anthocyanin content was bigger than the average of fresh petals (33.06±0.626).

In all rose petal jams from this rose variety, total anthocyanin content was significant lower than in fresh or dry petals.

Table 4. Total soluble solids, dry matter and total anthocyanin on dehydrated rose petals and rose petal jam of 'Falstaff' variety

Variant	Dry matter content (D.M.) (%)	Water content (U) (%)	Total soluble solids (TSS) (% Brix)	Total anthocyanin content (mg/100 g)
Fd	92.40	7.60	-	39.76±0.272
D2	21.00	79.00	68.70	6.67±0.009
D5	29.00	71.00	58.60	7.20±0.329
D8	27.0	73.00	61.80	5.96±0.399
Average D.n	***25.67***	***74.33***	***63.03***	***6.61±0.599***

CONCLUSIONS

This study aimed to determine and compare different parameters analyzed in rose petals and rose jams, as total carotenoids, total anthocyanins, total soluble solids and dry matter influenced by the three varieties of roses and the applied organic growing technologies.

Water content was similar in all variants both for the two rose variety 'Crown Princess Margareta' and 'Falstaff', with an average of 84.14%. Total soluble solid content varied with the mulch variants (wood chips and wool) and also with the plant ameliorative species. The lower values was in V7 (7.93%) variant and the biggest in V3 for 'Crown Princess Margareta' (9.20%). The maximum value was obtain in V4.3 with 10.20%. For the 'Falstaff' the lower value was in V2 variant (7.57%) and the biggest in V6 (9.60%). The maximum value was obtain in V6.2 with 11.10%. Both rose varieties have close TSS values on average.

Total carotenoids content in fresh petals for all 'Crown Princess Margareta' variants was influenced by the mulch and ameliorative variants. V4 variant, *Sinapis* + *Tagetes* registered the lowest value of total carotenoids content in average (0.84) and V3, *Phacelia*, variant the maximum values (2.00). Wood chip mulch variants had a positive influence, total carotenoids content was higher in these variants. Dried petals preserved the carotenoids contents while in the rose petal jam was very low. Total anthocyanin content in fresh petals of 'Falstaff' variants were in average at 33.06 mg/100 g, more than in many fruits as blueberry, apple (Bezdadea Cătuneanu et al., 2017). V7, *Tagetes*, and V3, *Phacelia*, variants registered the lowest values of total anthocyanin in average (30.06, respectively 30.09) while the mixed species variants V5 and V4 registered the maximum values of total anthocyanin in average (36.35, respectively 36.03). Wool was beneficial, the maximum values on anthocyanin being registered in these variants. Dried petals preserved the anthocyanins while in the rose petal jam they are very low.

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CULTIVATION TECHNOLOGY OF ORGANIC ROSES FOR PETAL PRODUCTION

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Abstract

The aim of this paper is to present a technology for the cultivation of organic edible roses. The basic principle followed is the creation of an organic production ecosystem considering the natural and anthropogenic factors. To increase the soil biologic activity and for soil disinfection and disinsection, three ameliorative species: Sinapis alba L., Phacelia tanacetifolia L. and Tagetes patula L. were used before and after planting. Microbiological and agrochemical analyses were made to monitor the soil activity and the influence on the rose plants. On the row, the soil was mulched with wood chips and wool and for irrigation, a drip systems was used. The rose climbing plants were supported by wire trellis. For plant protection, a strategy to alternate copper and sulfur based products with alternative ones like sodium bicarbonate, pepper, garlic and propolis tincture and other products based on plant and animal extracts (Mimosa sp., chitosan) was followed. Different bio-stimulators and caw milk were used to increase the plant immunity system. The cultivation technology of organic roses can be implemented on large scale farms with good economical results.

Key words*: soil management, plant canopy, fertilization, plant protection, picking, storage.*

INTRODUCTION

Rose is one of the most mankind beloved plant specie since the ancient times. It is a symbol of love and victory, sign of nobility and refinement. Its therapeutic properties are usually forgotten in favor of the ornamental role (Lambraki, 2001; Milică et al., 2010; Vasilva Mozăceni, 2002). It is important to rediscover them and for an appropriate analyze an organic culture is needed.

The aim of this paper is to present a technology for the cultivation of organic roses for petal production.

The basic principle followed is the creation of an organic production ecosystem considering the natural and anthropogenic factors (Milică et al., 2010; Wagner, 2010).

The cultivation technology of organic roses for petal production detailed in this paper, can be implemented on large scale farms with good economical results.

MATERIALS AND METHODS

At the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, an organic rose culture with three climbing varieties: 'Falstaff', 'Brother Cadfael' and 'Crown Princess Margareta', was established (Austin, 2012).

The rose crop was protected by planting a poplar (*Populus balsamifera*) wind break.

At the beginning, to increase the soil biologic activity and for soil disinfection and disinsection, three ameliorative species: *Sinapis alba* L.*, Phacelia tanacetifolia* L. and *Tagetes patula* L. were used before and after planting.

Microbiological and agrochemical analyses were made to monitor the soil activity and the influence on the rose plants (Butcaru et al., 2016; Butcaru et al., 2017)

On the row, the soil was mulched with saw dust and wool and for irrigation, a drip systems was used (Matei et al., 2017; Butcaru et al., 2017). The inter-row was kept grassy through repeated mowing.

The rose climbing plants were supported by wire trellis and starting with the second growing year, a specific pruning scheme was applied. Each plant was formed with three parallel, vertical shoots (Trident). In the first year, from the beginning of flowering, the flower buds were eliminated until the principal shoots reached more than 1 m height. After that, the roses were left to flower.

The same organic crop management regarding fertilization and plant protection was applied for all experimental variants. Manure was applied at planting, while other organic fertilizers on different phenological stages. Wool mulch applied on plant rows proved to have high qualities as organic fertilizer too. (Butcaru et al., 2017)

For plant protection, a strategy to alternate copper and sulfur based products with alternative ones like sodium bicarbonate, pepper, garlic and propolis tincture and other products based on plant and animal extracts (*Mimosa* sp., chitosan) was followed. Different bio-stimulators and caw milk were used to increase the plant immunity system.

The petals were collected in plastic bags during the full flowering period (from May to October), in the morning (7.00 - 10.00 a.m.). After sorting and cleaning, petals were used for drying or processing as jam. The unprocessed petals were kept in cold storage at 1-2^{0}C and 85-95% humidity for 7-10 days. Diferent rose petal jam variants were tested for production (Butcaru et al., 2017).

RESULTS AND DISCUSSIONS

The technology for the cultivation of organic roses for petal production consists in several stages, presented below.

Stage 1. Preparing the land for planting

1.1. Land clearing of existing vegetation and clean the other existing rubbish

This operation consists in cleaning the land from the older vegetable debris of the last culture and in the same time eliminates stones, boulders, glass etc. (Figure 1).

Figure 1. Collecting debris (stones, boulders, glass)

1.2. Plotting the land

Depending on the size of the land taken in the crop, it can be divided into several plots for ease of maintenance. Usually the parcels are rectangular, the rows of roses being parallel to the small side of the plot. At the same time, roads and return areas are usually arranged, which are usually ground or sown.

1.3. Fencing the culture

Fencing the rose culture is intended to protect against wild animals. It is possible to use a galvanized net, stretched on concrete pillars, 2.5 m high, fixed at 2.0 m between them. From 50 to 50 m the pillars will be provided with oblique counter blades in order to stretch the corresponding net.

The net will have a height of 2.0 m. Its base has to be buried 10 to 15 cm in the ground to prevent the rabbits or other animals from entering under it.

1.4. Creating the windbreak

The organic edible rose culture is well suited to having a windbreak both in creating a microclimate where external influences are diminished (possibly polluting substances from neighboring crops) and to create a shelter for useful avifauna.

In the established culture within the Experimental Field of UASMV Bucharest, a poplar windbreak (*Populus balsamifera*) was planted (Figure 2). In March 2015, a grass band 2.0-2.5 m wide was sown on all four sides of the cultivated land (1,350 m^2). In December 2015, the boreholes were dug and planted poplar (*Populus balsamifera*) on each side, 1 m apart, respectively, in the center of the strip.

Figure 2. Leveling band with drainage slope; Poplar windbreak (*Populus balsamifera*)

1.5. The proper preparation of the land

1.5.1. Scarification of the land

Scarification of the land is done in two directions, with a scarifier with active organs at

a depth of 60-80 cm. It has the role of deflecting the land in depth and removing any roots from the ground. It is good to be done a few months ahead.

1.5.2. Ground leveling

It can be done either by plant soil intake or by leveling the existing one. In the land taken in culture, in March 2015, the small oscillation of level were leveled by moving a small amount of surface soil vegetation between different points.

1.5.3. Depth plowing 25-30 cm

It is usually done in autumn with specific machines.

1.5.4. Soil preparation with a rotary cutter

It has the role of creating a perfectly flat surface with well-grounded soil.

1.5.5. Natural soil disinfection and disinsection

An important stage in the soil preparation in an organic system consists in the actions carried out in order to increase the biological activity in the soil. One step in this direction is the sowing of ameliorative plants with a role in soil restructuring, the elimination of dangerous soil diseases and pests, the potentiation of fungi and favorable soil bacteria (Figure 3). It is recommended to use the herbaceous plant species: mustard (*Sinapis alba*) at 12 kg/ha, *Tagetes* sp., 6-8 kg/ha, *Phacelia tanacetifolia* in the amount of 8-10 kg/ha. They are sown early spring (March - early April). After blossoming (50 to 55 days for mustard and *Phacelia*; 70 days for *Tagetes* sp.) the plants are incorporated in the soil.

The best results on the potentiation of microbiological activity in soil were observed when plants were used in combinations of 2 or 3 species. Since they have different flowering periods, sowing is recommended in strips 1-3 meters wide.

After incorporation of the plants into the soil, the soil is kept free of weeds until the establishment of the crop, by mowing them and leaving them on the ground.

Figure 3. Ameliorative plants details with soil incorporation

Stage 2. Establishing the organic edible rose culture

2.1. Establishing the culture

2.1.1. Supply of seedlings

In order to establish an organic rose culture, seedlings must be procured in advance. In organic farming, these must be certified. If organic suppliers of organic planting material are not available, conventional seedlings can be used with the proving the absence of certified seedlings. If the plants arrive long before planting, they are stratified into ditch or containers.

2.1.2. Establishment of planting distances and land plotting

Depending on the varieties of roses to be planted, the distance between the rows and the row is then determined, followed by picketing on the field.

For the used varieties of roses, planting distances were 2 m between rows and 1 m per row. To make the plotting, pickets of 0.5 m length, 50 m long roulette and 0.5 m long knotted wire of about 50 m long are used.

When land is picked up, it is meant to mark the place where each rose will be grown. Direction of rows is recommended to be north - south.

Considering the 1 m distance between plants, it is recommended to open planting ditches.

2.1.3. Basic fertilization at planting

After opening the planting ditch, well fermented manure (10-15 t/ha) is applied. Other fertilizers in organic farming can also be applied.

In areas where it is difficult to purchase manure, it can be replaced by other types of fertilizer such as Organofert (based on poultry manure) (2 t/ha).

2.1.4. Installing trellis system

The trellis system is usually installed prior to planting. The variant chosen for the crop was made up of: acacia pillars and counter pillars of diameters 80-100 cm, height 300 cm and pillars for interior of diameter 60-80 cm, height 300 cm. The pits can be done with the help of the motor or manual drill 100 cm (Figure 4).

The pillars are fixed to the rows of roses at a distance of about 7 m between them and at a depth of 60 cm. It is first burned at the base with the burner at a length of 1 m.

Three galvanized wires of 2.5 mm are fastened with bows, the first one at 70 cm of soil, the second one at 130 cm and the third one at 200 cm of soil. The second wire is recommended to be doubled to direct the branches of roses inside the created space.

Figure 4. Trellis system details

2.1.5. Install fertigation system

The fertigation system consists of a main pipeline and several secondary pipes for each row of roses. The drip tubes were lifted on the first wire of the support system (Figure 5).

Figure 5. Fertigation system details

2.1.6. Rose planting

Planting period. The most suitable period for planting is autumn after the fall of the leaves.

Preparing plant material. The plants have to be hydrate 24 hours before the planting time. Check the condition of the roots, where dry or injured portions are eliminated until to the healthy portion. Then the roots are dipped in a mud made from fresh dung, to which the addition of bio stimulators for root formation (eg Rootip mix 2-3 l/ha) is recommended.

Planting. The plants are planted with the grafting point located 10-15 cm above ground level. Place it with its roots in the planting ditch on a loose soil mound. Draw soil from the soil fertile ground; rotate evenly around the plant from the outside to the inside.

An organic-mineral fertilizer can be applied at this time to stimulate the rapid growth of the roots or later to a few days application of Rootip mix through the fertilization system.

Then the rest amount of soil is added and wet with at least 15-20 liters of water/plant.

In the case of autumn plantings, the roses are then mussed by pulling the ground at the base of the roots, forming a 20-30 cm high hill. Early spring (end of February - beginning of March) breaks down to favor growing buds.

Figure 6. Planting details

2.1.7. Soil maintenance

It is recommended to mulch the roses. Wool, wood chips or different wraps can be used. Wool has the advantage of being a very good fertilizer. It is recommended to use it in a slightly processed form to avoid the holes that leave the weeds to grow (Figure 7).

Figure 7. Mulch details (wool and wood chips)

Between rows, the vegetation is regularly mowed, keeping it at low heights.

2.2. Training pruning

Year 1. Early spring pruning works are carried out, following the development of the bushes and the distribution of uniform branches. Remove the blooms until the bush reaches an average height of 1m, and then let it bloom.

As a form of branch leadership, it can be chosen three vigorous branches that make up the skeleton elements, the rest shortening.

Year 2. In the second year, the three branches of the previous year remain, springing down the anticipated branches. Shorten to the ring or very short the other branches of the bush (Figure 8).

Figure 8. Pruning details (first and second year)

2.3. Organic treatment

Table 1. Scheme of organic treatments for edible roses

No.	Phenophase	Pathogen/pest	Recommended plant protection products
1	The beginning of vegetation	Aphids, mites (hibernate forms)	- vegetable oil (Ovipron 2000 - 0,5%) - Oleorgan – 0,3% - Laser 240 – 0,25% - Canelys – 0,3%
		Mildew, Black spot, Rust	- Bouille bordelaise – 0,5-1,0% - Microthiol – 0,3% - sodium bicarbonate – 0,5% - Mimox – 0,3% - Altosan – 0,3-0,8%
2	Growing shoots (April - May)	Diseases produced by fungi and bacteria (Black spot, mildew, rust)	- Bouille bordelaise – 0,5% - Microthiol – 0,3% - sodium bicarbonate – 0,5% - Mimox – 0,3% - Altosan – 0,3-0,8%
		Aphids, other pests	- Oleorgan – 0,3% - Laser 240 – 0,25% - Canelys – 0,3%
3	June - September	Diseases produced by fungi and bacteria (Black spot, mildew, rust)	- Bouille bordelaise – 0,5% - Microthiol – 0,3% - sodium bicarbonate – 0,5% - Mimox – 0,3% - Altosan – 0,3-0,8%
		Aphids	- Oleorgan – 0,3% - Laser 240 – 0,25% - Canelys – 0,3% - propolis tincture – 0,15% - hot pepper tincture – 0,3% -garlic tincture – 0,3%
4	Fall of the leaves (November - December)	Mycotic pathogens and bacteria	Bouille bordelaise – 0,5% - 1,0%
5	Winter period	Mycotic pathogens and bacteria, pest (hibernating forms)	- vegetable oil (Ovipron 2000 - 0,5%) - Bouille bordelaise – 1,0%

2.4. Fertilization

Table 2. Scheme of fertilization for the organic edible rose culture

No	Phenophase	Recommended plant protection products
1	Înfrunzit, creşterea lăstarilor (aprilie - mai)	Alga 0,3% (foliar or radicular) Magnetic fertilizer Cropmax 0,2% Caw milk 1,0%
2	June - September	Alga 0,3% (foliar or radicular) Cropmax 0,2% Caw milk 1,0%
3	At the fall of the leaves, during the winter period	Bentonite 1,5% (frost protection)

2.5. Rose petal harvest

In the first year, the harvesting of flowers begins in July, after the plants have reached an average height of 1 m. Since the second year, the flowers can be harvested since May (Figure 9).

Petals are harvested in the morning, after dew, in pots or baskets. It is sorted immediately, eliminating the various impurities.

They are packaged in vacuum bags of convenient size for the assigned destination (according to the specific quantities).

The vacuum bags are kept in a cold store at temperatures of 1-2°C when the petals are processed for the next 5-7 days or in the freezer at temperatures of -18 to -20°C for extended periods.

Figure 9. Flowering details (second year)

Table 3. Technology to establish an organic edible rose culture

No.	Operation	Period	Equipment / Supplies
1	Land clearing of existing vegetation and clean the other existing rubbish.	March- September, year I	Backhoe/bags / Containers/Tractor with trailer
2	Plotting	March- September, year I	Pickets, roulette
3	Fencing	March- September, year I	Drilling tractor/Concrete pillars (h = 2.5 m), galvanized mesh (h = 2 m)
4	Land scarification	March- September, year I	Scarifier
5	Ground leveling	March- September, year I	Backhoe
6	Depth plowing 25-30 cm	September – October, year I	Tractor
7	Soil preparation with a rotary cutter	February – March, year II	Tractor, rotary cutter
8	Creating the windbreak	March- September, year I	Drilling tractor/Poplars (*Populus balsamifera)* manure
9	Natural soil disinfection and disinsection	March– April, year II	Sowing equipment/ seeds: mustard (*Sinapis alba*) - 12 kg/ha, T*agetes* sp. - 6-8 kg/ha, phacelia (*Phacelia tanacetifolia*) - 8-10 kg/ha. Tractor
10	Supply of seedlings	September, year II	Seedlings
11	Establishment of planting distances and land plotting	October – November, year II	Pickets, roulette, string
12	Installing trellis system	October – November, year II	Drill 100 mm/ acacia pillars, burner, wire 2,5 mm, staples, wire tensioners
13	Install fertigation system	October – November, year II	Principal pipelines, drip, elements
14	Opening the planting ditch	October – November, year II	Tractor
15	Basic fertilization at planting	October – November, year II	Fertilization distribution equipment / manure (10-15 t/ha) or other (ex. Orgafert 2 t/ha)
16	Rose planting	October – November, year II	scissors/fresh dung mud/Rootip mix (2-3 l/ha)

Table 4. Maintenance of an organic rooting plant

No.	Operation	Period	Equipment/Supplies
1	Winter treatment	January- February	- vegetable oil (Ovipron 2000 - 0,5%) - Bouille bordelaise – 1,0% - bentonite 1,5% (frost protection)
2	Eliminate the winter soil hills	February - March	- dig/ dibble
3	Pruning and trellising branches	February - March	- Scissors/trellis elements
4	Eliminate dried leaves, branches (cultural hygiene)	March	- Scissors
5	Spring treatment	March	***Insecticides:*** - Oleorgan – 0,3% - Laser 240 – 0,25% - Canelys – 0,3% ***Fungicide/Bio-stimulators:*** -Bouille bordelaise – 0,5-1,0% - Microthiol – 0,3% - Sodium Bicarbonate – 0,5% - Mimox – 0,3% - Altosan – 0,3-0,8%
6	Soil maintenance	March	- Wool/twood chips/ processed wool - trimer/ mowing equipment
7	Ameliorative plants sowing	March – April	-Sowing equipment/ ameliorative plants seeds
8	Treatment sprout growing and fertilization	April – May	***Fungicide/Bio-stimulators*** - Bouille bordelaise – 0,5% - Microthiol – 0,3% - sodium bicarbonate – 0,5% - Mimox – 0,3% - Altosan – 0,3-0,8% ***Insecticides*** - Oleorgan – 0,3% - Laser 240 – 0,25% - Canelys – 0,3% ***Fertilizers*** - Alga 0,3% (foliar or radicular) - Magnetic fertilizer - Cropmax 0,2% - Cow milk 1,0%
9	Remove the blooms (year I)	May – June	
10	Soil maintenance	May – October	- trimer/ mowing equipment
11	Irrigation	May – September	Irrigation system
12	Trellising new sprouts	June – September	- trellising elements
13	Protection and fertilization treatments	June – September	***Fungicide/Bio-stimulators*** - Bouille bordelaise – 0,5% - Microthiol – 0,3% - sodium bicarbonate – 0,5% - Mimox – 0,3% - Altosan – 0,3-0,8% ***Insecticides*** - Oleorgan – 0,3% - Laser 240 – 0,25% - Canelys – 0,3% - propolis tincture – 0,15% - hot pepper tincture – 0,3% -garlic tincture – 0,3% ***Fertilizers*** - Alga 0,3% (foliar or radicular) - Cropmax 0,2% - Caw milk 1,0% - Bombardier 0,25%
14	Rose petals harvesting	July - October	- Boxes/baskets/scissors
15	Complete plants	October - December	- drill, scissors/manure/fresh dung/ seedlings/Rootip mix
16	Falling leaves treatments	November - December	- Bouille bordelaise – 0,5% - 1,0%

CONCLUSIONS

The study aimed to present the technology for an organic edible rose crop. It had, as basic principle, the creation and maintenence of an organic production ecosystem considering the natural and anthropogenic factors.

The technology focus in the first stages of establishment of the organic edible rose crop on the increase of soil biological activity.

Using three ameliorative species (*Sinapis alba*, *Phacelia tanacetifolia* and *Tagetes patula*) the soil bacteria and fungi increased at important levels.

Climbing rose varieties used proved several advantages towards the classical edible rose: trellising on the vertical position, repeated flowering since May to October and fragrance varieties with multiple way of valorisation.

An innovative pruning system refer to a 2 year rotation scheme of branches, giving the possibility to have a constant production every year.

The average quantity of petals per hectare was 9.44 t/ha at 'Crown Princess Margareta' variety, 7.54 t/ha at 'Falstaff' variety and 5.62 t/ha at 'Brother Cadfael' variety.

The best variant of mulch used was wool, which proved to have special qualities as a fertiliser too.

The best temperature for maintain the harvested petals were 1-2°C when the petals are processed in the next 7-10 days.

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MORUS SP. FOR REVIGORATING SILKWORM BREEDING IN ROMANIA AND PROMOTING HEALTH BENEFITS OF LEAVES AND FRUITS

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Abstract

Besides supplying food for silkworm rearing, initiation of a mulberry plantation is an important business, which may bring important income in rural areas. Plants of the genus Morus are known to be a rich source of bioactive compounds from flavonoids and phenolic acids, compounds with known antioxidant and other benefic properties. The aim of the present study was the characterization of mulberry extracts (from leaves and fruits) regarding their chemical composition and determination of some bioactive properties of the mentioned matrices. Sugar profile, total protein and lipid content, total phenolics and flavonoids, mineral composition and aminoacid profile were determined. Mulberry leaves are important protein sources (being the principal feed for silkworm) and also important source of free aminoacids, including the essential ones. 25 free aminoacids (aa), were identified and quantified by internal standard method using LC-MS and EZ: faast Phenomenex kit. The main aa were alanine (144 mg/100g) and Gama-aminobutiric acid (153 mg/100g). The main minerals from mulberry extracts were: iron, zinc and calcium. Total identified simple sugars ranged between 8 and 13% and lipid content between 0.5 to 1.5%.

Key words*: Morus sp., agriculture, mulberry leaves; bioactive properties, silkworm*

INTRODUCTION

Mulberry (*Morus* sp.) are grown worldwide for sericulture. Their leafs being the only natural food for silkworms, but also for other purposes, among them being fruit production. The most popular species are *Morus alba* and *Morus nigra*, and the intensive selection and mutation breeding have resulted in thousands of cultivars, hybrids and polyploids (Yongkang, 2000). Mulberry trees are valuable species not only for silkworm rearing, but also for gardening and landscaping, street shade and reduction of pollution level in the environment and soil (Rafati et al., 2011).

The fruits are important sources of bioactive compounds such as simple sugars (glucose and fructose) (Imran et al., 2010), alkaloids (Kim et al., 2014), carotenoids, polyphenols, anthocianins and minerals (Dimitrijevic et al., 2014; Okatan et al., 2016).

The bioactivity of mulberry fruits was determined in many studies, having antioxidant properties (Imran et al., 2010; Okatan et al., 2016), blood glucose reductor (Shin et al., 2016), antibacterial, anti-inflammatory and anticancer activity, as well as cardiovascular and cardioprotective activity (Gryn-Rynko et al., 2016). Even the phytochemical composition of leaves and fruits of mulberry are studied worldwide, new promising sources of natural antioxidants or other phytotherapeuticals can be found in this valuable matrix.

The aim of our study was to evaluate the chemical composition of extracts from fresh and dry leaves and fruits of *Morus nigra*, Ukraina variety.

MATERIALS AND METHODS

Chemicals and materials. All chemicals and reagents were analytical grade or chromategraphic grade and ultrapure water, purchased from Sigma, Merck and Fluka.

Mulberry leafs, belonging to *Morus nigra* specie, 'Ukraina' vartiety, were harvested in

2017, placed at -18°C or air dried in the dark and grounded in fine powder and kept at 4°C until analysis. Mulberry fruits were harvested in 2017, and kept at -18°C in plastic bags or dried in the oven at 60°C until constant weight. After drying, the fruits were grounded in fine powder and refrigerated in plastic bags until determinations.

Sample preparation. Mulberry fruits and leafs were prepared according to the parameter to be determined: water extracts, ethanol extracts or calcinations for mineral determination.

Sugar spectrum. Method used for determination of sugar spectrum is high performance liquid chromatography with refractive index detection (Bonta et al., 2008).

Operational parameters of chromatographic system were: column: modified Alltima Amino 100Å, 5 µm, 250 x 4.6 mm; mobile phase flow: 1.3 ml/min; mobile phase: acetonitrile/water (75/25; v/v); column temperature: 30°C; injection volume : 20 µl; separation time: 60 min. Sugar standard solutions are prepared like the analyzed sample. Standards are injected and analysed separately to determine the retention time of each sugar and in mixture for the calibration curve construction. Results are expressed in g/100 g sample.

*Lipid content***.** Total lipids were determined by extraction with organic solvents using Soxhlet method. Two grams of sample were weighted on filter paper, which will be packed and placed in the paper cartridge. Dry and clean extraction glasses containing 2 boiling stones, will be weighted and together with the cartridge and the solvent (70 ml n-hexane) will be fixed in PTFE cylinders. The method is set from the multistat: extraction temperature 140°C, extraction time 3 h, 25 min, washing 30 min, solvent evaporation in hot air flow 10 min. Extraction glasses, dried in the oven at 60°C and cooling, are weighted and the result were expressed as percent.

Protein content. From the homogenized sample, 1g is weighted in paper bags handled with a tweezer so that they are not contaminated with different substances that may contain nitrogen. Paper bags are placed in digestion unit vialse (Buchi Digesion Unit K-424), with 2 Kjeldahl tablets and 20 ml concentrated sulphuric acid (95-98%). Digestion lasts 2 h, until the solution turns to green. Distilation is made with Büchi, KjelFlex K-360 unit, every determination is made with a mixture of reagents (50 ml H_2O: 90 ml NaOH: 60 ml H_3BO_3). Titration is made with automatic titrator TitroLine Eeasy (Schott), using 0.05M sulphuric acid for low protein matrices, until pH of 4.65.

Determination of mineral content. To determine the levels of micro and macroelements: Na, Mg, K, Ca, Fe, Pb from studied plant matrices, the atomic absorption spectrometry method was used. The mineralization of the samples was performed in a microwave furnace, Berghof digestion system MWS-2. Approximately 0.3 grams of the homogenized samples were placed in special Teflon tubes, 2 ml of 65% HNO_3 was added and let to react for 15 minutes, after which 3 ml of H_2O_2 was added before the container was sealed (Quinn et al., 1994; Finger et al., 2014). At the end of the initiated program, the solution is transferred into plastic containers and the sample is diluted with ultrapure water to a volume of 125 ml.

An Aanalyst 800 Atomic Absorption Spectrometer from Perkin-Elmer was used, equipped with a cross-linked graphite furnace. It is electrically heated and the voltage will be applied transversely to the tube, perpendicular to the light beam, and finally the electromagnet will generate a magnetic field parallel to the radiation beam emitted by the lamp (Farrukh, 2012).

Free aminoacid profile. Aminoacid profile was determined by liquid chromatography – mass detection (LC-MS), as a method with high selectivity and sensitivity. Determinations were made on a Shimadzu LC-MS (Japan), with electrospray ionization, opedrating in positive mode. Operational parameters of the method were: EZ: faast AAA-MS, 250 x 3.0 mm chromatographic column, mobile phases: ammonium formate 10 mM in water (A) and ammonium formate 10 mM in methanol (B), 0.3 ml/min flow, column temperature 35ºC, injection volume 1 µL, detector tension 1.7 KV, acquisition time 33 min.

Polyphenolic and Flavones Contents. For the total polyphenol content determination, the Folin-Ciocâlteu method was used (Folin and Ciocâlteu, 1927), modified by various authors and adapted to all types of matrices (Duda et al., 2015; Mihaylova et al., 2013; Singleton and

Rossi, 1965; Kim et al., 2003). A volume of 25 µl of each ethanolic extract was mixed for 5 minutes with 125 µl of 0.2 N Folin-Ciocâlteu. The samples were incubated in the dark for 120 min. The absorbance was measured at 760 nm, using a Sinergy 2 Biotek Multichannel spectrophotometer. The standard curve was prepared by using different concentrations of gallic acid and the results were expressed as gallic acid equivalents/100 g).

The quantification of flavones in the samples was made using Dowd method (1959) based on the reaction of aluminium chloride, as specific reagent, with the flavonoids present in the sample giving a yellow color, proportionally with the concentration of the compounds, determined spectrophotometrically at 415 nm.

Radical Scavenging Activity Assay (DPPH).

The antioxidant activity is the primary step in determining the biological activity of any natural matrix. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving a discoloration of solution, proportionally with the amount of antioxidant present in the sample (Paşca et al., 2016; Duda et al., 2015). The free radical scavenging ability of the ethanolic extracts was measured in terms of hydrogen donation or radical scavenging ability using this method. Thus, 5 µl of 1% plant alcoholic extract was mixed with 295 µl of 0.02 mg/ml DPPH solution in methanol, stirred and incubated in the dark for 20 minutes. The absorbance changes were monitored at 517 nm using a Sinergy 2 Biotek multichanel spectrophotometer. The percent inhibition of DPPH free radical was calculated by the formula: Percentage inhibition (% I) = $[(A_{blank} - A_{sample})/A_{blank}] \times 100$, where, A_{blank} is the absorbance of the control reaction (DPPH alone) and A_{sample} is the absorbance of DPPH solution in the presence of the test compound.

RESULTS AND DISCUSSIONS

Mulberry fresh leaves and fresh fruits have a high content of water (62.07% in leaves and 40.89% in fruits).

Chemical composition of leaves and fruits of *Morus* sp. are presented in Table 1.

Table 1. Sugar spectrum, total lipids and total nitrogen (%) of fruits and leaves of *Morus nigra*

Specification	Fresh leaves	Dry leaves	Fresh fruits	Dry fruits
Glucose	0.56	1.48	5.96	27.09
Fructose	0.89	1.77	6.16	29.65
Zaharoza	1.43	3.06	-	-
Galactoza	1.02	2.03	-	-
Total lipids	2.41	3.40	1.51	3.65
Total nitrogen	11.56	19.70	0.96	1.57

Leaves of mulberry contain fructose and glucose in small amounts, but a higher content of sucrose. Also galactose is present in this matrix. Instead, the fruits contain only fructose (in higher amounts) and glucose. The amounts determined in our study were in accordance with other studies of *Morus nigra* fruits (Okatan et al., 2016). Dry fruits are important simple sugar sources, as seen in Table 1.

Leaves of mulberry contain high amounts of lipids, several times higher than determined by different authors (Gryn-Rynko et al., 2016; Imran et al. 2010), but smaller than those of Iqubal et al. (2012). However, the presence of an appreciable content of lipids demonstrates the potential of these leaves to have dietary purposes with promising nutritional attributes.

Higher amounts of lipids were obtained in the fruits of *Morus nigra*, compared with literature studies (Imran et al., 2010).

The contents of some minerals and lead from mulberry leaves and fruits are given in Table 2.

Table 2. Mineral content of leaves and fruits of *Morus nigra*

Specification	Fresh leaves	Dry leaves	Fresh fruits	Dry fruits
Na (mg/kg)	23.41	50.51	71.65	81.51
Mg (mg/kg)	2.54	4.03	41.80	56.76
Ca (mg/kg)	542.25	908.79	2693.82	7102.44
Fe (mg/kg)	50.41	76.21	67.52	86.86
K (mg/kg)	1756.22	3308.46	3333.33	7009.18
Pb (mg/kg)	0.00	0.00	0.00	0.00

The distribution of the elements among the leaves and fruits of mulberry were in favour of fruits, much higher amounts being determined in fruits compared to leaves.

No lead was determined in leaves and fruits of mulberry, indicating no pollution in the area of mulberry plantation.

Dimitrijevic et al. (2014) found in mulberry fruits different heavy metals in fruits of mulberry grown in Serbia (9 mg/kg lead and 36 mg/kg nichel).

Very high amounts of potassium were determined both in leaves and in fruits of mulberry. If the leaves are good source of pota-

ssium for silkworm, the fruits are very good candidates for potassium supplementation in human diet. High amounts of calcium (7102.44 mg/kg) were determined in dry fruits. This mineral is very important in diet, and having such a high content in the fruits of *Morus nigra*, makes this fruit even more valuable.

Iron and magnesium determined in our study was similar with other studies (Imran et al., 2010). As can be seen in Table 2, mulberry fruits may be considered as good sources of magnesium.

Very different amounts were determined by Yigit et al. (2010) in some varieties of mulberry (leaves and fruits) drom Turkey. Similar results were obtained only for calcium and sodium.

Taking into consideration the high amount of protein from mulberry leaves, we conducted a study of determining the free aminoacid profile from this matrix (Table 3).

Table 3. Aminoacid profile of mulberry leaves

Aminoacid	Leaf (base)	Leaf (middle)	Leaf (top)
Arginine	33.46	20.48	45.54
Serine	164.43	16.25	17.05
Asparagine	75.67	43.33	46.63
1-metil-histidine	0.18	0.14	0.21
4-hidroxiproline	46.29	27.25	28.82
Glicine	13.21	15.07	8.85
Glicina-proline (dipeptide)	0.28	0.23	0.27
Treonine	29.80	31.10	23.61
Alanine	144.01	128.96	66.54
Gama-aminobutiric acid	153.30	129.07	105.78
Alfa-aminobutiric acid	0.32	0.51	0.00
Ornitine	0.55	0.38	0.48
Metionine	0.21	0.13	0.17
Proline	60.66	47.63	42.90
Lizine	23.20	21.49	23.46
Aspartic acid	11.39	15.36	12.52
Histidine	6.72	6.14	5.64
Thiaproline	0.06	0.08	0.02
Valine	25.69	26.93	12.80
Glutamic acid	27.29	33.07	20.18
Triptophan	13.58	12.57	12.60
Leucine	24.94	22.75	21.07
Phenilalanine	18.71	16.00	20.12
Izoleucine	11.45	12.29	8.71
Tyrozine	17.71	16.07	17.64

Twenty five free aminoacids were detedrmined in the leaves of mulberry, harvested from three different parts of the tree. No significant differences were observed in the three locations of the tree in respect of free aminoacid profile, but the total amount of free aminoacids were determined in leaves from the base of the tree (903.1 mg/100 g), followed by the leaves from middle (643.2 mg/100 g) and the leaves from the top of the tree (541.6 mg/100 g).

High amount of alanine and Gama-aminobutiric acid were determined (Table 3), followed by asparagine and proline.

Phenolic compounds are secondary metabolites of plants and contribute to different flavours (sweet, bitter, astringent), and determine the antioxidant activity of the matrix (Thomas-Barberan and Espin, 2001).

The amount of total polyphenols and total flavonoids are presented in Figure 1.

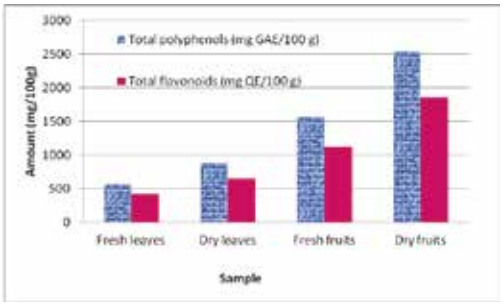

Figure 1. Polyphenolic and flavonoid content in mulberry leaves and fruits

Higher amounts of phenolics (total polyphenols and flavonoids) were determined in fruits, compared to leaves.

The obtained results were in accordance with literature studies (Dimitrijevic et al., 2014; Popescu et al., 2014; Okatan et al., 2016)

Radical scavenging activity of the ethanolic extracts from leaves and fruits of mulberry ranged between 45 - 67% inhibition percent, similar to literature studies (Iqbal et al., 2012; Dimitrijevic et al., 2014; Okatan et al., 2016).

CONCLUSIONS

The high polyphenolic content, together with the high amount of simple sugars, total lipids and total proteins, underline the nutritive and phytopharmaceutical potential of mulberry.

This study suggested that mulberry fruits, but not only, may be used as a potential healthy food, or an important antioxidant carrier for different pharmaceutical applications and food supplements.

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IN VITRO EFFECT OF GENOTYPE, GROWTH SEASON AND CYTOKININES ON PEACH VARIETIES (*Prunus persica* (L.) Batsch) PROPAGATION

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Abstract

In vitro propagation is one of the most convenient methods for plant material multiplication in order to obtain virus free planting material in high quantity and short time. The paper presents the influences of genotype, growth season and hormones on in vitro propagation of some new Romanian peach varieties with very good prospects on the market. Three peach genotypes: 'Florin', 'Filip' and 'Mimi' from the Didactic Field of Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest were tested. In the protocol for the in vitro establishment, winter and spring growth season were chosen. Initial shoot explants were obtained in winter by forcing the dormant shoots in the growing chamber and in spring (April-May), directly from the field. The explants were cultured on MS (Murashige and Skoog, 1962) basal medium supplemented with 30 g sucrose and 7 g agar, as carbon source. Benzyl aminopurine (BAP) on four variants with 0, 1, 5 and, respectively, 10 mg/l was used as cytokinine. The results show that Florin genotype was superior to the rest of the varieties used in the experiment in terms of the number of formed shoots and the strength of their growth. For the in vitro culture initiation, the shoots taken in spring, during the growth season, gave the best results compared to the winter period. The results showed a significant correlation between the concentration of BAP and the shoots number (multiplication rate) and height. The concentration of 5 mg/l BAP (V3), gave the best rate of shoot formation and the highest elongation rate.

Keywords: *benzyl aminopurine, culture media, shoots, multiplication rate, elongation rate*

INTRODUCTION

Peach (*Prunus persica* (L.) Batsch) is one of the most popular stone fruits. Peaches belong to *Prunoideae*, *Prunus* genus a subfamily of *Rosaceae*. It is one of the most popular deciduous fruits and ranks second to apple among temperate zone deciduous fruit trees from the standpoint of production and value (Childers, 1978; USDA, 2017).

China is the centre of origin for peaches and nectarines and was domesticated there 4000 years ago (Wang and Zhuang, 2001). Peach fruits are of high nutritional value because they contain high levels of carbohydrates, fats, salts and vitamins and are used in the treatment of anaemia, poor digestion and nourishment of the nervous systems (Al-Sheikh, 2003). Micropropagation is one form of tissue culture which allows the production of large number of plants from small pieces of the mother plant in relatively short period of time and limited space. It is an aseptic process which requires sophisticated laboratory procedure with unique facilities and special skills (Hartmann et al., 2004; Sathyanarayana, 2007). Micropropagation is affected by many factors such as genotype, plant growth regulators (PGRs), agar, type of explants, culture medium and light conditions etc. For example, cytokinins are major factors in the induction of somatic organs (George, 1993; Feyissa et al., 2005).

Several experiments were carried out for the multiplication of wood plants by tissue culture such as Ferradini N. et al. (1996) on apple rootstock and Peticila A.G. (2012) on kiwi. In addition, the difficulty of regenerating plants from mature tissues of woody plants is well established (Smigocki et al., 1991).

Peach is one of the most recalcitrant species with regard to micropropagation (Bhansali et al., 1990; Padilla et al., 2006). Successful regeneration of peach plants were from immature seeds (Meng and Zhou., 1981; Hammerschlag et al., 1985; Scorza et al., 1990;

Bhansali et al., 1991; Smigocki et al., 1991; Svircev et al., 1993; Pérez- Clemente et al., 2004). Also regenerated from leaves explants excised from shoots apexs culture (Gentile et al., 2002).

Therefore, the main goal of this study was to establish a micropropagation protocol for 'Florin', 'Filip' and 'Mimi' peach varieties in order to produce a large scale of plants in a short period. Plant hormones are the most important effect factors in shoot regeneration (Bhojwani and Razdan, 1996). Cytokinins are a type of plant growth regulators (PGRs). Contributed to in many processes of plants growth, like cells division, Shoot and root morphogenesis. PGRs are regulating axillary bud growth. Considering the importance of these PGRs, among growth regulators used in peach tissue culture media are the cytokinins: TDZ, kinetin and BAP, for auxins: IBA, IAA and NAA (Hammerschlag, 1985; Mante et al., 1989). This study aimed to evaluate different concentrations of BAP cytokinins for *in vitro* shoot development in peach**.**

MATERIALS AND METHODS

The study was conducted at the tissue culture laboratory of the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest during the period October 2016 - June 2017 on Peach (*Prunus persica* L.). Three peach varieties ('Florin', 'Filip' and 'Mimi') were included in the experiment. Explants were taken from trees planted in the field of experiments to the Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine in Bucharest. Two explants types namely shoot-tips and nodes (one node with a single axillary buds) were taken at 0.5-1cm length, were tested on their ability to maintain and initiate shoots on MS medium without any hormone supplements during initiation stage and added 0, 1, 5, respectively, 10 mg/l Benzyl aminopurine (BAP) during multiplication stage. V1=0 mg/l (control); V2=1 mg/l; V3=5 mg/l; V4=10 mg/l.

Shoot-tips and nodes were obtained from two sources (last year's growths, modern growths). The explants were taken in the winter season (dormancy buds) 15-20 cm long and placed in jars containing water to stimulate the sprouts to grow and break the dormancy (Figure 1). After two-three weeks were taken buds formed and used in the experiment, while the nodes were used directly in the experiment. In the growing season (spring: April-May) the explants were taken and used directly in the experiment.

Media and culture conditions

All explants were rinsed with ethanol 70% for 2-3 min then were washed with distilled water three times for 2-3 min, Explants were surface sterilized with NaOCl (10% v/v) for 10-15 min. Then explants were washed with sterile distilled water at least three times for 5 min. MS (Moorashige and Skoog, 1962) consisting of 30 g/l sucrose and 7 g/l agar without any hormone supplements during initiation stage and added 0, 1, 5, respectively, 10 mg/l Benzyl aminopurine (BAP) during multiplication stage. The pH of medium was adjusted to 5.6 with HCl 0.1 N or NaOH 0.1 N before sterilization by autoclaving at 121°C for 15 minutes. Sterilized explants were inoculated on culture media and then placed in an incubation room at 22±2°C, and 16 hours daily (Stănică et al., 2002).

Statistical analysis

The experiment was repeated two times, each treatment contained 10 replicates initiation stage and 5 replicates tested on multiplication stage. All experiments were arranged in a completely randomized design (CRD). Culture period ranged between four to eight weeks depending on individual experiment. Data were recorded on shoots number formed, shoots length and leaves number were analyzed. Significance of differences between the results was estimated by Analysis of Variance (ANOVA) on SPSS version 14 (SPSS 2005) program with the means compared with LSD test at < 0.05.

Figure 1. Peach branches placed in water for break dormancy

RESULTS AND DISCUSSIONS

Analysis of variance (Tables 1 and 2) revealed that the treatment had highly significant effect on mean leaves number and shoots length. The results of means mean leaves number and shoots length formed developing in response to all the varieties of the experiment for the tissue culture and there were significant differences between the varieties, where Florin variety had the largest number of leaves formed 5.00 leaf per explants in winter and 5.80 leaf per explants in spring while the 'Fillip' and 'Mimi' varieties gave the least number of leaves. Also the 'Florin' variety gave the longest shoots formed (2.37 cm in winter and 4.77 cm in spring) while the 'Filip' variety gave the shortest shoots formed (1.69 cm in winter and 2.77 cm in spring).

Figure 2. Hoot-tips of peach on MS after 2 week from culture

There were no significant differences in shoot number per explants in initiation stage. There was a single shoot in all explants (Figure 2). Growth of roots in the hormone-free medium was not observed. In multiplication stage also there were significant differences between the varieties. 'Florin' variety superior on 'Filip' and 'Mimi' varieties in the number and length of shoots formed (Figure 3). The effect of genotype on successful tissue culture has been previously reported (Gubis et al., 2003; Blinstrubiene et al., 2004). Cotton callus initiation (Zouzou et al., 1997; Zouzou et al., 2000) at all the genotypes were cultured onto hormone-free medium. It can be assumed that the differences in their response in tissue culture were determined by the balance of their endogenous hormones (Razdan, 2003). The difference might be due to intra-metabolism of plant which affected cell division and differentiation (Techato et al., 2002).

The results showed that there were statistical differences in the shoots length of and leaves number formed by shoot-tips and nodes. Results showed superiority shoot-tips on nodes (Tables 1 and 2). Results showed that each type of explants are characterized by a certain regeneration potential, depending on the species of plant and its degree of maturity, which is of physiological state of explants. Several types of explants have been widely used for *in vitro* such as Citrus lemon (Rathore et al., 2004); young leaves on French bean (Kamal and Praven, 1991); terminal buds of renewal on gladioli (Rumynin et al., 1990); apical buds on hybrid of mountain ash (Suvorova et al., 1990) and Rough lemon (Ali and Mizra, 2006).

Analysis of the results showed that there were significant differences between leaves number formed and shoots length from explants which were taken from different seasons (Table. 2). Explants taken in the growth season (spring) gave the best results, as opposed to explants which were taken in the winter for all varieties (Table.1).

Previous studies have confirmed that there is a relationship between phenolic compounds and the age of the plant used, a common problem reported in tissue culture of woody species (Mc Cown, 2000; Mathur et al., 1999). Ozyigit (2008) indicated a positive direct relationship between age of explants and phenolic exudation in tissue culture of cotton.

Different combinations of cytokinins (BAP) interacted significantly in terms of the shoots number (Table 3). Variant V3 (BAP 5 mg/l) gave the maximum shoots number ('Florin' 8.00, 'Filip' 7.40 and 'Mimi' 5.40). Lowest number of shoot (1.00) were obtained in control medium (without any plant growth regulators). Increasing BAP doses in combination had an increasing effect up to a certain level V4 (BAP10 mg/l). Data presented in higher shoots length were obtained in control medium (without any plant growth regulators).

This data shows that the shoot's lengths were markedly affected by various combinations of cytokinins. Statistically, after treatment V4 (BAP 10 mg/l) with elevated levels of cytokinins, shoot length decreased ('Florin' 2.59 cm, 'Filip' 1.77 cm and 'Mimi' 1.94 cm). Analysis of LSD values between varieties and variants showed that the effect of BAP on the shoots number and shoots lengths were significant at $p \leq 0.05$ (Tables 4 and 5).

Similar regeneration behaviours of BAP in pear (Kadata and Numi, 2003); in peach rootstock GF 677 (Ahmad et al., 2003); in bananas by (Vuylsteke, 1989; Arinaitwe et al., 2000). Previous researchers Vuylsteke and De Langhe (1985); Bairu et al. (2008) indicated that 5 mg/l BAP was the best concentration for banana varieties.

In vitro multiplication rate was largely controlled by interaction the varieties and cytokinins concentration and BAP is the most economical cytokinins (Gaspar et al., 1996; Augusto,. 2001; Silveira et al., 2009). Rapid growth and multiplication of shoots are based on the quantity and quality of cytokinins and auxins in media as well as on their endogenous levels in plants. Histological studies showed that the inclusion of BAP in shoot proliferation media enhanced the growth of axial shoots and promoted the multiplication of shoots from the basal tissues of explants (Ohki and Sawaki, 1999). A decline in the number of shoots with higher BAP levels has also been reported. Waseem et al. (2009) showed that the use of higher concentrations of PGRs may result in plant weakness and decreased growth (Panjaitan et al., 2007).

Al-Sulaiman and Barakat (2010) cytokinins had a positive effect on the production of lateral shoots of *Ziziphus spinachristi*. The appropriate addition of cytokinin promotes the growth of shoots and reduces the dominance of the apical bud (Asaad et al., 2009). In the *Prunus* species the type of citokinine and its concentration are important factors for multiplication and elongation rate (Leontiev-Orlov et al., 2000a). Increasing (BAP) causing a rise in the numbers of buds primordia in chrysanthemum (Karim et al., 2002, 2003). PGRs for bud break and shoot differentiation due to their role in cell multiplication and the breakdown of apical dominance (Casimiro et al., 2001). In woody plants, BAP is paramount for growth compared to kinetin (Fráguas et al., 2004).

A B C

Figure 3. Peach genotypes after 3 weeks on V3 (MS+ BAP 5 mg/l): 'Florin' (A); 'Fillip' (B); 'Mimi' (C)

Table 1. Analysis of Means and Std. Deviation for the effect of genotype, growth season and explants on leaves number and shoots length after 4 weeks cultivation on three peach varieties ('Florin', 'Filip' and 'Mimi')

Genotype	Season	Explants	Mean leaves number	Std. deviation	Mean shoots length	Std. deviation
Florin	Winter	Shoot-tips	5.0000	1.00000	2.3740	.80640
		Nodes	3.0000	1.22474	2.0180	.90502
		Total	4.0000	1.49071	2.1960	.82961
	Spring	Shoot-tips	5.8000	1.09545	4.7760	.92802
		Nodes	8.0000	2.34521	3.0220	.31260
		Total	6.9000	2.07900	3.8990	1.13172
	Total	Shoot-tips	5.4000	1.07497	3.5750	1.50813
		Nodes	5.5000	3.17105	2.5200	.82914
		Total	5.4500	2.30503	3.0475	1.30227
Filip	Winter	Shoot-tips	3.8000	.83666	1.6900	.48974
		Nodes	3.2000	1.30384	1.3960	.20671
		Total	3.5000	1.08012	1.5430	.38678
	Spring	Shoot-tips	4.4000	.54772	2.7700	.53810
		Nodes	4.6000	.54772	2.2960	.54344
		Total	4.5000	.52705	2.5330	.56776
	Total	Shoot-tips	4.1000	.73786	2.2300	.74786
		Nodes	3.9000	1.19722	1.8460	.61258
		Total	4.0000	.97333	2.0380	.69389
Mimi	Winter	Shoot-tips	2.6000	.54772	1.6140	.68090
		Nodes	2.4000	1.14018	1.2900	.29589
		Total	2.5000	.84984	1.4520	.52357
	Spring	Shoot-tips	3.8000	1.30384	2.9420	.40752
		Nodes	2.6000	.54772	1.9780	.13554
		Total	3.2000	1.13529	2.4600	.58319
	Total	Shoot-tips	3.2000	1.13529	2.2780	.87735
		Nodes	2.5000	.84984	1.6340	.42256
		Total	2.8500	1.03999	1.9560	.74722
Total	Winter	Shoot-tips	3.8000	1.26491	1.8927	.71550
		Nodes	2.8667	1.18723	1.5680	.61784
		Total	3.3333	1.29544	1.7303	.67726
	Spring	Shoot-tips	4.6667	1.29099	3.4960	1.12216
		Nodes	5.0667	2.65832	2.4320	.56753
		Total	4.8667	2.06336	2.9640	1.02771
	Total	Shoot-tips	4.2333	1.33089	2.6943	1.23283
		Nodes	3.9667	2.31164	2.0000	.72996
		Total	4.1000	1.87490	2.3472	1.06373

Table.2. Analysis of variance (ANOVA) for the effect of genotype, growth season and explants on leaves number and shoots length after 4 weeks cultivation on three peach varieties ('Florin', 'Filip' and 'Mimi')

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Leaves number	144.200[a]	11	13.109	9.956	.000
	Shoots length cm	50.635[b]	11	4.603	13.702	.000
Intercept	Leaves number	1008.600	1	1008.600	766.025	.000
	Shoots length cm	330.551	1	330.551	983.935	.000
Varieties	Leaves number	67.900	2	33.950	25.785	.000
	Shoots length cm	14.781	2	7.391	21.999	.000
Season	Leaves number	35.267	1	35.267	26.785	.000
	Shoots length cm	22.829	1	22.829	67.954	.000
Explants	Leaves number	1.067	1	1.067	.810	.373
	Shoots length cm	7.231	1	7.231	21.526	.000
Genotype *Season *Explants	Leaves number	17.433	2	8.717	6.620	.003
	Shoots length cm	.946	2	.473	1.408	.255
Genotype *Season	Leaves number	14.233	2	7.117	5.405	.008
	Shoots length cm	1.653	2	.826	2.460	.096
Genotype *Explants	Leaves number	1.633	2	.817	.620	.542
	Shoots length cm	1.145	2	.572	1.704	.193
Season *Explants	Leaves number	6.667	1	6.667	5.063	.029
	Shoots length cm	2.050	1	2.050	6.102	.017
Error	Leaves number	63.200	48	1.317		
	Shoots length cm	16.126	48	.336		
Total	Leaves number	1216.000	60			
	Shoots length cm	397.312	60			
Corrected Total	Leaves number	207.400	59			
	Shoots length cm	66.760	59			

Table. 3. Combined effect of genotype and concentrations of BAP on shoots number and shoots length per explants

Genotypes	Variants	Mean shoots number	Std. deviation	Mean shoots length (cm)	Std. deviation
Florin	V1	1.0000	.00000	5.7380	.67887
	V2	4.0000	.70711	3.8440	.69540
	V3	8.0000	1.87083	4.7760	.92802
	V4	3.8000	1.30384	2.5920	.91319
	Total	4.2000	2.78341	4.2375	1.40635
Filip	V1	1.0000	.00000	3.3340	.69346
	V2	2.4000	.54772	2.7700	.53810
	V3	7.4000	1.51658	3.1440	.62616
	V4	1.6000	.89443	1.7680	.43275
	Total	3.1000	2.73188	2.7540	.81770
Mimi	V1	1.0000	.00000	4.0980	.43390
	V2	2.2000	.83666	2.9420	.40752
	V3	5.4000	1.14018	3.8880	.44263
	V4	1.2000	.44721	1.9400	.69199
	Total	2.4500	1.93241	3.2170	.99420
Total	V1	1.0000	.00000	4.3900	1.18345
	V2	2.8667	1.06010	3.1853	.71137
	V3	6.9333	1.83095	3.9360	.94388
	V4	2.2000	1.47358	2.1000	.75069
	Total	3.2500	2.57514	3.4028	1.24945

Table 4. LSD values between genotype on shoots number and shoots length per explants at $P \leq 0.05$

(I) Genotypes	(J) Varieties	Mean difference (I-J) Shoots number	Std. Error	Sig.	Mean difference (I-J) Shoots length (cm)	Std. Error	Sig.
Florin	Filip	1.1000*	.30687	.001	1.4835*	.20450	.000
	Mimi	1.7500*	.30687	.000	1.0205*	.20450	.000
Filip	Florin	-1.1000*	.30687	.001	-1.4835*	.20450	.000
	Mimi	.6500*	.30687	.039	-.4630*	.20450	.028
Mimi	Florin	-1.7500*	.30687	.000	-1.0205*	.20450	.000
	Filip	-.6500*	.30687	.039	.4630*	.20450	.028

Based on observed means. The error term is mean square (error)=0.942. Shoots number. The error term is mean square (error)=0.418. Shoots lengths (cm). *The mean difference is significant at the 0.05 level.

Table 5. LSD values between variants on shoots number and shoots length per explants at $P \leq 0.05$

(I) Variants	(J) Variants	Mean difference (I-J) Shoots number	Std. Error	Sig.	Mean difference (I-J) Shoots length (cm)	Std. Error	Sig.
V1	V2	-1.8667*	.35434	.000	1.2047*	.23614	.000
	V3	-5.9333*	.35434	.000	.4540	.23614	.060
	V4	-1.2000*	.35434	.001	2.2900*	.23614	.000
V2	V1	1.8667*	.35434	.000	-1.2047*	.23614	.000
	V3	-4.0667*	.35434	.000	-.7507*	.23614	.003
	V4	.6667	.35434	.066	1.0853*	.23614	.000
V3	V1	5.9333*	.35434	.000	-.4540	.23614	.060
	V2	4.0667*	.35434	.000	.7507*	.23614	.003
	V4	4.7333*	.35434	.000	1.8360*	.23614	.000
V4	V1	1.2000*	.35434	.001	-2.2900*	.23614	.000
	V2	-.6667	.35434	.066	-1.0853*	.23614	.000
	V3	-4.7333*	.35434	.000	-1.8360*	.23614	.000

Based on observed means. The error term is mean square (error)=0.942. Shoots number. The error term is mean square (error)=0.418. Shoots lengths (cm). *The mean difference is significant at the 0.05 level.

CONCLUSIONS

The regenerative activity in the studied three varieties depends on the highest level from the genotype, type of the explants and growing season were better regeneration activity is registered with shoot-tip and spring season. The results showed a significant correlation between the concentration of BAP and the shoots number (multiplication rate) and height. The best rate of shoot formation and the highest elongation rate was obtained at a concentration of 5 mg/l BAP (V3).

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IN VITRO EFFECT OF VARIOUS STERILIZATION TECHNIQUES ON PEACH (*Prunus persica* (L.) Batsch) EXPLANTS

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Abstract

One of the major challenges in Romania in fruit growing sector is the production of certified planting material, considering the specificities of the local climate conditions and the consumers' preferences. Due to the damages produced by hard winters and late spring frosts and the lack of resistant and suitable peach varieties, in the last decades, this species was almost eliminated from the producers choices in establishing new orchards. One of the major research projects of the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest is to identify and multiply the best peach varieties (Prunus persica L. Batsch) adapted to the Romanian harsh conditions. This paper presents different sterilization techniques applied to peach explants necessary for the initiation of the in vitro culture. The research was conducted at the Micropropagation Laboratory within the Faculty of Horticulture. For peach tissue culture initiation, four sterilization agents where tested in 18 different variants: Sodium hypochlorite (NaOCl) in three concentrations: 5%, 10% and 15%, for 5 and 10 min; Hydrogen peroxide (H_2O_2), in two concentrations: 5% and 10%, for 10 and 20 min; Captan (50%) fungicide, in four concentrations: 1%, 2%, 3% and 4%, for 5 min and Boric acid ($B(OH)_3$), in two concentrations: 1% and 2%, for 5 and 10 min. The explants (shoots-tip and nodes) were cultured in MS (Murashige and Skoog, 1962) basal medium supplemented with 30g sucrose, as carbon source and 7g agar. The growth chamber for the in vitro cultures had 22±2°C temperature and 70 to 80% relative humidity, with a photoperiod of 16 h day light and 8 h dark. The percentage of contamination, survival rate and shoot growth were studied during the initiation phase. Among the different sterilization variants, sodium hypochlorite was the most effective treatment with 50% survival rate at V5 (15% for 5 min) and 60% at V4 (10% for 10 min). After sterilization, shoots continued to grow vigorously and the multiplication phase initiated.

Key words*: shoot-tip, node, sodium hypochlorite, hydrogen peroxide, boric acid.*

INTRODUCTION

Peach (*Prunus persica* L. Batsch) is one of the most important stone fruits which are grown extensively in different parts of the world. Peaches are native to China and their planting dates refer to at least 4000 years (Wang and Zhuang, 2001). Peach trees are infected by numerous different pests and diseases. Micropropagation which encompasses cell culture, tissue culture, organ and embryo culture, has been a vital technique for considerable multiplication of plants, elimination of plant diseases through tissue culture technique, plant conservation and improvement production through gene transfer (Sarasan et al., 2011).

Tissue culture techniques are used for commercial and research purposes extensively to grow many different plants (Hussain et al, 2012). Aseptic conditions are usually practiced in micropropagation, plant tissues on their surfaces inherently have various bacteria and fungi, moulds etc. It is necessary that the explants be free from any surface contaminants prior to tissue culture since contaminants can grow in the culture medium, rendering the culture non activity (Hiremath, 2006). There is so much pathogen (microbial contaminants) which has been a major threat to tissue cultures due to their rapid proliferation characteristics, microbes can be come from explants, laboratory instruments, conditions in the laboratory and contaminants may be introduced with the staff during manipulations in the laboratory (Leifert and Cassells, 2001; Enjalric et al., 1998).

Contamination is a real problem that opposes the progress and development of tissue culture technology (Webster et al., 2003). These microbes compete adversely with explants for nutrients, and their presence often results in

variable growth or increased culture mortality or can also result, reduced shoot proliferation, tissue necrosis and reduced rooting (Oyebanji et al., 2009). A successful *in vitro* culture protocol, starts with effective explants sterilization, the sterilization chosen for an experiment depend on the type of explants, Sterilize material and plant genotype (Dodds and Roberts, 1985; Rezadost et al., 2013). There are several various sterilization factors are used to sterilize tissues, these disinfect materials are also toxic to explants tissues, and therefore select the correct concentration and the times of exposure to explants, must be elected to reduce the injury of plants (CPRI, 1992).

So there is a state of balance between sterilizing explants and killing the explants themselves (Qin et al., 2012; Olew et al., 2014). Many researchers have used these sterilizing agents successfully; also there are studies on the effect of fungicides and antibiotics on these kinds of contaminants (George, 1993; Rashid et al., 2008; Maqbool et al., 2010; Bakhsh et al., 2012). Several different mechanism are used to eliminate fungal and bacterial contamination, including the use of inactivation by heat and light, fungicides and antibiotics, the time of sterilization is dependent on the type of tissue (Haldeman et al., 1987; Kneifel and Leonhardt, 1992; Leifert et al., 1992).

Explants are commonly surface-sterilized using ethanol, sodium hypochlorite, mercuric chloride, hydrogen peroxide, fungicides and antibiotics. Therefore, the present study was conducted to compare different sterilizing protocols for peach micropropagation and to find out the best, efficient and cost effective sterilization procedure that may result in least or no contamination in peach tissue culture. In our study we compared several modifications of four surface sterilization methods based on the use of, sodium hypochlorite, hydrogen peroxide, captan (50%) and boric acid with using explants of peach accessions with different degrees of contamination.

However, in this study we did not used the mercuric chloride, this material is very dangerous because of more difficult to dispose and high toxicity (Li et al., 2005; Jean-Philippe et al., 2012). Sodium hypochlorite (bleach) is the most common sterilization agent used for seed and explants sterilization in many plants. Hypochlorite was used to surface sterilize wheat seeds (Sauer and Burroughs, 1986).

Also sodium hypochlorite has been reported to be very effective factors different types of bacterial strains (Nakagawara et al., 1998). Hydrogen peroxide solution as sterilizing agent has been reported for plants (Dumroese et al., 1988; Ogawa and Masaki, 2001; Rosner et al., 2003). It is recommended to use in the initial sterilization as ethanol (Stănică et al., 2002). Hydrogen peroxide solution as sterilizing factor and improved germination on many plants like wax, cotton, barley, pines, safflower and currant (Dumroese et al., 1988; Rosner et al., 2003; Cram and Fraedrich, 2009; Çavusoglu and Kabar, 2010; Lizarraga-Paulin et al., 2013). A report confirmed by Dolatabadian and Modarressanavy (2008) hydrogen peroxide is more effective than other sterilization factors for plant tissue. However, there are several studies indicates that hydrogen peroxide was ineffective for surface sterilization of explants and seeds (Miche and Balandreau, 2001). Captan 50% WP, agricultural fungicide for the control of certain fungus diseases of fruit, vegetables and ornamental crops are used in surface sterilization *in vitro*.

There are several studies like reported by Sohnle et al. (1998). Also the effectiveness of Bavistin was confirmed by Garla et al. (2011). Reported by Altan, et al. (2010), it failed to inhibit the microorganism and the activity of these sterilizing factors. Shields et al. (1984) analyzed the effects of fungicides against *in vitro* on tobacco cultures. They recommend two fungicides, carbendazim and fenbendazole. Boric acid in agriculture is used as an insecticide, herbicide and fungicide in food crops and orchards (EPA. U.S., 1993). Used in the United States as a fungicide on citrus (Olkowski et al. 1993). The results by Jan H. et al. (2002) indicate that use of boric acid (3% solution) as pre-plant seed treatment on potato was gave the lowest percent incidence of powdery scab diseases 4.3%.

MATERIALS AND METHODS

Explants surface sterilization

Shoot tips and nodes (0.5-1 cm in size) of peach (*Prunus persica* L. Batsch cv 'Florin')

were collected from the orchard of trees planted in the field of experiments to the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest to used as explants for *in vitro* culture establishment.

Explants were placed under running tap water with detergent for 30 min to remove any foreign contaminants.

After washing, explants were dissected and surface sterilized in a laminar air flow hood with rinsed with ethanol 70% for 2-3 min then were washed with distilled water three times for 2-3 min after that. For peach tissue culture initiation, four sterilization agents where tested in 18 different variants: Sodium hypochlorite (NaOCl) in three concentrations: 5%, 10% and 15%, for 5 and 10 min (Figures 3, 4); Hydrogen peroxide (H_2O_2), in two concentrations: 5% and 10%, for 10 and 20 min (Figure 1); Captan (50%) WP fungicide, in four concentrations: 1%, 2%, 3% and 4%, for 5 min (Figure 2) and Boric acid ($B(OH)_3$) in two concentrations: 1% and 2%, for 5 and 10 min. (Table 1).

Culture media and culture conditions

The explants (shoots-tips and nodes) were cultured in MS (Murashige and Skoog, 1962) basal medium supplemented with 30 g sucrose, as carbon source and 7 g agar. pH was adjusted between 5.7 and 5.8 by using either 1 N HCl or 1 N NaOH before the agar was added.

Media was then heated on a hot plate with continuous stirring using a magnetic stirrer until agar is dissolved and media put in the culture tubes. The culture tubes were covered with lids and put in trays and autoclaved. Autoclave was adjusted at a temperature of 121°C for 15 min. The growth chamber for the *in vitro* cultures had 22±2°C temperature and 80-85% relative humidity, with a photoperiod of 16 h day light and 8h dark. (Stănică et al., 2002)

Data collection

In the experiment ten replicates (one explants in one tube culture) were used for each treatment and the experiment was repeated twice.

Results were taken after 2, 4 weeks of planting and the following data were recorded; % of contamination (fungus+ bacteria and sterilizer); % of explants survived; mean length of shoots (cm); mean number of leaves per explant. Experiments were conducted as factorial experiments based on Completely Randomized Design (CRD).

RESULTS AND DISCUSSIONS

Effect of sterilization factors

The study showed there is an effect of substances used in sterilization, sodium hypochlorite the most effective treatment with 50% survival rate in 15% for 5 min and 60% in 10% for 10 min and has outperformed the rest of the other sterilizers which their results were not satisfactory as the results were hydrogen peroxide (H_2O_2) with 25% survival rate in 10% for 20 min; Captan 50% with 25% survival rate in 4% for 5 min; Boric acid ($B(OH)_3$) with 20% survival rate in 2% for 10 min (Table 2).

These results are similar to the studies Satish et al. (2012) on sugarcane; Siddique et al. (2018) on *Skimmia laureola*, when they used different substances in sterilization, where the results differed according to the sterilizers.

Studied by many researchers, the solution of sodium hypochlorite for superficial sterilization of explant was efficient and didn't injury the explants at appropriate focus (Gertlowski K. and Petersen M., 1993).

These results are similar with those of Hippolyte (2000), which reference that the high focus of sodium hypochlorite can be effective in sterilizing the superficial explants cultivated *in vitro*, but it is accompanied by the death of explants.

Many researchers have found these sterilizing agents successfully (Rashid et al., 2008; Maqbool et al., 2010; Bakhsh et al., 2012).

Effect of concentration and exposure time

The study showed that there was a correlation between the dipping period and the concentration of the substance used in the sterilization on the extent of their effect on the percentage of explants survived, explants contaminated by fungi, bacteria and explants dead due to the increased concentration of the material used (Tables 2, 3 and 4).

Increasing the exposure duration and sterile concentration had reduced the contamination

rate but highest number of loss explants resulted.

The influence of sterilizing chemical ruin the shape and functions of microbe's enzymes (George et al., 2008).

But the increasing exposure duration and concentration of sterilizes above certain optimum limit cause loss of explants because of the oxidant chemical ingredient ruin the plant tissue as well (Danso et al., 2011).

These findings are similar of the negative effects of Sodium hypochloride at high concentration were observed (Colgecen et al., 2011).

And a higher concentration of hydrogen peroxide 5% was reported to negatively affect in sunflower (Dolatabadian and Modarressanavy, 2008).

Figure 1. Fungal contamination on peach explants after 14 day from sterilization V9 (H_2O_2 10% for 10 min)

Figure 2. Fungal contamination on peach explants after 14 day from sterilization V11 (captan 1% for 5 min)

Effect of explants

The study showed there are differences in the extent of the response of the explants used in rate of plant growth (shoots length and leaves number), contaminated rate and survival rate (Tables 3 and 4). Also, the shoots had been registered the lowest rate of infection and the most effect to increase the concentration of sterile material compared to the contract also explained that the shoots and nodes gave the best rate of shoots length and leaves number formed when using concentrations less (Figures 3 and 4).

These results are similar with (Rezadost et al, 2013) who confirmed that the surface sterilize used for an experiment typically depend on the explants and plant species.

Figure 3. Peach explants (shoots-tip) after 28 day from sterilization V10 (NaOCl 10% for 10 min). There are leaves damaged because of the sterilizer

Figure 4. Peach explants (nodes) after 28 day from sterilization V10 (NaOCl 10% for 10 min)

Table 1. Types of sterilizing agents used in a different concentration with varying time of sterilizings on peach explants

Variants	Pre-sterilization Substance disinfectants	Concentration %	Exposure time (min)	Surface sterilizer	Concentration %	Exposure time (min)
V1	Ethanol	70%	2-3	Sodium hypochlorite	5	5
V2	Ethanol	70%	2-3	Sodium hypochlorite	5	10
V3	Ethanol	70%	2-3	Sodium hypochlorite	10	5
V4	Ethanol	70%	2-3	Sodium hypochlorite	10	10
V5	Ethanol	70%	2-3	Sodium hypochlorite	15	5
V6	Ethanol	70%	2-3	Sodium hypochlorite	15	10
V7	Ethanol	70%	2-3	Hydrogen peroxide	5	10
V8	Ethanol	70%	2-3	Hydrogen peroxide	5	20
V9	Ethanol	70%	2-3	Hydrogen peroxide	10	10
V10	Ethanol	70%	2-3	Hydrogen peroxide	10	20
V11	Ethanol	70%	2-3	Captan 50%	1	5
V12	Ethanol	70%	2-3	Captan 50%	2	5
V13	Ethanol	70%	2-3	Captan 50%	3	5
V14	Ethanol	70%	2-3	Captan 50%	4	5
V15	Ethanol	70%	2-3	Boric acid	1	5
V16	Ethanol	70%	2-3	Boric acid	1	10
V17	Ethanol	70%	2-3	Boric acid	2	5
V18	Ethanol	70%	2-3	Boric acid	2	10

Table 2. Effect of surface sterilizer, various concentrations and time exposure on % of contamination and % of survived on explants after 14, 28 days from culture

Variants	Surface sterilizer	Concentration %	Exposure time (min)	Shoot-tips			Nodes		
				Contamination %		Survived %	Contamination %		Survived %
				after 14 days	after 28 days		after 14 days	after 28 days	
V1	Sodium hypochlorite	5	5	70	100	00	80	100	00
V2	Sodium hypochlorite	5	10	50	90	10	70	100	00
V3	Sodium hypochlorite	10	5	45	70	30	50	70	30
V4	Sodium hypochlorite	10	10	20	40	60	35	40	60
V5	Sodium hypochlorite	15	5	25	50	50	40	50	50
V6	Sodium hypochlorite	15	10	50	70	30	70	80	20
V7	Hydrogen peroxide	5	10	70	85	15	70	85	15
V8	Hydrogen peroxide	5	20	65	85	15	65	85	15
V9	Hydrogen peroxide	10	10	65	80	20	65	80	20
V10	Hydrogen peroxide	10	20	50	75	25	60	80	20
V11	Captan 50%	1	5	35	90	10	50	100	00
V12	Captan 50%	2	5	35	90	10	70	100	00
V13	Captan 50%	3	5	30	80	20	40	85	15
V14	Captan 50%	4	5	30	75	25	45	85	15
V15	Boric acid	1	5	50	100	00	70	100	00
V16	Boric acid	1	10	50	85	15	60	100	00
V17	Boric acid	2	5	40	80	20	70	90	10
V18	Boric acid	2	10	45	80	20	80	85	15

Table 3. Effect of surface sterilizer, various concentrations and time exposure on % of contamination (fungus + bacteria and sterilizers) on explants after 28 days from culture

Variants	Surface sterilizer	Concentration %	Exposure time (min)	Shoot-tips			Nodes		
				Contamination %			Contamination %		
				after 28 days	fungus+ bacteria	sterilizer	after 28 days	fungus+ bacteria	sterilizer
V1	Sodium hypochlorite	5	5	100	100	00	100	100	00
V2	Sodium hypochlorite	5	10	90	90	00	100	100	00
V3	Sodium hypochlorite	10	5	70	59.5	10.5	70	63	7
V4	Sodium hypochlorite	10	10	40	30	10	40	36	4
V5	Sodium hypochlorite	15	5	50	15	35	50	42.50	7.50
V6	Sodium hypochlorite	15	10	70	30	40	80	24	56
V7	Hydrogen peroxide	5	10	85	85	00	85	85	00
V8	Hydrogen peroxide	5	20	85	85	00	85	85	00
V9	Hydrogen peroxide	10	10	80	64	16	80	72	8
V10	Hydrogen peroxide	10	20	75	52.5	22.5	80	68	12
V11	Captan 50%	1	5	90	90	00	100	100	00
V12	Captan 50%	2	5	90	90	00	100	100	00
V13	Captan 50%	3	5	80	56.25	23.75	85	76.50	8.50
V14	Captan 50%	4	5	75	56.25	18.75	85	67.25	12.75
V15	Boric acid	1	5	100	100	00	100	100	00
V16	Boric acid	1	10	85	85	00	100	100	00
V17	Boric acid	2	5	80	60	20	90	65.50	4.50
V18	Boric acid	2	10	80	60	20	85	67.25	12.75

Table 4. Effect of surface sterilizer, various concentrations and time exposure on mean shoots length (cm) and mean (no) leaves shoot on explants after 28 days from culture

Variants	Surface sterilizer	Concentration %	Exposure time (min)	Shoot-tips			Nodes		
				Survived after 28%	Mean shoot length (cm)	Mean shoot Leaves (on)	Survived after 28%	Mean shoots length (cm)	Mean shoot Leaves (on)
V1	Sodium hypochlorite	5	5	00	0.00	0.00	00	0.00	0.00
V2	Sodium hypochlorite	5	10	10	4.64	5.70	00	3.12	7.88
V3	Sodium hypochlorite	10	5	30	3.32	4.22	30	3.12	7.10
V4	Sodium hypochlorite	10	10	60	3.17	4.05	60	2.78	5.87
V5	Sodium hypochlorite	15	5	50	3.25	3.50	50	2.55	4.65
V6	Sodium hypochlorite	15	10	30	2.64	3.12	20	2.06	4.50
V7	Hydrogen peroxide	5	10	15	4.34	4.44	10	3.45	7.33
V8	Hydrogen peroxide	5	20	15	3.89	4.21	10	3.12	7.02
V9	Hydrogen peroxide	10	10	20	3.11	4.00	20	3.00	5.16
V10	Hydrogen peroxide	10	20	25	2.64	3.66	20	2.77	5.00
V11	Captan 50%	1	5	10	4.50	5.99	00	0.00	0.00
V12	Captan 50%	2	5	10	4.22	5.78	00	3.13	7.23
V13	Captan 50%	3	5	20	3.80	4.43	15	2.98	7.11
V14	Captan 50%	4	5	25	3.62	3.68	15	2.77	6.78
V15	Boric acid	1	5	00	0.00	0.00	00	0.00	0.00
V16	Boric acid	1	10	15	4.17	4.16	00	0.00	0.00
V17	Boric acid	2	5	20	3.87	4.20	10	3.01	6.89
V18	Boric acid	2	10	20	3.45	3.80	15	2.48	6.22

CONCLUSIONS

Among the different sterilization protocols tested for the successful establishment of *in vitro* culture of peach tissue culture. Our results showed that during the sterilization were different depend on the sterilization factors, exposure time and explants type was used for micro- propagation.

It is recommended for this study to be used among the different sterilization variants, sodium hypochlorite was the most effective treatment with 50% survival rate at V5 (15% for 5 min) and 60% survival rate at V4 (10% for 10 min). Recommended also hydrogen peroxide (H_2O_2) 10% for 20 min; Captan 50% 4% for 5 min; Boric acid ($B(OH)_3$) 2% for 10 min recommended to use in the initial sterilization.

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SENSORY ANALYSIS OF THE DEHYDRATED PRODUCT OBTAINED FROM APPLES HARVESTED FROM THE TRADITIONAL ORCHARDS OF THE BRAN - ZĂRNEȘTI AREA

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Abstract

Having a scientific foundation of more than two decades, sensory analysis is an easy method for assessing the quality of a food product by bringing its characteristic features to the public consumers' attention. As a complementary method in food control and expertise, sensory analysis mirrors the outcome of certain physiological and psychological aspects of the tasters, which involve such actions as reception, recognition, ordering, description, and appreciation. Relying on the consumer's first contact with the product, the analysis measures its sensory properties that affect the subjects' choice power as well as their purchase decision. By describing the results obtained from the sensory analysis of apple chips, the present paper seeks to emphasize the importance of tasting in the presentation, refinement and promotion of the product. This objective was accomplished at the INDAGRA International Fair, which took place at Romexpo Bucharest on 4th November 2016. The apple chips and other food products were featured by the stand of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. 191 people, 21-60 years old, were invited to visit the stand of the University of Agronomic Sciences and Veterinary Medicine Bucharest and taste the products. The apple chips were evaluated for different organoleptic features like appearance, taste and aroma as well as for the general impression to simulate a possible authentication of the product as a preferred registered trademark. At the same time, the experiment aimed to emphasize the importance of nutraceutical fruit consumption by attracting the consumer's attention with the present analysis and through raising awareness of healthy food and eating. The paper presents the results, being an important step to follow with the serial production of the tested products.

Key words*: apple chips, taste, appearance, aroma.*

INTRODUCTION

Having a scientific foundation of more than two decades, sensory analysis is an easy method for assessing the quality of a food product by bringing its characteristic features to the public consumers' attention.

As a complementary method in food control and expertise, sensory analysis mirrors the outcome of certain physiological and psychological aspects of the tasters, which involve such actions as reception, recognition, ordering, description, and appreciation.

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By describing the results obtained from the sensory analysis of apple chips, the present paper seeks to emphasize the importance of tasting in the presentation, refinement and promotion of the product.

This objective was accomplished at the INDAGRA International Fair, which took place at Romexpo Bucharest on 4th November 2016. The apple chips and other food products were featured by the stand of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

To attain the objective, we planned on questioning the visitors open to taste the products and participate in the survey that highlights the sensory properties of these products.

MATERIALS AND METHODS

Apples chips

Drying has been used in the United States for more than a century (Downing, 1989) and

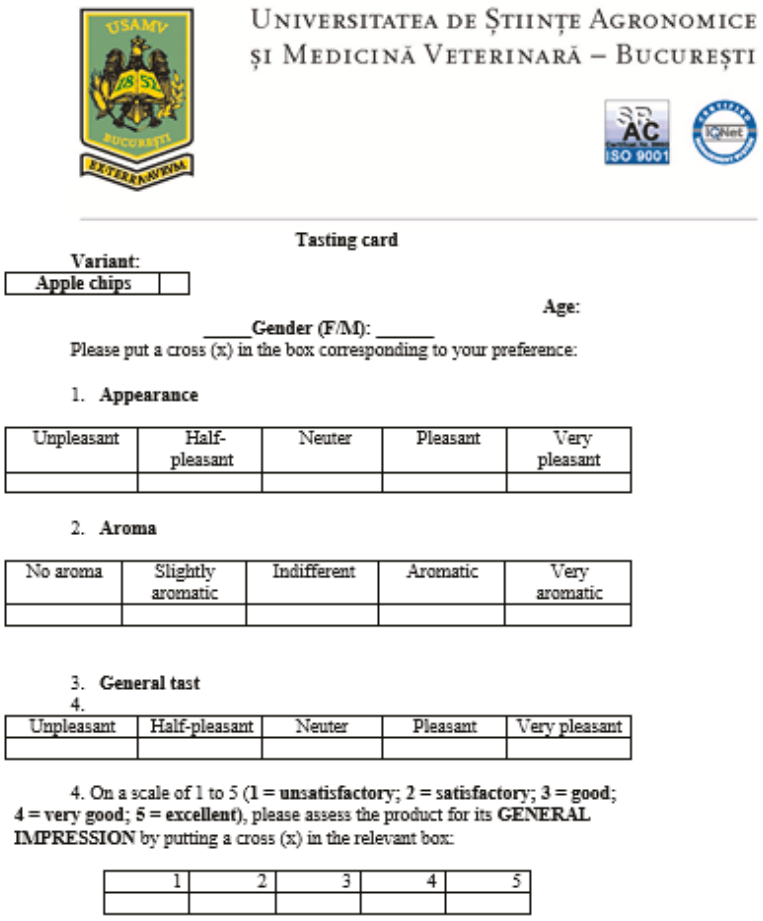
represents the process that underlies the obtaining of different food products like apple chips. Researcher S. Gould reported that at the turn of the 19th century fruit dehydration had been common for several years in the western part of the New York state.
Shaped as rings or slices, apple chips are the result of drying healthy, ripened fruit.
Their thickness and shape are often irregular, dehydration being a procedure that transforms the fresh apple pieces in a particular way.
The drying proper implies the vaporization of water within the fruit and its removal from the used drying system.
As important as dehydration is product packaging, which must ensure long-term storage.
At the same time, packaging must meet the requirements of regular handling during transportation and storage as well as of keeping product integrity by removing moisture, oxygen, light and foreign odour. Observance of packaging standards and norms guarantees product quality.

The sensory analysis
As mentioned in the introduction, the quality measurement of the dehydrated products was achieved through the sensory analysis method, which recorded a wide range of customers' preferences.
The apple chips were evaluated for different organoleptic features like appearance, taste and aroma as well as for the general impression to simulate a possible authentication of the product as a preferred registered trademark.
At the same time, the experiment aimed to emphasize the importance of nutraceutical fruit consumption by attracting the consumer's attention with the present analysis and through raising awareness of healthy food and eating.

The target consumers
Intending to achieve a highly objective and complex result, the sensory analysis targeted subjects of different age, gender and class. Consequently, the realization of this selection was sought within the INDAGRA International Fair.
191 people, 21-60 years old, were invited to visit the stand of the University of Agronomic Sciences and Veterinary Medicine of Bucharest and taste the products. Most of them, mainly males, gladly answered the invitation, some of them showing an interest in forming or enriching their healthy diet culture.
As expected, the subjects 40-60 years old were present in the highest number, 70%, the younger people being responsive in a lower percentage, 30%. As already mentioned, the male subjects outnumbered the female ones by 68.06%.

Tasting the chips
In key with the particularity of the Bran - Zărneşti area, the apple chips obtained from the Jonathan and Scortos cultivars were manually packed in small plastic bags decked with strips featuring traditional folk themes. Likewise, the products were exhibited in wattle baskets made by local craftsmen. The apple chips were offered in servings of four, as half-round slices.
The tasters were free to choose their preferred products, which they did by picking one or more food samples.
To measure and record the analysis results, the tasting was required to occur on the spot.

The tasting card
The research method of the present experiment was the hedonic analysis, or the preference test represented by the tasting card (Figure 1).



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ȘI MEDICINĂ VETERINARĂ – BUCUREȘTI

Tasting card

Variant:

Apple chips	

Age:

____Gender (F/M): _____

Please put a cross (x) in the box corresponding to your preference:

1. **Appearance**

Unpleasant	Half-pleasant	Neuter	Pleasant	Very pleasant

2. **Aroma**

No aroma	Slightly aromatic	Indifferent	Aromatic	Very aromatic

3. **General tast**
4.

Unpleasant	Half-pleasant	Neuter	Pleasant	Very pleasant

4. On a scale of 1 to 5 (**1 = unsatisfactory**; **2 = satisfactory**; **3 = good**; **4 = very good**; **5 = excellent**), please assess the product for its **GENERAL IMPRESSION** by putting a cross (x) in the relevant box:

1	2	3	4	5

Figure 1. Tasting card - research method

Figure 1 shows that the tasting card was conceived by following certain parameters. By putting a cross in a certain box, each taster expressed their opinion about the appearance, aroma, taste and general impression of the product.

While the first three characteristics were associated with parameters like unpleasant, half-pleasant, neuter, pleasant, very pleasant, or no aroma, slightly aromatic, indifferent, aromatic, very aromatic, the general impression was measured in figures on a 1 to 5 scale.

For a correct assessment, the figures were associated with the following ratings: unsatisfactory, satisfactory, good, very good and excellent.

Aiming at a highly objective result, the tasting card did not require the subject's name, except their age and gender.

RESULTS AND DISCUSSIONS

The results were measured via a questionnaire, whose attitude scale interpreted the obtained information statistically.

The survey recorded relative and absolute frequencies, which were associated with percentage and numerical results (Clinciu, 2013).

Accordingly, organoleptic dimensions like aroma and taste recorded the results shown in the following tables (Tables 1, 2).

On the strength of Table 1, one can analyse the tested consumers' opinion about the aroma of the prepared and presented chips.
It is noticeable that the female respondents accept these chips more easily as they might become new and healthy prepared food for both adults and children.

Table 1. Aroma determination

Respondents	No aroma	Slightly aromatic	Indifferent	Aromatic	Very aromatic
Male %	0.76	5.38	0.76	49.24	43.84
Female %	-	1.63	-	47.54	50.82

In Figure 2, one can statistically notice that females become more attracted by these natural products as compared to males.

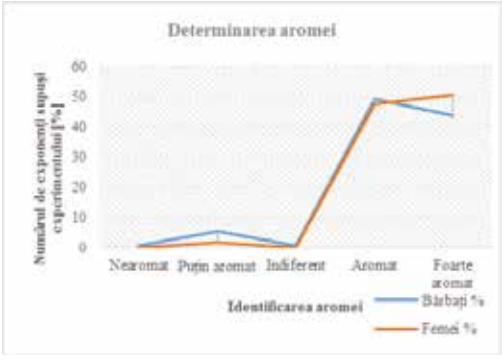

Figure 2. Determination of apple chip aroma by the male and female respondents

Chip taste determination leads to the observation that there is only a slight difference between the male and female respondents. As a conclusion, the respondents' taste does not vary too much.
Figure 3 is a graphical representation, according to male and female respondents, of the individual taste of the analysed chips.
One can therefore say that both genders have close taste regarding the apple chips prepared in the laboratory. As more than 50% of the respondents described the taste as being very pleasant and more than 40% as pleasant, one can gather that these apple chips are appreciated by the consumers and their market production might be successful.

Table 2. General taste determination

Respondents	Taste determination				
	Unpleasant	Half-pleasant	Neuter	Pleasant	Very pleasant
Male (%)	-	0.76	1.54	40.78	56.92
Female (%)	-	-	1.63	44.17	54.10

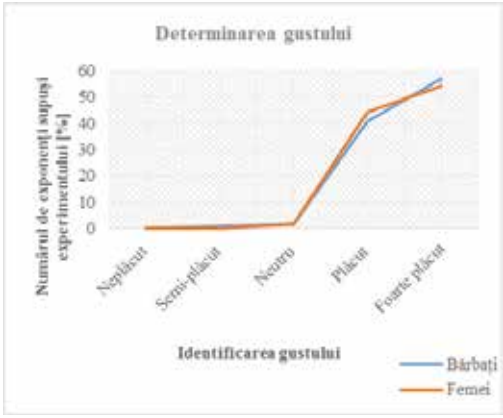

Figure 3. Chip taste determination by male and female respondents

Except for one taster, who thought that the product had no aroma, the others appreciated its quality. The vast majority considered that its features were pleasant and very pleasant.
According to gender and age, the opinions varied from one subject to another, as the above tables clearly show.
It was a welcome surprise to find that the appearance and general impression of the product recorded no parameters for unpleasant and half-pleasant. Almost 100 percent of the subjects opted for the pleasant and very pleasant appearance and general impression of the product.
It is worth remarking that more than 50% of the female subjects had a positive attitude, classifying the product as very pleasant and excellent (Tables 3, 4).

Table 3 recorded the data regarding the appearance of the chips obtained through dehydration and drying.
Following the data obtained from the examined respondents, one can notice that the majority are satisfied with the appearance of the consumed chips, which are very similar to the classic chips such as those made from potatoes.

Table 3. Appearance determination

Respondents	Chip appearance determination				
	Unpleasant	Half-pleasant	Neuter	Pleasant	Very pleasant
Male (%)	-	-	3.08	43.84	53.08
Female (%)	-	-	-	42.62	57.38

Statistically, as shown in the Figure 4 below, both the male and female consumers share a common opinion about the appearance of apple chips.

Figure 4. Determination of the chips exterior appearance by the male and female respondents

Feedback

The feedback obtained from the subjects of our experiments shows that the females are more pleased with the new apple chips even as compared to the ones made from potatoes, while the males, at close quarters, are also pleasantly impressed by the new apple products, which might be produced soon and are healthy to boot. Likewise, the male and female subjects appreciate all the quality characteristics of these chips as well as the aroma and the pleasant taste of the traditional Romanian apples which, compared to the imported ones, are usually tasteless and have no aroma, except for a pleasant appearance.

Table 4 statistically indicates that about 60% of the subjects are very impressed by the new food products, while about 40% of them are only pleasantly impressed.

Table 4. General impression

Respondents	Unpleasant	Half-pleasant	Neuter	Pleasant	Very pleasant
	1	2	3	4	5
Male (%)	-	-	2,30	39.24	58.46
Female (%)	-	-	-	37.71	62.29

Figure 5 graphically demonstrates the same tendency towards the appreciation of the remarkable quality of these Romanian apple chips.

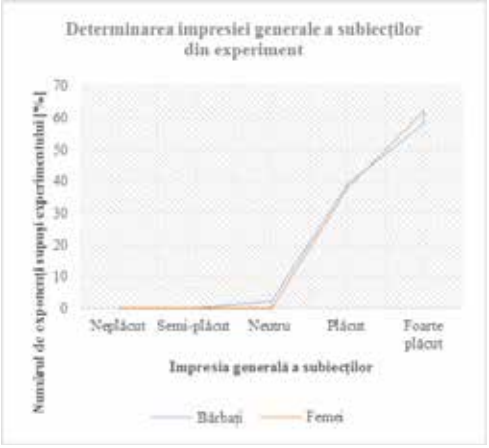

Figure 5. Determination of the subjects' general impression regarding the chips obtained from apples specific to the Bran - Zărnești area

CONCLUSIONS

The Bran - Zărnești area is traditionally appropriate for the growth of apple cultivars that have aromatic fruit and are resistant to the cold climate of the region.
The aim of this research was to test the consumer's preferences on dried apples in order to promote locally new possibilities of valorisation of apple fruits.
The results showed that more than 62% of the women and 58% of men appreciated this product with the highest scores.

Both women and men evaluated the taste of the product with „pleasant" and „very pleasant" scores, being a very important parameter of the sensorial analysis.
For the dried apples it was chosen a package with national traditional aspects. The consumers liked the theme, this parameter being noted with the highest score also.
For the aroma parameter, the women showed more interest than men, most of them appreciated it.
The positive results of this study are an important step for continuing the research in the valorisation of the local products in the Bran - Zărnești area.

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CUTTINGS PROPAGATION OF SOME FIG GENOTYPES (*FICUS CARICA*)

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Abstract

This paper presents the first result of the cuttings propagation of some genotypes of fig (Ficus carica) using an integrated system of basal heating with artificial fog. Fig plants are spread in many regions of Romania, especially in the south parts, and their fruits are very much appreciated by the consumers. Unfortunately, the offer of plant material is very low on the market. In the research project of identification, monitoring and propagation of valuable fig genotypes, more cuttings were collected from different genotypes and different regions of Romania and Iraq. Green and dry cuttings were used and planted in benches for rooting with basal heating, in the greenhouses of the Research Center for Studies of Food and Agricultural Products Quality at the UASVM Bucharest. Different substrates: sand + perlite 70:30, sand + perlite +sawdust, perlite + peat + marc, wood chips with perlite, sand + perlite 60:40 were used. The best results were obtained in the sand with perlite variants 70:30 (61.87%). After rooting, the new obtained plants were transferred in pots and grown under controlled conditions (cold glass houses).

Key words*: artificial mist, fig genotypes, rooted cuttings.*

INTRODUCTION

Fig plants are cultivated on a large scale in tropical, subtropical and warm temperate areas of the world, especially in Morocco, Syria and Italy. Turkey is, however, the largest producer of dried figs (Sinha, 2003).

Despite its varietal diversity, fresh fig is still considered as a minor fruit in trade with Mediterranean countries (Mavsar et al., 2008).

Fig plants are spread in many regions of Romania, especially in the south parts, and their fruits are very much appreciated by the consumers. Unfortunately, the offer of plant material is very low on the market. Some of the major challenges for the fig plants are the resistance to winter frost and the quality of fruits. Being considered a species of interest for Romania, many authors have presented over the years the biology and the peculiarities of the fig tree culture.

Cepoiu et al. (2005) mention in their book „Practical Fruit Growing" that in the climatic conditions of Romania, the fig plant can be cultivated only protected over winter with different materials or at the shelter of some buildings.

In „Pomology", Hoza (2000) states that in our country the fig tree culture has developed in southern Banat, southern Oltenia and its Sub-Carpathian depression, Dobrogea, as well as around the main cities in the west and south of the country. In these areas, local varieties or varieties are grown, producing 1-2 crops per year depending on climatic conditions. In most crop areas, the fig plant is protected during winter and, if planted in sheltered places, it can withstand even without protection.

In plantations, plants need 4-5 m between rows and 2.5-4.0 m per row. In Romania it is grown in the garden next to the house, with promising results in winter protected areas (Stănică et al., 2011).

A perennial woody plant, the fig plant behaves like a shrub or even a tree in the conditions of Romania, and in the pedoclimatic conditions in Iraq it can reach heights up to 12 m (Ghena et al., 2004). Due to the natural capacity of the species to form roots on sliced and rooted portions of the plant, the fig plant can be successfully multiplied by the vegetative method by means of coventional methods, such as by dry or green cuttings (Stănică, 2002).

Fig plant is multiplied vegetative by slips, cuttings or grafting. Because it has great rooting capacity, dry cuttings is the most used method in nurseries (Hoza, 2002; Grădinariu, 2002)

This paper presents the first result of the cuttings propagation of some genotypes of fig (*Ficus carica*) using an integrated system of basal heating with artificial fog.

MATERIALS AND METHODS

In the research project of identification, monitoring and propagation of valuable fig genotypes, more cuttings were collected from different fig genotypes and different regions of Romania and Iraq.

Green and dry cuttings were used and planted in benches for rooting with basal heating, in the greenhouses of the Research Center for Studies of Food and Agricultural Products Quality at the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Different substrates: sand + perlite 70:30, sand + perlite +sawdust, perlite + peat + marc, wood chips with perlite, sand + perlite 60:40 were used. After rooting, the new obtained plants were transferred in pots and grown under controlled conditions (cold glass houses). In the same time, part of the collected biotypes are planted in four testing plots at Pietroasa Viticulture and Wine Processing Research Station, Dăbuleni Research Station, Fruit Nursery and Farm Istrita in Buzau county and Svinița Village, Mehedinți. All the plants are monitored.

***Green cuttings**:* consists in cutting some pieces of seedlings of 13-15 cm, leaving the upper leaves unsteady and the rest of the leaves out. The cuttings thus obtained are inserted into the rooting bed at a depth of 10-13 cm. They are regularly wetted, requiring a humidity of 96-98% and a temperature at the cutting level of 20-22 degrees Celsius.

Cuttings are kept inside the rooting bench for 150-160 days after which they are carefully removed without breaking the roots. Depending on the degree of root development, the cuttings are planted in pots with a diameter of 15-20 centimeters.

***Dry cuttings**:* it is harvested after the fall of the leaves from November to the beginning of March. An important aspect to be taken into account when harvesting shoots during the cold period is the degree of freezing during the winter. The cuttings are shorten to 13-15 cm and inserted into the substrate at a depth of 11-13 cm. The rooting bench in the dry place is placed in a heated greenhouse, watering can be done manually or automated with a sprinkler system.

The rooting bank for green cuttings is made of an aluminum vat about 20 centimeters deep above which is mounted an arcade system that supports a shading net that ensures future plants maintain humidity and a temperature easier to control. Also on the arcade system is mounted the artificial fog system which is connected with a device called „artificial leaf" with the purpose of starting and stopping the irrigation system. The biological material studied consists in different genotypes (Table 1).

Table 1. Fig genotypes

Variant	Genotype	Origin
V1	Secuilor 1	Bucharest, Romania
V2	Secuilor 2	Bucharest, Romania
V3	Negoiești 01	Prahova, Romania
V4	Str. Ștefan cel Mare	Bucharest, Romania
V5	Muzeul Storck	Bucharest, Romania
V6	PH Corno	Prahova, Romania
V9	Sebus	Oradea, Bihor, Romania
V10	Stoica Dan	Mangalia, Romania
V11	Galben mare Giurgiu (Braniștea G.)	Giurgiu, Romania
V17	Olimpia Tm	Timișoara, Romania
V18	Smochin negru	Ploiesti, Prahova, Romania
V21	Str. Părintele Stăniloae	Bucharest, Romania
V28	Brazi	Brazi, Prahova, România
V34	Rot negru Otiman	Svinița, Caras-Severin, Romania
V42	Irak 1	Kerkuk, Irak

RESULTS AND DISCUSSIONS

The results obtained were synthesized in the following tables and graphs.

Five months after planting in rooting lawns (Figure 1), the cuttings were transplanted into pots and analyzed the following: the percentage of viability of the plants obtained, the number of roots formed and their length, the size of the cuttings.

Figure 1. Fig plant cuttings detail

Table 2 presents the number of roots formed by genotype and their size, 5 months after planting in the rooting bed (Figure 2).

Figure 2. Rooted cuttings detail

Table 2. Rooting results on fig genotypes

Variant	Lenght (cm)	Roots (no)	Average length (cm)
V5	40.33	7.33	5.23
V9	86.67	9.67	8.57
V11	87.50	10.00	5.54
V17	35.40	5.80	6.04
V21	95.50	11.10	8.78
V28	75.33	11.67	6.54
V34	84.67	9.00	9.77

V21 genotype presented the best parameters followed closed by V11, V9 and V34 genotypes.

As a result of the vegetative propagation activities of fig plants, the influence made by the composition of the substrate in the rooting process was made (Table 3).

Table 3. Rooting percentage of cuttings depending on the substrate used in the bench

No.	Number of banks (substrate type)	Percentage of success (%)
1.	Bank I sand + perlite (70:30)	61.87
2.	Bank II sand + perlite + sawdust	30.00
3.	Bank III perlite + peat + marc	21.76
4.	Bank IV sand + perlite (60:40)	44.50

From the measurements made it was found that the substrate in which perlite and sand is in the ratio of 30:70 proved to be the best substrate for this type of propagation, confirming the authors cited (Stănică et al, 2002; Hoza, 2000)

In order to observe the most effective method of propagation, the percentage of those who formed viable roots was transcribed when rooted cuttings were planted in pots.

Thus, for the genotypes studied as a vegetative propagation method, the dry one, which had a rate of 42.07%, is favorable as opposed to the one in the green which has a percentage of 14.69%. (Table 4)

Table 4. The degree of rooting according to the type of cuttings

No.	Cuttings type	Initial cuttings (no)	Final rooted cuttings (no)	Rooting percentage (%)
1.	Green	490	72	14.69
2.	Dry	920	387	42.07

Depending on the sun's exposure during the first two weeks of transplantation, the plants responded differently.

They have undergone two different situations. One of the situations requires the plant to be exposed in full sunshine, and in the second plants it is protected from the sun's rays (Table 5).

Table 5. The influence of shading on survival percentage of transplanted cuttings

No.	Growth conditions	Cuttings into pots (no)	Viable cuttings after two weeks (no)	Rate (%)
1.	Sun	55	34	61.81%
2.	Shadow	144	129	89.91%

As a result of the measurements made it was found that the sun exposure of the plants transplanted on the pots is not indicated, so that at this stage the fig plants have to be sheltered from the direct sun rays in accordance with the results presented by Stănică et al., 2002.

Two years later measurements were made on plant growths, foliar surfaces, and the number of fruits obtained.

In Table 6 one can observe the measurements made on pot plants.

Table 6. Measurements made of potted plants

Variant	Length (cm)	Shoots (no.)	Leaves (no.)	Fruit (no.)
V1	52.60	3.20	12.80	2.60
V2	54.40	4.20	14.80	1.00
V3	63.00	1.62	14.00	4.25
V4	53.40	4.20	17.80	2.20
V5	60.85	1.57	13.43	1.28
V6	71.87	1.50	10.37	2.50
V10	54.20	1.40	15.40	2.40
V11	71.80	1.60	12.40	2.40
V18	4.20	2.20	8.30	4.25
V34	46.25	2.00	11.50	1.75
V42	21.75	1.50	8.00	1.25

It can be noticed that the largest length of the plants was made at genotypes V6 and V11 (71.8 cm).
The largest number of shoots was formed at genotypes V2 and V4 (4.2). The average number of leaves per plant had values between 8.0 for genotype V42 and 17.80 for genotype V4. Most genotypes have fruits, but the average value did not exceed 5 fruits per plant. The highest value was recorded in genotypes V3 and V18 (4.25).

CONCLUSIONS

For the fig genotypes studied the best vegetative propagation method was dry cuttings instead of green cuttings.
The substrate in which perlite and sand is in the ratio of 30:70 proved to be the best substrate for this type of propagation. After transplanting the plants in pot, they have to be put in a shadow space for better results.
V21 genotype presented the best rooting parameters followed closed by V11, V9 and V34 genotypes.

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CHARACTERIZATION OF GRAPE AND WINE QUALITY INFLUENCED BY TERROIR IN DIFFERENT ECOSYSTEMS FROM ROMANIA CULTIVATED WITH FETEASCĂ NEAGRĂ

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Abstract

Grapes and especially wine from same variety may be very different, depending on many factors. These attributes have the advantage that, different tastes, curiosities and demands of wine connoisseurs can be satisfied. The experiments concerning the influence of terroir on the grapes and wine quality in 'Feteasca Neagră' variety were located in the main vineyards from western Romania (Minis-Maderat, Recas and Buzias-Silagiu). The aim of the research was to found the influence of the ecological resources, type of pruning, fruit loading and age plantation on grapes quality (sugar, acidity, must yield), but also on the wine quantity and quality (liters per hectare, alcohol concentration, organoleptic characteristics, anthocyanin concentration, acidity, full-bodied). Although belongs to the same variety, the resulted wines had different characteristics on most parameters analyzed. Recas wines are fruity; those from Buzias are more obvious full bodied, while those from Minis-Maderat have higher anthocyanin pigmentation. After results analyzing, it can be concluded that in all three areas of research, terroir allowed obtaining quality wines, but with different characteristics, each bearing the mark of the origin area.

Key words: *acidity, grapes, sugar, terroir, wine.*

INTRODUCTION

The soil, climatic and technological diversity in which the grapevine is cultivated have major impact on wine quality - even in the same variety - so that wines with certain quality and typicality can be obtained, that can satisfy a wide range of consumers (Gladstones, 2011). Quality cannot be achieved only through a harmonious combination of factors: soil-climate-soil-technology (Dobrei et al., 2016a). In red wines, the influence of terroir on quality is high, resulting in wines with different properties (body and balance, anthocyanin pigmentation, tannins etc. - Dobrei A. et al., 2010).

Wine texture qualities are essential in determining wine quality classes and are determined in accordance with wine tasting and typicity which reflects varietal origins (Dobrei et al., 2016a).

One of the most valuable red varieties is the 'Feteasca neagră', considered by many scientists and consumers, the most valuable Romanian variety. However, although not highly advertised in last decades, through a proper technology and winemaking, remarkable results have been achieved (Dobrei et al., 2016b).

A harmonious correlation of the crop technology (fruit breeding, green pruning and training, optimum harvesting time), with the ecological resources and climatic conditions of the crop year, most often resulted in the 'Feteasca neagră' grapes of high quality, without which it is not possible to obtain reference wines (Belda et al., 2017; Cichi et al., 2016).

A harmonious correlation of the crop technology (crop load, green pruning, favorable harvesting time) with the ecological resources of the area and with the climatic conditions, have often resulted in obtaining grapes of special quality in 'Feteasca neagră' variety, without which it is not possible to obtain special wines (Rotaru et al., 2010; Rotaru et al., 2013).

MATERIALS AND METHODS

Resarch was carried out during 2016-2017 in four different locations: Buziaș-Silagiu, Recaș,

Miniş-Măderat and Mocrea vineyards. Mocrea was recognized from 1199 as wine-growing settlement, now part of Miniş-Măderat vineyards.

The complex influences of terroir - climate, soils, slope, and vineyard growing technology - on grapes and wine quality from 'Fetească neagră' variety was investigated.

For more accurate results, the experiments were located in vineyards with different characteristics (vine age, vine training system, crop load) placed on different soil types and land with various exposure to sunlight (Table 1). Research years were climatically different: 2017 has been marked by high temperatures and low rainfall, while 2016 was a warmer year and more rainfall, but without exceeding the normal.

Temperature associated with vineyard placement and soil, has strong control on ripening and then on grape and wine quality. Located not far away from each other, all three vineyards produced wine for long time. However, several geo-morphological variations which induce different micro-climatic conditions between vineyards affect the grapevine development and growth.

Grape maturity during ripening was determined by sugar concentration and titratable acidity, in the third decade of September in 2016 and second decade of September in 2017.

Grape samples were harvested from experimental plots from each vineyard in which the research was carried out.

After berries crushing, the samples of must were subjected to analyze for sugars and total acidity determination.

Sugar concentration (g/l) was measured using a digital refractometer at 20^{o}C.

The acidity of the samples was performed by the titrimetric method by neutralizing the acidity of the samples with a known 0.1 M NaOH solution (F = 0.9527). The obtained results are expressed in g/l tartaric acid. Winemaking technology has not been the subject of our research, this being carried by a standard protocol specific to each vineyard. Wine analyses were done after finishing the winemaking process.

Total content of monomeric anthocyanins (cyanidin-3-glucoside equivalents / L sample) was calculated according to the following relation equivalents / L sample) and was calculated according to the following relation:

$$\text{Anthocyani ns (mg/l)} = \frac{(A \cdot MW \cdot DF \cdot 1000)}{\varepsilon \cdot L}$$

where: A = (A520 nm pH 1.0 - A700 nm pH 1.0) - (A520 pH 4.5 nm - A700 nm pH 4.5); MW = molecular weight of cyanidin-3-glucoside (449.2); DF = dilution factor; ε = molar absorbance of cyanidin-3-glucoside in acidic aqueous solution; L = optical path (1 cm).

The method of wine colour determination with the highest accuracy was done by the spectrophotometric method. This involves raising VIS spectra (in the visible wavelength: 380-720 nm) in reflectance or transmittance.

The sensory analysis was carried out in accordance with the Regulation of the Association of Authorized Tasters in Romania and according to the International Vine and Wine Office.

According to the OIV (International Vine and Wine Organization) quality assessment system, wine is assessed on three main characteristics and nine sub-characteristics, as well as a global assessment called harmony.

The main characteristics that were evaluated are: appearance (turbidity or haze, color), aroma, taste and mouthfeel (acidity, sweetness, bitterness, astringency, any new flavors or aromas perceived). The tasting was done by a five member group, under laboratory conditions, using tasting glasses, wines having a temperature of 18°C.

The rating was divided into the following categories, based on the average of the points received, obtained by adding the five notes and dividing them to five.

The score obtained was as follows: excellent (full body); very good (medium to full body); good (medium).

Results concerning the influence of terroir from experimental variants on 'Fetească neagră' grapes production and quality (sugar, acidity, must yield), but also on the wine quantity and quality (wine yield per hectare, alcohol by volume, wine sensory properties and texture, anthocyanin pigmentation) were statistically analyzed. All experiments were repeated three times.

Table 1. Experimental variants

Vineyard	Soil type	Exposure to sunlight	Vine age (years)	Vine training system	Crop load (buds/vine)
V_1 - Recaș	Reddish brown	S-E	20	Guyot	20
V_2 - Buziaș	Luvisols Podzoluvisols	E	12	Cazenave	25
V_3 - Mocrea	Rendzina soils	S-E	6	Cazenave	16
V_4 - Miniș	Rendzina soils	S	15	Guyot	18

Average and standard deviations of the data were calculated. The average comparison test and the Fisher test (LSD) were applied to compare the results of must and wine characteristics. The confidence interval was set at 5%. For all statistical tests, the SPSS (SPSS Inc., Chicago, IL) for Windows, version 22.0 was used.

RESULTS AND DISCUSSIONS

Year 2016 was normal for grapevine growing, without extremely favourable climatic conditions due to the high rainfall in July, August and September.

Grape yields were high, close to the maximum crop potential, especially in the Buziaș and Recaș vineyards (Table 2). In these areas the soil fertility correlated with rainfall and crop load increased the production to 11375 kg/ha at Buziaș and 10758 kg/ha at Recaș. In Miniș and Mocrea vineyards, yields were lower compared to those from Buziaș and Recaș, but in normal limits for 'Fetească neagră' variety. In these areas, rendzina soils are considered to have limited potential for grapevine production.

Concerning the grapes sugar content, results were good considering the climatic conditions variation during the year; the highest sugar content was recorded in Mocrea vineyard, followed by results from Miniș and Recaș. Higher sugar concentrations recorded in the Mocrea and Miniș areas are statistically significant, as result of the rendzina soils properties which lowered the crop load. Regarding the must yield, differences between investigated variants are insignificant, with limits between 75-79%, values considered normal.

Table 2. The influence of terroir on production quality during 2016

Experimental variant	Production (kg/ha)	Sugars (g/l must)	Acidity (g/l H_2SO_4)	Must yield (%)	Difference to control (sugars g/l)	Significance
V_1 - Recaș	10758	209	4.9	79	-4	-
V_2 - Buziaș	11375	198	5.2	78	-15	00
V_3 - Mocrea	8773	226	4.6	75	+13	**
V_4 - Miniș	9145	218	4.8	76	+5	*
Mean (Mt)	10013	213	4.8	77	-	-
DL	5% - 4.93		1% - 10.17		0.1% - 18.32	

During 2017 climate conditions were high temperatures and low rainfall, with positive influence on grape quality, but with lower yields than the previous year (Table 3). The yields ranged between 10873 kg/ha at Buziaș and 8152 kg/ha at Mocrea. The sugar concentration increased in grape berries from all four vineyards experimental plots, ranging from 249 grams per liter in Mocrea and 218 grams per liter in Buziaș.

Table 3. The influence of terroir on production quality during 2017

Experimental variant	Production (kg/ha)	Sugars (g/l must)	Acidity (g/l H_2SO_4)	Must yield (%)	Difference to control (sugars g/l)	Significance
V_1 - Recaș	10120	228	4.7	78.0	-4	-
V_2 - Buziaș	10873	218	5.0	76.0	-14	00
V_3 - Mocrea	8152	249	4.4	73.0	+17	***
V_4 - Miniș	8207	233	4.6	74.0	+1	-
Mean (Mt)	9338	232	4.6	75.2	-	-
DL	5% - 4.03		1% - 9.24		0.1% - 16.92	

Table 4. The influence of terroir on production quality during 2016 - 2017

Experimental variant	Production (kg/ha)	Sugars (g/l must)	Acidity (g/l H_2SO_4)	Must yield (%)	Difference to control (sugars g/l)	Significance
V_1 - Recaș	10439	219	4.8	78.5	-4	-
V_2 - Buziaș	11124	208	5.1	77.0	-15	00
V_3 - Mocrea	8463	238	4.5	74.0	+15	**
V_4 - Miniș	8676	226	4.7	75.0	+3	-
Mean (Mt)	9676	223	4.7	76.1	-	-
DL	5% - 4.64		1% - 9.43		0.1% - 17.20	

Due to the climate variability from both years, for the accuracy of the results is presented an average of the two years of research in Table 4.
Regarding the production, were recorded average and high values for this variety in correlation with climatic conditions, soil type and crop loads. The smallest productions were obtained on less fertile rendzina soils and on lower crop loads from Mocrea and Miniș vineyards. The more fertile soils from Buziaș and Recaș, in correlation with higher crop loads, create a positive effect on the average production potential of this variety and higher yields were recorded.
With respect to grapes quality, all four grape-growing areas have proven to be very favourable to 'Fetească neagră' variety, sugar concentration recorded in grape berries being high or very high.
Rendzina soils correlated with the favourable exposure to sunlight and smaller crop loads, favoured the accumulation of very high amounts of sugar (238 g/l) in the grape must from Mocrea and 226 g/l in grape must from Miniș. Very good grape quality was also recorded in Recaș vineyards, where the reddish-brown soil associated with the southern exposure of the vine rows and a moderate crop load on long spurs/canes, favoured the accumulation of 219 g/l sugar.
In Buziaș vineyards, the luvisols in correlation with a higher crop load on short canes favoured the accumulation of medium amounts of sugars in must (208 g/l), which are lower compared to the other three grape-growing areas. This value for the sugars is the only one with negative statistical significance compared to the average results found during the research.
During 2016, the wine yield per hectare varied between 8 531 l/ha at Buziaș and 6 404 l/ha at Mocrea vineyards (Table 5). The more fertile soils from Buziaș and Recaș favoured a higher amount of wine per hectare compared with less fertile rendzina soils from Miniș and Mocrea. In contrast, rendzina soil provided higher alcohol concentration and higher anthocyanin content compared to fertile soils.
In 2017, high temperatures and low rainfall decreased the amount of wine yield per hectare in all grape-growing areas investigated, but instead had a positive influence on alcohol concentration and anthocyanin content. This year, the smallest wine yields per hectare were produced on rendzina soils from Miniș (5 826 l/ ha) and Mocrea (5 787 l/ha) respectively (Table 6).
Concerning the sensory wine properties, in both years, wines were marked by their clarity, colour and body, with a plus for those produced in 2017 (Table 7). These wines, by proper storing and vintage can become reference wines with real chances of assertion in the future national or international wine contests.

Table 5. The influence of terroir on wine quality during 2016

Experimental variant	Wine yield (l/ha)	Alcohol (%)	Anthocyanin (mg/l)	Sensory properties qualities		
				Limpidity	Color	Body
V_1 - Recaș	8176	12.0	233.0	Clear	Red ruby	Medium to full
V_2 - Buziaș	8531	11.5	218.0	Clear	Red ruby	Medium
V_3 - Mocrea	6404	13.1	236.0	Limpid	Red ruby	Full
V_4 - Miniș	6675	12.7	238.0	Limpid	Dark red	Full
Mean (Mt)	7446	12.3	231.2	-	-	-

Table 6. The influence of terroir on wine quality during 2017

Experimental variant	Wine yield (l/ha)	Alcohol (%)	Anthocyanin (mg/l)	Sensory properties qualities		
				Limpidity	Color	Body
V_1 - Recaş	7590	13.2	243	Clear	Red ruby	Medium to full
V_2 - Buziaş	7937	12.7	224	Clear	Red ruby	Medium
V_3 - Mocrea	5787	14.5	245	Limpid	Dark red	Full
V_4 - Miniş	5826	13.5	248	Limpid	Dark red	Full
Mean (Mt)	6785	13.4	240	-	-	-

Table 7. The influence of terroir on wine quality during 2016 -2017

Experimental variant	Wine yield (l/ha)	Alcohol (%)	Anthocyanin (mg/l)
V_1 - Recaş	7883	12.6	238.0
V_2 – Buziaş	8234	12.1	221.0
V_3 - Mocrea	6096	13.8	240.5
V_4 – Miniş	6251	13.1	243.0

During the research made by Artem et al. (2014) on four red wine varieties, they found in 'Feteasc㠠neagră', highest values for acidity (7.72 g/l), and for anthocyanins (325.92 mg/l). The alcohol concentration found in the same research (13.55%), was quite similar with the average of 'Feteasc㠠neagră' from our investigation (13.4%). Mori et al. (2007) mention in their research that temperature over 35^0C decreased the anthocyanin content in red grape berries. In a study carried out during 2014-2015, Coldea et al., found out in 'Feteasc㠠neagră' variety a lower total acidity, between 3.28 and 3.72 mg/l, and alcohol content with limits between 11.78 and 13.70%. In the research carried out by Artem et al. (2015) in Murfatlar vineyards on 'Feteasc㠠neagră' variety, they found out close results for alcohol concentration (12.8 - 13.4%), higher values for acidity (6.33 - 5.77 mg/l) and for anthocyanins (409 - 531 mg/l).

Wine components are much or less correlated with each other. Alcohol concentration (%) from 'Feteasc㠠neagră' during 2016-2017 is highly and negatively influenced by the wine yield (l/ha) and by the crop load. The higher the crop load, the lowest is the alcohol concentration in the wine. The wine yield is strong influenced by the crop load. The anthocyanin content in the wine is low correlated with crop load, while alcohol concentration in the wine is negative correlated with the anthocyanin content and crop load (Table 8). Strong positive correlation was recorded between wine yield and crop load.

Table 8. Correlation between wine yield, anthocyanin and crop load in 'Feteasc㠠neagră' variety, during 2016-2017

		Wine yield (l/ha)	Alcohol (%)	Anthocyanin (mg/l)	Crop load (buds/vine)
Wine yield (l/ha)	7883	1	-0.936946137	0.545198709	0.989044201
Alcohol (%)	12.6	-0.936946137	1	-0.217855545	-0.978270352
Anthocyanin (mg/ l)	243	0.545198709	-0.217855545	1	0.415475039
Crop load (buds/vine)	20	0.989044201	-0.978270352	0.415475039	1

Table 9. Variability in Feteasc㠠neagră variety traits during 2016-2017, in research locations

	Wine yield (l/ha)	Alcohol (%)	Anthocyanin (mg/l)	Crop load (buds/vine)
Mean	6860.33	13.00	233.17	19.67
Standard Error	688.28	0.49	6.13	2.73
Standard Deviation	1192.15	0.85	10.61	4.73
Minimum	6096.00	12.10	221.00	16.00
Maximum	8234.00	13.80	240.50	25.00
CV %	17.37.00	6.57	4.55	24.05

As Jackson and Lombard (1993) said, the quality of the wine "is not easy to define", but it is sure that a good wine must have good taste, special aroma and sensory properties above average for each type of wine.

From Table 9 can be found that the highest variability was registered in the crop load (CV=24.05%), with high level of dispersion around the mean. Low coefficient of variation of 4.55% show that anthocyanins content in 'Fetească neagră' wine, is uniform during research years (2016-2017). In the same time anthocyanins is uniform in vineyards where researches were carried out. Low coefficient of correlation for alcohol concentration (CV=6.57), show the versatility and high adaptability of 'Fetească neagră' variety in different ecosystems and terroir from the west of Romania. Studding the Montenegrin autochthonous red varieties, Košmerl et al. (2013) concluded that poor correlation was found among yield and quality parameters. In their research, lower crop yield didn't result in higher levels of anthocyanins, polyphenols, or sugars.

CONCLUSIONS

'Fetească neagră' variety proved to be a well-adapted in all four grape-growing areas, both grapes and wine being of very good quality.

The different climatic conditions during the years, different soil types from one area to another, the crop load and vine age, were factors that influenced the quality of grapes and wine and highlighted the particular adaptability of this variety.

Variety gives good results both on deep and fertile soils which ensure acceptable production and both on thin and rocky soil type like rendzina soil, on which are highlighted the variety quality potential.

Research results are confirmed by other previous research (Dobrei et al., 2010), according to which 'Fetească neagră' produce pigmented wines with the highest content in anthocyanins on rendzina soils in Miniș and Mocrea vineyards, followed by reddish brown soils from Recaș and Buziaș.

Linking of growing technology with the soil type and environmental resources of the area, make it possible to achieve special wine that ensures customer demands through the special sensory qualities, typicity and authenticity.

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STUDY OF THE ANTHOCYANIC POTENTIAL OF GRAPES VARIETIES FOR RED WINES IN DRANIC WINE CENTER

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Abstract

The Dranic plantation is one of the youngest in Dolj county and was founded in 2010 through the process of reconversion and restructuring of the wine sector in our country. Under the favorable ecologically conditions for viticulture in the Oltenia area, the varieties for red wines besides the superior potential of glucidic accumulation, have a remarkable ability for biosynthesis of anthocyanins. The chromatic structures of the anthocyanin extracts represented by the yellow, red and blue pigments are balanced, attractive and fully in line with current requirements. They are dimensioned by colour intensity values, but especially by the qualitative chromatic indicators: the colour tone and the proportions of the flavylium cations. This study may be the start for the suitability of this area for obtaining high-quality red wines.

Key words*: anthocyanin, grape maturity, red, wines, extractability, chromatic structure.*

INTRODUCTION

The Dranic plantation is one of the youngest in Dolj county and was founded in 2010 through the process of reconversion and restructuring of the wine sector in our country. In the absence of clear data from the literature showing the direct relationship between natural factors and the evolution of the red wine grape ripening process, this detailed study was needed. This study may be the start for the suitability of this area for obtaining high-quality red wines (Muntean et al., 2017).

Phenolics, such as flavonoids, phenolic acids, and tannins, are considered to be the main antioxidants in fruits and vegetables (Mnari et al., 2016).

Phenolic compounds, such as phenols, phenolic acids, flavonoids, tannins, and anthocyanins, have received considerable attention for their high antioxidant activity (Karakaya et al., 2001; Rice-Evans et al., 1996). Phenolic compounds are free radical scavengers because they are nucleophiles that inhibit lipid peroxidation and chelators of metal ions that induce oxidation (Han and Baik, 2008).

Anthocyanins, the pigments responsible for the red colour of grapes, are widely used to carry out classification and chemotaxonomic studies in flavonoid metabolism.

Anthocyanins are contained in the grape skin, except in the case of a few cultivars whose pulp is also pigmented. Thus, a close relationship can be expected between anthocyanin composition and visual appearance (Fernandez-Lopez et al., 1998). The International Office of the Vine plants (OIV) has a descriptor list for grape vine varieties and *Vitis* species which classifies the grape varieties in seven groups according to their external colour: 1 (green-yellow), 2 (pink), 3 (red), 4 (red-grey), 5 (red-dark violet), 6 (blue-black) and 7 (red-black) (OIV, 1988).

Anthocyanins are gradually accumulated in berry skins from veraison through grape ripening (Gomez-Plaza, 2006; Fournand et al., 2006) malvidin-3-glucoside being the most abundant anthocyanin in almost all red grape varieties. However, the anthocyanin concentration may decline just before harvest and/or during over-ripening (Rolle et al., 2011). The relationship between cell wall composition and extractability of anthocyanins from red grape skins was assessed in Tempranillo grape samples harvested at three stages of ripening (pre-harvest, harvest and over-ripening) and three different contents of soluble solids (22, 24 and 26 °Brix) within each stage (Hernandez-Hierro et al., 2014).

The extractability of anthocyanins from wine-grapes with different skin hardness at two different ripening stages was evaluated by Role et al (2009). Significant interactions between ripening stage and skin hardness were found in the composition of individual anthocyanins present in the extract.

Some researchers Romero-Cascales et al., in 2005, were studying the differences in anthocyanin extractability from grapes to wines according to variety. The anthocyanin concentration of *Vitis vinifera* L. cvs. 'Cabernet Sauvignon', 'Merlot', 'Syrah' and 'Monastrell' grape skins was determined together with anthocyanin extractability at the exact time of harvest (measured by an extractability assay based on the comparison of the anthocyanin concentration of two different solutions obtained after macerating the grapes for four hours at two different pH values). These data were compared with the anthocyanin concentration and chromatic characteristics of the resulting wines.

The purpose of this study was to investigate the evolution of anthocyanin content during grapes maturation and the degree of extraction in wines

MATERIALS AND METHODS

Grape and wine sample

This study was carried out with grapes of the three cultivars *Vitis vinifera* cv. 'Cabernet Sauvignon', 'Merlot' and 'Feteasca neagră' from the wine-growing center Dranic-Dolj.

Grape samples of three red cultivars (*V. vinifera* L.) were collected at full maturity (FM) and at different other date after full maturity, means FM+10 days and FM+15 days. The grapes was harvest at FM+10 days which corespond with technological maturity.

The wine was obtained under the same biotechnological conditions of red vinification which was SO_2 - 50 mg/l; ADY addition 15 g/hl; 7 days maceration time; maceration temperature 26-28^{0}C.

Determination of anthocyanins from grapes and wine, pigments structure and flavilium cation from grapes and also chromatic composition and intensity and tonality of wine was done.

These analyzes were conducted in 3 consecutive viticultural years, 2015, 2016 and 2017.

Anthocynins extraction from grapes

Grape anthocyanins determined by the method of Poissant Leon. An average sample of 50 grape berries, weighed than the skin are carefully detached from the grape pulp. In order to remove excess moisture, the skins were wiped with filter paper and dried in a hot air source.

Dry skins are powdered quartz fine sand after which they are passed into a flask with ground glass stopper by repeated washing with 1% HCl solution (approx. 10 ml concentrated HCl/liter). In order to extract anthocynins first wash fraction (50 ml) to be in contact with the skins to about 12 hours. The reafter, the filtered or centrifuged extraction liquid is brought to a constant volume of 200 ml by mixing and the fractions resulting from repeated acid addition and kept in contact with the extract for at least one hour.

After obtaining the skin sample and the anthocynins extract, the filtered extract is read on a 1 cm cuvette spectrophotometer at the optical density (OD) of 520 nm. The amount of anthocynins is determined on the basis of the formula:

$$mg/kg\ grapes = \frac{OD520 \times 22.76 \times 0.4}{berries\ weight} \times 1000$$

Anthocynins from wines

Anthocyanins in wine are determined with spectrophotometer by pH difference (method Ribereau Gayon - Stonestreet - 1968). The difference between the optical densities read at 520 nm of a solution at two different pH is proportional to the amount of anthocynins contains the wine. From the optical density of the solution of pH 0.6, the optical density of the solution of pH 3.5 is decreased, thus obtaining the PA. The concentration in anthocynins is determined from the calibration curve, based on the data, and the expression is expressed in mg/l.

The colour of red wines (Glories, 1984) is determined with a T 70+UV/Vis spectrometer PG Instruments Ltd. in the visible field at wave lengths of 420, 520 and 620 nm.

After reading the yellow component at 420 nm (attributed to tannins and anthocyanin

degradation products), the red component at 520 nm (attributed mainly to the free anthocyanins as favylium cations and to the polymeric red pigments) and the blue component at 620 nm (attributed to anthocyanin quinoidal bases and copigmentation derived from a non-covalent complex copigment - anthocyanin), the colour intensity (Ic), the colour tone (Tc) and the flavylium cation-brilliance of red (dA%) ratios are calculated according to the following formulas:

$$Ic = OD420 + OD520 + OD620$$

$$Tc = \frac{OD420}{OD520}$$

$$dA\% = \left[OD520 - \frac{OD420 + OD620}{2} \right] \times \frac{1}{OD520} \times 100$$

RESULTS AND DISCUSSIONS

The anthocyanic potential and the chromatic structure of the grapes of the main varieties for high quality red wines grown in the center of the Winery are listed in Table 1.

By its genetic nature, the histological structure of the berry and the ability to harness the natural conditions superior to the 'Cabernet Sauvignon', accumulate the highest anthocyanin content, at the technological maturity ranging from 1394 - 1493 mg/kg of fresh berries. The second place is occupied by the Merlot variety, containing a concentration in anthocyanin ranging from 1205 to 1273 mg/kg of fresh berries. Modest contents of anthocyanin accumulates in grapes of 'Feteasc㠠neagră' variety being below 1200 mg/kg of fresh berries.

The chromatic structure, given by the proportions of the different categories of pigments, varies from one species to another and from one year to the next. Thus, in the anthocyanin complex of 'Cabernet Sauvignon' grapes, the yellow-orange pigments have the lowest values among the study varieties (31.5 - 33.9%) while the blue pigments are the most abundant (9.2 - 9.7%). The largest proportions of red and blue pigments have been accumulated, and the intensity of the anthocyanins extracts from the 'Cabernet Sauvignon' grapes is explained, as well as the values of the parameter for brilliance for red is high (dA% - 76.5 - 79.3). The 'Feteasc㠠naegră' variety showed the lowest anthocyanin content and the highest yellow pigments (39.5-41.7), while the blue component showed the lowest value (8.1-8.9%).

On the other hand Merlot variety, showed an intermediate characteristics, achieving a balanced chromatic structure with positive visual valences.

In the three years of study, the phenolic maturity was performed after full maturity, at about 10 days, between 15.X. and 25.X. for the 'Cabernet Sauvignon' variety, between 10.X. and 20.X. for the 'Merlot' variety. The maximum amount of anthocyanins in grapes does not correspond to the maximum amount of anthocyanins in wines. Correlated with phenolic maturity, the phenomenon is explained by the increase of extracts of anthocyanins from the skins to the over ripening of the grapes. At over-ripening, anthocyanin content in berries is lower, but wine has higher anthocyanin content.

Table 2 shows the evolution of anthocyanin content in grapes and their degree of extraction at full maturity and over-ripening in 2017, a year of excessively high temperatures.

In all 3 varieties observed, although total anthocyanin content decreases continuously, it increases, in turn, its extraction due to the degradation of the walls of the anthocyanin blasts of the hypodermis, under the action of its own pectolytic enzymes.

Thus, the proportion of extracted anthocyanins increases from the 41.5% to 54.1% ('Cabernet Sauvignon'), from the 41.7% to 52.5% ('Merlot') and from the 40.8% to 53.5% ('Feteasc㠠neagră').

In the wines obtained in 2017 the chromatic structure of the obtained wines has a different configuration (Table 3). As far as the over ripening of the grapes - raw material, in the anthocyanic complex extracted under the action of biotechnological factors, uniformly applied, they increase the proportions of yellow and red pigments and decrease the proportions of blue pigments, which is still a qualitative advantage of wine coloring, even when younger.

Values optical density at 420 nm wavelengths, 520 nm and 620 nm specific to different types

of pigments used to calculate the qualities chromaticity wines, listed in Table 3 reveals differences in some cases quite important. They are mostly of the genetic nature of variety and less of primary winemaking technology.

Yellow component showed the lowest value in wines produced from 'Cabernet Sauvignon', and the largest values in wines from 'Feteasca neagră' while the red component showed the highest values in wines of 'Cabernet Sauvignon' and the lowest value in wines of 'Feteasca neagră'. The blue component of wines is well and positively correlated with the red component.

An accurate image of the quantity and quality of material coloured wine is obtained from: absolute content of anthocyanins, the participation percentage of different types of pigments and values qualities chromaticity for their definition being considered optical density values (referred to in Table 4).

Table 1. Anthocyanin content and chromatic structure in grapes, technological maturity (Average 2015-2017)

Varieties	Antocyanin, mg/kg fresh berries		Yellow pigments OD 420 nm		Red pigments OD 520 nm		Blue pigments OD 620 nm		Brilliance of red, dA %	
	Variation	Average	Variation	Average	Variation	Average	Variation	Average	Variation	Average
Cabernet Sauvignon	1394.0 - 1493.0	1443.5	31.5-33.9	32.7	56.8-59.0	57.9	9.2-9.7	9.4	76.5-79.3	77.9
Merlot	1205.0 - 1273.0	1239.0	34.5-35.8	35.1	54.6-56.8	55.7	8.9-9.5	9.2	74.6-75.5	75.0
Feteasca neagră	1099.0 - 1140.0	1119.5	39.5-41.7	40.0	49.5-53.4	51.4	8.1-8.9	8.5	72.5-74.2	73.3

Table 2. Anthocyanin content of grapes and their degree extraction, depending on the harvesting period – 2017

Varieties	STAGE OF MATURITY								
	Full maturity (FM)			FM + 10 days			FM + 15 days		
	Total anthocyanin, mg/kg of fresh berries	Extractable anthocyanin, mg/kg of fresh berries	Degree of extr., %	Total anthocyanin, mg/kg of fresh berries	Extractable anthocyanin, mg/kg of fresh berries	Degree of extr., %	Total anthocyanin, mg/kg of fresh berries	Extractable anthocyanin, mg/kg of fresh berries	Degree of extr., %
Cabernet Sauvignon	1498.0	623.2	41.5	1493.0	744.5	49.8	1469.0	795.4	54.1
Merlot	1281.0	534.7	41.7	1273.0	598.0	46.9	1192.0	625.8	52.5
Feteasca neagră	1146.0	468.5	40.8	1140.0	584.6	51.2	1132.0	605.8	53.5

Table 3. The optical densities of red wines varieties 2017

Varieties	OD 420 nm (yellow pigments)	OD 520 nm (red pigments)	OD 620 nm (blue pigments)
Cabernet Sauvignon	0.489	0.832	0.140
Merlot	0.492	0.737	0.125
Feteasca neagră	0.522	0.658	0.115

Table 4. Chromatic composition of red wines 2017

Varieties	Composition of pigments %			Intensity of colour (Ic)	Tonality of colour (Tc)
	Yellow pigm. (OD 420 nm)	Red pigm. (OD 520 nm)	Blue pigm. (OD 620 nm)		
Cabernet Sauvignon	33.35	57.05	9.60	1.46	0.58
Merlot	36.50	54.30	9.20	1.35	0.67
Feteasca neagră	40.30	50.80	8.90	1.29	0.79

Data on participation of various types of pigment in composition of coloring matters and levels of characteristics chromatic of complex anthocyanins, show structures chromatic very

favorable to all wines and proportions of different types of pigments are able to provide levels of color corresponding total over the claims current.

CONCLUSIONS

The natural conditions that are particularly favorable to the vineyards in the Dranic vineyard and significantly mark the anthocyanin - rich content of the grapes of the 'Cabernet Sauvignon', 'Merlot' and even of the native 'Fetească neagră' variety.

During the over-ripening process of grapes - a condition that is generally required for the production of high class red wines, anthocyanin content decreases with the progress of process, but in the same time it was observed an increase of the extraction degree and, on the other hand, chromatic characteristics of the resulted wines are improved substantially.

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THE BEHAVIOR OF THREE CABERNET SAUVIGNON CLONES IN VALEA CĂLUGĂREASCĂ AREA

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Abstract

Cabernet Sauvignon variety is one of the most widespread grapevine for wine grown in Romania. By application of clonal selection, the only method to avoid the degeneration phenomenon of varieties to biotypes, five clones of Cabernet Sauvignon have been obtained and homologated in Romania. The present study aimed to analyse the behaviour of three Cabernet Sauvignon clones, namely Cabernet Sauvignon 30 Vl., 131 St. and 54 Mn. in the Valea Calugareasca viticultural area, considering the extension of their culture. The experiments were carried out during 2016-2017 harvest seasons, and were referred to the phenotypic spectrum, the fertility and productivity characteristics, the biological potential, the maturation dynamics of the grapes, the qualitative and quantitative evaluation of the grapes at harvest. These three Cabernet Sauvignon clones showed a similar phenological cycle and the behaviour to biotic factors, resistance to the pathogen attack being good and very good. During ripening, the sugar accumulation was similar at all clones (between 2.38 and 2.63 g/day) the amount of sugar accumulated on maturation period being between 49.9g and 55.2g. The glucoacidimetric ratio recorded very high values, characteristic for exceptional years (over 60%), mainly due to the very low value of total acidity. Acids metabolism has been achieved on average with 1.59-1.73 g/day. In terms of grape weight, the clone Cabernet Sauvignon 30 Vl. was highlighted with a greater weight of bunches with 11.5% compared with the Cabernet Sauvignon 54 Mn. and with 12.41% compared with the Cabernet Sauvignon 131 St. From the point of view of the polyphenolic composition, Cabernet Sauvignon 54 Mn. was noted by the value of total polyphenol index and the anthocyanic potential.

Key words*: biotypes, clonal selection, Cabernet Sauvignon, glucoacidimetric ratio, polyphenol index.*

INTRODUCTION

Cabernet Sauvignon is one of the most widespread vinegrapes varieties for wine grown in Romania (Șerdinescu et al., 2006; Urucu, 2014). By application of clonal selection, the only method to avoid the degeneration phenomenon of varieties to biotypes, five clones of Cabernet Sauvignon have been obtained and homologated in Romania. Many authors have reported the distinction of some Cabernet Sauvignon clones from the point of view of wines quality, distinctive flavour of fruit aroma, higher content of tannins, anthocyanins (Jones and Davis, 2000; Fidelibus et al., 2006; Dejeu, 1986).

Clones of one variety differ from the population in better features of the grape and better quality of wines obtained (Stefanini et al., 2000; Tebeica et al., 2005). From a great number of Cabernet Sauvignon clones, wines with distinctive flavour of fruit aroma, higher content of tannins, anthocyanins etc. are produced in France (Catalogue, 2009), Italy (Fidelibus et al., 2006), Australia and other countries.

The present study aimed to analyse the behaviour of three Cabernet Sauvignon clones, in the Valea Calugareasca viticultural area, namely Cabernet Sauvignon 30 Vl. obtained by ICDVV (Research-Development Institute for Viticulture and Oenology, Valea Calugareasca) and approved in 2010, Cabernet Sauvignon 131 St., obtained by INCDBH (National Research and Development Institute for Biotechnology in Horticulture, Stefanesti Arges) and approved in

2000 and Cabernet Sauvignon 54 Mn. obtained by SCDVV (Research and Development Station for Viticulture and Oenology, Minis Arad) and approved in 2006, considering the extension of their culture.

MATERIALS AND METHODS

Three Cabernet Sauvignon clones, namely Cabernet Sauvignon 30 Vl., Cabernet Sauvignon 131 St., and Cabernet Sauvignon 54 Mn., were taken into study.

The vines, were grafted on the SO4 (Oppenheim Selection 4) rootstock, were planted in 2014 in the germplasm collection belonging to the Research and Development Institute for Viticulture and Oenology, Valea Calugareasca. 24 vines per genotype were taken into study. The evaluation of clonal accessions focused on the duration of their phenological cycles, grape fertility and productivity, resistance to diseases, quantity and quality of the grapes production.

The recording of the vegetation phenophases was carried out weekly, following the methodology defined by the OIV (International Organization of Vine and Wine) descriptor list for grape varieties and *Vitis* species (2009).

Time of bud burst was noticed when 50% of the buds are in green shoot tip stage C of Baggiolini, stage 7 to 9 of BBCH (Biologische, Bundesanstalt, Bundessortenamt und CHemische Industrie scale). Time of full bloom was noticed when 50% of flowers are open. When 50% of the red grape clusters show changes in colour, or when about 50% of the berries of the white cluster start softening was noticed the time of beginning of berry ripening (veraison). Time of physiological stage of full maturity of the berry is related to the maximum sugar content of the berry due to photosynthesis. Mean value of all bunches of 10 shoots was taken into account.

The behaviour to biotic factors was assessed by using OIV ampelographic descriptor method, the notation being done through attribution of figures depending on the level of expression.

Production and the quality of grape harvest was calculated based on fertility coefficient and productivity index, the average length and average weight of bunch grapes and berries, sugar content and the total acidity of must.

Absolute and relative fertility coefficients were calculated using computation formulas based on the number of inflorescences, total shoots and fertile shoots. The productivity of varieties was determined at full grape maturation, using absolute and relative productivity indices and the weight of the grapes (Pop, 2003). The average weight of grape at full maturity was calculated by weighting 50 grapes per clones.

The sugar concentration was determined by a hand held digital refractometer and the results were expressed as an absolute value and as a percentage by mass of sucrose (OIV-MA-AS2-02 method). Titratable acidity was determined by titration with 0.1M NaOH, with 1% phenolphthalein and the results were expressed in gL^{-1} H_2SO_4 (OIV-MA-AS313-01 method).

The behaviour to biotic factors was assessed by using OIV ampelographic descriptor method, the notation being done through attribution of figures depending on the level of expression.

The phenolic potential was assessed by the standard ITV (Institute Technique de la Vigne-France) method (www.vignevin-sudouest.com) based on the following analytical parameters: anthocyanins, total anthocyanin potential and total polyphenol index.

Extraction of phenolic compounds from the grapes was done with an aqueous acid solution. Maceration of the samples was done for one hour at room temperature. Fifteen milliliters of ethanol (95%) and 85 mL of 0.1% HCl were added to the 50 g of grape juice. The samples were shacked for one minute from quarter to quarter. Coloured extracts are filtered through glasswool (or quantitative filter paper) in order to obtain clear solutions.

The dilution of the samples to 1/100 was done in double-distilled water followed by the measurement of the absorbance at 280 nm in a 1 cm quartz vat, nm against a blank of distilled water and the total polyphenolic index (IPT) was determined as follow:

Total polyphenolic index (IPT) = DO 280 x 100 x [(weight of marc + 100)/ weight of marc].

The samples were diluted 1/20 in 1% hydrochloric acid solution and absorbance was measured at DO 520 nm against a blank of distilled water. The concentration of anthocyanin and the total anthocyanic potential were determined following formulas:

Anthocyanins (mg/l) = DO 520 x 22.75 x 20
Total anthocyanic potential (mg/kg) = anthocyanins (mg/l) x [(weight of marc + 100)/ weight of marc].

RESULTS AND DISCUSSIONS

Compared to multiannual averages, the wine year 2016-2017 is characterized by a higher thermal regime, due to a relatively normal rainfall regime. During the winter, there were no minimum temperatures to affect the viability of the fruit buds, with an absolute minimum of -12.8°C, recorded in January, compared to -13.6°C multiannual average.
Both medium and absolute highs temperatures were (with a few exceptions) much higher compared to multi-year averages. It should be noted the high temperature regime in the winter months, when active temperature values of 11.6°C were recorded in December (compared to 5.7°C multiannual average) and 15.3°C in February 2016 (versus 14.7°C multiannual average).
The precipitation rate was very low in the winter months, when it was 0.2 mm in December compared to 44.5 mm in the multiannual average, but it became surplus in the spring months (87.6 mm versus 35.3 mm multiannual average recorded in March) and in July, favouring the development of cryptogamic diseases. In the warmest months (June, July and August) there were 81.8 mm, 70.2 mm and 78.2 mm.
The sum of average temperature °C for 2010-2016 ranged from -4.3°C in January to 134.7°C in September.
Compared to multiannual averages, the 2016-2017 wine years can be characterized as a year with reduced heliothermal resources on the background of rich water resources. The low heat regime recorded in April ($\Sigma°t_{actives}$ = 226.7°C) caused a late start (about 10 days) and uneven vegetation in vineyard, a delay that was not recovered in May and June.
The average annual air temperature is 14.5°C and the monthly average is negative only in January (-3.3°C). The 18.4°C blooming temperature, the average July temperature of 21.2°C, the average August maximum temperature of 31.6°C and the average air humidity in August at 1:00 p.m. of 46-48% indicates the presence of a relatively warm and drier climate. The active heat balance of the vegetation period records an average value of 3379°C and the thermal balance is 1779°C.
The precipitation from the vegetation period amounted to an average of 411.3 mm.
The high heliothermic regime in July, August and September, on a normal rainfall regime, even low in August and September, caused a good accumulation of sugars in grapes (Matei et al., 2009).

The development of the vegetation phenophases

Related to the climatic conditions of 2016 year, in Valea Calugareasca viticultural centre, it has been noticed that all the clones entered in vegetation at the end of March.
The beginning of budburst occurred on 30th March at Cabernet Sauvignon 30 Vl. and two days later in case of the other two clones. This phenophase ended in the second decade of the month of April, after sixteen days at Cabernet Sauvignon 30 Vl. and after fourteen days at the other two.
The two days difference was also observed in the case of blossoming, 50% of the inflorescences at Cabernet Sauvignon 30 Vl. being blossomed on 7th June, respectively on 9th June in case of the other two clones.
The ripening of the grapes started from 15th August. 50% of the grape clusters showed changes in colour on 21st August, date when was noticed the time of beginning of berry ripening (veraison), the same for the all clones. In comparison with Cabernet Sauvignon 30 Vl. which reached the maturation of the grapes at the end of September, the other two ended the maturation 6 - 7 days early, in the first decade of October.
Related to the climatic conditions of 2017 year, the budding occurred between 07 April and 27 April, 50% of the buds being in green shoot tip stage (stage C of Baggiolini, stage 7 to 9 of BBCH scale) (Baggiolini, 1952) were registered ten days later in comparison with 2016 year. Little differences were observed in the duration of budding and blossoming phases for the three evaluated clones. Difference was registered from ripening of grapes and harvest, full maturity of the berry being reached at the end of September for Cabernet Sauvignon 30

Vl. and seven days later in case of the other two clones.

Fertility and productivity

The high percentage of fertile branches in case of Cabernet Sauvignon 54 Mn. and Cabernet Sauvignon 131 St. ranged values between 70.90% and 77.08%, and Cabernet Sauvignon 30 Vl., with values higher than 82%. All three clones are characterized by medium fertility and productivity (Table 1).

Diseases tolerance

According to the OIV norms, the Cabernet Sauvignon clones presented a good level of resistance to Mildew and a very good level to the attack of *Oidium* and *Botrytis cinerea* (Table 2).

Table 1. The fertility and productivity of the Cabernet Sauvignon clones in the Valea Calugareasca vineyard

Clones	Fertile branches (%)		Relative fertility coefficient		Absolute fertility coefficient		Relative productivity coefficient		Absolute productivity coefficient	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Cabernet Sauvignon 30 Vl.	82.40	82.12	1.05	1.03	1.50	1.26	111.49	153.88	159.57	188.09
Cabernet Sauvignon 54 Mn.	70.90	71.80	1.07	0.98	1.50	1.37	140.14	131.52	196.85	188.69
Cabernet Sauvignon 131 St.	75.50	77.08	1.20	1.04	1.59	1.36	108.69	138.49	143.71	180.36

Table 2. Resistance to diseases at the Cabernet Sauvignon clones in Valea Calugareasca vineyard (years 2016 - 2017)

Clones	Diseases	Attack level (%)			
		Leaves		Grapes	
		2016	2017	2016	2017
Cabernet Sauvignon 30 Vl.	Mildew	1,05±0.1	1.8±0.1	0,94±0.1	1.4±0.1
	Oidium	0,88±0.1	0.8±0.1	0,72±0.1	0.9±0.1
	Botrytis	-	-	1,00±0.1	0.6±0.1
Cabernet Sauvignon 54 Mn.	Mildew	0,77±0.1	1.9±0.1	1,15±0.1	1.3±0.1
	Oidium	0,95±0.1	0.8±0.1	0,99±0.1	0.8±0.1
	Botrytis	-	-	0,98±0.1	0.5±0.1
Cabernet Sauvignon 131 St.	Mildew	0,92±0.1	1.8±0.1	1,30±0.1	1.5±0.2
	Oidium	0,9±0.16	0.9±0.1	0,76±0.1	0.7±0.1
	Botrytis	-	-	0,95±0.1	0.5±0.1

Grapes quality

As a result of the evolution of climatic factors in the maturation period, weight grains were 11.17% for the Cabernet Sauvignon 30 Vl. clone and a maximum of 45.95% at the clone Cabernet Sauvignon 131 St.

The degree of sugar accumulation was close to Cabernet Sauvignon clones (between 2.38 and 2.63 g L^{-1}/day). Acid metabolism was averaged 1.59-1.73 g L^{-1}/day.

Compared to the other two clones, the average weight of a cluster at Cabernet Sauvignon 30 Vl. was higher with 18.92% - 31.06% in the climatic conditions of 2016 year and with 11.04% - 13.11% in 2017. Cabernet Sauvignon 30 Vl. was distinguished by a greater average weight of the berry (126.92 g - 145.44 g) compared to Cabernet Sauvignon 54 Mn. and Cabernet Sauvignon 131 St. (88.11 g - 128.68 g) (Table 3).

The differences in berry weight had an effect on their structure (Table 4). At Cabernet Sauvignon 30 Vl. the skin ranged between, 16.4 - 18.6% and seeds between 4.2 - 4.7%, correlated with a higher content in pulp (77.2-78.9%).

Table 3. Mechanical analysis of grapes at harvest

Clone	Grape				Cluster		Berry	
	Weight (g)		Volume (ml)		Weight (g)		Weight (g)	
	2016	2017	2016	2017	2016	2017	2016	2017
Cabernet Sauvignon 30.Vl	130.97	149.40	120	136.67	4.05	3.97	126.92	145.44
Cabernet Sauvignon 54 Mn.	106.18	133.79	98.33	118.33	3.84	4.27	102.35	129.52
Cabernet Sauvignon 131 St.	90.29	132.91	78.33	118.33	2.18	4.23	88.11	128.68

Table 4. Berry structure

Clone	Skin (%)		Seeds (%)		Pulp (%)	
	2016	2017	2016	2017	2016	2017
Cabernet Sauvignon 30 Vl.	16.4	18.6	4.7	4.2	78.9	77.2
Cabernet Sauvignon 54 Mn.	18.4	22.2	5.1	4.2	76.4	73.5
Cabernet Sauvignon 131 St.	25.8	20.0	5.8	4.8	68.5	75.2

Chemical composition of the stum

The potential for the accumulation of sugars in the stum, a characteristic of the variety, influenced by the climatic factors of the grape maturation period, was very high at all genotypes, in terms of acidity and low pH (Table 5).Grapes from the vintage 2017 had better sugar accumulation potential due to favourable weather conditions during the ripening stage.

Analysis of the polyphenolic potential of grapes

From the point of view of the polyphenolic composition, the Cabernet Sauvignon 54 Mn. clone is evidenced by the total polyphenol index and the anthocyanin potential.

The lowest values were obtained for clone 131 St. (average data over the two years of study) (Figure 1).

Table 5 Chemical composition of the stum

Clones	Weight of 100 berries (g)		Volume 100 berries		Sugar (g/l)		Acidity (g/l H_2SO_4)		pH	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Cabernet Sauvignon 30 Vl.	110.86	120.84	100	105	245.9	223.6	3.28	3.61	3.22	2.17
Cabernet Sauvignon 54 Mn.	106.97	108.58	95	95	224.9	219.4	2.95	3.21	2.64	2.29
Cabernet Sauvignon 131 St.	106.68	129.81	95	115	227.9	227.9	2.99	3.36	2.62	2.20

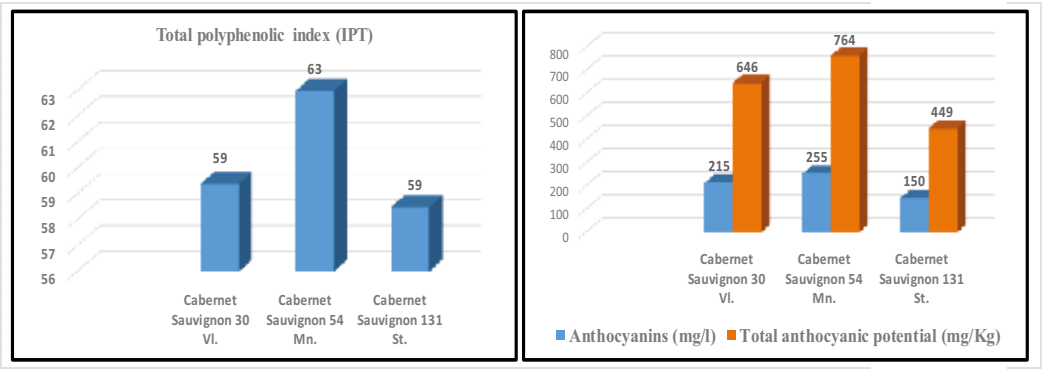

Figure 1. The polyphenolic potential of grapes (Average values 2016-2017)

The glucoacidimetric ratio records very high values of exceptional years (over 60%). The increase in this ratio is mainly due to the very low value of total acidity.

Between the degree of tolerance to cryptogamic diseases and the values of total polyphenol index (IPT) was recorded a very tight correlation.

The degree of mildew attack on grapes (Figure 2), with values ranging between 1.3% and 1.5%, influenced the value of the total polyphenolic index (IPT) of the grape must at harvesting by almost 80%, the value of the coefficient of determination R squared being 0.7961.

The intensity of the *Oidium* attack influenced the value of the total polyphenolic index to almost 90% ($R^2 = 0.898$) (Figure 3).

The degree of correlation shows a very high value in the case of the *Botrytis* attack (Figure 4), the value of the total polyphenolic index being determined almost entirely (99 %) by the frequency of the attack (Urucu, 2014).

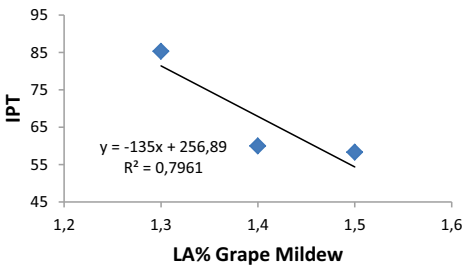

Figure 2. The Polyphenol Index (IPT) in Grape Mildew

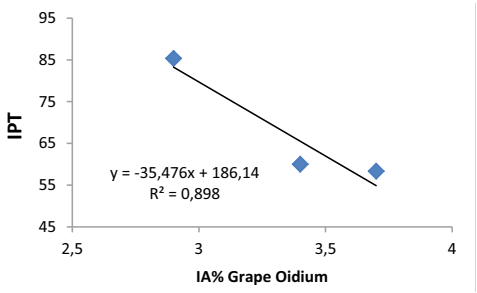

Figure 3. The Polyphenol Index (IPT) in *Oidium* attack

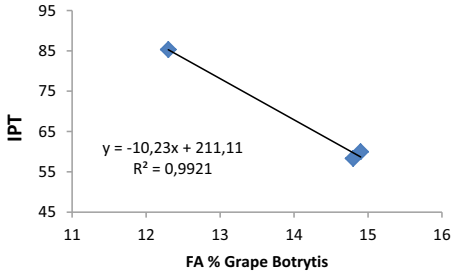

Figure 4. The Polyphenol Index (IPT) in *Botrytis* attack

CONCLUSIONS

The development of the vegetative phenophases depends on the climatic conditions during 2016 and 2017 harvest. Differences were registered concerning the maturation of the grapes which occurred 6-7 days later in case of Cabernet Sauvignon 54 Mn. and Cabernet Sauvignon 131 St.

Cabernet Sauvignon 30 Vl. highlighted by a greater weight of the cluster and berries.

The potential of sugar accumulation in the must was high in case of all clones, while the acidity and pH values were low.

Concerning the polyphenolic composition, Cabernet Sauvignon 54 Mn. was noted by total polyphenolic index (IPT) and Anthocyanin potential. The degree of correlation between polyphenolic index and frequency of the mains diseases attack shows a very high value in the case of the *Botrytis* attack (99%).

Further studies will be done concerning the quality of wines in order to establish the degree of maintenance of wines tipicity.

ACKNOWLEDGEMENTS

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TEMPERATURE AND RAINFALL INFLUENCE ON SHOOT LENGTH IN PINOT NOIR, MERLOT AND CABERNET SAUVIGNON VARIETIES

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Abstract

Climate changes from last decades influenced plants growth including grapevine. The objective of the research was to evaluate the influence of temperature and rainfall on shoot growth in Pinot Noir, Cabernet Sauvignon and Merlot varieties located in two vineyards, Recaş and Buziaş-Silagiu, during five years (2011-2015). The vines were trained on vertical trellis by bi-lateral cordon and vertical shoot positioned. For research were selected the main shoots and lateral shoots from 25 vines from each variety. Shoots length was measured from the base to the growing tip. Shoots cease growth earlier in dry years 2012 and 2015 compared with the wettest years 2013 and 2014. The warm weather and moderate rainfall in 2011 favor shoots growth in all three varieties and both locations. Measurements show significant differences between shoots length. The longest shoots were found in Cabernet Sauvignon variety in Buziaş-Silagiu vineyard and the shortest in Pinot Noir variety from Recaş vineyard. Results also show that vines subjected to water stress and high temperatures had shorter main and lateral shoots.

Key words: *grapevine, length, rainfall, shoots, temperature.*

INTRODUCTION

Leaf area from canopy have a major importance in photosynthesis and therefore in vine development, creating the microclimate for grape berry development and ripening (Andreini et al., 2009; Deloire A., 2009). An excessive shoot length and dense canopy influence negative the grape veraison and ripening by shading and increase the humidity (Fournioux, 1997; Keller, 2015). Dense canopy favours diseases as rots and mildew. Canopy includes the shoots, leaves and flowers respectively berry bunches (Petrie et al. 2000; Dobrei et al., 2016b). Vine canopy depends on grapevine variety, cane pruning, plant vigor, growing area and is described by number of shoots/trunk, length, width, height and leaf area (Dokoozlian and Kliewer, 1995). Shoots emerge in the spring from buds and grow fast before bloom due to the energy reserves from roots and then their growing slow down as grape berries develops (Lorenz et al., 2005; Dobrei et al., 2014). Usually in well-balanced vineyards, shoots growth stop near veraison; if the shoot growing continues the canopy will need more green pruning to improve berries development and ripening, including leaf removal (Kliewer and Dokoozlian, 2005). The vine shoot length is important because they have poorly developed mechanical tissues, and when they reach a certain length, more than 40-60 cm, can no longer sustain themselves, bends over, which favor the pests and diseases, and prevents further maintenance work in vineyards (Sánchez and Dokoozlian, 2005).

Most vineyards around the world are found in the strip drawn by parallels 30 and 50 in both northern and southern hemispheres (Keller et al., 2005). Within these areas, climate has a great influence on the development of vineyards (Santesteban et al., 2010). The vine is quite resistant to drought; works well in areas with annual rainfall of 500-650 mm, but like most plants, vines need a decent amount of water to survive and grow (Smart et al., 1991; Schultz and Matthews, 1988).

Each physiological phenomenon is conditioned by reaching a certain level of temperature and humidity that marks the beginning or end of a

biological stage (Hendrickson et al., 2004). Favorable temperature is correlated with a large amount of fruitful buds and more leaves (Gris et al., 2010). Both spring rainfall and higher temperatures greatly contribute to keeping the plant vigorous (Matthews et al., 1987).
The objective of the work was to evaluate the temperature and rainfall influence on main and lateral shoots growth in first three phenological stages in three grapewine varieties from Buzias-Silagiu and Recas vineyards, in the west of Romania area, during climate changing years 2011-2015.

MATERIALS AND METHODS

The experiment was carried out in two well-known vineyards from west of Romania: Recas and Buzias-Silagiu, during 2011-2015. All three varieties (Pinot Noir, Merlot and Cabernet Sauvignon) were trained on vertical trellis by bi-lateral cordon and vertical shoot positioned. In each variety were selected for measurements the main and two lateral shoots from 25 vines. Grapevine shoots monitoring begun each year in the last decade of April and ended in July, during all five years of experiment. Plant material was selected from vines of 6 year-old, planted on east-west orientated rows. Lateral shoots were selected from node positions 2-3. Shoots length was measured from the base to the growing tip, in the first three stages: before bloom, full bloom and fruit set, until the summer trimming. Measurements were made using a metric tape. In both vineyards the vine management was uniform. In recent years, the temperature increased from the beginning of the spring, while rainfall are very heavy or are very small quantity in summer; often very wet and hot years (like 2014) alternate with very warm and dry years. From one area to another, during the winter, cold days alternate with frosty periods. Climatic variability influences blooming, growth and grapevine development, grape production and its quality, through the late spring frosts, high summer temperatures, heavy rains or hailstorms.
In May 2011 the average temperature was 0.1°C above normal. From May 1-8, the weather was colder than usual. Monthly rainfall was 18% lower than normal. There were heavy rains (on May 24, 25 and 28) often associated with thunderstorms and hail. In June the average temperature was 1.1°C higher than normal. During June 19-24, the weather was very hot. Between 25 and 30 June the temperature dropped significantly, reaching below normal values for this period. In July the average temperature was 1.2°C higher than normal. From 9 to 20 July, the weather was very hot (Figure 1).

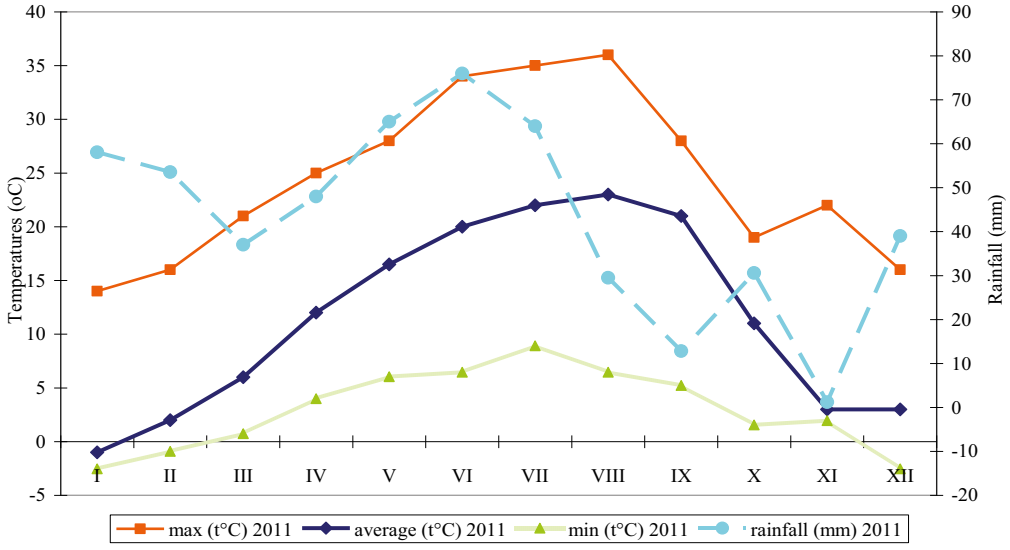

Figure 1. Temperatures and rainfall (2011)

Among 13-18 May 2012, the weather becomes very cold in the west. There were heavy rains, often thunderstorms and hail. In June, the average temperature was 3°C above normal. Monthly rainfall was 38% below standard normal. In July, the temperature was 4.5°C higher than normal standard. In 13-15 July and 28-30 July, the weather was very hot. Monthly rainfall was 48% below standard normal. May 2012 was characterized by a higher air temperature than normal. Often thunderstorms and hailstorms were recorded (Figure 2).

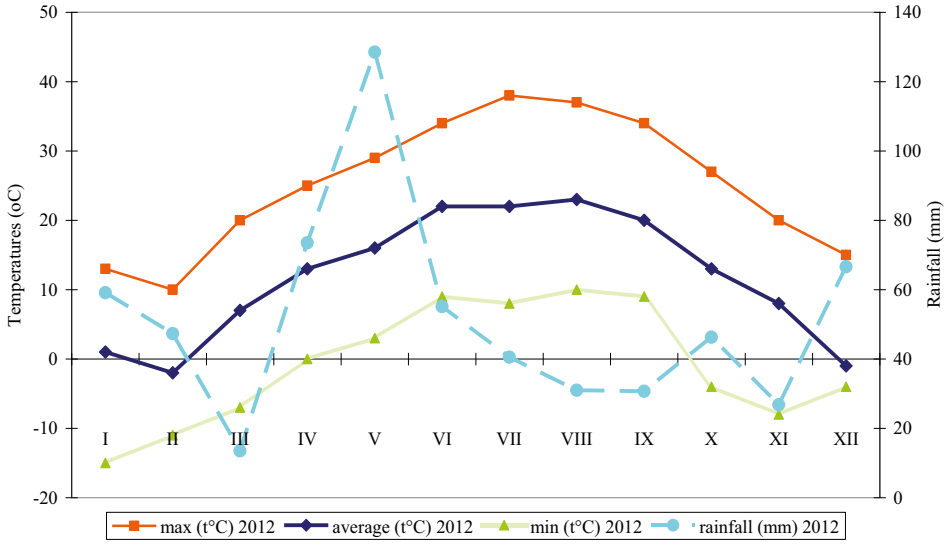

Figure 2. Temperatures and rainfall (2012)

The rainfall between 1st and 31 May (2013) was normal (26-50 l/m^2) in the north of Banat and around 51-100 l/m^2 in the other areas of the region. In June 2013 normal thermal days alternate with periods with higher air temperature than usual. The average daily air temperature values ranged from 17 to 29°C during the warmest periods. During June, there were rainy days, thunderstorms and hailstorms that partially/totally affected the vine by breaking canes and shoots (Figure 3).

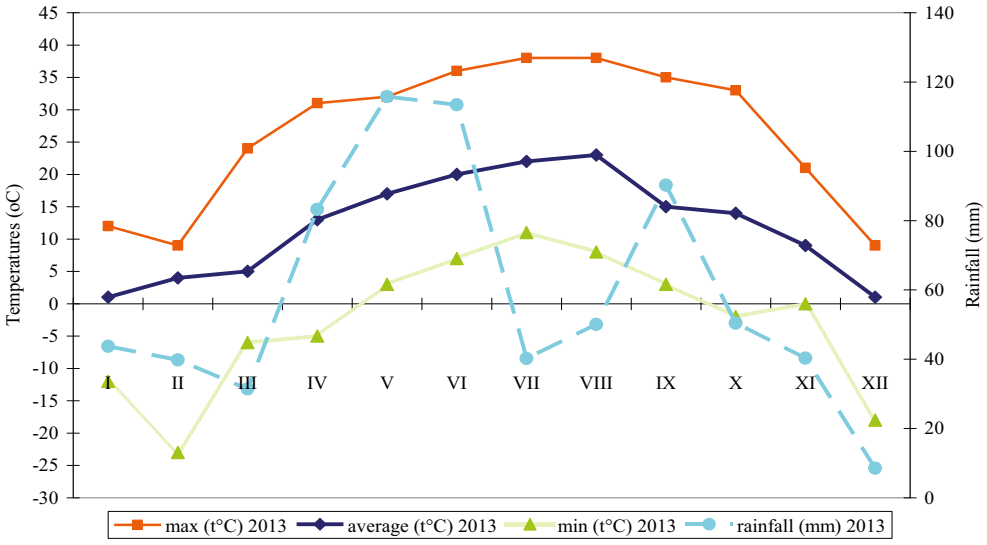

Figure 3. Temperatures and rainfall (2013)

In May 2014 normal days alternate with intervals in which the air temperature was lower than usual. Precipitations were among 51-100 l/m^2, in the south of Timis County, abundant (101-125 l/m^2) and even excessive (126-212 l/m^2) on large area in west of Romania. June 2014 had warmer days, with periods in which the air temperature was normal. During June 2014 heavy rainfall were recorded in large areas of the western part of the country, which led to an improvement of the soil moisture.

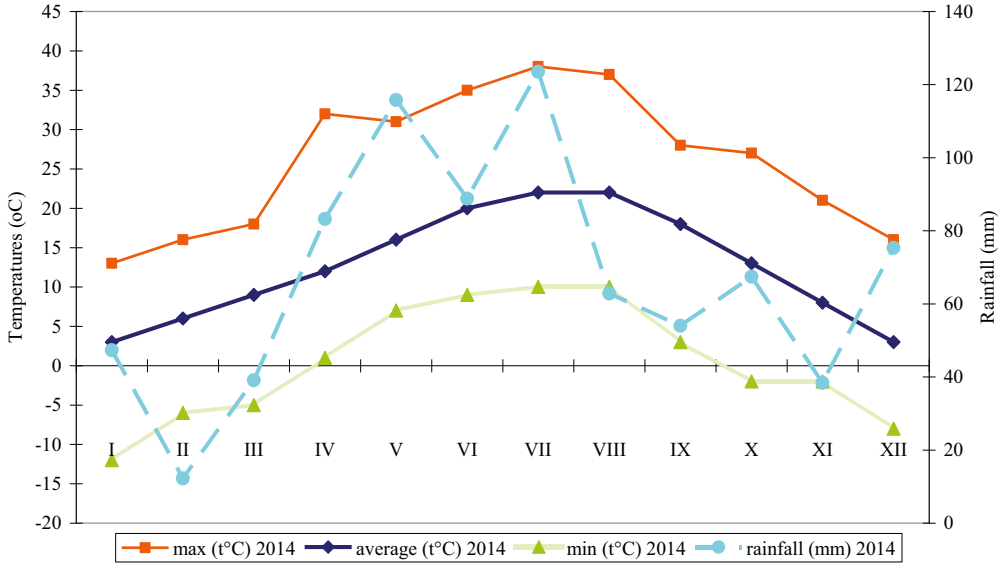

Figure 4. Temperatures and rainfall (2014)

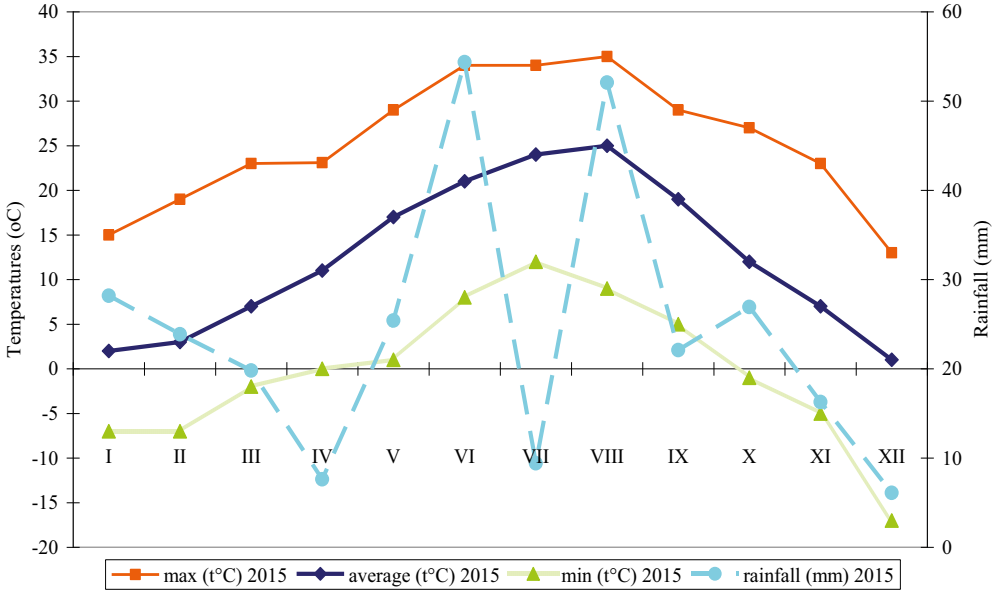

Figure 5. Temperature and rainfall (2015)

Quantities of water in the interval 01-30 June 2014 were reduced (12-50 l/m^2), in the western Timis County, normal (51-100 l/m^2) on extended surfaces and very high (01-150 l/m^2), in the south. Weather conditions were favorable for vine growth and development in the vine.

First decade of July 2014 was warm weather, after which the temperature increased gradually. The higher temperature ranged between 19 ... 35°C and the lowest between 10 ... 26°C. There were heavy rainfalls but also thunderstorms, and isolated hailstorms. The rainfall in the period 01-31 July 2014 was among 51-100 l/m^2, in the southwest and central of the Timis County. On extended areas rainfall were heavy (101-125 l/m^2) and over normal (126-241 l/m^2), which favored the downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*) (Figure 4). In May 2015, in the center of the region, normal (26-50 l/m^2), high (51-100 l/m^2), abundant (101-125 l/m^2) and excess rainfall (126-200 l/m^2) were reported on extended areas of Banat. Moisture in the 0-100 cm of soil at the end of May 2015, in Timis County, was low values. The summer season of 2015 was characterized by a warmer weather than normal. Between May and June, the amount of rainfall was deficient (< 150 l/m^2) (Figure 5). Data were subjected to statistical analysis using Statistica 13.0.159.7 software for Windows (One way ANOVA).

RESULTS AND DISCUSSION

Results concerning the length measurements of main shoots in Pinot Noir, Cabernet Sauvignon and Merlot varieties, from Buzias-Silagiu and Recas vineyards, among 2011 and 2015, are shown in Figure 6. In Pinot Noir the longest main shoot before bloom was in 2014 (27 cm) when were recorded daily temperatures of 31^{0}C and nights with no less than 7^{0}C. The same behavior was observed in the other two varieties, Cabernet Sauvignon (64 cm in 2014) and Merlot respectively (49 cm). The spring of 2015 (similar to 2011) was cool, with temperatures slightly below the average of the period, which delayed the blooming with about 7-10 days without major influence on the vine phenology. However the shortest main shoots before bloom, in all three varieties, were registered in 2015 followed by results from 2011.

Higher grow rates were observed before full bloom, followed by slow grow after fruit set due to the competition for nutrients and water among canopy and bunches (Figure 6). In full bloom stage, the longest main shoot was recorded in Cabernet Sauvignon (104 cm) in 2014. In fruit set stage, remains the same rank of main shoot length for all varieties. Young shoots were green, healthy and vigorous, and flower bunches equally distributed on the canes. In 2014 the constant rainfalls at regular intervals, alternating with sunny and low wind days helped to air the vineyard and decreased humidity around flowers.

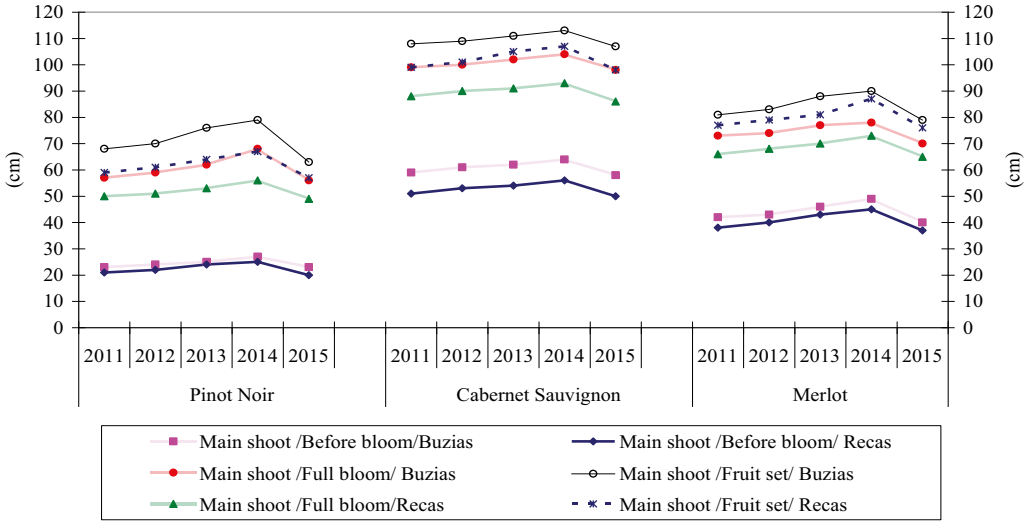

Figure 6. Main shoot length before bloom, full bloom and fruit set stages, in Pinot Noir, Cabernet Sauvignon and Merlot varieties, from Buzias -Silagiu and Recas vineyards (2011-2015)

Cheng et al. (2014), in a study concerning the influence of soil properties and climatic conditions of 2011 and 2012 years, on Cabernet Sauvignon variety, found that the average shoot length at harvest, in two vineyards from China, was between 122 and 136 cm, length that can be reached and exceeded by main shoots of Cabernet Sauvignon from Buzias-Silagiu and Recas until the harvest time.

Sabbatini and Schilder (2012) found in their research from Michigan vineyards, longest main shoot in Pinot Noir variety in all three stages: 67 cm (before bloom), 76 cm (full bloom) and 95 cm (fruit set) respectively. Reynolds and Naylor (1994) investigate Pinot Noir variety in a glasshouse situated in British Columbia, Canada.

Lateral shoots measured between 47 and 108 days after full bloom was among 6 and 143 cm. Lateral shoots from the Pinot Noir grown in both vineyards from the west of Romania are included in these limits and are much shorter. Schreiner et al. (2013), studied the impact of NPK supply on leaf area and shoot growth before bloom stage in pinot Noir variety. According to their research results, the shoot length was between 84-117 cm, and little influenced by different NPK supply.

Pinot Noir is a medium vigor variety and temperatures above 35^0C in 2014 and 2013 summer days slow the shoot growing due to the shutting down of the photosynthesis.

Cabernet Sauvignon has large vigor and medium fertility (65-70% fertile shoots). It has good tolerance to frost (-20, -22^0C), very resistant to drought, good tolerance to oidium, gray mold and rots (Dobrei et al., 2016b).

The vegetative vigour of Cabernet Sauvignon from high altitude vineyards of Southern Brazil was studied by Rufato et al. (2014) after application of prohexadione-calcium for reducing shoot growing. Despite the treatment, the main shoot final length range among 209.7 and 258.1 cm).

During 2006 -2009, Borghezan et al. (2012), evaluate shoot growth in Merlot and Cabernet Sauvignon, cultivated in São Joaquim vineyards from Brazil. They found out that the main shoots average length in both varieties was quite similar for around 130 days, until ripening stage. The final main shoot length was in average 3.22 m for Merlot variety and 2.90 m for Cabernet Sauvignon. Growing rate observed was 5.0 cm per week before blooming and around 25.0 cm per week after until grow decrease after fruit setting. In Leal G.R. (2007) studies, on Pinot Noir variety from New Zealand, main shoot final growth was 89.1 m.

Hunter and Visse (1990), in their research developed in South Africa, concerning the effect of defoliation on Cabernet Sauvignon growth, found longer lateral shoot length in berry set stage, amongst 63 - 91 cm, very significant longer compared to lateral shoots of Pinot Noir from Buzias-Silagiu or Recas vineyards. Longer lateral shoots are the result of partial defoliation which improved the light environment and leaves photosynthesis in the vine canopy (Dobrei et al., 2016c).

Merlot is a medium - large vigor. It is adapted to different soil and climate except arid areas. Variety resistance to frosts is low (-16°C - 18°C) and drought, and medium to diseases. Sprouting takes place early, and is consequently sensitive to spring frosts Unfavorable weather during flowering leads to millerandage (Dobrei et al., 2015).

Variety is also susceptible to mold (although it has a better mold resistance than other varieties) (Robinson J., 2003). It is a poorly tolerant to drought and frost-resistant (-18^0C) (Dobrei A., 2004). Similar results to those from Buzias-Silagiu and Recas vineyards, concerning the main shoot length in Merlot variety (from 37 to 90 cm) were found in two trials conducted during 1995-1998 in the Research station Agroscope Changins-Wädenswil from Switzerland.

Jemini et al. (2010) found a shoot length in Merlot variety affected by downy mildew, between 12.30 cm in the second decade of May and 81.99 cm after 30 days in June in the 1996 trial and between 25.91 and 78.59 in 1998.

Lateral shoots length before bloom, full bloom and fruit set stages, in Pinot Noir, Cabernet Sauvignon and Merlot varieties, from Buzias-Silagiu and Recas vineyards (2011-2015) are shown in Figure 7.

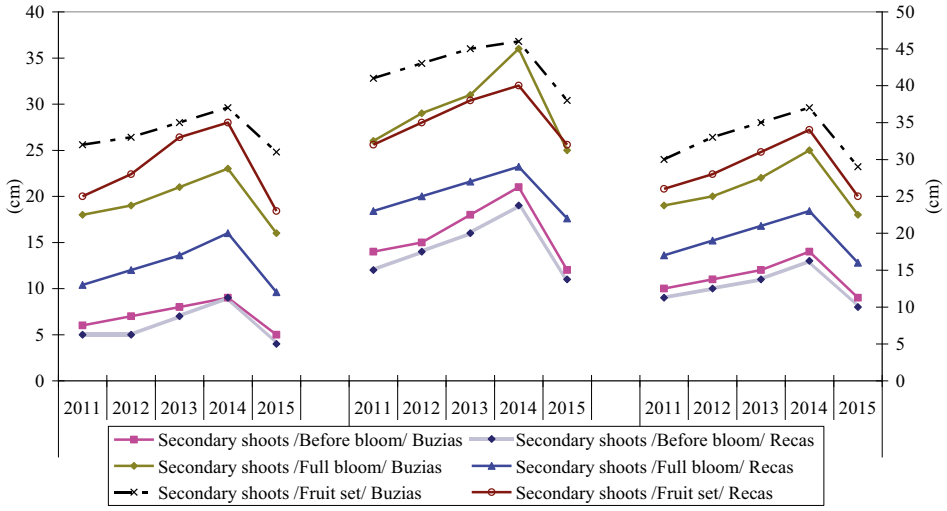

Figure 7. Secondary shoots length before bloom, full bloom and fruit set stages, in Pinot Noir, Cabernet sauvignon and Merlot varieties, from Buzias-Silagiu and Recas vineyards (2011-2015)

In the first stages of development, lateral shoots compete for nutrients and water with the other components of the vine, but after a 30 cm length they can be a source of nutrient compounds for the bunches. Lateral shoots length measured by Leal G.R. (2007) in Pinot Noir from New Zealand ranged in the same limits (5-10.4 cm) like those from Buzias-Silagiu and Recas.

CONCLUSIONS

Both low and very high temperatures influence the grapevine canopy, by decreasing shoot growth and photosynthesis. Pinot Noir had in 2015 the shortest main and lateral shoots because although it is a frost-resistant variety and drought, high humidity associated with low temperatures during the flowering (end of May) affects berries setting, significantly and influencing considerably the next harvest. The longest shoots were found in Cabernet Sauvignon variety in Buziaș-Silagiu vineyard and the shortest in Pinot Noir variety from Recaș vineyard. Although between the two vineyards there were not significant differences concerning the shoots length, Cabernet sauvignon had longer main and lateral shoots in each phenological stage. Results also show that vines subjected to water stress and high temperatures had shorter main and lateral shoots.

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VOLATILE PROFILE OF FETEASCĂ NEAGRĂ WINES OBTAINED IN MURFTALAR REGION AND THE INFLUENCE OF THE ORGANIC AND CLUSTER THINNING VITICULTURAL PRACTICES

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Abstract

'Feteasca neagră' is a versatile grape variety, with results highly dependent on the cultivation region and viticultural practices. In Murfatlar this variety is cultivated both organically and conventionally, thus providing a good opportunity to study the behaviour of the variety in the two cultivation systems. Furthermore, in both culture systems, in some experimental variants 30% of the grapes from each vine were removed after the cluster formation - a practice often employed with an aim to obtaining a better-quality crop. The grapes harvested in autumn at technological maturity were vinified in 4 variants, in accordance to the viticultural practices applied: organic and conventional cultivation, with or without cluster thinning. In this work the influences of these viticultural practices on the volatile profile of the obtained wines were evaluated by using a flash GC-electronic nose. Although some differences are present when 30% cluster thinning is applied, the main influence in the volatile profile of wines is still induced by the cultivation system, irrespective of the grape reduction practice. The variability among the volatile profiles of the 4 groups of wines is explained in proportion of 85.3% by the cultivation system and only 12.4% by the grape reduction practice. The wines made from conventionally grown grapes display a more complex volatile profile as compared to the wines obtained from organic grapes.

Key words: '*Fetească neagră', electronic nose, volatile profile, organic viticulture, cluster thinning.*

INTRODUCTION

'Fetească neagră' is a versatile grape variety, with results highly dependent on the cultivation region and viticultural practices. Considering the increasing consumption of organic wine and the constant demand for quality wines, it is logical that practices such as organic cultivation or the reduction of yield by cluster thinning practice should be taken into account. Due to the particularities of the Murfatlar region, 'Fetească neagră' finds here conditions to be cultivated not only by the conventional technology, but also organically. In previous studies (Ranca et al., 2010), 'Fetească neagră' of Murfatlar showed that it is suitable for organic cultivation, several good results being already reported (Artem et al, 2014a).

Also, to improve the concentration of the anthocyanins accumulated in the grapes, a special practice, used by other authors too (Bubola et al., 2011), was applied in the Murfatlar vineyards, consisting of the reduction of the clusters on each vine by 30%. While some improvement in polyphenolic quality was obtained by this cluster thinning (Artem et al., 2015), the organic cultivation also showed some increase in phenolic compounds important for health (Artem et al., 2014b) and wine ageing.

However, both practices are costly and should be applied only when the wines can be perceived by the consumers as being different. For this reason, comparing the volatile profiles with an electronic nose based on flash chromatography is an easy way of determining if the wines resulted from grapes produced with different viticulture technologies can be clearly discriminated.

Even though the taste of wine is not evaluated in this work, the volatile profiles determined can explain some of the effects induced by the viticulture technologies and could also be a basis for further analysis.

MATERIALS AND METHODS

In Murfatlar, 'Fetească neagră' variety is cultivated both organically and conventionally, thus providing a good opportunity to study the behaviour of the variety in the two cultivation systems. The technologies for the cultivation in both systems are those usually applied in the region and they were detailed elsewhere (Artem, 2017).

In order to assess if a better quality of grapes can be obtained by reducing the yield, in both culture systems, variants with 30% of the grape reduction were also produced. The grape thinning was performed on each vine, 30% of the clusters being removed after the cluster formation.

The grapes harvested in autumn at technological maturity were vinified in 4 variants, in accordance to the viticultural practices applied: organic and conventional cultivation, with or without cluster thinning.

The grapes obtained from conventional and organic growth system, both from vines in which grape thinning was practiced or not, were prepared in triplicate.

The resulted wines are coded as described in Table 1:

Table 1. 'Fetească neagră' wine samples

Wine code	Organic cultivation	Conventional cultivation	Grape thinning	Group Colour
FN_Eco_B1_M FN_Eco_B2_M FN_Eco_B3_M	x			light blue
FN_Eco_B1_R FN_Eco_B2_R FN_Eco_B3_R	x		x	dark blue
FN_Con_B1_M FN_Con_B2_M FN_Con_B3_M		x		light brown
FN_Con_B1_R FN_Con_B2_R FN_Con_B3_R		x	x	dark brown

The influences of these viticultural practices on the volatile profile of the obtained wines were evaluated by using a flash GC-electronic nose (Heracles I, Alpha-MOS, France) equipped with two short chromatographic columns of different polarities (1A: DB5 and 2A: DB1701). The equipment, software and the method applied are described in detail in previous papers (Antoce and Namolosanu, 2011; Antoce 2012a, b; Antoce, 2013; Antoce et al., 2015; Antoce and Cojocaru, 2017).

For the electronic nose analysis, 4 ml samples from each wine bottle were taken in 3 vials of 10 ml volume. Thus, each group of wines consisted in 9 analyzed samples.

RESULTS AND DISCUSSIONS

In order to separate the groups of wines based on some of the chromatographic peaks recorded by the electronic nose, from among all the peaks observed only some were selected, namely those with a discrimination power above 0.5 (more exactly from 0.835 down to 0.497). Also used were those peaks with lower overall discrimination power but automatically selected by the software of the apparatus as being of importance.

As a result, the four groups of wines were separated by the electronic nose based on the multivariate statistical analysis of the discriminant factors (Figure 1).

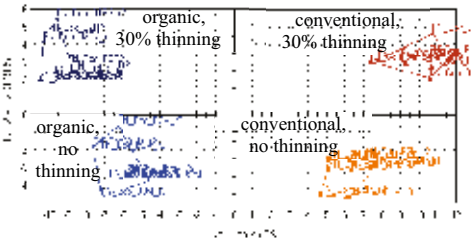

Figure 1. Discriminant Factor Analysis (DFA) diagram of the 'Fetească neagră' wine groups obtained from grapes with different cultivation technologies (light blue = organic cultivation; dark blue = organic cultivation and grape thinning, light brown = conventional cultivation; dark brown = conventional cultivation and grape thinning)

In Figure 1 it can be seen that the groups of wines were separated mostly on the basis of DF1 axis (responsible for 85.26% of the variability observed), which represents the cultivation system (organic towards the left side of the diagram and conventional - towards the right side of the diagram). The grape thinning accounted only for 12.38% of the total variability, but still, it can be said that this viticultural practice did have a specific influence on the resulted wines.

More specifically, the separation of the groups of wines from organic and conventional grapes, respectively, was determined by certain volatile compounds. In Figure 2, the vectors

corresponding to the selected discriminant chromatographic peaks are also included in the diagram and, as it can be seen, the loadings of most of these vectors on the horizontal axis tend to be higher for the wines from conventional grapes. This also suggests an increased aromatic complexity of those wine samples

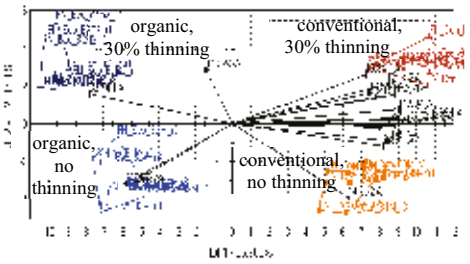

Figure 2. Discriminant Factor Analysis Diagram of the 'Fetească neagră' wine groups with the discriminant peaks (light blue = organic cultivation; dark blue = organic cultivation and grape thinning, light brown = conventional cultivation; dark brown = conventional cultivation and grape thinning)

With the use of the Arochembase software of the electronic-nose, some of the compounds corresponding to important discriminant peaks were identified.

Table 2 contains the volatile compounds identified for the peaks found to be discriminating for the wines obtained with grapes from the conventional culture with or without grape thinning.

As it can be seen, the specific discriminant volatile profile of the wines obtained from conventional grapes is quite complex, with several compounds conferring fruity aroma, with some green and floral notes too.

Although very close to the 'Fetească neagră' conventional group with 30% grape thinning, the group of wines from conventional grapes without yield reduction had fewer discriminant volatile compounds. With a 0.772 discrimination power the peak 14.32-2A (on DC1701 column) is the only one strongly correlated to conventional 'Fetească neagră' without grape reduction. The peak is close to 4-methoxy-2-methyl-2-butanethiol, which is a compound with aroma of black currant, one of the most specific flavours for this grape variety.

Table 2. Volatile compounds found to be discriminant for the wines obtained with 'Fetească neagră' grapes conventionally cultivated

Column	Peak (retention time)	Discri-mination Power	Compound identified	Type of aroma*
1A (DB5)	8.49	0.557	acetic acid	acid, fruit, pungent, sour, vinegar
1A (DB5)	8.99	0.556	not identified	
1A (DB5)	10.71	0.497	ethyl butyrate	apple, butter, cheese, pineapple, strawberry
1A (DB5)	11.90	0.612	2-methyl ethyl butyrate	apple, fruit, pineapple
1A (DB5)	15.94	0.633	3-hexen-1-ol	green
1A (DB5)	16.50	0.529	amino-benzaldehyde	-
1A (DB5)	18.69	0.835	3-hexenyl acetate	banana, candy, floral, green
1A (DB5)	19.49	0.667	ethyl hexanoate	apple peel, brandy, overripe fruit, pineapple
1A (DB5)	21.67	0.578	not identified	
1A (DB5)	23.91	0.518	2-isopropyl-3-methoxypyrazine	green pepper; bell pepper
2A (DB1701)	12.14	0.663	close to trans-3-hexen-1-ol	
2A (DB1701)	20.22	0.562	3-hexenyl acetate	banana, candy, floral, green
2A (DB1701)	22.44	0.758	2-phenyl-ethanol	fruit, honey, lilac, rose, wine

*The flavour profiles of the identified substances, included in the table to generally describe the possible expected aroma, are taken from Pubchem Open Chemistry database (https://pubchem.ncbi.nlm.nih.gov).

The volatile compounds mostly correlated to wines from organic culture are included in Table 3.

Table 3. Volatile compounds found to be discriminant for the wines obtained with 'Fetească neagră' grapes organically cultivated

Column	Peak (retention time)	Discrimi-nation Power	Compound identified	Type of aroma*
1A (DB5)	10.37	butyl acetate	0.559	apple, banana, glue, pungent
1A (DB5)	32.74	ethyl decanoate	0.086	brandy, grape, pear
2A (DB1701)	11.32	trans-3-hexen-1-ol	0.271	green
2A (DB1701)	13.87	ethyl-2-methyl-butyrate	0.559	apple, ester, green apple, kiwi, strawberry

Basically, the influence of the grape thinning on the wines form organic grapes are not very much discriminated. However, with a 0.521 discrimination power the peak 32.74-1A (on column BD5) is strongly correlated to FN organic without grape thinning. The peak is close to ethyl-decanoate, which mostly confer general, non-specific wine/grape aroma.

Overall, the discriminant peaks can differentiate the groups of wine by statistical analysis, but the fingerprint of the wines, consisting of the peaks with their height or area, are not very different. Figure 3 shows that the fingerprints of the wines are quite similar, and especially the wines from grapes where cluster thinning was performed are not easily differentiated from those produced without thinning. However, the samples from organic grapes (blue colour on the diagram) have clear different fingerprints from those from conventionally cultivated grapes (brown colour on the diagram), irrespective of the application of cluster thinning. To verify if the cluster thinning practice caused a significant influence on the volatile profile of the final wines, the distances between the groups of wines were calculated in odor units. Figure 4 shows that the groups from grapes organically cultivated (blue) are relatively separated from those from grapes conventionally cultivated (brown), but inside each type of technology, the odor distances between samples from grapes with and without cluster thinning is not significant (groups of light and dark wine samples of the same colour overlap).

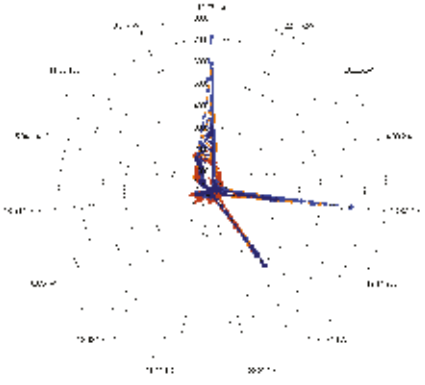

Figure 3. 'Fetească neagră' wines fingerprints based on the height of the discriminant chromatographic peaks (blue = samples from grapes organically cultivated, brown = samples grapes conventionally cultivated)

Thus, it may be safely said that the cluster thinning techniques applied in the vineyards for 'Fetească neagră' do not sufficiently affect the volatile profile of the final wines, so that the consumer will most likely not be able to perceive a clear difference in the aroma.

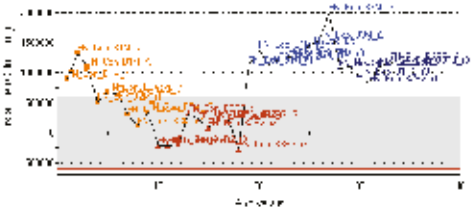

Figure 4. Odor distances of 'Fetească neagră' wines groups produced with grapes from different viticultural technologies (blue = wines from grapes organically cultivated, brown = wines grapes conventionally cultivated)

Aside of the chemical composition of the grapes, the winemaking and the post-fermentative evolution are also influencing the final wine profile. For example, the discrimination of the wine groups was initially performed based on all the peaks with a discrimination power over 0.500. However, by sensory and chemical analysis some samples, but not all, were found to have slightly higher volatile acidity. To eliminate this bias from the discrimination analysis, the statistical DFA analysis was repeated without the peaks related to acetic acid (volatile acidity found at retention times 8.49 and 8.99).

Figure 5 (a and b), obtained based on all peaks with a discrimination power over 0.500, except those for volatile acidity, shows that the groups of wines are separated even better than earlier, still most of the variability being explained by the culture system (DF1 = 82.22%), while the grape thinning accounted for 15.9% (DF2) of the total variability. This slight increase in DF2 (from 12.38% to 15.9%) means that without the volatile acidity which developed only in some of the samples after winemaking, the differences between samples with or without cluster thinning should be a bit more evident. However, the discriminant peaks (Figure 5. b) are not modified, the compounds and their importance in discrimination remaining the same as described in the tables above.

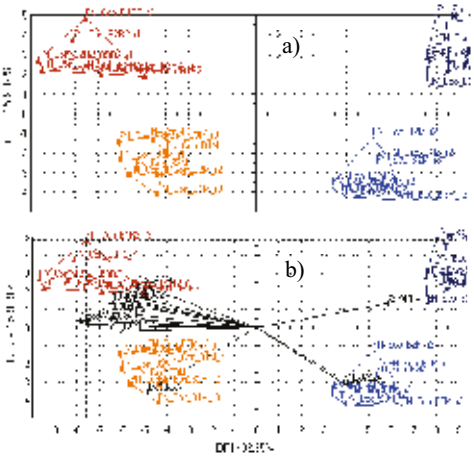

Figure 5. Discriminant Factor Analysis Diagram of the 'Fetească neagră' wine groups after the elimination of bias induced by volatile acidity

CONCLUSIONS

This work shows that the flash GC-electronic nose can distinguish wines produced from grapes obtained by different viticultural practices, simply on the basis of their volatile profile. Although some differences are present when 30% cluster thinning is applied, the main influence in the volatile profile of wines is still induced by the cultivation system, irrespective of the grape reduction practice. The variability among the volatile profiles of the 4 groups of wines is explained in proportion of 85.3% by the cultivation system and only 12.4% by the grape reduction practice (these figures change, but only slightly, when the peaks corresponding to volatile acidity are excluded from analysis). For the particular case of 'Fetească neagră' produced in Murfatlar region, the wines made from conventionally grown grapes display a more complex volatile profile as compared to the wines obtained from organic grapes.

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RESEARCHES REGARDING QUALITY AND QUANTITATIV PERFORMANCE OF SOME TABLE GRAPES IN THE EXPERIMENTAL FIELD OF USAMV BUCHAREST

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Abstract

The main processes of fecundation and pollination on vine are largely influenced by the genetic profile of the variety, external factors involved like - temperature, precipitation, insolation, relative humidity and also by special crop techonology applied. The aim of this research was to study some of the most grown table grapes varieties in Romania (and also worldwide), varieties which belong to a the same kin group - 'Muscat Hamburg' cv., 'Muscat d'Adda' cv., 'Afuz Ali' cv. and 'Victoria' cv. For example, 'Muscat d'Adda' cv. has been obtained by self-pollination of 'Muscat Hamburg' variety and 'Victoria' variety has been obtained by crossing of two cultivars Cardinal and 'Afuz Ali'. The results showed that the quantity and qualitative performance of these varieties, expressed by productive indices, carpometric values of grapes and berry and also organoleptic qualities has been influenced both by special crop technology (isolation of inflorescences and pinching fertile shoots a few days before blooming) and by environmental factors recorded during the study. In the study, has been obtained higher average values of the most analyzed indicators and the best results have been recorded by: 'Muscat Hamburg' cv., 'Muscat d'Adda' cv., 'Afuz Ali' and lesser, but also in positive limits, 'Victoria' variety.

Key words*: cluster, inflorescences, phenology, table grape, yield.*

INTRODUCTION

It is well known that the quantitative and qualitative parameters of the harvested crops are influenced by the environmental factors, like the soil type, the technology applied, the genetic nature of the varieties, but from all these factors, the greatest impact has the climate, (Jones, 2005; Cleland et. al., 2007; Gladstones, 2011; Shinomiya et al., 2015; Van Leeuwen and Darriet, 2016). Practically, in the most wine-growing areas, there are recorded more and more changes in the climate as a results of the high average temperatures, due to low precipitations followed by extreme events (Tomasi et al., 2017). Even though, the annual sequence of phenological stages of grapevine is commonly observed to be accelerated with an increase in temperature (Duchêne and Schneider, 2005; Duchêne et al., 2010; Parker et al., 2013; Blanco-Ward et al., 2017), the annual cycle of the vine, the flowering, fruit set and also the ripening of the grapes depends very much on the factors mentioned above.

Due to unfavourable conditions during flowering and the way the pollination process has been carried out, the percentage of fruit growth will be different and the fruit set will have a different evolution developing the millerandage phenomenon (Dobrei et al., 2005). This phenomenon is quite common on the grape varieties that require foreign pollen, like 'Muscat Hamburg', due to the low pollen germination and in this case the growth of berries it stops at 6-7 mm, due to the lack of hormone substance, this it may also occur in grapes varieties that develop incompatibility of its own pollen (Constantinescu et al., 1959; Lepadatu, 1979; Stroe et al, 2014). After this phenomen occurs, the berries start ripening earlier and gain higher quantities of sugar, but, at the time of harvesting, the quality of grapes is shortcoming, especially in table grapes varieties. Even more, various studies have shown that gaining excessive sugar, it is not

followed anymore by an optimal polyphenolic and aromatic ripening; the intake of grapes is alterated; in fact, the difference between aromatic and phenolic content and tehnological ripening (ratio between sugars/acids) is always higher. This may lead to the conclusion that obtaining well-balanced yield in terms of quantitative and qualitative parameters is a constant challenge for the viticulturist. Based on this consideration, the aim of this study has been to analyze the evolution and influence of climatic factors in 2017, in a critical period for the vine (20 May - 20 June), in conjunction with 2 special cultural techniques - the isolation of the inflorescences and pinch of the growth peak of fertile shoots on table grapes. The result of the experience has been analyzed also by quality parametres of the grapevine products (yield) influenced by this factors.

MATERIALS AND METHODS

2.1. Plant material and growth conditions

Four grape varieties were taken in the study: 'Muscat Hamburg' cv., 'Muscat d'Adda' cv., 'Afuz Ali' cv., from the world collection and 'Victoria' cv., variety obtained in Romania in 1978 by 'Victoria' Lepădatu and Gh. Condei (Table 1). The main updated data on these varieties can be found in the Vitis International Variety Catalog (www.vivc.de). In Romania, the first three listed varieties occupies the largest areas, although all three varieties have proven over the years to be sensitive to temperature and humidity factors during flowering phenophase. Grape table varieties are located in the experimental field of the ampelographic collection from the University of Agronomic Sciences and Veterinary Medicine of Bucharest. During the study, has been made basic observations and determinations, commonly used in the current technology, but attention was directed to the carpometric elements that define the productive and qualitative potential: average weight of a grape (g), average weight of a berries (g), yield (kg/vine), ^{0}Brix, (refractometric), total acidity (g tartaric acid/L by titration), sugar-acidity indices.

2.2. Phenological and temperature data

During the study, meteorological parameters (temperature, precipitation, relative humidity) have been analyzed during 20 may - 20 june 2017, using the daily average from 6 hourly data (05:00, 08:00, 11:00, 14:00, 17:00, 20:00, 23:00) registered at Bucharest - Baneasa, Romania. The forecast was performed over a longer period just to ensure a better accuracy of the results, although the flowering phenophase of the studied varieties lasted 12 days (29 May-10 June 2017). For the acknowledgement of the flowering phenophase, the study used the updated version of the universal scale for the description of Monocots and Dicotyledons numbered 00-97, with a special look at the main growth stage 6: Flowering, stages 61 and 67 and main growth stage 7: Development of fruits, 71 and 77 (Pierot and Rochard, 2013). Three observations have been made at different growing stage (Table 1), taking into account all the inflorescences on 6 vines but the isolation of the inflorescences in waxed paper bags and pinching fertile shoots were applied on three vine a few days before flowering for each variety, resulting in a total of 8 experimental variants. Harvesting of the grapes has been carried out at the full ripening stage of each variety.

RESULTS AND DISCUSSIONS

The studied varieties have reacted differently, indifferent by cultural applied techniques, in terms of the percentage of fruit set, the percentage of shaking of the formed flowers and the manifestation of millerandage phenomenon. The distinct character of the wine year 2017 in terms of climatic peculiarities led to an early age in terms of flowering onset (Cleland et al., 2007; Stroe et al., 2017), noting that all analyzed varieties bloomed at the end of the third decade of May. The phenophase period has been lasting for approximately 12 days (29 May-10 June 2017), with a gap of 2-3 days between varieties, as follows: on 'Muscat d'Adda' cv. and 'Afuz Ali' cv., blooming started on May 29, and at 'Victoria' cv. started later in two days. During the period of interest, the daily average temperature values have had significant fluctuations (Figure 1), but the 18.59ºC average, was even lower than the optimum of varieties in the first decade of May, only 15.5ºC, but in full flowering the value was

19.84ºC, even recording a ceiling of 20ºC between 3rd and 8th of June.

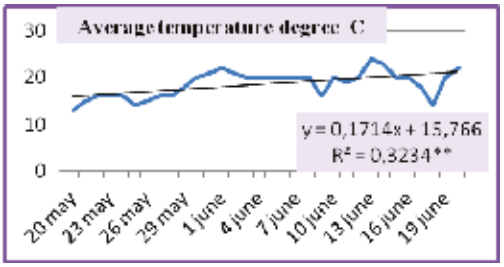

Figure 1. Evolution of the average temperature (°C) for flowering stage

In this matter, it can be seen that in the period mentioned above, the first three varieties were in the process of flowering, being known in the viticultural practice that a percentage of 20-30% of the total flowers bloom in the first 2-3 days of flowering, in the next 3-4 days bloom 60-70% and only a small percentage of flowers open at the end of the phenophase.

One important note is that the temperature at the end of phenophase for 'Victoria' variety was only 16°C (June 9), and this resulted in forming of a larger number of uneven berries, whatever experimental variant had analyzed. Figure 2 analysis the relative humidity values and their impact to the studied varieties. The average value had been 67.25% and the highest was 91% registered in the first decade of May. Practically, optimal values, close to the normal blooming range, 55-65%, had been registered on 26th of May.

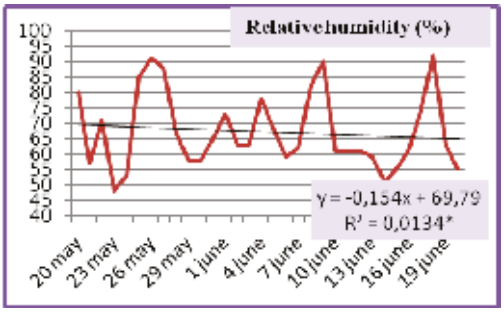

Figure 2. Evolution of the average relative humidity (%) for the period 20 may-20 june 2017

In the same period mention above, rainfall records a value of 37 mm, and in full flowering process, 24 mm (Figure 3).

Regarding to the special vine techniques - the isolation of the inflorescences and the pinch of the growth peak of fertile shoots - applied on

Table 1. The genetic origin of studied varieties

Prime name	Muscat Hamburg	Muscat d'Adda	Afuz Ali	'Victoria'
Variety number VIVC	8226	8050	122	13031
Country of origin of the variety	U. K.	Italy	Liban	România
Species	*Vitis vinifera L.*	*Vitis vinifera* L.	*Vitis vinifera* L.	*Vitis vinifera* L.
Pedigree as given by breeder/bibliography	Trollinger x Muscat Alexandria	Muscat Hamburg SP	-	-
Pedigree confirmed by markers	Schiava grossa x Muscat Alexandria	-	-	Cardinal x 'Afuz Ali'
Prime name of pedigree parent 1	Schiava grossa	-	-	Cardinal
Prime name of pedigree parent 2	Muscat Alexandria	-	-	'Afuz Ali'
Year of crossing	1850	-	-	1964
Last update	18.01.2018		18.01.2018	18.01.2018
Principal growth stage 6: Flowering				
61: Beginning of flowering: 10% of flower hoods fallen				
67: 70% of flowerhoods fallen				
Principal growth stage 7: Development of fruits				
71: Fruit set: young fruits begin to swell, remains of flowers lost				
77: Berries beginning to touch (if bunch are tight) I observation (31.05.2017) 70% of flowerhoods fallen; II observation (10.06.2017) Small-berry grape only formats; III observation (01.07.2017) Berries beginning to touch				
Muscat Hamburg	**'Muscat d'Adda'**	**'Afuz Ali'**	**'Victoria'**	
Isolation of the inflorescences + pinching fertile shoots 'Muscat Hamburg' (a)	Isolation of the inflorescences + pinching fertile shoots 'Muscat d'Adda' (a)	Isolation of the inflorescences + pinching fertile shoots 'Afuz Ali' (a)	Isolation of the inflorescences + pinching fertile shoots 'Victoria' (a)	
Pollination open 'Muscat Hamburg' (b)	Pollination open 'Muscat d'Adda' (b)	Pollination open 'Afuz Ali' (b)	Pollination open 'Victoria' (b)	

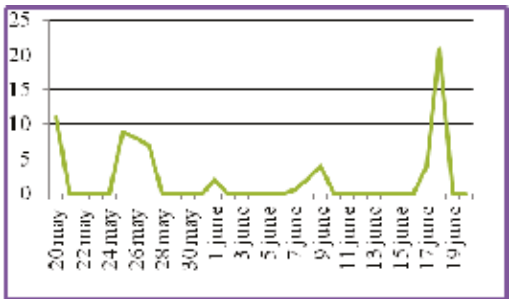

Figure 3. Evolution of the rainfall (%)
for the period 20 may-20 june 2017

27th of May 2017 for all experimental variants, in order to observe the percentage of berries formed (fruit set), self-pollination and open pollination, on one hand, and the uniformity of their size, on the other hand, it was observed that the percentage of berries binding (fruit set) varies from one species to another (Figure 4a), as follows: 'Muscat d'Adda' varieties and 'Muscat Hamburg' recorded the lowest percentage (21% and 23%, respectively), followed by 'Afuz Ali' (31%) and the highest percentage of berries formed by the 'Victoria' variety (48%), in the case of self-pollination and pinching fertile (a).

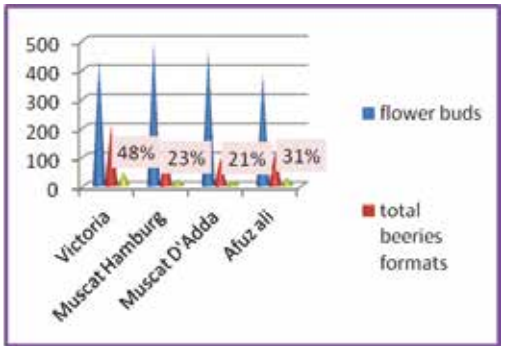

Figure 4a. Evolution of fruit set (a)

In the case of open pollination, a similar evolution had been observed, but the percentages registered a higher value for all four grape varieties (Figure 4b).
This shows that some varieties, even if they have normal functional hermaphrodite flowers, they have proved to have incompatibility with their own pollen (Lepadatu, 1979) as seen in the 'Afuz Ali' variety.

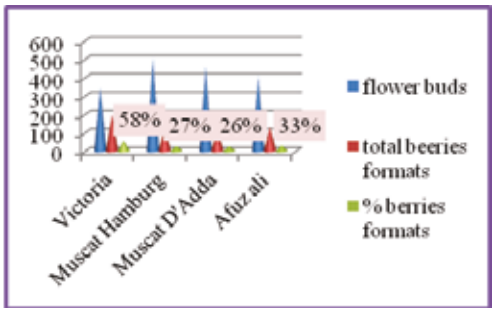

Figure 4b. Evolution of fruit set (b)

Figures 5a, 5b provide details of the size categories in which the berries are engraved, expressed as a percentage, and it was noticed that 'Muscat Hamburg' had developed very high percentage of normal berries (76.1%), followed by the 'Muscat d'Adda' (74%) and 'Afuz Ali' (71.1%), and the lowest percentage of normal berries was registered by 'Victoria' variety with only 55.5%, when the two cultural techniques were applied.

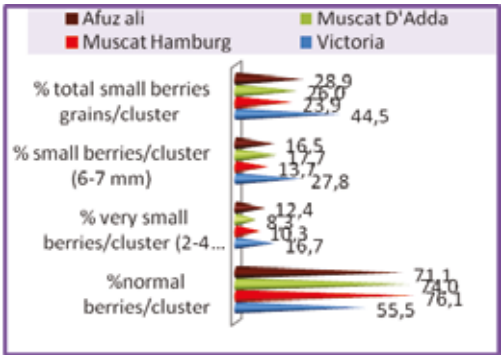

Figure 5a. Evolution of the categories
of grains formed (a)

For experimental variants with open pollination, the same things have been noticed, but with slightly higher values. Interesting and also surprising is that although 'Afuz Ali' and 'Victoria', for the two experimental variants, had the highest percentage of berries formed (fruit set), the proportion of grapes with a very small berries (2-4 mm millet grain size) and small berries (6-7 mm pea size) was more pronounced. Thus, the 'Victoria' variety had recorded 44% non-uniform berries in the case of the isolation of the inflorescences and 42% in the case of open pollination.

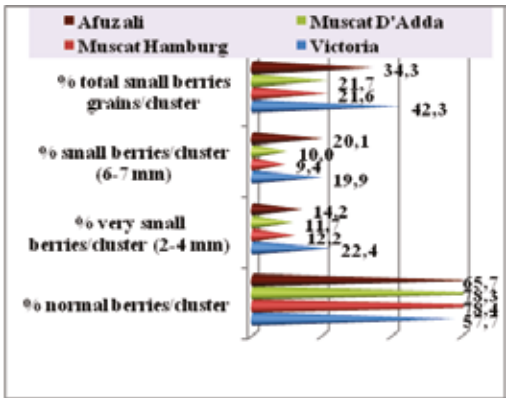

Figure 5b. Evolution of the categories of berries formed (b)

Variety 'Afuz Ali' had recorded smaller values, but different in both studied variants, a precentage of 29% in the isolation of the inflorescence and 34% in open pollination. This things indicates that it had been influenced by the climatic conditions on the last days of flowering phenophase when temperature recorded was 16ºC. The results obtained at this stage of development (flowering) are outlined in the quantitative and qualitative parameters of the obtained products (Table 2), reaching a high productive potential due to the large grape sizes, whose values are within the performance limits of the varieties analyzed. Regarding the quality, which is appreciated by the size of the berries and their uniformity, the data show at least for the 'Victoria' variety that the remaining berries are large, uniform in size, especially for grapes of the 'Victoria' variant (a) were the applied technology resulted in notable production increases (Paolicelli M. et al., 2013). To the other three varieties exposed to free pollination were found a similar evolution, with greater uniformity and bigger berries. (Rolle L. et al., 2015). The total content of sugars at full ripening expressed by °Brix or Total Soluble Solids shows that the varieties obtain optimal quantities, given by factors such as the gene of the variety, ripening period and also by the footprint of the climate factors, the values varies between 14.12 ^{0}Brix - 'Victoria' (s) and 22,13 ^{0}Brix - 'Muscat Hamburg' (b). In general, the values recorded are within the limits specified by O.I.V. and CODEX STAN 255 (2007, 2008b). According to CODEX STAN 255 (2007), table grapes can be harvested when the refractometric index reaches at least 16 ^{0}Brix. Grapes with a lower refractometric index are accepted provided the sugar/acid ratio (Total Soluble Solids/Tritatable Acidity – TSS/TA) is at least equal to 20:1 if the Brix level is comprised between 12.5 ° and 14 °Brix, 18:1 if the Brix level is comprised between 14° and 16 ^{0}Brix. Some table grapes, like the varieties chosen in this study can accumulate TSS higher than 16 °Brix, with low levels of acidity, thus leading to a TSS/AT ratio even greater than 30:1, (Antonacci et al., 2017). Some table grapes, as in the present case, can accumulate TSS higher than 16 ° Brix with low levels of acidity, leading to a TSS/AT ratio even greater than 30: 1.The concentrated values of sugars are followed by an acidity that gives the varieties balanced sugar-acidity indices, the highest values being recorded in 'Afuz Ali' (b) 5.52, and the lowest value for 'Victoria' 3.61, 'Victoria' variant (a).

CONCLUSIONS

The special cultural techniques for table grape varieties have a positive impact on quantity and quality parameters, but all of them on the background of a set of generous climatic factors, recorded in critical phenophase of vine (flowering, ripening). In all experimental variants, the quantities of sugar accumulated had been adequate, being in correlation with acidity, ensuring in this balanced sugar-acidity indices and also in line with the standards demanded by the consumer in the market.

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Table 2. Evolution of quality parameters on the experimental varieties

Experimental Varieties		^{0}Brix (as Total Soluble Solids, g/L)	Total acidity (g tartaric acid/L)	Gluco-acidometric index	Average weight of a berries (g)	Average weight of a grape (g)	Yield (kg/vine)
'Victoria'	a	12.68 109.87	3.51	3.61	6.44	889.47	9.33
	b	**14.12** 124.74	3.63	3.89	5.0	673.1	7.06
'Muscat Hamburg'	a	18.66 172.59	5.04	3.70	2.96	460.42	14.04
	b	**22.13** 209.7	4.65	4.76	3.34	364.06	8.00
Muscat d.Adda	a	19.22 178.9	5.3	3.63	3.97	502.23	11.80
	b	19.6 183.15	5.1	3.84	4.61	433.34	6.39
'Afuz Ali'	a	18.26 168.28	3.67	4.98	4.20	432.65	5.98
	b	20.7 194.83	3.75	5.52	5.55	447.88	6.19

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INFLUENCES OF GRAPE CULTIVATION TECHNOLOGY ON CERTAIN AROMA COMPOUNDS SIGNIFICANT FOR THE DIFFERENTIATION OF 'CABERNET SAUVIGNON' AND 'FETEASCĂ NEAGRĂ' WINES

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Abstract

Red wine grape varieties 'Feteasca neagră' and 'Cabernet Sauvignon' were produced in Murfatlar wine region under organic and conventional viticultural systems. For both varieties and viticultural systems, 30% cluster thinning was performed in some variants during summer, at the beginning of veraison. The resulted grapes, harvested in the autumn of 2014, were vinified using the same winemaking protocol and the differences in aromatic profile of the wine samples were assessed by using a GC e-nose from Alpha MOS. The chromatographic peaks were recorded and analysed with Alpha Soft ver. 12.42 and AroChemBase. The discrimination of the samples was based on multivariate DFA statistical analysis. The results showed that 30% cluster thinning did not influence significantly the major wine aroma compounds, only small differences being observed in the DFA diagram for samples with and without cluster thinning. However, the grape variety and the application of organic cultivation technology led to a good discrimination, with a validation score of 92, of the samples on the DFA diagram. An even better discrimination, with a validation score of 96, was observed on wine samples from grapes produced in conventional and organic system when the cluster thinning was disregarded and samples with or without this intervention in the vineyard were included in the same group. These results show that, as far as the aromatic profile is concerned, the wines produced from grapes to which cluster thinning was applied are difficult to differentiate from the controls produced from vines with normal yield, even with a sensitive equipment such as an e-nose. On the other hand, the organic and conventional growing led to wine samples easily differentiated by the electronic nose, suggesting that these differences can also be perceived by consumers. Some of the aroma compounds significant for the discrimination of wines produced from organic or conventional grapes were identified and their expected sensory effect is discussed.

Key words*: 'Feteasca neagră', 'Cabernet Sauvignon', electronic nose, aroma profile, organic viticulture, cluster thinning.*

INTRODUCTION

The global quality of wine aroma is well related with grape-growing conditions and healthy grapes, along with the winemaking technology involved, being an important attribute for consumers buying decisions and a challenge for the winemakers. The primary aroma of wines is derived from grapes and implicitly from cultivation technology, along with climate and soil variables, while the secondary aroma is related to the winemaking processing techniques and enzymatic transformations of grape compounds by microorganisms, especially during alcoholic fermentation. Viticultural practices and ecosystem factors influence the expression of certain genes involved in production of aroma compounds and precursors in grapes. Limited soil fertility influences the ripening process favouring aroma maturation and lowering the vegetative growth, which in turn favours the formation of flavours (Jackson, 2008). On the other hand, an increased potassium content in soil may lead to an increase in C_{13} norisoprenoids in grapes and of certain acetate esters produced during fermentation in wines (Falcao et al., 2008). Type of soil and drainage show an influence on the aroma composition of grapes and in the resulted wines, such as metoxypyrazines decrease in grapes on gravelly soil (Ribéreau-Gayon et al., 2006), more floral, sweet and fruity aromas develop in wines derived from clay soils, solvent-like and green notes appear in wines derived from sandy soils (Gómez-Míguez et al., 2007) while more terpenoids, sesquiterpenoids, and C_{13} norisoprenoids are found in wines derived from calcareous soils (Coelho et al., 2009). However, the type of soil and drainage, directly

influence the soil temperature which appear to influence aroma compounds in wines with the highest accumulation of *β*-damascenone and geraniol in warmer soils and conversely, a lower accumulation *β*-damascenone but higher accumulation in *β*-ionone in cold soils. Dry weather at the end of summer improves fruit quality through the advance of ripening and favours the flavour formation (Jackson, 2008), while the temperature influences the development of C_6 aldehydes in cool regions and higher concentrations of monoterpenes in warmer regions (Ji and Dami, 2008). Excessive sunlight exposure or partially shading of grape clusters modify the terpenol contents. In Muscat cultivars, the highest concentrations of terpenols were found in artificially 50% shade (Belancic et al., 1997), while a 90% shade decreased the terpenols and C_{13} norisoprenoids (Bureau et al., 2000a, 2000b). The concentration of TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) increased with sunlight exposure in Riesling (Gerdes et al., 2002), while in the other study on Shyrah, an extreme shading lead to a decrease in concentration of TDN (Ristic et al., 2007). Viticultural practices on different varieties regarding cluster thinning and basal leaf removal showed an increase of free terpenols and glycosidically bond terpenols (Reynolds et al., 1996b; Roberts et al., 2007; Hernandez-Orte et al., 2014; González-Barreiro et al., 2015). However, different training systems studied by various authors showed variations in terpenols concentrations (Ji and Dami, 2008; Reynolds et al., 1996a; Zoecklein et al., 2008). Water stress may increase blackberry, jam, raisin or dried fruit aroma in 'Cabernet Sauvignon' wines (Chapman et al., 2005), increase of cysteinylated thiol precursors in grapes of 'Sauvignon blanc' (Ribéreau-Gayon et al., 2006), increase of glycoconjugates of the main aromatic compounds observed in Agiorgitiko vines (Koundouras et al., 2006), increase of vitispirane, guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-vinylguaiacol observed in Merlot vines (Qian et al., 2009). Fertilization of vineyard with nitrogen lead to higher cysteinylated thiol precursors in 'Sauvignon blanc' grapes (Choné et al., 2006), while in Riesling wines a rise in 1-butanol, *trans*-3-hexen-1-ol, benzyl alcohol and the majority of esters was observed (Webster et al., 1993). Plant protection with fungicides may lead to residues on the grapes that affect the yeasts and fermentative performance which in turn influence aroma quality (Noguerol-Pato et al., 2011; Gonzales-Rodriguez et al., 2011; Gonzalez-Alvarez et al., 2012a and 2012b).

MATERIALS AND METHODS

The grapes used for this study were from the 'Cabernet Sauvignon' and 'Fetească neagră' cultivars, vintage 2014, cultivated in Murfatlar wine region. The vine parcels used in this study are located close to each other, in order to reduce the variability induced by soil and climate. The organic cultivation technology for both varieties are subject to actual regulations and controlled by Council Regulation (EEC) 834/2007. An additional viticultural practice applied consisted in 30% cluster thinning during summer, at the beginning of veraison, being evaluated against the control with no cluster thinning. The grapes were harvested on 16th September for the 'Fetească neagră' and on 24th September for 'Cabernet Sauvignon'. In this manner eight experimental variants were produced, according with viticultural practices briefly described in Table 1. More details regarding the grapes physico-chemical composition and other grape related parameters are described in other paper (Artem et al., 2015). The grape processing was done for batches of grapes of 50 kg, in 3 repetitions resulting 24 samples. The vinification process was the classical one, with maceration and fermentation on skins for 5-8 days for 'Fetească neagră' and for 8-10 days for 'Cabernet Sauvignon'. The fermentation was conducted at room temperature at about 22-24°C with native yeasts (no added yeast), without added nutrients or enzymes. At the end of fermentation the macerated grape marc of each variant was pressed using a laboratory press. After one month of clarification and cold stabilization, the resulted wines were racked off and treated with a dose of 50 mg/L of SO_2 to prevent oxidation and development of spoilage microorganisms. The resulted wine samples were analysed in other 3 repetitions on 7th May 2015, assessed with a GC e-nose from Alpha MOS in order to establish the differences

among certain aroma compounds between experimental variants.

Alpha MOS Heracles e-nose analyser is a Fast GC using a Tenax trap for pre-concentration of volatile organic compounds, designed with two short capillary columns working together having a different polarity, one being non-polar DB5 (5% diphenyl, 95% dimethylpolysiloxane) and the other with a low/mid polarity DB1701 (14% cyanopropylphenyl, 86% dimethylpolysiloxane).

Table 1. Experimental variants and the related codes attributed

Variety	Grape cultivation technology	Additional practice	Variant code
'Cabernet Sauvignon'	Conventional	No cluster thinning	CS_Con_N
		30% Cluster thinning	CS_Con_CT
	Organic	No cluster thinning	CS_Org_N
		30% Cluster thinning	CS_Org_CT
'Fetească neagră'	Conventional	No cluster thinning	FN_Con_N
		30% Cluster thinning	FN_Con_CT
	Organic	No cluster thinning	FN_Org_N
		30% Cluster thinning	FN_Org_CT

The separated volatile organic compounds are detected by means of simultaneous dual flame ionization detectors (FID), located at the end of each chromatographic column. The injection mode was made with 2.5 ml HS syringe, extraction of volatiles from the head-space of vials, using a 250°C injector temperature with an initial column temperature 40°C with an increase rate of a 5°C/s up to 200°C and hydrogen in constant pressure (16 psi) as a carrier gas. The oven agitator temperature for sample preparation was 10 minutes at 60°C and 500 rpm. The flame ionization detectors temperature have been set at 220°C and a fuel pressure of 35 psi. Other details regarding the methodology and Alpha MOS Heracles can be found in other papers (Antoce et al., 2015; Antoce and Namolosanu, 2011). The identification of peaks was based on retention index (Kovats), calibrated by using a known standard n-alkane mixture (n-C_6 to n-C_{16}) with the same determination conditions as for the analysed wine samples. The discriminant factor analysis (DFA) was selected in Alpha Soft v 12.42 software as the most relevant statistical method to separate the experimental variants and groups.

RESULTS AND DISCUSSIONS

In order to differentiate wine samples, discriminant factor analysis was applied to certain chromatographic peaks area (sensors), selected by using an extensive iterative process and their discriminant power provided by Alpha Soft v 12.42. Using this process, the main volatile organic compounds identified to differentiate wine samples in this study are briefly described in Table 2. The results show small differences between the experimental variants with 30% cluster thinning as compared to no cluster thinning variants, this being especially evident for conventional 'Fetească neagră' (Figure 1). This clearly led to the idea that cluster thinning practice did not significantly influence the major volatile organic compounds in wine samples and only small changes being observed, which are hardly recognized even by a powerful tool such as GC-e-nose. However a good validation score of 92 in DFA diagram (Figure 1) is explained by the higher variability induced by both grape cultivation technology (organic or conventional) and variety. According to Figure 1, the DFA bi-plot explained 96.554% of the total variance in the data through the first two dimensions, with 85.069% and 11.485% explained by DF1 (mostly grape variety related) and DF2, respectively. In general in Figure 1 and 4, no cluster thinning, appear to be associated with more negative volatile organic compounds as 2,4-hexadienal, 2,3-octanedione and valeric acid, while 30% cluster thinning, appear to be associated with more positive volatile organic compounds as 3-methyl-1-pentanol, dehydro-*p*-cymene, *β*-phenylethanol, nerol oxide and (*E*)-2-hexenal. The results regarding cluster thinning practice did not show a clear difference between those samples as can be seen in Figure 4, where DFA bi-plot have a validation score of 69.

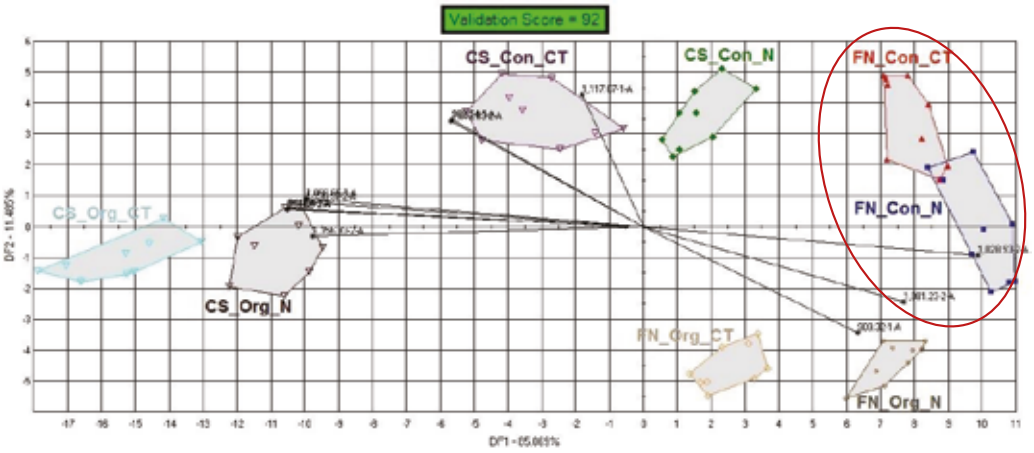

Figure 1. DFA bi-plot of aroma compounds peaks used for differentiation of wine sample groups in accordance to grape variety, grape cultivation technology (organic/conventional) and cluster thinning practice

Table 2. Relevant aroma compounds identified as being discriminative in wine samples of 'Cabernet Sauvignon' and 'Fetească neagră'

*Retention time	Column	*Sample Kovats	Database Kovats	Sensor number	Identified volatile organic compounds	Sensory descriptor
8.05	DB5	844,04	843	844,04-1-A	3-methyl-1-pentanol	chocolate, wine-like, green
10.36	DB5	909,32	908	909,32-1-A	2,4-hexadienal	green, citrus, floral
12.54	DB5	962,54	962	962,54-1-A	γ-valerolactone	anise, herbal, sweet, warm, tabacco, cocoa, woody, hay-like, coumarinic odor,
17.72	DB5	1090,66	1094	1090,66-1-A	dehydro-p-cymene	citrus, pine
18.70	DB5	1117,07	1117	1117,07-1-A	β-phenylethanol	sweet, floral, fresh, bready, rose, honey
11.25	DB1701	953,38	956	953,38-2-A	(*E*)-2-Hexenal	sweet, vegetable, almond, apple, green, plum
14.34	DB1701	1028,93	1031	1028,93-2-A	valeric acid	cheese, sweat
16.10	DB1701	1072,03	1073	1072,03-2-A	γ-valerolactone	anise, herbal, sweet, warm, tabacco, cocoa, woody, hay-like, coumarinic odor,
16.49	DB1701	1081,23	1082	1081,23-2-A	2,3-octanedione	dill, cooke, broccoli, buttery
19.58	DB1701	1156,97	1157	1156,97-2-A	dehydro-p-cymene	citrus, pine
27.26	DB1701	1356,93	1353	1356,93-2-A	nerol oxide	flower, oil

*average values resulted from all chromatograms;

Nevertheless, the cultivation technology and variety are the most important variables in this study, for which reason we have redefined the groups according to only cultivation technology and variety, removing the cluster thinning variable (Figure 2). Using this approach, a better validation score (96) is obtained and a very good separation on the DFA bi-plot (Figure 2). In Figure 2, the DFA bi-plot explained 96.852% of the total variance in the data through the first two discriminant functions, with DF1=76.516% and DF2=20.336%. From the loadings of the discriminant peaks also included in Figure 2 it can be seen that conventional 'Fetească neagră' is associated with more valeric acid than the organically cultivated 'Fetească neagră', which is associated with 2,4-hexadienal, while the conventional 'Cabernet Sauvignon' is associated with β-phenylethanol and organic 'Cabernet Sauvignon' with nerol oxide, dehydro-*p*-cymene and (*E*)-2-hexenal.

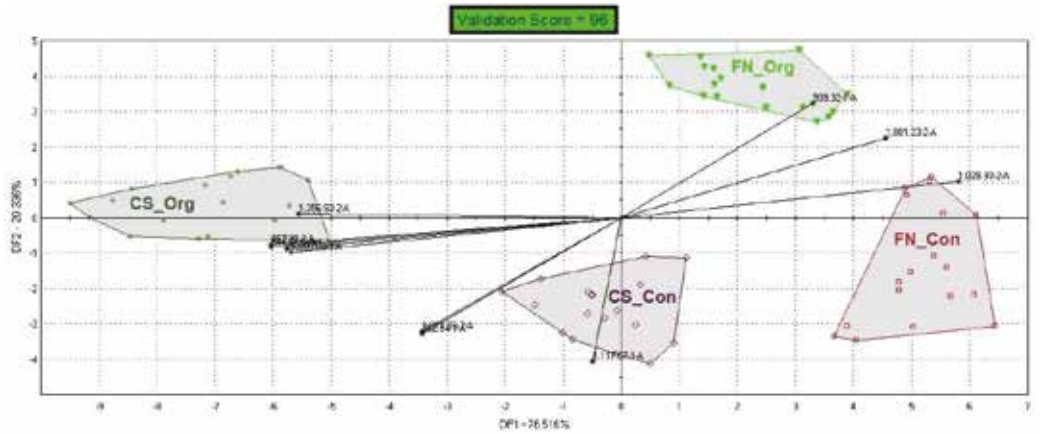

Figure 2. DFA bi-plot of aroma compounds peaks used for differentiation of wine samples depending on grape variety and grape cultivation technology (organic/conventional)

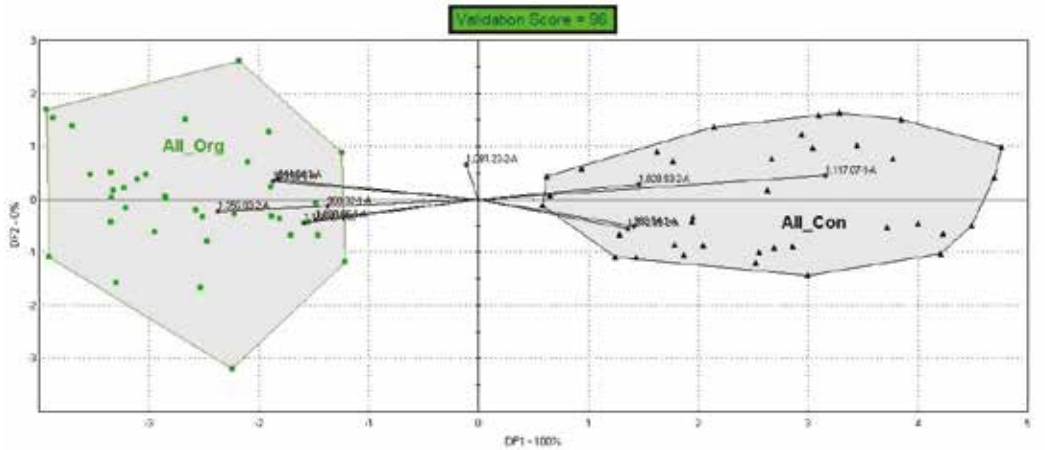

Figure 3. DFA bi-plot of aroma compounds peaks used for differentiation of wine samples depending on grape cultivation technology (organic/conventional)

When we have redefined the groups according to only cultivation technology removing the variety and cluster thinning variables (Figure 3), all organic samples are well differentiated by conventional samples with a good validation score of 96 observed in the DFA bi-plot. In figure 3 we can observe the main differences between organic and conventional samples and the DFA bi-plot explained 100% of the total variance in the data through the first dimension. The results from the figure 3 reveal that conventional growing technology in this study is associated with more *β*-phenylethanol, *γ*-valerolactone and valeric acid, while the organic grape production is associated with more nerol oxide, dehydro-*p*-cymene, 3-methyl-1-pentanol, 2,4-hexadienal and (*E*)-2-hexenal and slightly with 2,3-octanedione. However, the organic and conventional growing led to wine samples easily differentiated by the electronic nose, suggesting that these differences can also be perceived by consumers. In one study of sensory evaluation of red wines deriving from organically and conventionally grown grapes, trained assessors and regular wine consumers attributed the differences mainly to sour and bitter taste, with astringency sensations, while aroma have a minor role (Pagliarini et al., 2013).

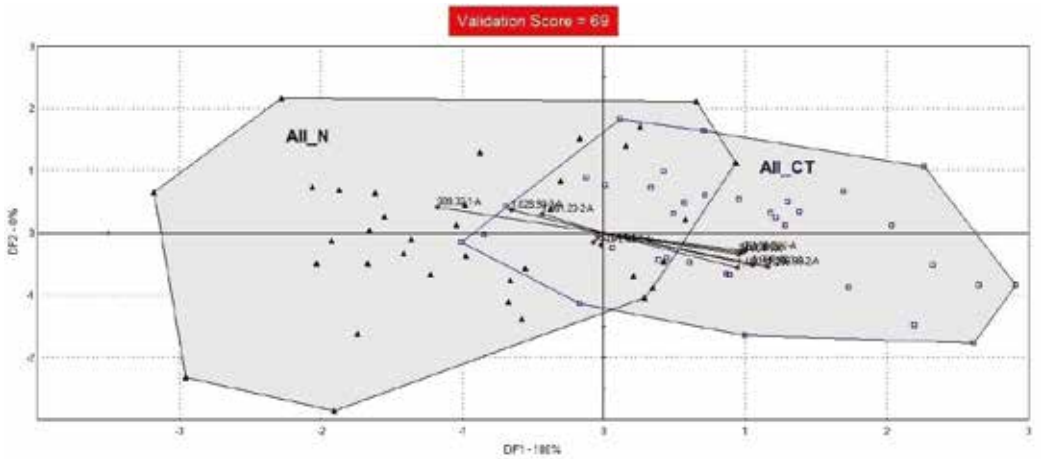

Figure 4. DFA bi-plot of aroma compounds peaks used for differentiation of wine samples depending on cluster thinning

CONCLUSIONS

Grape variety followed by the type of grape technology (organic/conventional) were the most discriminative factors for the wines produced and evaluated in this study, allowing for a good separation of wine samples in the DFA bi-plots, with high validation scores (96). This means that the grape growing technology has a significant impact on grape aroma compounds and implicitly in wines, meaning that these modifications are probably perceived even by regular wine consumers. The 30% cluster thinning did not influence significantly these aroma compounds compared with the samples with no cluster thinning and in this case, certainly, discrimination of wine samples by the electronic nose could not be achieved in this experiment, leading us to the idea that this viticultural practice did not have sufficient impact on the overall aroma quality. The organic grape technology affected more the wine aroma, resulting in associations with monoterpenes (nerol oxide and dehydro-*p*-cymene), higher alcohols (3-methyl-1-pentanol), aldehydes (2,4-hexadienal and 2-hexenal) derived from grape lipid enzymatic oxidation, and slightly with some ketones (2,3-octanedione). On the other hand, the conventional grape technology impact on wine aroma resulted in associations with other higher alcohols (*β*-phenylethanol), lactones (*γ*-valerolactone) and volatile acids (valeric acid). All these compounds may not be perceptible in wine as the pure substances are perceived and described in literature, as their mixture in the wine matrix changes very much this perception. However, these findings serve as hints to winemaker to find ways to improve the quality of grapes and wines.

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INCREASE OF ANTHOCYANINS ACCUMULATION BY PRE-HARVEST 24-EPIBRASSINOLID (24-EBL) APPLICATIONS IN 'HOROZ KARASI' GRAPE CULTIVAR

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Abstract

In this study, the effect of pre-harvest 24-eBL (a BR analogue) applications on the accumulation of anthocyanin of 'Horoz Karasi' grape cultivar was examined. 24-eBL was applied to vines with 13 combinations including different application times (veraison, 7 days after berry set + veraison, 7 days after berry set + veraison + 30 days after veraison) and 4 different concentrations of 24-eBL (0.2, 0.4, 0.6 and 0.8 mg/L) and control. As a result of the study, the highest anthocyanin content was obtained from the vines applied with 0.2 mg/L of 24-eBL at 7 days after fruit set and at veraison stages in both years (respectively, 47.98 and 71.16 mg/100 g FW).

Key words: *anthocyanin, brassinosteroid, Grape 24-eBL, pre-harvest application.*

INTRODUCTION

Grapes are one of the most consumed fruits, and due to its rich phenolic compounds, such as antiradical and antioxidant properties, interest in grapes and grape products (especially food additives, pharmaceutical industry and natural cosmetic products) is increasing (Bourgaud et al., 2001; Ghafoor et al., 2009).

Anthocyanins in grapes have well-known many beneficial effects for human health including the reduction in the incidence of coronary heart disease, enhancement of visual acuity, maintenance of normal vascular activity, as well as pharmacological properties and strong biological functions such as antiinflammatory and antioxidant activities (Hohnova et al., 2008). Also, anthocyanins in grapes play an important role in the quality of colour and they are successfully used as food colourants and nutraceuticals (Espín et al., 2007). Therefore, the determination of anthocyanins in red grapes has acquired of increasing interest during last decade. Many different applications can be made in cultivation in order to enrich the nutrient and antioxidant contents of grapes. The most important treatments in grape growing for this purpose are the usage of plant growth regulators.

Brassinosteroids (BRs), known as the sixth group of hormones, have highly beneficial effects on plant growth, development and physiological aspects in plants such as seed germination, rhizogenesis, flowering, rooting, senescence, abscission, cell expansion and elongation and they are considered as plant hormones with having pleiotropic effects (Clouse and Sasse, 1998; Vardhini and Rao, 2002; Luan et al., 2013).

Moreover exogenous applications of BRs increase the synthesis of secondary metabolites in plants (Biesaga-Koscielniak et al., 2014; Ghorbani et al., 2017).

Symons et al. (2006) and Luan et al. (2013) showed that the exogenous application of BR to grape berries evidently enhanced skin coloration and anthocyanin accumulation. Although there are some studies showing that BRs influence the expression of anthocyanin biosynthesis genes in grapes (Luan et al., 2013; Xi et al., 2013), little is known about the possible roles of BRs involved in anthocyanin accumulation. In this study we investigated the effect of BRs on anthocyanin accumulation in grapes.

Thus, the objective of this study was to determine the effect of pre-harvest 24-eBL application at different doses and application

times on anthocyanin accumulation in 'Horoz Karasi' grape cultivars.

MATERIALS AND METHODS

The experimental vineyard was located in Senirkent-Isparta Province in the Mediterranean region of Turkey. Nine-year old *Vitis vinifera* L. cv. 'Horoz Karasi' grapevines grafted 41 B.M.G. were used. The vines were planted at 2 x 3 m spacing trained on a bilateral cordon system. 24-eBL was sprayed during two years at concentrations of 0, 0.2, 0.4, 0.6 and 0.8 mg/L and prepared by dissolving in DMSO. Tween 20 at the rate of 0.1% (v/v), as a surfactant was added to 24-eBL solution. The prepared solutions were sprayed directly onto the vine (1L per vine) at veraison (approximately 10% of the berries of 50% of the clusters become soft and at colour break), at 7 days after fruit set (3-5 mm diameter berry size) + at veraison and 7 days after fruit set and at veraison + 30 days after veraison by a hand pump sprayer until run-off early in the morning. Grapes were harvested at commercial maturity and bunches were packed in plastic crates and transported to the laboratory where they were frozen in liquid nitrogen and stored at -80°C until the analyses were performed.

Total anthocyanin content determination was based on a pH differential method and expressed as malvidin 3-glucoside equivalents (Wrostad, 1976). For this purpose, aliquots of the extracts were adjusted to pH 1.0 and 4.5 with buffers. The absorbance of each solution was measured at wavelength of 520 and 700 nm.

The experiment was conducted in randomized complete block design with 3 (application time; at veraison, at 7 days after fruit set + at veraison and 7 days after fruit set + at veraison + 30 days after veraison) × 5 (24-eBL concentration; 0, 0.2, 0.4, 0.6 and 0.8 mg/L) × 2 (years; first and second) factorial arrangements. Each treatment had three replications of eight vines.

Statistical analyses were performed with LSD multiple range test.

Differences were considered statistically significant at the $p \leq 0.05$ levels. The software JMP 8 (SAS Institute, Inc., Cary, NC) was used for carrying out statistical analyses of the data.

RESULTS AND DISCUSSIONS

Effects of 24-eBL concentrations and its application periods on anthocyanin contents were given in Table 1. 24-eBL applications generally increased the anthocyanin accumulation. In the both years, interaction effects of 24-eBL concentrations and application periods on the anthocyanin contents were statistically significant ($p<0.05$).

In the first year, the highest anthocyanin content was observed at 7 days after fruit set and at veraison periods with 0.2 mg/L 24-eBL (47.98 mg/100 g FW) and at 7 days after fruit set + at veraison periods with 0.6 mg/L 24-eBL (45.35 mg/100 g FW) and at 7 days after fruit set + at veraison + at 30 days after verasion periods with 0.6 mg/L 24-eBL (45.35 mg/100 g FW).

In the second year, the treated with 0.2 mg/L 24-eBL at 7 days after fruit set and at veraison periods grapes had the highest anthocyanin contents. The lowest anthocyanin contents were observed in control grapes for both years.

Table 1. Anthocyanin contents (mg/100 g FW) in 'Horoz Karasi' grapes as effected by 24-eBL concentrations and application periods

Application period (AP)	Concentrations (C) (mg/L)	1st year	2nd year
Veraison (V)	0	29.36 g	39.73 e
	0.2	33.38 f	41.24 de
	0.4	36.40 d	57.14 abcd
	0.6	35.22 e	62.34 abc
	0.8	22.73 j	51.46 bcde
7 days after fruit set + veraison (FS+V)	0	29.38 g	39.73 e
	0.2	47.98 a	71.16 a
	0.4	21.19 l	42.23 de
	0.6	45.35 b	65.35 ab
	0.8	29.41 g	47.33 cde
7 days after fruit set + veraison + 30 days after veraison (FS+V+30V)	0	29.38 g	39.73 e
	0.2	27.50 h	42.78 de
	0.4	22.40 k	52.02 bcde
	0.6	39.75 c	41.08 de
	0.8	23.86 l	60.83 abc
Main effect (Stages)			
V		31.42	50.38
FS+V		34.66	53.16
FS+V+V30		28.58	47.29
Main effect (Concentrations)			
	0	29.38	39.73
	0.2	36.29	51.73
	0.4	26.66	50.46
	0.6	40.11	56.26
	0.8	25.33	53.21
p values			
AP		<0.0001	0.2991
C		<0.0001	0.0197
AP x C		<0.0001	0.0035

Different letters indicate significant differences between groups ($p<0.05$).

Also, the main effect of 24-eBL application periods and concentrations on anthocyanin contents were shown in Table 1, too. In both years, when the main effects of 24-eBL application periods and concentrations examined, the application at 7 days after fruit set + at veraison periods and 0.6 mg/L 24-eBL concentration had the highest anthocyanin contents, respectively. Anthocyanins are synthesized by the biosynthetic way regulated by enzymes such as PAL, chalcone synthase (CHS), chalcone isomerase (CHI), F3H, DFR, leucoanthocyanidin dioxygenase (LDOX) (UFGT). The rise in these enzymes causes the synthesis and accumulation of anthocyanin. BRs can increase the amount of anthocyanin as a result of the enhancement in the enzymes on this synthetic route, and increase in the expressions of the genes encoding these enzymes. Indeed, it was determined that BRs can stimulate the expressions of the genes involved in the synthesis of anthocyanin, thus increasing the amount of anthocyanin in grapes (Xi et al., 2013). Peng et al. (2011) reported that BRs affect the accumulation of anthocyanin induced by jasmonates, through the regulation of late anthocyanin biosynthetic genes such as BRL, DFR, LDOX and UFGT. Similarly, previous studies showed that pre-harvest BRs applications increase the amount of total anthocyanin compared to control application in grapes, too (Luan et al., 2013; Xi et al., 2013; Champa et al., 2015).

CONCLUSIONS

As a result of investigations, it was seen that all pre-harvest 24-eBL applications increased the amount of anthocyanin compared to the control application. Accordingly, it is revealed that among the 24-eBL applications, the better results were obtained from 0.2 mg/L 24-eBL applicated at 7 days after fruit set and at veraison periods. So, this application could be recommended for 'Horoz Karasi' cultivar.

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THE INFLUENCE OF PRE-HARVEST BORIC ACID APPLICATIONS ON THE ACCUMULATION OF SOME ANTIOXIDANT COMPONENTS IN ALPHONSE LAVALLÉE GRAPE CULTIVAR

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Abstract

The aim of the research was carried out to determine the effects of boric acid (BA) applications on the accumulation of anthocyanins, β-carotene, ascorbic acid and total phenolics in 'Alphonse Lavallée' grape cultivar. BA was applied to vines with four different concentrations (250, 500, 750 and 1000 mg/L) and at two different periods (at blooming and at blooming+fruit set). At the end of the study, it was determined that the BA applications consist of 250mg/L and 750 mg/L of boric acid concentrations during both blooming and fruit set stages were the most effective applications providing the highest anthocyanins, β-carotene and total phenolics. On the other hand, boric acid applications had no important effects on the ascorbic acid contents in berries.

Key words: *'Alphonse Lavallée', antioxidant components, boric acid, pre-harvest applications.*

INTRODUCTION

Antioxidants are substances that, at relatively low concentrations, acting to scavenge and stabilize of free radicals. The benefits of antioxidants are very important to good health. Antioxidants can protect the human body free radicals that may cause some chronic diseases including cancer, cardiovascular diseases and cataract (Shahidi, 2000; Pham-Huy et al., 2008). Consumers are becoming increasingly prefer aware antioxidant substances rich in fruits and vegetables because of their beneficial effects on health. Grape is one of the most important fruit commodities as widely grown through the world. Also, it is a significant source of nutritional antioxidants, such as polyphenols, anthocyanins as well as biologically active dietary components (Orak, 2007). Therefore, due to its rich phenolic compounds, such as antiradical and antioxidant properties, interest in grapes and grape products (especially food additives, pharmaceutical industry and natural cosmetic products) is increasing (Bourgaud et al., 2001; Ghafoor et al., 2009). To enhance the production of antioxidant substances, strategies such as treating with elicitors (UV, salicylic acid, methyl jasmonate, ozone) and invoking biotic and abiotic stresses have been used in both plant cell culture and intact plants (Dong et al., 2010; Krzyzanowska et al., 2012). However, optimizing the agricultural practices also provide a rapid and efficient way to increase the antioxidant content. Mineral nutrients are essential for the growth, survival and reproductive success of plants. Among the essential elements for healthy plants, boron (B) is responsible for activation of dehydrogenase enzymes, sugar translocation, nucleic acids, plant hormones and effect on cell wall structure cell elongation, root growth and transfer of sugar and altered production of a wide range of phenol content and metabolism. In addition to it is helpful in growth and productivity, fruit setting and yield. B deficiency has visual symptom on root and leaves growth, flower, cluster and berry development in grapevine. Severely reduced in internodes and shoot length, shoot tip death, low fruit set, and tiny berries are all common symptoms of B deficiency (Fleischer et al., 1998; Ruiz et al., 1998; Ebadive et al., 2001; Brown et al., 2002; Christensen et al., 2006; Singh et al., 2012). Several studies have demonstrated that B application increased the yield in grapes

(Mostafa et al., 2006; Er et al., 2011; Ally et al., 2015; Asci et al., 2017). However, although there is limited information on the effect of B on the antioxidant components of plants and there is no information about grapes. Therefore, the major objective of this study was to determine the effect pre-harvest B application on the accumulation of some antioxidant components (total phenolics, ascorbic acid, β-carotene, anthocyanins) in 'Alphonse Lavallée' grape cultivars.

MATERIALS AND METHODS

The experiment was performed on 9-year old *Vitis vinifera* L. cv. 'Alphonse Lavallée' grafted on 41 B.M.G. planted at 2 x 3 m spacing trained on a bilateral cordon system in a commercial vineyard in the Senirkent-Isparta Province in the Mediterranean region of Turkey (lat. 38º 11' 8" N, long. 30º 40' 55" E and elevation 981 m). Eight vine at similar conditions were used. Boric acid (BA) was sprayed at concentrations of 0, 250, 500, 750 and 1000 mg/L. Tween 20 at the rate of 0.1% (v/v), as a surfactant was added to BA solution. The prepared solutions were sprayed directly onto the vine (1 L per vine) at blooming and at blooming + fruit set stages by a hand pump sprayer until run-off early in the morning. Grapes were harvested at commercial maturity and bunches were packed in plastic crates and transported to the laboratory where they were frozen in liquid nitrogen and stored at -80°C until the analyses were performed. Total phenolic content was estimated by the Folin-Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi (1965), using gallic acid as a standard phenolic compound. The reduction of the Folin-Ciocalteu reagent by phenolic compounds under alkali conditions, which resulted in the development of a blue colour, was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated with the use of a calibration curve and results were expressed as mg gallic acid equivalent per g dry weight. L-Ascorbic acid content was determined spectrophotometrically at 520 nm using a standart curve according to the procedure described by Pearson and Churchill (1970). Each determination was carried out in triplicate. β-carotene content of the sample was measured using the method of Association of Official Analytical Chemists (AOAC, 1980). The absorbance of the extracts was measured at 436 nm wavelength by using a spectrophotometer. The concentration of β-carotene was calculated using Bear-Lamberts Law. Total anthocyanin content determination was based on a pH differential method and expressed as malvidin 3-glucoside equivalents (Wrolstad, 1976). For this purpose, aliquots of the extracts were adjusted to pH 1.0 and 4.5 with buffers. The absorbance of each solution was measured at wavelength of 520 and 700 nm. The experiment was conducted in 2 (application time; at blooming, at blooming + at fruit set) × 5 (BA concentration; 0, 250, 500, 750 and 1000 mg/L) factorial arrangements with completely randomized block design. Each treatment had three replications of 8 vines. Statistical analyses were performed with LSD multiple range test. Differences were considered statistically significant at the $p \leq 0.05$ levels. Statistical analyses were performed by using JMP 8 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSIONS

In concerning with influences of BA spraying on antioxidant properties of Alphonse Lavallée grape, data was shown in Table 1 and Table 2. Data showed that the interaction effects of BA concentrations and application periods had a significant ($p \leq 0.0001$) influence on total phenolic, β-carotene and anthocyanin contents. Grapes tretaed with 250 and 750 mg/L BA at blooming and at fruit set stages produced more total phenolic compound (16.84 mg/g and 16.32 mg/g, respectively) compared to control and the other applications. Similarly, the highest anthocyanin content was observed with 250 and 750 mg/L BA at blooming and at fruit set stage, too (66.89 and 66.55 mg/100 g) (Table 1).

As shown Table 2, β-carotene content was also higher 250 mg/L BA at blooming and at fruit set stage (698.32 µg/kg) followed by 750 mg/L BA at blooming and at fruit set stage (422.75 µg/kg). Data showed that there was no significant difference observed among the treatments for ascorbic acid content. Ascorbic acid content varied from 1.97 to 2.73 mg/100 g.

Table 1. Response of BA spraying at different stage on total phenolic (mg/g DW) and anthocyanins (mg/100 g FW) of 'Alphonse Lavallée'

Application period (AP)	Concentrations (C) (mg/L)	Total phenolics (mg/g DW)	Anthocyanins (mg/100 g FW)
Blooming (B)	**0**	7.59 f	35.24 de
	250	11.33 e	46.67 b
	500	14.40 b	30.33 e
	750	12.13 cde	42.03 bc
	1000	13.03 c	35.78 cde
Blooming + Fruit set (B+FS)	**0**	7.59 f	35.24 de
	250	16.84 a	66.89 a
	500	12.73 cd	41.60 bcd
	750	16.32 a	69.55 a
	1000	11.88 de	37.39 cd
Main effect (Stages)			
B		11.70	38.01
B + FS		13.07	50.13
	Main effect (Concentrations)		
	0	7.59	35.24
	250	14.09	56.78
	500	13.56	35.96
	750	14.22	55.79
	1000	12.46	36.59
p values			
AP		<0.0001	<0.0001
C		<0.0001	<0.0001
AP x C		<0.0001	<0.0001

Different letters indicate significant differences between groups ($p<0.05$).

Also, the main effect of BA application periods and concentrations on antioxidant properties were shown in Table 1, too. When the main effects of BA application periods and concentrations examined, they were obtained that at blooming + at fruit set periods and 250 mg/L BA concentrations had the highest total phenolics, ascorbic acid, anthocyanins and β-carotene contents (Tables 1 and 2).

Antioxidants, such as total phenolics and anthocyanins, are important indicators in grape and wine, and their contents are considered as the most important nutritional value parameters. B is one of the nutrients responsible for the changes in concentration and metabolism of phenolic compounds in vascular plants. It is well known that B deficiency causes an accumulation of phenolics through the stimulation of the enzyme phenylalanine-ammonium lyase (PAL) (Cakmak et al., 1995). B is rapidly absorbed by flowers (Sarrwy et al., 2012) and the B application effects can be attributed to its effect on fruit set and development or other metabolic processes such as carbohydrate transport, which are enhanced by its application (Marschner, 2012; Davarpanah et al., 2016). Due to the fact sucrose is a positive regulator of the biosynthesis of phenolics, the improvement of B treatment on photosynthesis and sugar accumulation could possibly enhance the biosynthesis of phenolics (Solfanelli et al., 2006). To our knowledge, there is very little studies examining the relationship between phenolics and B. There were no comprehensive and detailed studies about the effect of sprayed B on the whole development process included antioxidant properties of grapes. But some investigators have observed a positive effect of B on total phenolic contents in fruits. For instance, foliar sprays of B increased total phenolic compounds in olive (Saadati et al., 2013) and in pungent pepper (Manas et al., 2014). The authors indicate that the increase was due to an increased expression of genes responsible to phenolic compound biosynthesis.

Table 2. Response of BA spraying at different stage on ascorbic acid (mg/100 g FW) and β-carotene (µg/kg FW) of 'Alphonse Lavallée'

Application period (AP)	Concentrations (C) (mg/L)	Ascorbic acid (mg/100 g FW)	β-carotene (µg/kg FW)
Blooming (B)	**0**	1.97	289.65 bcd
	250	2.60	321.04 bcd
	500	2.62	352.34 bcd
	750	2.69	281.56 cd
	1000	1.79	392.61 bc
Blooming + Fruit set (B+FS)	**0**	1.97	289.65 bcd
	250	2.73	698.32 a
	500	2.19	273.21 cd
	750	2.52	422.75 b
	1000	2.25	245.90 d
Main effect (Stages)			
B		2.33	327.44
B + FS		2.33	385.96
	Main effect (Concentrations)		
	0	1.97	289.65
	250	2.66	509.68
	500	2.41	312.78
	750	2.60	352.15
	1000	2.02	319.25
p values			
AP		0.9965	0.0626
C		0.1000	0.0010
AP x C		0.6670	0.0001

Different letters indicate significant differences between groups ($p<0.05$).

Ascorbic acid acts as an antioxidant in plants. According to some researchers its levels are responsive to a variety of environmental or stress factors such as light, temperature, salt and drought, pollution, metals or herbicides (Hancock and Viola, 2005; Nicolle et al., 2004). Lukaszewski and Blevins (1996) reported that adequate level of B is required for the accumulation of ascorbic acid. Although there was no significant difference observed among the B treatments for ascorbic acid content in our investigation, Mondi and Munsi (1993) reported that applied B significantly increased the ascorbic acid concentration in potatoes. According to the study, since B plays an important role in the translocation of carbohydrates from leaves to other portions of the plant, greater concentrations of ascorbic acid may have been translocated to the tuber. Also, Govindan (1950) reported that the ascorbic acid concentration of tomatoes rose with increased B uptake in the plant. Cakmak and Romheld (1997) have shown that the decline in vitamin C in some plant parts such as shoot tips and young leaves of sunflower under B deficient conditions.

It is known that B is important for the structural and functional integrity of plasma membranes. The lack or additional of B may also influence efflux of other macro and micro elements (Camacho-Cristobal et al., 2008; Hajiboland and Farhanghi, 2010). Singh et al. (2012) found that the accumulation of P, K, Mg, S, Na, Al and Mn in the carrot storage roots increased when additional B was not supplied to the plants. This alteration of mineral levels could either directly or indirectly affect the accumulation of phytonutrients, such β-carotenes.

CONCLUSIONS

Previous studies were mostly concerned the effect of B on the quality of grapes but, there were no comprehensive and detailed studies about the effect of sprayed B on the whole development process included antioxidant properties of grapes. Obtained results are important to increase the antioxidant properties of 'Alphonse Lavallée' grapes. As a result of investigations, the better results were obtained with 250 mg/L BA applicated at blooming and at fruit set periods and B application can be recommended for 'Alphonse Lavallée' grape cultivar.

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MOLECULAR DETECTION OF BLACK *ASPERGILLUS* AND *PENICILLIUM* SPECIES FROM DEALU MARE VINEYARD

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Abstract

The aim of this study was to determine the incidence and diversity of black Aspergillus and Penicillium species on white and red grape varieties in Dealu Mare vineyard (in two vilticultural centres - Valea Calugareasca and Pietroasa), during the harvest time of 2014 and 2015. A total of 61 fungal strains belonging to black Aspergillus (28 strains) and Penicillium (33 strains) were isolated. An RFLP analysis of the 5.8 S-ITS region was performed by using different combinations of endonucleases for molecular identification of fungal isolates at species level. A. niger (accounting 60.71% of all isolates) and P. expansum (66.66%) were the predominant species identified, followed by A. tubingensis (17.85%) and P. chrysogenum (21.21%). Higher biodiversity of fungal isolates has been found in Pietroasa Centre than from Valea Calugareasca.

Key words*: black Aspergillus, Penicillium sp., PCR-ITS RFLP, grapes, Dealu Mare vineyard.*

INTRODUCTION

Filamentous fungi (or molds) are communally contaminants of grapes. In recent years, particular attention has been drawn of black *Aspergillus* and *Penicillium* on grapes, being considered the main spoilage agents of grapes and grape-derived products and is responsible for significant economic losses. Rousseaux et al. (2014) reviewed thirty-six species of *Aspergillus* and fifty-nine different species of *Penicillium* identified on grapes in vineyards around the world.

The incidence of the species of black *Aspergillus* and *Penicillium* has been studied on grape varieties from vineyards worldwide: France (La Guerche et al., 2004; Garcia et al., 2006; Bejaoui et al., 2006; Diguță et al., 2011; Ahmed et al., 2015); Portugal (Serra et al., 2006); Spain (Martínez-Culebras and Ramón, 2007; Sardinas et al., 2011; Sempere et al., 2011; Garcia-Cela et al., 2014); Italy (Ayoub et al., 2010; De Rossi et al., 2011; Spadaro et al., 2012; Somma et al., 2012); Greece (Kizis et al., 2014; Kogkaki et al., 2015); Slovakia (Felsociova et al., 2013; Santini et al., 2014; Felsociova et al., 2015); Romania (Diguță et al., 2011; Diguță et al., 2015; Diguță et al., 2016).

Also, several species of *Aspergillus* section *Nigri* and *Penicillium* are recognized as potentially producers of mycotoxins (ochratoxin A, patulin etc.) and volatile compounds (geosmin, 2-methyl-isoborneol etc.) (La Guerche et al., 2004; Garcia et al., 2006; Martinez-Culebras and Ramon, 2007; Somma et al., 2012; Rousseaux et al., 2014). Among the black aspergilli, *A. carbonarius* has been considered as the main OTA producer (Belli et al., 2006), followed by species belonging *A. niger* aggregate (*A. niger* and *A. tubingensis*) (Bejaoui et al., 2006; Perrone et al., 2006). Among *Penicillium* species, *P. expansum* is the major causal agents of the blue mold rot of grapes and the main source of volatile compounds production which determinate off-flavours (La Guerche et al., 2004, Garcia et al., 2006).

The detection of mycotoxins and volatile compounds in grape wines can influence not only detrimental to the sanitary quality, but also to the organoleptic quality (La Guerche et al., 2004; Garcia et al., 2006; Martinez-Culebras and Ramon, 2007; Somma et al., 2012; Rousseaux et al., 2014). In Romania, Geana et al. (2012) reported the detection to traceable OTA levels (ranging from 0.06 ng/mL to 0.45 ng/mL) in 7 wine commercialized on the

Romanian market, however, the concentrations found being far below the proposed European limit (2 ng/mL). Black aspergilli and *Pencillium* species are difficult to taxonomical identification by classical methods. Among molecular approaches, restriction fragment length polymorphism (RFLP) based on ITS-5.8S region have been proved useful tool to the identification and classification the species belonging to *Aspergillus* section *Nigri* (Accensi et al., 1999; Bau et al., 2006; Martínez-Culebras and Ramón, 2007; Kizis et al., 2014); to differentiate *P. expansum* among fungal grape species (Garcia et al., 2006); to discriminate 22 species belonging to *Penicillium* genus and 7 species belonging to *Aspergillus* (Diguţă et al., 2011).

The latest available data of The International Organisation of Vine and Wine (OIV) shows that Romania is considered in top viticultural production potential with an area under vines the 191 thousands of hectares of overall in European vineyards (OIV, 2017a). After two poor harvests, Romania returned to a high level of production (5.3 millions of hectoliters) (OIV, 2017b).

Consequently, there is a special need to manage and combat the spoilage molds directly in the vineyard in order to obtain high quality of the Romanian wines which can compete with high quoted wines coming from other traditional wine-making countries (France, Italy, Spain etc.) or more recent producers as USA, Argentina or South Africa.

In this context, this study was focused to determinate the incidence and diversity of black *Aspergillus* and *Penicillium* species on white and red grape varieties in Dealu Mare vineyard (in two vilticultural centres - Valea Calugareasca and Pietroasa), during the harvest time of 2014 and 2015.

MATERIALS AND METHODS

Fungal isolation

The grape samples (white and red grape varieties) were randomly and aseptically collected from Dealu Mare vineyard (in two vilticultural centres - Valea Calugareasca and Pietroasa) at harvest time in 2014 and 2015 season (Table 1). Fungal isolation was performed according to the method described by Diguţă et al. (2011). The suspension was serially diluted and dilutions were plated on Dichloran Rose-Bengale Agar (Mecconti, Poland) supplemented with Chloramphenicol. Plates were incubated at 25°C in the dark for 5-7 days. Only fungal strains considered to represent black *Aspergillus* and *Penicillium* species were isolated and maintained to Potato dextrose agar (PDA, Mecconti, Poland). The pure cultures were preliminary screened by colony characteristics (mycelium type, color and growth type) as well as microscopic characteristics (conidiophores and spore) (Pitt and Hocking, 2009; Varga et al., 2011).

Table 1. Grape samples used in this study

Vilticultural centres	Grape variety	Acronym
Valea Călugărească	Fetească Regală (white grape)	FRV
	Cabernet Sauvignon (red grape)	CSV
Pietroasa	Fetească Regală Conventional (white grape)	FRCP
	Cabernet Sauvignon Conventional (red grape)	CSCONP
	Cabernet Sauvignon Ecologic* (red grape)	CEP
	Tămâioasă Românească Conventional (white grape)	TăRCP

*The vineyard is under certified ecological conditions.

DNA extraction

Fungal isolates were grown in 10 ml of PDB Broth (Mecconti, Poland) at 25ºC for 72 h. Mycelia were collected after centrifugation (10000 rpm for 5 minutes) and washed with sterile saline water then frozen at -25°C until use. Approximately 200mg of fungal biomass was used for DNA extraction by the use of ZR Fungal/Bacterial MiniPrep™ Kit (Zymo Research, USA), according to the manufacturer's instructions.

PCR-RFLP conditions

- Amplification of the 5.8S-ITS region was performed with universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3', forward) or ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3', forward) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3', reverse) in order to identify fungal strains (White et al., 1990). The PCR program for the amplification with ITS1/ITS4 was as follows: 2 min at 94°C, 34 cycles with 1 min at 94°C, 1 min at 55°C, 2

min at 72°C, with a final extension for 7 min at 72°C. The PCR program for the amplification with ITS5/ITS4 was as follows: 2 min at 95°C, 35 cycles consisting of 1 min at 95°C, 1 min at 52°C and 1 min at 72°C, with a final extension for 10 min at 72°C.

PCR products were digested with different combination of restriction enzymes *Cfr9*I, *Hpy*188I, *Hinf*I, *Hha*I, *Mse*I, *Nla*III, *Rsa*I and *Sdu*I (Thermo Scientific, USA), according to PCR-ITS-RFLP methods developed by Martínez-Culebras and Ramón (2007) and Diguţă et al. (2011) (Figure 1).

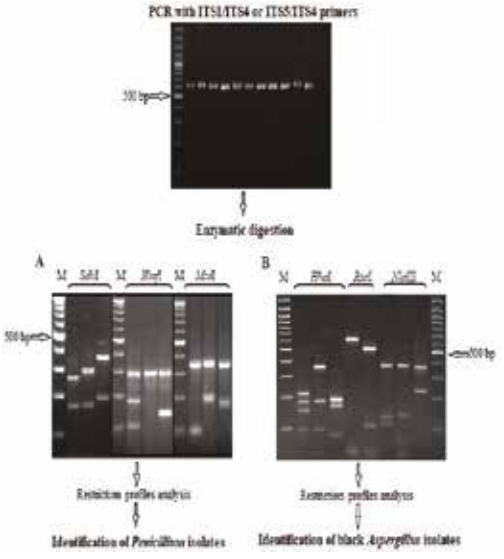

Figure 1. Identification of black *Aspergillus* and *Penicillium* isolates by PCR-RFLP based 5.8S-ITS region, adapted after Diguţă et al. (2011) (**A**) and Martinez-Culebras and Ramón (2007) (B); M - GeneRuler 100bp Plus DNA Ladder

PCR- RFLP analysis

The amplification and digestion results have been visualized under UV (254 nm) after electrophoresis in agarose gel 2% (90V/60 minutes). All fragment sizes were evaluated by the fluorescence intensity and were approximated using the DNA ladder (GeneRuler 100bp Plus DNA Ladder, Thermo Scientific, USA). Restriction fragments are considered to be distinguished when the difference between them is more than 20bp and considered to be fragments in common if migrated the same distance during agarose gel electrophoresis, respectively. Restriction fragments smaller than 70bp could not be clearly visualized and were not included in our results. Restriction patterns were compared with RFLP patterns obtained by Martínez-Culebras and Ramón (2007) and Diguţă et al. (2011).

RESULTS AND DISCUSSIONS

A total of 61 fungal strains including 28 strains black *Aspergillus* and 33 strains *Penicillium* were isolated in this study. All the isolates have been taxonomically identified by molecular methods using PCR-RFLP. Twenty eight fungal strains belonging to *Aspergillus* section *Nigri* have been isolated. The primer pairs ITS5/ITS4 were used to amplify the 5.8S-ITS region, followed by restriction enzyme digestion with three endonucleases *Hha*I, *Nla*III and *Rsa*I, according to ITS-RFLP method developed by Martínez-Culebras and Ramón (2007) (Table 2). Based on RFLP types, four species belonging to black *Aspergillus* have been identified: *A. niger aggregate (A. niger* and *A. tubingensis), A. aculeatus, A. japonicus* (Table 2).

A. niger (17 of all the isolates) was dominated among isolated fungus from black aspergilli, followed by *A. tubingensis* (5 isolates). Moreover, two strains belonging to the uniseriate species *Aspergillus aculeatus* and *A. japonicus* showed the same PCR-RFLP. Digestion with *Hinf*I showed two distinct restriction patterns: 110+180+270 (for *A. aculeatus*) and 270+290 (for *A. japonicus*) (personal data). Based on RFLP profile, 4 isolates have been identified as *A. japonicus.*

Thirty-three fungal strains belonging to *Penicillium* genus have been isolated. Amplification of the 5.8S-ITS region was performed with the universal primers ITS1/ITS4 followed by digestion with the combination of three endonucleases *Sdu*I, *Hinf*I and *Mse*I to discriminate *Penicillium* isolates at species level, according to PCR-ITS-RFLP method developed by Diguta et al. (2011) (Table 3).

According to Table 3, of the *Penicillium* isolates, 22 isolates showed a RFLP profile corresponding to *P. expansum*. Seven isolates have been identified as *P. chrysogenum*. This

result has been confirmed with other endonucleases *Cfr9*I or *Hpy*188I to differentiate *P. chrysogenum* from *P. crustosum* and *P. commune* (personal data). In addition, other 4 isolates of *Penicillium* sp. showed new RFLP profiles. To validate our results, sequencing is required.

The distribution of identified species on grapes varieties in Dealu Mare vineyard is given in Figure 2. Higher biodiversity of fungal isolates has been found in Pietroasa Centre than from Valea Calugareasca (Figure 2 A and B).

The distribution of *Aspergillus* sp. and *Penicillium* sp. on the grape varieties can vary from one vineyard to another, from one year to another, grape maturity, climatic conditions and viticultural practices being key factors, which leads to difficulty in generalizing the management and combating of these molds directly from the vineyard. However, the impacts of climate changes and viticultural practices on the occurrence of black *Aspergillus* and *Penicillium* on grapes have been not taken into account, when have been analysed the results obtained in this study.

According to figure 2 we found that *A. niger* was the predominant species isolated from grapes. *A. carbonarius*, the main species of ochratoxigenic species, have not been isolated. However, in another study, *A. carbonarius* has been detected and quantified by qPCR in all naturally grape samples tacking in account in this study (Diguță et al. 2016).

Table 2. RFLP analysis of 5.8S-ITS region exhibited by the *Aspergillus* isolates

No.	*Aspergillus* sp.	Number isolates	Restriction fragments		
			*Hha*I (pb)	*Nla*III (pb)	*Rsa*I (pb)
1.	*A. niger*	17	90 120 180 210	110+130+360	520
2.	*A. tubingensis*	5	90 120 180 210	110 130 360	80+480
3.	*A. aculeatus*	1	70 140 180 180	220+350	80+480
4.	*A. japonicus*	4	70 140 180 180	220 +350	80 +480

Table 3. RFLP analysis of 5.8S-ITS region exhibited by the *Penicillium* isolates

No.	*Penicillium* sp.	Number isolates	Restriction fragments		
			*Sdu*I (pb)	*Hinf*I (pb)	*Mse*I(pb)
1.	*P. expansum*	22	170 270	110 180 290	110 110 360
2.	*P. chrysogenum*	7	170 270	290 290	110 110 360
3.	*Penicillium* 1	1	170 270	110 180 290	560
4.	*Penicillium* 2	1	170 270	290 290	560
5.	*Penicillium* 3	1	190 320	290 290	110 110 360
6.	*Penicillium* 4	1	560	130 160 290	190 240

On the Fetească Regală grape variety (in 2014) and Cabernet Sauvignon (in 2015), no strain of *Aspergillus* sp. or *Penicillium* sp. have been isolated (Figure 2A). Other species commonly found on grapes, namely *Alternaria alternata, Cladoporium cladosporioides, Epicoccum nigrum* on both varieties were identified (personal data). A certain fungal diversity was observed on Fetească Regală grape variety (in 2015), predominantly *P. expansum*, followed by other new *Penicillium* species, A. *tubingensis* (Figure 2A).

A special attention has been given to fungal mycobiota of two autochthonous grape varieties (Fetească Regală and Tămâioasă Românească) and international grape variety Cabernet Sauvignon from Pietroasa Centre (Figure 2B). Diversity of *Aspergillus* sp. and *Penicillium* sp. is higher from Tămâioasă Românească variety (TăRCP - for 2014) and Fetească Regală variety (FRCP - for 2014 and 2015) from Pietroasa Centre (Figure 2 B). *P. expansum* was detected on FRCP (both years season), TăRCP (2014) and CSCONP (2015). Also, *P. chrysogenum* was another highly isolated species on the two grape varieties analyzed (TăRCP, for 2014 and FRCP, for 2015). *A. niger* was isolated predominantly on

the Cabernet Sauvignon Conventional Variety (CSCONP) in 2014 season. Also, *A. niger* was identified on the Cabernet Sauvignon Ecological (CSEP) variety in 2015. *A. tubingensis* was predominated CSEP (2014) and TăRCP (2015) (Figure 2B).

Two other uniseriate *Aspergillus* species, *A. japonicus* and *A. aculeatus* were punctually isolated on CSCONP (both seasons), TăRCP (2014) and FRCP (2015) (Figure 2B).

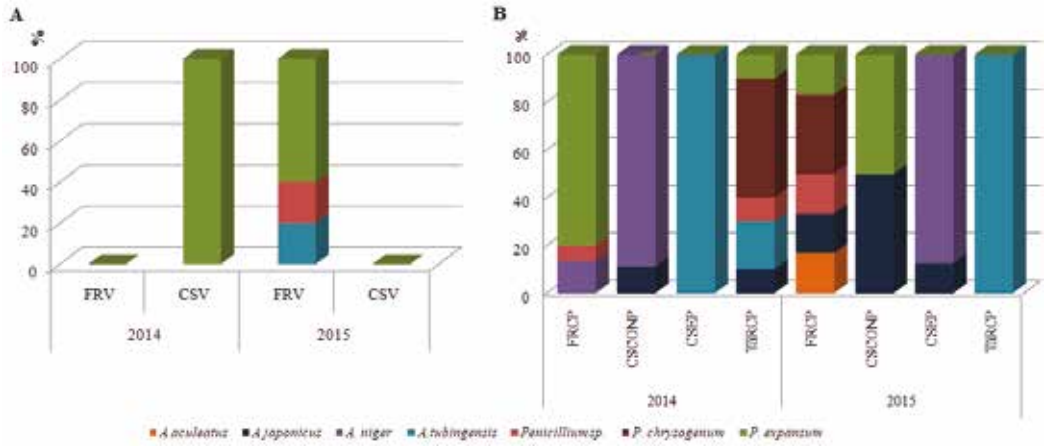

Figure 2. Distribution of black *Aspergillus* and *Penicillium* isolates in two vilticultural centres - Valea Călugărească (**A**) and Pietroasa Centre (**B**) on different grape varieties

CONCLUSIONS

A total of 61 fungal isolates have been isolated from red and white wines cultivated in Dealu Mare vineyard under conventional and ecological conditions.

A. niger and *P. expansum* were the predominant species identified among the isolates. No significant difference in fungal biodiversity of the grapes has been detected between conventional and ecological cultures.

In our work four fungal isolates couldn't be clearly identified because of the lack of information in the enzymatic profiles in databases. However, PCR-RFLP can be a useful tool for the identification of different species belonging to black *Aspergillus* and *Penicillium*.

When this identification is performed in early stage, better prevention measures and controls can be performed for a good quality management of the final product, the wine.

Future work will be focused on mycotoxin and volatile compounds production of isolated strains.

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INFLORESCENCE AND FLOWER DIFFERENTIATION IN THE GRAPEVINE (*V. VINIFERA* L.) VARIETIES 'ŽILAVKA' AND 'BLATINA'

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Abstract

The different dynamics of flower differentiation i.e. flowering and the functional capacity of reproductive organs in grapevine must be studied as genotypic specificity under particular agroenvironmental conditions and brought to a level at which standardized biological control of fruiting is exercised in order to define sustainable production systems. Research on the functional morphology of the inflorescence and flower in the indigenous grapevine varieties 'Žilavka' and 'Blatina' as the major ones used for grape production in Bosnia and Herzegovina (B&H) was conducted in 2010-2011 through analysis of permanent histological slides. The morphological characteristics of the inflorescence, as observed through the presence and distribution of flowers, and the flowering dynamics of individual flowers in the inflorescence pave the way for the normal course of flowering and pollination in the studied varieties. During inflorescence development, the degree of flower congregation in the distal part of the inflorescence is defined as a varietal characteristic. The inflorescence in 'Žilavka' and 'Blatina' grapevines forms a cone-shaped panicle due to the different structure of side branches i.e. different morphology and structure of branching on the axes of simple botryose inflorescences along the inflorescence axis, when observed from the top down to the base. The sequence and dynamics of differentiation of the flower generative elements clearly indicate protandry in both varieties.

Key words*: grapevine, inflorescence, flower, characteristic*

INTRODUCTION

The grapevine inflorescence is a panicle belonging to compound racemose inflorescences with the axis growing forward relatively long at the tip forming side branches with flowers arranged in simple racemose inflorescences, and terminating in a flower (closed inflorescence). The grapevine inflorescence differentiates in the bud from the end of May until September in a single growing season (Mulins et al., 1992; Burić, 1981) and continues to develop in the spring of the following year. As stressed by Carmona et al. (2008), this fact is the main constraint to many studies aiming to explore all differentiation sequences and finally execute the genetic control of grapevine reproduction and yield. A more detailed analysis of flower differentiation in the inflorescence in accordance with the classification proposed by Mulins et al. (1992) has been provided by Caporali et al. (2003). 'Blatina' is an indigenous grapevine variety producing functionally female flowers that have a large effect on yield, which varies depending on the year. The production of a single-variety wine from this variety has not been a regular winemaking practice. As the 'Blatina' varietal wine does not exhibit long-lasting color stability, it is recommended to blend wines made from varieties used as pollenizers in plantings (Blesić, 2001). On the other hand, 'Žilavka' has been the leading grapevine variety in B&H for many years now (Mijatović, 1988; Tarailo, 1991). As estimated by Tarailo and Kovačina (1983), the important role 'Žilavka' plays in grapevine production and its heterogeneity in cluster and berry appearance make it possible to select a range of genotypes. However, when analyzing the genetic variability of 'Žilavka' by the AFLP technique Tomić (2009) determined the genetic profile characteristic of 'Žilavka' at 14 microsatellite loci and found no genetic background for the selection of 'Žilavka' clones or new genotypes with improved varietal

properties. The objective of this study was to examine the functional morphology of the inflorescence and flower in 'Žilavka' and 'Blatina' grapevines as the basis of success in micro- and macrosporogenesis.

MATERIALS AND METHODS

Anatomical, morphological and cytohistological characteristics of reproductive organs were evaluated in two major indigenous varieties in B&H - 'Blatina' and 'Žilavka'. Part of the experimental research and sampling for the anatomical, morphological and cytohistological analyses of reproductive organs were conducted in a commercial vineyard of the Aluminium Plant, Mostar (southeastern B&H). The varieties were grafted on Kober 5BB rootstock, with vines trained to the Moser cordon system and subjected to short pruning. Standard agricultural and viticultural practices were used, which along with growing conditions (modified Mediterranean climate) ensured steady yields. Samples for the preparation of histological slides were collected from initial cluster emergence until fertilization. The cytohistological analysis of the reproductive organs involved preparation of permanent histological slides. Sampling for the preparation of histological slides was performed during shoot and inflorescence growth and at early flowering and pollination stages. Inflorescences were sampled every two days between 9 and 10:30 a. m. from 27 April until 25 May in 2009 and 2010. In the field, immediately after removal from the shoots, the inflorescences were placed into bottles and fixed in Navashin's fixative. The fixed paraffin-embedded specimens were sectioned using a microtome at a thickness of 9 *ηm*. The differential staining of the histological sections was performed by Delafield's hematoxylin. The sections were analyzed under an Olympus/DP/SOFT light microscope. Images were documented using an Olympus/DP camera and edited by the Image Analyzer software.

RESULTS AND DISCUSSIONS

During inflorescence development, the degree of flower congregation in the distal part of the inflorescence is defined as a varietal characteristic. This aggregation and grouping of flowers in simple botryose inflorescences on side branches in the basal half of the panicle is the result of the progressive shortening of the internodes on the side branches of the inflorescence. Reduction in internode length on side branches which carry simple botryose inflorescences is a variety-specific morphological character in ampelography as it results in variable compactness of the infructescence (the arrangement of berries in the cluster), and at preflowering it can be associated with the mechanical pressure on petals, that is, this compactness among flowers can have a role towards flower caps in terms of flower opening-cap fall.

Figure 1. Structural analysis of side branches of an inflorescence histologically (left) and morphologically (right) showing flowers grouped in simple clusters in the following arrangement - the apical part of the side branch terminating in three flowers which have bract rudiments at their base; remaining flowers grouped in simple botryose - racemose inflorescences whose number and distribution on the shortened to compact axes of the side branches of the inflorescence can be observed primarily through bract presence

The morphological characteristics of the inflorescence cannot be directly associated with functional morphology as the presence and distribution of flowers and the flowering dynamics of individual flowers in the inflorescence cannot be associated with pollination or fruit set. The first branch of the inflorescence closest

to the base is often quite distant from the other branches and constitutes a distinct part of the inflorescence. During further growth and development, this branch of the inflorescence can develop into a small cluster called a lateral cluster or a wing, which can reach the length of the main inflorescence in some varieties. This inflorescence character has been established in 'Žilavka' grapevine and is considered its varietal characteristic as regards the ampelographic characterization of the cluster (Tarailo and Kovačina, 1983; Mijatović, 1988; Tarailo, 1991). The differentiation of flower primordia was observed dynamically from the microstage at which the emergence of inflorescence axes on the shoots was visible until fully formed flowers (Carmona et al., 2008). The research performed at this level of observation showed no anatomical, morphological or histological differences in the differentiation of individual flowers in the studied varieties. The difference in the differentiation dynamics between the varieties and among the inflorescences differently positioned on the shoots at this point of the research was analytically generalized so that more extensive histological and cytological analyses of individual flowers could be executed for the evaluation of their individual elements. The flower of the grapevine is pentagonal, composed of 5 sepals arranged along the margin of the receptacle. As the sepals are poorly developed, they can be considered rudimentary. The inside of the calyx holds five petals which are fused along their entire length. There are 5 stamens, completely free and practically located in the axils of the petals. Each stamen consists of an anther supported by a filament. The pistil occupies the center of the flower, and comprises the ovary, stigma and style. The ovary is bilocular, having two carpels, morphologically two chambers, each containing two ovules. The histological analysis of flower primordia becoming visible as a small pinhead shows the differentiation of sepal primordia enclosing the less conspicuous petal primordia, which form a roof over dome-shaped anther initials (Figure 2a). At the microstage when the petals surpass the sepals by their whole length, all anther primordia elements differentiate, and initial carpel differentiation is observed (Figure 2b).

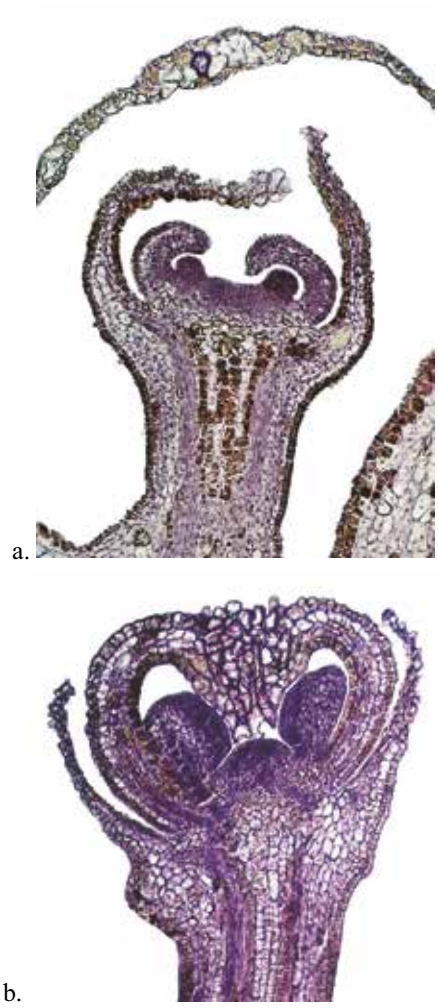

Figure 2. Microstages of flower differentiation in 'Blatina': a) sepals, petals and anther primordia differentiated on the flower axis; b) microstages of the differentiation dynamics of flower elements observed in relation to the sepals whose development is arrested as early as the beginning of flower primordium development, due to which they practically remain rudimentary. The petals fuse at the tips and cover anther initials, keeping them leaning against the stigma of the ovary

The differentiation sequence and dynamics of the flower generative elements clearly indicate that both varieties are protandrous. Protandry is fully visible in the histological sections of the anthers and ovaries in the same flower initials (Figure 3).

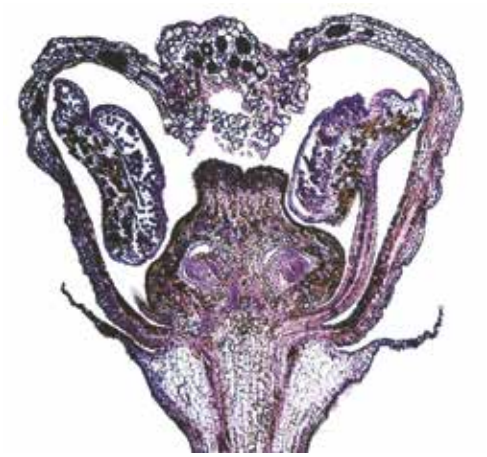

Figure 3. Microstages of flower differentiation in 'Blatina'. At this microstage of flower primordium development, the final stages of microsporogenesis and initial stages of macrosporogenesis are observed

The results on the dynamics of flower differentiation in the inflorescences of 'Žilavka' and 'Blatina' grapevines, as observed on the histological slides, are in complete agreement with the flower differentiation dynamics reported by Caporali et al. (2003) based on the classification proposed by Mulins et al. (1992). The cross-section through the center of the flower shows the position of the anthers relative to the petals and the manner in which the petals fuse to form the flower cap in the studied varieties. Minor differences are observed in the length of the fusion zone between the varieties (the fusion zone being somewhat longer in 'Žilavka' than in 'Blatina'), which can affect the rate of cap detachment during bloom (Figure 4 a, b). The center of the flower is occupied by the pistil made up of two fused carpels - microsporophylls. The ovary is bilocular - it consists of two chambers, each containing two ovules.

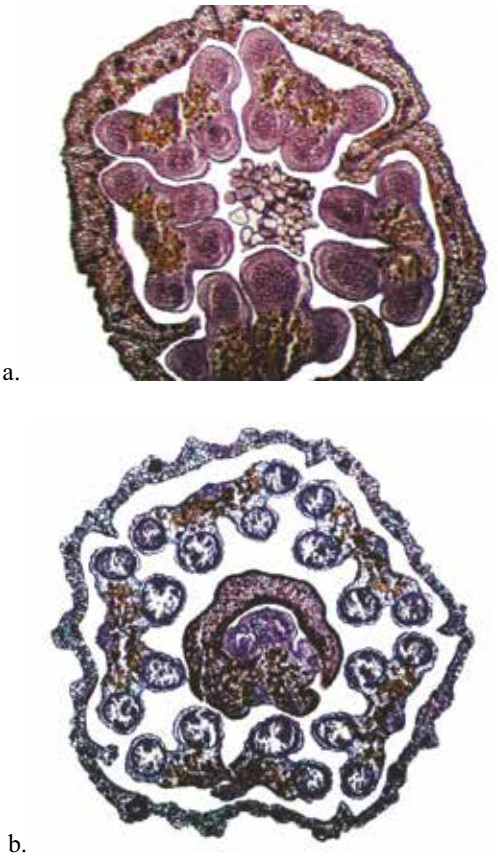

Figure 4. Histological cross-sections of flower initials in 'Žilavka' (a) and 'Blatina' (b) clearly showing the petal fusion zone

The partition between the chambers is formed by the bending and curving of the carpel margins inwards towards the interior of the ovary. In this way, the carpels fuse laterally, dividing the ovary into two chambers. All ovules are initiated perpendicular to the margin, and then nucellar and integumentary cells grow and develop at different rates, giving rise to anatropous ovules with the micropyle facing the base of the ovary. In this way, four anatropous ovules in marginal placentation are formed in the ovary of each variety (Figures 5 and 6). The central part of the floral structure is occupied by the ovary composed of two chambers, each holding two ovules.

Figure 5. Histological representation of the cross-section of the bottom third of an individual flower, in 'Žilavka'. The cross-section displays the formed ovules at the integument differentiation stage. Cross-sections of the filaments are visible in the zone between the ovary and the petals. The cross-section of the filament in 'Žilavka' clearly shows epidermal and parenchyma cells located at the periphery of the filament and somewhat smaller central cells of the vascular tissue). This anatomical structure of the filament is typical of bisexual flowers in the grapevine

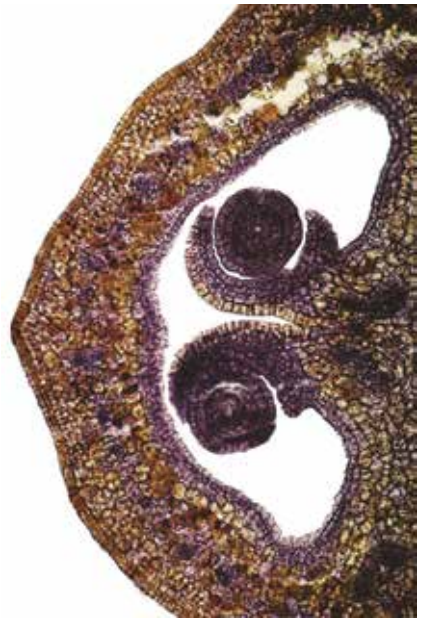

Figure 6. Histological representation of the morphological and anatomical structure of a portion of the ovary in 'Žilavka'. The ovule develops in the interior of the ovary and has marginal placentation. Later on, the ovule develops into a seed. The cross-section shows the ovule the size of a small bulge covered by the integument

The analysis of inflorescence and flower differentiation in the tested varieties is consistent with the findings of Caporali et al. (2003) who reported that functional differences between the (male and female) flowers are commonly recorded at this stage, when no style and stigma formation occurs on the pistil of male flowers. Due to these morphological changes, the pistils of male and female flowers are quite different at the next stage of development, whereas their stamens still remain very similar in appearance.

CONCLUSIONS

The inflorescence in 'Žilavka' and 'Blatina' grapevines forms a cone-shaped panicle due to the different structure of side branches i.e. different morphology and structure of branching on the axes of simple botryose inflorescences along the inflorescence axis, when observed from the top down to the base. The degree of flower congregation in the distal part of the inflorescence can be defined as a varietal characteristic. This aggregation and grouping of flowers of simple botryose inflorescences on side branches in the basal half of the panicle is the result of the progressive shortening of internodes on the side branches of the inflorescence. The histological analysis of the flower primordium visible as a small pinhead at the morphological stage shows differentiated primordia of sepals enclosing the less conspicuous primordia of petals, which form a roof over dome-shaped anther initials. The differentiation sequence and dynamics of the flower generative elements clearly show that both varieties are protandrous. All inflorescence and flower differentiation processes in the tested varieties take their normal course, with no anomalies potentially adversely affecting pollination and fertilization found at this level of observation.

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DETERMINATION OF THE PHENOLIC COMPOUNDS, ANTIOXIDANT AND ANTIRADICAL ACTIVITIES OF SENIRKENT KARASI GRAPE CULTIVAR'S SKIN AND SEEDS

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Abstract

Purpose of this study was to determine total phenolic content, phenolic composition, antioxidant and antiradical activities of 'Senirkent Karası' grape cultivar's skin and seeds. While total phenolic contents of grape skin and seeds were determined by Folin-Ciocalteu method spectrophotometrically expressing the results in terms of gallic acid (GAE), phenolic composition was analyzed by HPLC (High Performance Liquid Chromatograph). Antioxidant activities of the grape skin and seeds were evaluated by reducing powers whereas antiradical activities were examined using DPPH (1-diphenyl-2- picrylhydrazyl). Results showed that total phenolic contents of seeds and skin were 52.32 and 1.89 mg g^{-1} GAE g^{-1} DM, respectively. Antiradical activities of seed and skin extracts (100 ppm) were 95.90 and 16.22 %, respectively. Reducing powers of seeds were 1.64 at 250 ppm, and were 2.39 at 1000 ppm whereas antioxidant activities) of skins were 0.08 at 250 ppm; 0.31 at 1000 ppm. Results showed that skin had higher amount of phenolic compounds than seeds and gallic acid, catechin, cafeic acid, syringic acid, resveratrol, quarcetin, kaempherol, p-cumaric acid were present in skin whereas only gallic acid, catechin, epicatechin were present in seeds. Seeds had the highest values of epicatechin (746.94 µg g^{-1}) while skins had the highest values of syringic acid (17.01µg g^{-1}) and gallic acid (5.29 µg g^{-1}).

Key words: *phenolics, antioxidant activity, antiradical activity, 'Senirkent Karası'.*

INTRODUCTION

There is an increasing interest on grape, grape products and other parts of grapevine due to rich chemical compounds they have. Increasing interest on natural antioxidants resulted in an increase in number of research on improvement and evaluation of natural products that are rich in phenolic compounds. Phenolic compounds that have very high antioxidant and antiradical properties are substances that have direct effect on quality, that give resistance ability to diseases and have pharmacologic features (Macheix et al., 1990; Clausen et al., 1992; Ayed et al., 1999; Yi et al., 2006). In addition, phenolic compounds reduce risk of cancer and heart diseases and lead to low density lipoprotein (LDL) due to their high antioxidant properties. There are studies showing that grape skin and seeds have a variety of polyphenol contents, high antioxidant property and contain flavonoids (catechin, epicatechin, procyanidins, anthocyanins), phenolic acids (gallic acid, ellagic acid) and stilbenes (resveratrol and piceids) (Jayaparakasha et al., 2003; Negro et al., 2003; Yılmaz and Toledo, 2006). However different parts of grape have different content of these above mentioned compounds.

'Senirkent Karasi' grape cultivar is a local grape grown in Isparta province that is mainly used as wine, table and dried consumption grape.

Thus, the purpose of this study was to determine the total phenolic content, phenolic composition, antioxidant and antiradical activities of 'Senirkent Karasi' grape cultivar's skin and seeds.

MATERIALS AND METHODS

Materials

In the study skin and seeds of 'Senirkent Karasi', a commonly grown cultivar in Isparta, was used. Fresh grapes were obtained from Isparta Directorate of Provincial Food Agriculture and Livestock, dried in the shade and later seeds and skin were separated to be analyzed. There were three replications for each analysis.

Phenolic extraction

Grape seeds and skins were manually separated from whole berries, seeds were dried at room temperature and then were crushed in a grinder for two min. In order to remove the fatty materials from seeds, the powdered grape seeds (100 g) were extracted in a *Soxhlet* extractor for 6 h with 150 ml of petroleum ether at 60°C. The defatted grape seed powder and also powdered skin were extracted in a *Soxhlet* apparatus for 8 h with 200 ml of acetone: water: acetic acid (90:9.5:0.5) at 60°C as described by Jayaprakasha et al (2003). The extracts were concentrated by rotary evaporator at 70°C to get crude extracts and stored in a desiccator.

Determination of total phenolic content

Total phenolic contents of the grape seed and skin extracts were determined spectrophotometrically using a PG Instruments T70 Plus Dual Beam Spectrophotometer (Arlington, MA, USA) according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965), calibrating against gallic acid standards and expressing the results as mg gallic acid equivalents (GAE g^{-1}) extract for seed and skin extracts. Data presented are average of three measurements.

HPLC determination of phenolic compounds

Chromatographic analyses were carried out on a Shimadzu model HPLC system (Shimadzu Corp., Kyoto, Japan). Separation of phenolics was performed by the modified method of Caponio et al. (1999). Reversed phase (RP)-HPLC analysis was done using a SCL-10Avp system controller, a SIL- 10AD vp autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater, and a Diode Array Detector with wavelengths set at 278 nm. The 250 x 4.6 mm i.d. 5 µm column used was filled with Agilent Eclipse XDB-C18 (Wallborn, Germany). The flow rate was 0.8 ml min^{-1}, the injection volume was 20 µl, and the column temperature was set at 30°C. For gradient elution, mobile phase A contained 3% acetic acid in water; solvent B contained methanol. The following gradient was used: 0-3 min, from 100% A to 95% A; 3-20 min, from 95% A to 80% A; 20-30 min, from 80% A to 75% A; 30-40 min, from 75% A to 70% A; 40-50 min 70% A to 60% A; 50-55 min, 60% A to 50% AB; 55-65 min, 50% A to 0% A. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The grape samples, standard solutions and mobile phases were filtered by a 0.45 µm pore size membrane filter (Millipore Co. Bedford, MA). The amount of phenolic compounds in the seed and skin extracts were calculated as mg 100 g^{-1} extract, separately, using external calibration curves obtained for each phenolic standard. Caffeic acid, (+)-catechin, chlorogenic acid, o-coumaric acid, p-coumaric acid, (-)-epicatechin, ferulic acid, gallic acid, kaempherol, trans-resveratrol, quercetin, syringic acid and vanillin acquired from Sigma (St. Louis, MO, USA) were used as standards and determined in the samples.

Determination of antiradical activity

The free radical scavenging activity of extracts were examined by comparing to those of known antioxidants such as BHT (butylated hydroxytoluene), BHA (Butylated hydroxyanisole) and trolox by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma (St. Louis, MO, USA) using the method of Shimada et al. (1992). Briefly, a 1.0 ml solution of the samples (seed and skin extracts) and standards at 100 µg ml in methanol was mixed with 1.0 ml of methanolic solution of DPPH (0.2 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm against methanol as the blank in a PG Instruments T70 Plus Dual Beam Spectrophotometer (Arlington, MA, USA). The addition of the samples to the DPPH solution caused a rapid decrease in the optical density at 517 nm.

The degrees of discoloration indicate the scavenging capacity of the samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity (Baumann et al., 1979). Antioxidants break the free radical chain of oxidation and donate hydrogen from the phenolic hydroxyl groups. Therefore, the resulting stable endproduct does not permit further oxidation of the lipid (Sherwin, 1978). All determinations were done in triplicate and the percent of DPPH decolouration of the samples were calculated according to the formula:

Antiradical activity (%) = 100x[(absorbance of control-absorbance of sample)/absorbance.

Determination of reducing power

The reducing power of samples were determined by Oyaizu method (1986). Absorbance of supernatant was measured at 700 nm and compared to three standards, BHA, BHT and trolox; any increase in absorbance is synonymous of an increase in reducing power.

RESULTS AND DISCUSSIONS

Total phenolic compound content, antiradical and antioxidant activity of seed and skin are presented in Table 1.

As it is observed in Table 1, the yields (dry weight) of grape seed and skin had 12.60% and 9.64 %, respectively.

Table 1. Yield, total phenolic compound content, antiradical and antioxidant activity of seed and skin of 'Senirkent Karasi' grape cultivar

Sample	Yield (%)	Total phenolic content (mg/g GAE)	DPPH (100 ppm extract (%)	Reducing power (μgl^{-1}) (Absorbance)	
				250 ppm	1000 ppm
Seed	12.60±0.63	52.32±3.25	95.90±1.03	1.64±0.18	2.39±0.20
Skin	9.64±0.68	1.89±0.29	16.22±0.80	0.08±0.00	0.31±0.01

Total phenolic contents of the samples were estimated with Folin-Ciocalteu colorimetric method. When total phenolic contents of seeds extracts were calculated as mg GAE g^{-1} (Table 1) it is found that seeds had higher total phenolic compound content than skins. Seeds and skin had total phenolic compound content of 52.32±3.25 and 1.89±0.29 mg/g, respectively, in terms of gallic acid. Results are in agreement with those found by (Negro et al., 2003; Yılmaz and Toledo, 2004; Iacopini et al., 2008). These researcher also found that seeds had higher total phenolic compound content than skin.

HPLC method for analyzing phenolics in the samples has some advantages, such as easy and time consuming procedure for preparation of the samples, possibilities of quantification of a greater amount of diverse phenolics, the precision, accuracy and detection limits obtained for the phenolics quantified by this method enabling its application to grape (Gomez Alonso et al., 2007). The amounts and variations of phenolic compounds in the seed and skin extracts were determined by HPLC and presented in Table 2.

Table 2. Phenolic compounds of seeds and skin of 'Senirkent Karasi' grape cultivar

Phenolic compound	Skin, µg. g^{-1}	Seeds, µg. g^{-1}
Gallic acid	5.29±0.10	144.76±0.45
(+)-Catechin	3.43±0.21	637.88±5.55
(-)-Epicatechin	nd	746.94±2.13
Caffeic acid	3.83±0.29	nd
Syringic acid	17.01±0.21	nd
p-coumaric acid	0.70±0.01	nd
Trans-Resveratrol	2.47±0.02	nd
Ouarcetin	2.49±0.20	nd
Kaemferol	0.50±0.02	nd

It is found that skin had higher number of phenolic compounds than seeds. In skin samples 8 compounds such as gallic acid, catechin, caffeic acid, syringic acid, p-coumaric acid, resveratrol, quarcetin and kaempferol were detected whereas in seed samples only 3 compounds such as gallic acid, epicatechin and catechin were detected. Gallic acid amount in seeds and skin were determined as 144.76±0.45 µg g^{-1} and 5.29 ±0.10 µg g^{-1}, respectively. In

the same manner catechin amount in seeds and skin were determined as 637.88 ±5.55 μg g^{-1} and 3.43 ±0.21 μg g^{-1}, respectively. As regards to the presence of catechin in skin and seeds, it is commonly known that flavan-3-ols are located in both grape skin and seeds; however, skin contains much lower concentrations of flavan-3-ols than seeds (Revilla and Ryan, 2000).

In addition, another flavonoid, epicatechin, amounted in seeds 746.94±2.13 μg g^{-1} and it was not detected in skin. The results agree with the studies of Cheynier (1998), Rodriquez Montealegre et al. (2006) and Baydar et al. (2011), who also found that grape seeds had higher flavanol contents than skins. Another study also found that there was presence of epicatechin in seeds, whereas there was no epicatechin in skin (Souquet et al., 2000).

Trans-Resveratrol, a phytoalexin that belongs to the group of compounds known as stilbenes, is known to occur in grapes and consequently in grape products and in wine. *Trans*-resveratrol was found in 2.47 μg g^{-1} in the skin extracts. Baydar et al. (2011) also found 1.82 and 4.02 mg 100 g^{-1} of *trans*-resveratrol in grape skin extract. Iacopini et al. (2008) explained this result as the consequence of the fact that grapes produce stilbenes in response to mold infections and physiological stresses. If these stresses are not present, the levels of stilbenes in grapes remain low.

Radical scavenging activities of grape extracts, and standards were tested by the DPPH method. When radical scavenging activities of seed and skin is examined, it is observed that seeds had 95.90% antiradical activity whereas skin had 16.22% antiradical activity. The radical scavenging activities of the seed extracts were considerably higher than those of skin extracts. Grape seed extracts almost completely inhibited DPPH absorbtion. Otherwise skin extract contained remarkably lower amounts of radical scavenging compounds. Some researcher reported that there was a correlation between DPPH activity and total phenolic compound content of seed (Guendez et al., 2005; Hua et al., 2008). In this research it is also found that seeds had higher total phenolic compound content than skins and seeds had higher DPPH activity than skin.

When the reducing powers of seeds and skin was examined it was found that seeds had 1.64 μg l^{-1} and 2.39 μg l^{-1}values at 250 and 1000 ppm, respectively, whereas skin had 0.08 μg l^{-1} and 0.31 μg l^{-1} reducing power ability values at 250 and 1000 ppm, respectively. Higher absorbance values correspond to higher reducing power thus it is found that seeds had higher reducing power than skin. Hua et al. (2008) reported that in seeds of grapes there was a correlation between reducing power. In this research it is also found that seeds had higher total phenolic compound content than skins and seeds had higher reducing power than skin.

CONCLUSIONS

In this research we determined phenolic compounds, antiradical and antioxidant activity in seeds and skin of 'Senirkent Karasi' grape cultivar which is commonly produced and consumed in Isparta province.

The results obtained in this study showed that large differences were found grape seed and skin in relation to the phenolics composition. Senirkent Karası grape's seeds, and skins contained different phenolics with different levels and these variations affected the antioxidant capacity of the samples. Total phenolic contents, reducing powers of grape seed extracts are higher than those of grape skin extracts.

The result of study is important because grape seeds and skin are a good source of phenolic compounds that have positive effect on health, and they are rich in natural antioxidants. Thus, determining these compounds in a local cultivar is important in terms of health issue.

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STUDY ON THE BEHAVIOR OF THE VICTORIA TABLE GRAPE VARIETY IN THE HINOVA VITICULTURAL AREA, MEHEDINTI COUNTY

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Abstract

This paper aims at studying the favorability of the cultivation of the 'Victoria' table grape variety in the Hinova viticultural area, Mehedinti County, in a non-irrigated system. The research was carried out in 2017. After determining the viability of the buds losses were found, so there were studied 3 different fruit loads to follow the number of buds started from the vegetation and to meet the number of shoots required for the study. Once this requirement has been fulfilled, the work of normalizing the inflorescences was carried out, taking into consideration 5 variants, namely: 6, 8, 10, 12, 14 inflorescences/vine. The evolution of these fruit variants was followed up until reaching the full maturity stage, with the harvesting of the grapes resulting in the deliverable production of each variant of the studied fruit load. The conclusion of this study is that Hinova viticultural area in Mehedinti County is favorable to the cultivation of the 'Victoria' vine variety in a non-irrigated system, only if a moderate fruit load is respected, thus obtaining quality grapes.

Key words: *favorability, Hinova, table grapes, 'Victoria' variety, viticultural area*

INTRODUCTION

Hinova viticulture area located in Mehedinti County enjoys a climate with Mediterranean influence, due to its proximity to the Danube, mild winters and dry summers (Gherasim et al., 1977).

This very important factor in the zoning of vine varieties allows the cultivation of table grape varieties in this area, who has been cultivated until 1989 with table grape varieties such as 'Perla de Csaba', 'Regina Viilor', 'Coarna alba', 'Coarna neagra', 'Chasselas doré', 'Chasselas rozé'. The table grape variety Victoria has special charactheristics (precocity, size, weight, taste, appearance), being grown on large surfaces both in Romania and in countries with a tradition in the cultivation of table grape varieties: Italy, Greece, Spain, Turkey (Giugea et al., 2015)

The study of the behavior of the 'Victoria' table grape variety in the Hinova viticultural area in a non-irrigated system is a novelty for this area, since it has not been cultivated in the past, so it can be noticed what technological measures must be taken to obtain the best productions, both quantitatively and qualitatively.

MATERIALS AND METHODS

In order to study the favorability of the cultivation of the Victoria table grape variety in the Hinova viticultual area located in Mehedinti County, the following elements were studied: the viability of the winter buds in order to determine the type of winter pruning that had to be carried out, the number of winter buds to be assigned to the fruit elements (2 canes + 2 spurs), the number of buds started in vegetation, the number of inflorescences assigned to each vine, the determination of the weight of the harvested grapes.

This study was conducted during the year 2017.

RESULTS AND DISCUSSIONS

Knowing the viability of the winter buds is an important element in determining the number of winter buds to be attributed to the fruit elements, with the occasion of the winer pruning. It is done by cross-section with the blade by the buds during the rest period of the vines, once the danger of other losses due to low temperatures has passed. Once the viability check has been carried out, it has resulted a

percentage of loss of the main buds due to winter frost of 60%.

Figure 1. Cross section made with the blade through the bud complex

The type of winter pruning is double Guyot (2 woody shoots + 2 spurs) .

Figure 2. Guyot type pruning applied to the 'Victoria' vine

In order to study the number of buds that will start in vegetation and to meet the number of shoots with fruit necessary for the study, we have studied 3 different variants of loads, which will be attributed to the fruit elements during the winter pruning, and namely: 16 winter buds/grape vine divided into 2 canes x 6 winter buds + 2 spurs , 20 winter eyes/grape vines divided by 2 canes x 8 winter buds + 2 spurs, 24 winter buds/grape vines divided by 2 canes x 10 winter eyes + 2 spurs.
The number of buds started in vegetation as well as the number of inflorescences / grape vine for each studied variant are presented in Table 1:

Table 1. Load variants/vine

No. variant	No. winter buds	No. buds started in vegetation	No. inflorescences/vine
1	16	13	20
2	20	12	19
3	24	14	23

Analyzing the data presented in this table, we can conclude that from of all the variants taken in the study resulted a number of similar shoots, the same conclusion can be made in the case of the number of inflorescences distributed on each grape vine. Next, there are 5 variants of fruit loads taken in study, obtained by the work of normalization of the inflorescences: 6, 8, 10, 12, 14 inflorescences/grape vine.

Figure 3. Buds widening phenophase

The evolution of the 5 variants was followed during the vegetation period, of interest being the period of ripeness and the period of maturity of the grapes (Table 2).

Figure 4. Phenophase of 2-3 leaves

Figure 5. Blooming phenophase

Figure 6. Ripness phenophase

Table 2. Ripeness/maturity date of grapes depending on the number of inflorences left on the vine

No.inflorences/vine	Ripeness starting date	Date of maturity
6	July 23-24	August 10
8	July 22-24	August 14
10	July 26-27	August 16
12	July 29-30	August 22
14	August 1-2	August 25

In Table 3 there are presented data showing the number of grapes harvested from the three variants taken into study as well as the total weight of grapes obtained/grape vine:

Table 3. Number of harvested grapes and weight/vine dending on the number of infloreces left on the vine

No.inflorescences/vine	No. of harvested grapes	Total grape weight/ vine
6	6	4.2
8	8	5.4
10	9	4.9
12	9	4.6
14	4	1.4

Figure 7. 'Victoria' vine with a load of 12 grapes/vine

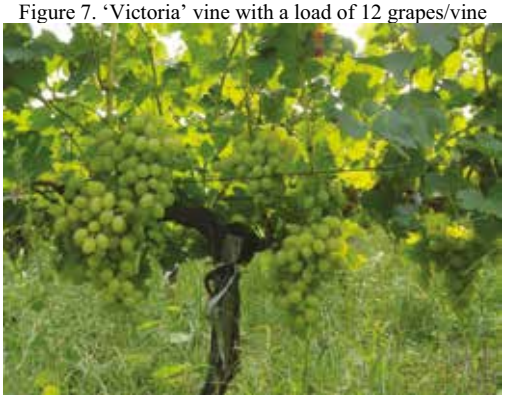

Figure 8. 'Victoria' vine with a load of 8 grapes/vine

CONCLUSIONS

This study highlights the fact that once the number of inflorescences / grapes on the grape vine increases, it increases also the date when the grapes reach maturity, and the number of grapes harvested from the grape vine decreases. It was noteworthy that the best results had the grape vines with a load of 8-10 grapes/vine. Also, it was observed that in the variants with fewer grapes/vine they were bigger and more compact, and in the variants with more grapes/vine they were smaller and less compact.

The conclusion of this study is that the Hinova viticultural area located in Mehedinti County is favorable to the cultivation of the 'Victoria' vine variety in a non-irrigated system, only if a moderate fruit load is respected, thus obtaining quality grapes.

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CONSIDERATIONS REGARDING THE USE OF ANTI-HAIL NETS FOR THE PROTECTION OF TABLE GRAPE PLANTATIONS IN HINOVA VITICULTURAL AREA, MEHEDINTI COUNTY

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Abstract

This paper aims to present the benefits of implementing a protection system with nets against hail in table grape vineyards, as well as the problems that may occur. The study was carried out between 2013 and 2017, in a table grape vineyard. in Hinova, Mehedinti County. It was possible to analyze during the 5 years of study the behavior of vegetative phenophases of the 'Victoria' vineyard grown in a system covered with the anti-hail net, compared to another 'Victoria' vineyard cultivated in an uncovered system. In the studied period, hailstones occurred in 2014, 2016 and 2017, the most severe hail fall occurring on June 20, 2014, where the damage to the unprotected 'Victoria' vineyard was 100%. In conclusion, the protection of the vineyards with anti-hail net is beneficial, it protects to a certain extent against late spring frost, but it also draws certain shortcomings or reservations in deciding to choose for a such protection: high-cost investment, more frequent application of treatments to fight diseases and pests.

Keywords: *anti-hail net, Hinova area, Mehedinti County, vine protection.*

INTRODUCTION

Hailstorms, according to their intensity, can have destructive effects on vine plantations, causing significant damage, compromising the grape production in that year, plant tissue damage, thus creating doorways for the diseases of the vine and negatively influencing the resistance to winter frosts (Bernaz, 2003).
Faced with these unpredictable natural phenomena, grape growers in general and table grape growers in particular can take a series of measures, one of which is the installation of nets to protect against hail. Taking this into account, the present work presents the benefits that such protection can offer, as well as the problems that can cause in a table grape vineyard.

MATERIALS AND METHODS

In order to highlight the advantages and disadvantages offered to vineyards by anti-hail net systems, two plots cultivated with 'Victoria' table grapes variety were studied, one having a net protection system against the hail installed, the other not having this system installed.

Figure 1. 'Victoria' vineyard protected by anti-hail net

Figure 2. Unprotected 'Victoria' vineyard

The analysis period in this study was 2013-2017.

RESULTS AND DISCUSSIONS

The hail protection system was installed in the summer of 2013. During the year 2013 no hailstones occurred.
The two plots behaved relatively similarly until ripeness (BBCH 81-85), when in the plot covered with the net against the hail there were noticed botrytis infested grapes, especially in the middle part of the plot, while on the uncovered vineyard the grapes were perfectly healthy. The net against hail was removed at the end of October.
In 2013, both plots were treated with identical phytosanitary treatments.
In 2014, the anti-hail net was installed in early April. Both plots were treated with identical phytosanitary treatments until the beginning of blooming. During the beginning of the blooming (BBCH 55-57), in the plot covered with the net there were reported inflorescences attacked by mildew, although the number of treatments and the interval between them was strictly respected, using only phytosanitary products with systemic action. Measures have been taken and additional treatments were administrated to keep the attack under control. There was no mildew attack reported in the uncovered plot.

Figure 3. 'Victoria' inflorescence attacked by mildew

On June 20, during a strong storm accompanied by massive hailstones, grape production in the uncovered 'Victoria' vineyard was totally compromised, destroying both grape bunches, leaves and vine shoots, who suffered deep tissue lesions.

Figure 4. Damage caused by heavy hail fall

In the protected vineyard, with the exception of the marginal rows, there were no problems caused by hail, thus protecting both the grape production and the health of the vines.

Figure 5. Hail stones on top of the anti-hail net system

Figure 6. Average-size hailstones reported after hail fall in 2014

In 2015, on the vine shoots from the 'Victoria' vineyard covered with anti-hail net traces of excoriosis attack was reported, vine wood disease never met before in the plantation. The uncovered plot did not show signs of this disease.

During the year, high-pressure infection of mildew, oidium and botrytis was reported in the anti-hail protected vineyard. In order to maintain the state of health of the vineyards, the interval between phytosanitary treatments was shortened, and at the same time in the disease control program there were introduced phytosanitary products with contact action for additional protection in critical moments (copper oxychloride against mildew and soluble sulfur against oidium). This increased the number of phytosanitary treatments applied to the vine throughout the wine year.

Hailstones did not occur in 2015.

The year 2016 was similar to 2015, on the plot covered with anti-hail net, the total number of treatments applied against diseases and pests increased to 10, compared to 7 treatments applied on the plot not covered with anti-hail net.

Two hailstorms occurred at the beginning of May and early July, of low intensity, resulting in minor damage to the unprotected plot. August was rainy, with cumulative rainfall reaching 70 L/sqm. These climatic conditions, which intersected with the time when the grapes reached maturity, gave rise to a strong botrytis attack, with considerable losses of production being reported in the covered plot with 60% compared to 20% - losses in the uncovered plot.

In the spring of 2017, on April 22, while the vine shoot was in the 2-3 leaf stage (BBCH10-12), the min-max thermometer recorded a minimum of 0^0C during the night, resulting in producing a late spring frost. As a result of this climatic accident, the vine shoots of the uncovered plot were affected in a proportion of 75-80%.

On the plot covered with the anti-hail net there was no significant damage, the production of grapes being a normal one, characteristic of the variety. The shoots on the plot uncovered against hail have been partially recovered, starting from the buds situated at the wrist of the leaves , but they were weak, had no fruit, so the production of grapes has been low and of poor quality.

Figure 7. Temperatures of 0^oC recorded by min-max thermometer on April 22, 2017

Figure 8. 'Victoria' young shoots affected by late-spring frost cultivated in unprotected vineyard

Figure 9. 'Victoria' young shoots unaffected by late-spring frost cultivated in net-protected vineyard

The number of phytosanitary treatments applied to the protected plot was 10, and in the unprotected plot 6.

Figure 10. Phytosanitary treatment applied in 'Victoria' vineyard protected by anti-hail nets

During the year 2017, several hailstorms of small and medium intensity occurred, affecting to some extent the quality of the poor grape production in the plot unprotected with anti-hail nets.

CONCLUSIONS

Protecting the vineyards with the anti-hail net is an ideal solution for table grape growers who want to obtain a high quality grape production, thus providing a system that can protect both grape production and vegetation year by year by the devastating effects of hailstones, good results also being obtained in the protection of the late spring frosts, when the temperatures are not well below the freezing threshold.

Among the less pleasant aspects of this study can be summarized: large initial investment, reduced ventilation at the level of the hub in the area covered with anti-hail net, high moisture content, as well as persistent dew for a longer time, compared to the plot uncovered with anti-hail net, which results in the need for a higher number of phytosanitary treatments.

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EVALUATING SOME OLFACTORY CHARACTERISTICS OF WINE IN THE CONTEXT OF GLOBAL WARMING IN THE "PLAIURILE DRÂNCEI" VITICULTURAL REGION

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Abstract

Global warming is recognized among the scientific community as a worrying fact for the environment, which generally speaking causes unwanted changes in the species' evolutionary accommodation mechanism to superior thermal conditions. In this context, this study's purpose is to point out some chemical transformations which positively contribute to the olfactory characteristics of wine. Global warming determines an increase in the alcohol levels, therefore causing a proportional increase of secondary fermentation products, which are helpful in giving a pleasant aroma. The samples used for the analysis were of the 'Sauvignon Blanc' variety from the "Plaiurile Drâncei" vineyard. The alcohol concentration of the samples varies between 11.8 and 13.6% v/v. The identification of chemical compounds was performed using the head space technique coupled with GC-MS. Following the analysis, there were identified chemical compounds which mainly give floral, fruity, citric notes and also not specific to fermentation, moreover giving a pleasant olfactory sensation to the final product. Therefore, the olfactory characteristics of wine depend on a series of factors beginning from climate parameters and ending with the fermentation process.

Key words*:* wine, *GS-MS, global warming*

INTRODUCTION

Romania is country rich in soil and diverse microclimates. It is for these reasons why from the country's territory there can be obtained from white wines characterized by freshness and lean fruity aromas to red wines, noble, suited for aging and also wines with low or high acidity and even wines suitable for producing sparkling wines. Therefore, unlike in other countries, the climate of Romania allows for variation in the types of vinification. (Stoian, 2011; Cotea et al., 2003).

The "Plaiurile Drancei" vineyard is located in the hilly terrain of the fields of "Oltenia", towards the Danube and is part of the vinicultural region of the "Getic" plateau and is one of the most southern vineyards of Romania. Documentary certified sources indicate the fact that viticulture began to develop in this area beginning around 1407, the most well-known vinicultural locations being "Oravița", "Rogova", "Drâncea" and "Oprișor". Around the year 1900, during the visit of King Charles I, the wines of this vineyard became renowned in the whole country, being sought after by every "collector" of good wines. These wines are characterized by high levels of alcoholic concentration and rich in mineral substances and extract.

The vineyard is situated on slopes, divided into two regions: the North West region of the fields of "Oltenia" which descends towards the Danube in several stages which have altitudes smaller than 200m; and the region around the "Bălăcița" plateau which has altitudes between 200m and 300m. The tilt of the slopes varies between 5 to 10 degrees. This placement is one of the factors that contribute to the superior quality of the wines. Another factor is the soil's composition. Their composition is made of: 60% brown-red strongly eroded soil, 15% ordinary brown-red soil, 10% brown-red

eroded soil, 10% ordinary leached chernozem and 5% ordinary alluvial soil.

Ion Ionescu de la Brad claimed in one of his papers that in "Oprișor" the sun falls on the vineyard all day long and that the soil in this locality was "red clay", referring to the brow-red soil. The hydrography is characterized by a network of small rivers and their tributaries.

In these vinicultural areas the clime is continental temperate with mediterranean influences and is characterized by hot summers and relatively mild and wet winters (the frost seldom happens or it is of weaker intensity). The average temperature is between -1 and -2 degrees Celsius in January and between 21 and 22 degrees Celsius in July. The average annual temperature is 11 degrees Celsius and the average annual precipitations are between 550 and 600 mm, while snow is relatively scarce. During the periods of drought there is a water deficit. By taking into account all these parameters of the natural setting, they add up to an ecosystem that is favourable for high quality viticulture (Cotea and Andreescu, 2008; Cotea et al., 2003; Toti et al., 2017; Ionescu, 1968).

According to the National Meteorology Administration's estimations, which were presented in a report, the average annual temperature of Romania increased during 1961-2014 by 0.5 degrees Celsius and regarding precipitations, their quantity resulted in an increase in the number of extremely droughty years. The forecasts on this subject show that in the following years (2021 - 2050) the summers will become hotter, draughty and the precipitations will decrease by up to 10-20% on average (Sandu, 2015).

The 'Sauvignon Blanc' variety is the second most popular white wine in the world and is native to France, Bordeaux but it is grown from the oldest times in the Loire Valley, in New Zeeland or in the USA. It belongs to the category of quality wines. Due to its adaptability, in present it is being grown in a lot of countries and on all the continents. It is assumed that the name "Sauvignon" comes from the French word "*sauvage*" which means untamed or wild, the association being made with the wild vines or with its special aroma. Generally, it is a wine with a soft aroma. Based on the grapes' degree of ripening they can give herbal, vegetable or green fruity tones, and when coming to a higher degree of ripening they can give floral and exotic fruity tones. Therefore the range of aromas can vary from forest apples, grapefruit, passion fruit, kiwi and well ripened cantaloupe. Furthermore, the specifics of this variety consist of the smell of "cat urine", but experienced in a pleasant way which is a designator of quality and is used as a means of identification. Although it is a variety that offers herbal (green leaves) tones, this changes when the level of alcoholic concentration rises or oak is used in the winemaking process and the exotic tones prevail (Old, 2013; Stoian, 2011; Cotea et al., 2003).

In the context of global warming this paper takes a more positive approach on the changes caused by the climate factors. The trend is that of the vineyard, through its characteristics, to be able to adapt to the sharp rise of temperatures, respectively resisting to the absence of precipitations. If a variety of grapes is developing naturally, while keeping its specifics, in a certain range of temperature, when the temperature rises on average even with as little as 1-2 degrees Celsius there is a risk of losing its defining attributes or not being able to adapt at all in these new climate conditions. In that case the approach taken by the winemaker is very important. The biggest change in wines that is caused by global warming is the increase in the alcoholic concentration (even with as much as 2-3%). Once this happens, the intensity of the aroma also increases, especially the aromas of oven baked fruits, exotic aromas, spicy or oak aromas. The current trend of the Romanian consumer is to drink white young wines which are as floral, fruity and fresh as possible. The formation of these aromas can be better studied through volatile chemical compounds, which are responsible of the product's final result (Jones, 2007; van Leeuwen, 2016).

MATERIALS AND METHODS

The samples belong to the 'Sauvignon Blanc' variety from the vinicultural region of the "Getic" plateau, "Plaiurile Drâncei" vineyard, Oprișor locality, and were obtained in three different years: 2013, 2014 and 2015. A number of five samples was chosen for this

study (denoted SB0-SB4) and the difference between each other consists of the type of yeast strain used for the fermentation process. Sample SB0 was obtained through gravitational clarification in the absence of yeast and the rest of the samples (SB1, SB2, SB3, SB4) were obtained through gravitational enzymatic clarification.

The samples from 2015 were chosen for a more thorough analysis, from which a volume of 7 mL was collected and analysed through gas chromatography using the head-space technique coupled to a mass spectrometer. The equipment used was a Shimadzu Head Space HS 20 trap - GC 2010plus-MS8040TQ. The compound identification was done by correlating with the software's database and the quantitative results were obtained using the internal standard method by comparing with 4-methyl-2-pentanol (Grigorică, 2017). The samples' alcoholic concentration was determined according to the OIV-MA-AS312-01 method (OIV, 2018).

RESULTS AND DISCUSSIONS

After determining the alcoholic concentration of all the samples, the values from Table 1 were obtained:

Table 1. Alcoholic concentration of samples for year of production 2013-2015

Year of production	Samples	Alcohol (%v/v)
2013	SB0	12.8
2013	SB1	13.1
2013	SB2	13.1
2013	SB3	13.0
2013	SB4	13.0
2014	SB0	11.8
2014	SB1	12.0
2014	SB2	12.1
2014	SB3	11.9
2014	SB4	12.0
2015	SB0	13.1
2015	SB1	13.6
2015	SB2	13.3
2015	SB3	13.3
2015	SB4	13.5

In 2015 there is an increase in alcoholic concentration of approximately 0.5% v/v across all samples, compared to 2013. This is somewhat expected given the climatic changes, especially the air temperature, which is strongly connected with the increase of alcoholic concentration. 2014 was an unusual year, being considered by the National Meteorology Administration as the fourth rainiest year from the last fifty years. This caused a decrease in temperatures and July did not bring any heat waves as it was expected (Polifronie, 2014).

Therefore, following a comparison between the samples' alcoholic concentration, it was expected that the wine from 2015 would be richer in volatile compounds which give more intense fruity aromas. This was confirmed by the results from the chromatographic analysis (Table 2). There were several volatile compounds that were identified by this analysis, but are showed only those for which the literature gives clear information of their flavour (Wu, 2016; *thegoodscentscompany) and especially those which have a positive impact and through which the influence of alcoholic concentration can be observed.

By analysing the aromatic profile it is obvious that the dominating aromas are fruity. Responsible for this are the chemical compounds from different classes such as alcohols, esters and carboxylic acids. Their concentration in this case is also influenced by the yeast that was used for fermentation - this explains the difference between the values of the same compound and with the same alcoholic concentration (for example in the case of ethyl octanoate for samples SB2 and SB3 or in the case of hexanoic acid for the same samples).

Most of the chemical compounds may have several sensorial characteristics (from pleasant fruity aroma to fatty and waxy repelling aromas), depending on the concentration in which they are present (Wu, 2016). From the numerical data obtained it follows that ethyl octanoate and ethyl decanoate have the biggest influence in forming the fruity aromas. The specifics of these aromas are apricot, banana, pear, pineapple, apple or wine.

Other compounds that present sweet and fruity aromas are 3-methyl-1-pentanol, 2-undecanol, ethyl decanoate, isoamylacetate (banana or pear), 3-methylbutyl decanoate (banana), decanoic acid (citrus), ethyl nonanoate (apple or banana), 3-hexenol acetate (banana or candy) or ethyl pentadecanoate (honey).

Table 2 Results of chromatographic analysis for wine samples from 2015

	Alcohol concentration (%v/v)	13.1	13.6	13.3	13.3	13.5
	Samples	SB0	SB1	SB2	SB3	SB4
Chemical compounds	Flavour	Samples concentration (microequivalents internal standard/L)				
isoamylacetate	banana, pear	ND	260.77	63.34	43.61	ND
2-methyl-1-propanol	ethereal, apple, bitter, cocoa, winey	ND	22.14	27.18	14.33	ND
2,6-dimethyl-4-heptanone	sweet, mint	37.19	52.54	ND	ND	44,4
3-hexenol acetate	banana, candy, floral, green	36.19	31.97	40.31	30.09	19.56
5-methyl-2-hexanol	grassy	7.36	7.63	6.2	6.48	17.9
3-methyl-1-pentanol	fruity	2.9	9.89	ND	ND	9.6
ethyl octanoate	sweet, fruity, apricot, banana, pear, pineapple, wine, waxy, dairy	1309.57	2266.25	1911.94	1057.88	2376.98
1-hexanol	floral, green, resin	4.92	24.9	17.93	20.24	14.35
3-hexenol	moss, fresh	13.89	93.62	59.48	47.4	ND
2-hydroxyethyl propanoate	butter	76.14	59.07	23.42	28.69	156.46
1-hydroxy-2-propanone	butter, grassy, malt, spicy	4.88	ND	4.11	ND	ND
ethyl nonanoate	fruity, apple, banana, rose, winey, cognac, waxy	5.99	20.08	13.49	9.4	17.4
2-undecanol	fruity	9.71	27.91	19.72	18.96	24.74
ethyl decanoate	sweet, fruity, apple, waxy	2141.92	1848.29	1828.46	1054.08	1211.38
isobutanoic acid	acidic, sour, cheesy, dairy, buttery, rancid	50.59	66.34	6.19	6.15	32.2
2,3-butanediol	fruity, buttery	35.53	29.41	ND	ND	11.11
butanoic acid	sharp, acidic, dairy, cheesy, sour, buttery with a fruity nuance	17.15	29.62	ND	ND	3.56
diethyl succinate	fruity, passion fruit, apple; apricot; cranberry, peach, pear, chocolate, grape, floral, musty, waxy, earthy	7	37.88	36.93	35.8	33.18
isovaleric acid	fruity, cheese, dairy, acidic, sour, pungent	18.27	36.97	28.98	25.3	30.18
ethyl palmitate	fruity, waxy, creamy and milky with a balsamic nuance	295.06	520.17	402.47	386.69	339.95
3-methylbutyl decanoate	sweet, fruity, banana, waxy, green nuance	5.91	10.49	9.31	9.42	4.14
hexanoic acid	sour, fatty, sweat, cheese	271.96	345.82	312.73	274.28	268.49
2-phenylethanol	sweet, floral, fresh and bready with a rose, honey nuance	225.19	237.77	251.38	251.17	255.68
ethyl myristate	sweet, violet, iris, waxy	ND	5.04	ND	31.48	ND
octanoic acid	waxy, dirty, cheese, phenolic, fatty, oily	728.65	864.24	784.73	792.78	703.07
ethyl pentadecanoate	sweet, honey	ND	12.03	ND	ND	6.21
decanoic acid	citrus, unpleasant, rancid, sour, fatty	312.55	162.56	141.03	114.26	99.43
ethyl tetradecanoate	mild, waxy	ND	10.13	13.71	11.12	8.89
ethyl linolate	fruity, mild, fatty	ND	8.3	5.35	5.83	7.2

*ND - not detected

Some compounds may have a specific wine aroma or in the case when the samples evolve in time (in gets older in a good way) there may be even aromas of brandy - in the case of 2-methyl-1-propanol, ethyl nonanoate.
The fact that the Sauvignon Blanc variety is also characterized by herbal aromas was also proved by the presence of compounds that can give this kind of aroma - 3-hexenol acetate, 1-hexanol, 3-methylbutyl decanoate. In most of these cases the samples which have a higher alcoholic concentration also have higher concentration of these compounds comparing with the other samples which have a lower alcoholic concentration. The fatty acids and their esters have a less pleasant contribution, but taking into account the relatively small concentrations in which they are present it is not disturbing. Most of them are responsible for the fatty, buttery, waxy and sometimes sour sensation - decanoic acid, octanoic acid, hexanoic acid, isobutanoic acid, ethyl tetradecanoate, ethyl linolate.
By categorizing the resulted aromas by the class of chemical compounds from which they come, one can notice that most of the pleasant, fruity aromas are owed to esters. The weight of the chemical compounds that give fruity aromas is larger than those that give unpleasant and disturbing smells.

CONCLUSIONS

The perception of an aroma largely depends on the experience and the sensitivity of the evaluator. However, the volatile chemical compounds which are responsible of aromas could be better highlighted if there is an alcoholic concentration which increases their volatility.

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HERITABILITY OF THE TOMATO GENOTYPES RESISTANCE TO THE HIGH AIR TEMPERATURES

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Abstract

Since the resistance of plants to stressful temperatures is a quantitatively controlled polygenetic feature, the manifestation and particularities of its inheritance in the system of the other quantitative characters, which usually have correlative relationships, are of interest. The aim of the research was to evaluate the heritability of the tomato genotypes resistance to the heat and to highlight the most prospects for use in breeding programs. Based on the growth capacity of the P1 and F1 hybrids, at optimal (25ºC) and high temperature (43ºC), it was found that at the sporophytic level, the resistance of tomatoes at high temperature is controlled by dominant factors, with varying degrees of expression and orientation (+/-) that most directly depend on the hybridization components. By calculating the multiple regression coefficient β regarding the contribution of the maternal and paternal form in the formation of the resistance of high temperature tomato hybrids F1 (+ 43°C), it was found that the analyzed parameter for the maternal parent is ~ 2 times higher than that of the paternal form, which reveals that resistance to heat stress in the case of tomato sporofite is inherited in particular by the maternal way.

Key words: *tomato, heritability, resistance, high temperature.*

INTRODUCTION

Lately, the effects of high temperature, known as thermal shock, exceed the specific level of normal temperature for agricultural plant species, including tomatoes, for which reason they have become a topical topic for fundamental and applied research (Hazra et al., 2007; Mihnea et al., 2011).

More and more obvious and alarming climate changes in the world, which frequently cause decline at crop yields, including tomatoes, have led to increased efforts to increase resistance in the breeding programs (Hazra P. et al., 2007). Although the tomatoes exhibit a high adaptability to environmental conditions, the heat stress can become a major limiting factor for the growth, reproduction and plant production level, the optimal temperature for cultivation of tomatoes being framed within the limits of 25-30ºC/20ºC - day/night (Camejo et al., 2005).

Most crop plants are subject to high temperature stress (HTS) at certain stages of development, this phenomenon being more and more frequently lately (Sato et al., 2000). Plant exposured to HTS reduces harvesting and its quality at many crop plants, including leguminous crops (Boote et al., 2005).

According to some opinions, heat stress can also be considered as the temperature level of 28-29°C which is only a few degrees higher than the optimal range of 21-24°C. Increasing the temperature to such a level does not lead to severe disturbances of the biochemical reactions in the plant that would affect the cell's functionality, the plants still developing normally, but the reduction in the number of fruits is the general response to this factor, the main cause being the various distortions of the reproductive processes.

The high temperature (32/26ºC - day/night), and in particular its association with drought, greatly affects the growth, development and productivity of tomatoes. This situation requires the orientation of tomato improvement programs towards the creation of heat-tolerant varieties (Venema et al., 2005; Hazra et al., 2007). Increasing the temperature even at 2-4ºC above the optimal level negatively influences the development of gametes and inhibits the ability of pollinated flowers to form fruit, thus significantly reducing the yield (Firon et al., 2006).

At higher temperatures - than 35°C, germination, flowering, meiotic processes, large fruit formation, egg development, its viability, and embryo development (Wahid et al., 2007) are affected to the majority of tomato varieties, and resistant tomato genotypes have the ability to form a much greater number of fruits than those sensitive under these conditions (Comlekcioglu et al., 2010).

MATERIALS AND METHODS

As a research material they have served varieties of different ecological and geographical origin and their F_1 hybrids.

High temperature reaction testing was carried out according to the method proposed by Ivakin (1979), based on plant growth capacity after maintaining it at elevated temperatures for 6 hours. For the analysis of the high temperature influence on the germination and the length of the plants, the thermal level of 43^0C was used. According to the method, between the resistance of the sporophyte and the tomato gametophyte there is a positive correlation.

The degree of domination (h_p) was calculated based on the formula (Briubeiker, 1966):

$h_p = F_1 - 0.5 (P_1+P_2)/ H_p - 0.5 (P_1+P_2)$, in which: F_1 - the mean value of the character in the F_1 generation; P_1, P_2 - the mean value of the character in the parental forms; H_p - average value of character rated at best parental component.

Clusterian analyzes were performed by constructing dendrograms based on the Ward method and the *k*-means method (Savary et al., 2010). In the *k*-means method, 3 clusters were programmed after the possible values of the characters: small, medium and high. The main purpose of these procedures is to find the similarities and differences between objects (genotypes) according to the parameters used and their distribution in groups so that objects in the same group are similar and those in different groups - special.

Multiple regression analysis. The overall importance of this analysis is to elucidate the links between several (or more) independent variables and a dependent variable.

Independent variables can correlate with each other and this is to be taken into account when determining regression coefficients to avoid misinterpretations caused by false correlations. The mathematical equation of multiple regression is as follows:

$$y = \beta_1 \cdot x_1 + \beta_2 \cdot x_2 + ... + \beta_n \cdot x_n + a,$$

where: y - dependent variable; β - the regression coefficient for each independent variable (x_1, x_2 ... x_n); a - constant.

After evaluating criterion (test) F, it is important to estimate the regression coefficient β which can be positive or negative, significant or insignificant. If the β coefficient is positive, it can be interpreted that for each increase of the predictor variable with 1 unit, the increase of the dependent variable will be equal to the value of the non-standardized coefficient β (Nathans et al., 2012).

The data obtained were statistically processed in the software package STATISTICA 7.

RESULTS AND DISCUSSIONS

One of the basic genetic indices that demonstrates the type of inheritance in F_1 generations is the degree of domination (h_p). To obtain F_1 hybrids, crossbreeds were made between parental forms with varying degrees of resistance / sensitivity to high temperatures.

The obtained data on the reaction of high-temperature hybrids of tomato populations show that hybrid combinations F_1 have values that show increased resistance of the obtained hybrids compared to the genitors, in some combinations the F_1 values are lower than the average of the parents. The analysis of the genitors and F_1 hybrids resistance at t = 42-43^0C showed a considerable variability of the studied character: 23.6 ... 56.0% (Table 1).

The degree of resistance of high temperature plants was high to the hybrids F_1: Visas x Sunmark, Katerina x Sunmark, A 90/7 x Gusar, Katerina x Danna, the values of which constituted 57.5, 53.6, 52.6, 52.2%, respectively.

Table 1. Heritability of the resistance/sensitivity of tomato genotypes at high temperature at the juvenile plant level

Nr.	Combination	Resistance of the plant to t=43^{0}C, %			
		P_1	P_2	F_1	h_p
1.	Onix x Saladette	28.9	31.3	33.6	2.92
2.	Katerina x Burnley Metro	41.8	38.1	35.5	-2.31
3.	A 90/7 x Gusar	38.6	20.4	52.6	2.54
4.	Narvic x Zastava	34.3	56.0	39.4	-0.53
5.	Cal J THM x Saladette	28.1	31.3	42.7	8.12
6.	Katerina x Zastava	41.8	56.0	49.6	0.09
7.	Nistru x Onix	30.9	28.9	40.9	11.0
8.	A 90/7 x Kredo	38.6	35.3	46.9	5.9
9.	Viza x Burnley Metro	38.5	38.1	23.6	-73.5
10.	Katerina x Danna	41.8	40.0	52.2	12.5
11.	Katerina x Sunmark	38.6	47.2	53.6	2.5
12.	A 90/7 x Costral	30.9	31.3	46.2	75.5
13.	Nistru x Saladette	38.5	64.0	46.4	-0.37
14.	Viza x Sunmark	41.8	64.0	57.5	-0.4

The appreciation of the high temperature influence on the growth of tomato plants has revealed considerable variability in both parental and hybrid combinations ranging from 2.2 ... 5.5 cm and 2.4 ... 5.8 cm, respectively (Figure 1).

At high temperatures, F_1 hybrids showed advanced positive resistance compared to the best genitor at the combinations: Onix x Saladette, A 90/7 x Gusar, Cal J THM x Saladette, A 90/7 x Kredo, Katerina x Danna, A 90/7 x Costral, Nistru x Onix.

The values of the h_p coefficient for the length of the plantlets reveal the overdominance of the resistance to 11 combinations: Onix x Saladette, Katerina x Burnley Metro, Narvic x Zastava, Cal J THM x Saladette, Katerina x Zastava, Nistru x Onix, A 90/7 x Kredo, A 90/7 x Credo, A 90/7 x Costral, Katerina x Sunmark, Nistru x Saladette, of the 14 studied. Dominance of sensitivity was recorded at the A 90/7 x Gusar combinations, and the intermediate sensitivity inheritance at Viza x Burnley Metro.

Considering that the phenomenon of overdominance is not inherited (because it is a result of allelic interactions), we can deduce that in the descendants of the combinations in which F_1 manifested resistance much higher than that of the best parent, more probably will not be found genotypes with this level of character manifestation.

The success of the selections will be determined only by the possibility of identifying homozygous resistance forms.

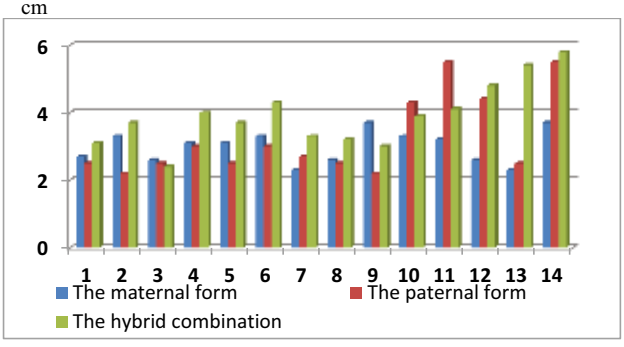
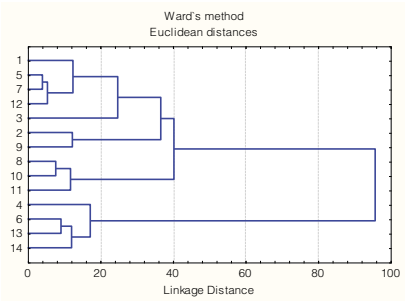
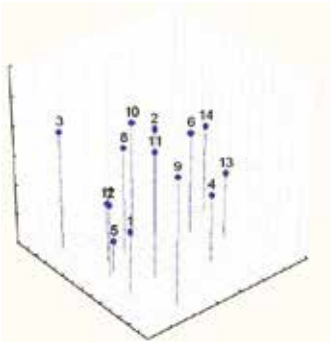


Figure 1. Comparative data of growing parental formulas and F_1 hybrids at high temperature (43°C):
1 - Onix x Saladette; 2 - Katerina x Burnley Metrou; 3 - A 90/7 x Gusar; 4 - Narvic x Zastava; 5 - Cal J THM x Saladette; 6 - Katerina x Zastava; 7 - Nistru x Onix; 8 - A 90/7 x Kredo; 9 - Viza x Burnley Metrou; 10 - Katerina x Danna; 11 - Katerina x Sunmark; 12 - A 90/7 x Costral; 13 - Nistru x Saladette; 14 - Viza x Sunmark

By cluster analysis (A) and multidimensional scaling (B), it was found that the investigated combinations differ greatly from the reaction of plantlets at + 43°C, forming clusters at different levels of aggregation or different location in the three-dimensional space (Figure 2).





Germination *Length of radicle, mm*

Figure 2. The dendrogram for the distribution of tomato combinations based on the reaction of parent plants (P_1, P_2) and high temperature hybrids F_1

The *k*-means clustering analysis of the combinations studied (based on the parental and hybrid F_1 reactions) in 3 clusters according to the possible values of the length of the plantula - large, medium, small, highlighted that the greatest differentiation capacity they showed the paternal form of the combination (Table 2, Figure 3).

Thus, the intercluster variance determined by the parental form (P_2) recorded the highest value: 2149.821.

Table 2. Analysis of cluster variance

Genotype	Intercluster variance	df	Intracluster variance	df	F	p
P_1	210.959	2	118.196	11	9.817	0.0004
P_2	2149.821	2	295.512	11	40.012	0.0001
F_1	221.282	2	872.150	11	1.396	0.2883

*- p≤0,05.

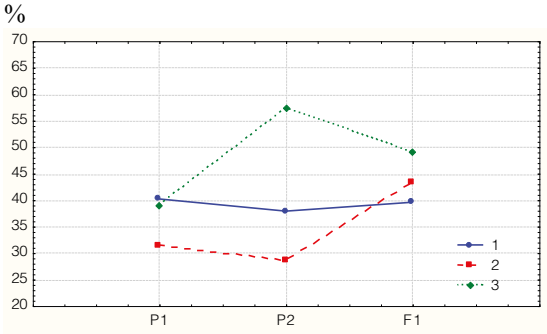


Figure 3. The ability to differentiate clusters (*k*-means method) by parental forms and tomato hybrid F_1

It should be noted that by calculating the multiple regression β coefficient regarding of the maternal and paternal form contribution in the formation of the phenotype of hybrid F_1 plants, the following regression equation was found: $y = 0.289\ P_1 + 0.139\ P_2$, in which: y - hybrid F_1; P_1 - maternal form; P_2 - paternal form. As it can be seen, the β coefficient in the case of the maternal is ~ 2 times higher than that of the paternal parent, which shows that the high temperature resistance in tomato plants is inherited in particular by the mother.

CONCLUSIONS

Based on the growth capacity of the P_1 and F_1 hybrids, at optimal temperature (25ºC) and high (43ºC), it was found that at the sporophytic level, the resistance of tomatoes at high temperature is controlled by dominant factors, with degrees variation of expression and orientation (+/-) that most directly depend on the hybridization components.

By calculating the multiple regression coefficient β regarding of the maternal and paternal form contribution in the resistance of high temperature tomato hybrids F_1 (+ 43ºC), it was found that the parameter analyzed for the maternal parent is ~ 2 times higher than the paternal parent, which reveals that the level of resistance of the maternal form as a hybridization component has to be taken into consideration in the elaboration of tomato improvement programs, especially the resistance to high temperatures.

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ESTIMATION OF VARIABILITY PARAMETERS OF SOME QUALITATIVES COMPONENTS AT A SET OF SWEET CORN LINES FROM AGRICULTURAL RESEARCH AND DEVELOPMENT STATION TURDA

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Abstract

Sweet corn is a recent crop in Romanian agriculture, being cultivated more and more. Modern sweet corn, like all crops, is the product of its own evolution, in which human intervention was the first unconscious phase, becoming conscious over last two centuries. Knowledge of this development may be useful for forming the basis for planning for future improvement of this plant. Purpose of this study was to analyze inbred maize lines from germplasm collection from Turda Agricultural Research Center in terms of variability of some chemical components. Biological material was represented by 27 inbred lines of sugar-1 gene. Each variant was sown on four rows, an average of 40,000 pl/ha. Quality analyzes were performed using TANGO NIR spectro-photometer. Chemical parameters analysed were: protein content, starch, sugar, NDF (neutral detergent fibers) and NCGD (neutral cellulose digestibility). Between analyzed lines there is an important variability of sugar content.

Key words*: lines, sugar, starch, sweet corn, variance.*

INTRODUCTION

Globally, sweet corn represents 7.7% of total vegetable consumption and ranks 2nd after tomatoes for US and Canada consumers (McMullen et al., 2009). Due to the high content of carbonates and other nutrients such as vitamins C, A, B_1, B_2, proteins, fats, starch, cellulose and mineral components (potassium, magnesium, calcium, phosphorus, iron, selenium, copper, nickel and chrom) and different uses for both fresh consumption and food industry, this species was quickly adopted worldwide (Hefny, 2011).

Recently, in U.S., use of high fructose syrup obtained from sweet corn and used as a sweetener gained a large market share (Revilla et al., 2005). Sweeteners made from sweet corn, belong to the best sweeteners class in US with a 52% market share due to their stability, high functionality and ease of use (Singh et al., 2014).

Sweet corn is one of the most recent crops in the landscape of Romanian agriculture, being cultivated more and more. Most of sweet corn production from Romania is obtained in the western part of the country and is not processed in the country, but taken to Hungary (www.agrointel.ro).

At Turda Agricultural Research Station, in 1971, a genetic improvement program was launched to obtain sweet corn on the basis of infusion of su1 recessive gene to a series of normal inbred lines. The obtained sugar lines were studied in a diaphragm crossing system, obtaining the general and specific combination capacity for the specific characters of sweet corn. Based on the behavior of studied inbred lines, the formula of DULCIN tri-linear hybrid was approved in 1988 (Căbulea et al., 1994; Has V., 2000).

Over past 15 years, Turda Agricultural Research Center has developed an intensive program to obtain new, early-quality inbred

lines with superior qualities, which has led to recording of new genetic advances in amelioration of sweet corn.

As a result of using initial material from very early American and Canadian origins, the parental forms of PRIMA hybrid were obtained, approved in 1991 and considered the earliest hybrid from sweet corn hybrids experimented in CIOS network (Has et al., 1994).

Modern sweet corn, like all crops, is the product of its own evolution, in which human intervention was the first unconscious phase, becoming conscious over last two centuries (Srdić et al., 2012). Knowledge of this development may be useful for forming the basis for planning for future improvement of this plant (Lazcano et al., 2011).

It is important to know basic materials (varieties, populations) from which modern inbred lines have been extracted to retrieve lost characters during inbreeding (Kashiani et al., 2010). Sweet corn breeders have paid attention to varieties, both as starting material for inbred lines and as new sources of favorable alleles (Huelsen, 1954; Tracy, 1994). The one who dealt with study of varieties, their origins, role played in sweet corn evolution of was Walton C. Galinat (1971) in the study: "The Evolution of Sweet Corn".

Over the last 50 years, production gain in sweet corn, attributed to genetic gain, was modest compared to that obtained with ordinary corn (Letrat and Pulam, 2007). Important reasons for this could be:

- narrow genetic basis of sweet corn which, according to some authors, is largely derived from Northern Flint and which in its evolution has gone towards a severe narrowing of variability;
- non-Northern Flint germplasm, represented by Stowell's Evergreen and County Gentleman varieties, emerged through the introgression of sugary-1 gene in germplasm dentiformis (Revilla et al., 2009);
- excessive use of "Golden Bantam" type in creation of inbred lines (as a source of yellow endosperm and taste qualities). Current germ cell gene mutation contains, to a greater or lesser extent, genes of Golden Bantam genus in pedigree (Romay et al., 2013).

Following this study, analysis of inbred maize lines from Turda germplasm collection in terms of variability of some chemical components (sugar, starch, protein, NDF and NCGD) was proposed.

MATERIALS AND METHODS

Biological material was represented by 27 inbred lines of sugary-1 gene. Each variant was sown on four rows of 5 m in length and at a distance of 0.70 m corresponding to an average of 40,000 plants/ha. This group of lines was subjected to quality analysis, which was performed with the TANGO NIR spectrophotometer (Bruker Optik Gmbh, Ettingen, Germany).

From each line, self-pollinated cobs were selected to eliminate effects of alopolen sources involvement in the analyzed chemical components. Sweet corn cobs were analyzed at full maturity, being allowed to lose their moisture by natural drying in laboratory, after which grain samples were taken from each line. For each line of self-pollinated corn cobs that have been harvested, samples of about 50 grams of grains were harvested, which were subsequently ground using a WZ-1 knife mill (Sandkiewicz Instruments - Poland), and then sifted with a sieve.

Chemical parameters analyzed were: protein content, starch, sugar, NDF (neutral detergent fibers) and NCGD (neutral cellulose digestibility).

Based on variance analysis, some derived genetic parameters were also calculated:

$$\mathrm{V}G(Genetic\ variance) = \frac{\text{genotype variance} - \text{enviroment variance (residual)}}{\text{number of repetitions (R)}}$$

$VE(enviroment\ variance)$ = residual variance;

$VP\ (phenotypic\ variance) = VG\ \frac{\mathrm{VM}}{\mathrm{R}}$;

CVG% (Coefficient of genetic variation) = $\frac{\sqrt{VG}}{\mathrm{X}}$x100;

CVP% (Coefficient of phenotypic variation) = $\frac{\sqrt{VP}}{\mathrm{X}}$x100;

CVM% (Coefficient of environment variation) = $\frac{\sqrt{VM}}{\mathrm{X}}$x100

(Singh and Chaudhary, 1985);

H (Heritability in a broad sense) = $\frac{VG}{VP}$

(Falconer, 1971);

X = character average.

Generated results will be chemometrically processed using main component analysis and cluster analysis; such charts can provide valuable information about possible correlations between studied variables or samples composition, especially when working with large sets of values. Also, resulting clusters provide valuable insights into possible similarities between lines and also their grouping according to these similarities or differences.

RESULTS AND DISCUSSIONS

Chemical composition of maize lines studied is shown in Table 1.

Table 1. Chemical composition of a set of sweet corn lines (Turda, 2016)

Lines	NCGD (%)	NDF (%)	Protein (%)	Starch (%)	Sugar (%)
Ta su 21Q206	86.18	14.26	11.33	49.68	5.67
TA su 22	87.95	12.09	11.78	47.66	4.83
Tsu 152	88.07	15.55	13.26	49.94	6.67
Tsu 345R	86.67	16.71	12.09	45.83	6.70
TA 26	87.75	13.35	12.07	52.73	3.72
TA 27	87.79	12.51	12.53	53.65	2.16
TA 28	87.41	14.19	11.64	47.54	2.83
TD 101	88.66	15.80	9.30	53.25	6.52
TD 102	87.39	14.18	12.47	46.81	4.40
TA 28	87.11	15.38	12.52	50.62	3.73
TD 110	85.68	14.07	14.43	49.77	1.41
TVa 638	87.73	15.14	12.58	49.53	2.72
Tva 642	87.66	15.53	11.08	51.57	1.92
TVa 647	87.59	13.06	13.63	49.55	0.28
TV a 670	87.76	16.63	13.25	46.51	5.04
T su 209	84.64	19.17	12.41	44.88	5.26
T Su 233	86.54	14.89	13.43	45.83	4.07
T Su 244	85.35	17.84	12.06	38.94	6.03
P 51	84.06	14.95	12.80	44.73	6.66
LC 154/74	85.14	16.35	11.53	47.29	6.33
SW 87	83.76	16.07	14.80	42.31	4.85
TC 179 su	86.86	12.08	12.48	49.59	4.00
TD 111 su	87.93	12.95	10.66	50.86	4.78
TD 112 su	88.94	11,73	10.41	54.01	3.89
TD 113 su	85.75	15.15	18.19	27.44	4.01
TD 114 su	84.81	16.40	19.88	25.73	4.09
TD 115 su	87.24	12.52	10.31	51.50	3.68

Higher sugar content was found in Tsu 152 and Tsu 345R lines, which can indicate them as possible genres (only after being tested for combining ability) in amelioration of sugar content in sweet corn lines with a high yield but a lower sugar content. On the opposite side are Tva 642, TVa 647 and TD 110, which probably should be included in a sugar content improvement program if they are valuable under other aspects (cob production, precocity, cob size uniformity etc.).

Analyzing data in Table 2 led to the conclusion that there are considerable differences between analyzed lines with respect to sugar content, having highest C.V. of 39.2%, which suggests the existence of a great variability of this important taste characteristic. As a matter of fact, wide range of minimum and maximum values indicates a significant variation in sugar content.

Table 2. Variability parameters of protein content, starch, NCGD sugar and NDF at a set of sweet corn lines (Turda, 2016)

Indicators	CV (%)	Mini-mum	Maxi-mum	Average	Standard deviation
Protein	17.4	9.3	19.88	12.70	2.21
Sugar	39.2	0.28	6.70	4.3	1.69
Starch	14.5	25.72	54.01	46.95	6.83
NDF	12.5	11.73	19.17	14.76	1.85
NCGD	1.6	83.76	88.94	86.75	1.4

CV% values reflect moderate variability within the group of lines analyzed for protein and starch content. Differences between minimum and maximum values of protein and starch content indicate an important variability of these components and the possibility of selecting valuable genres both as variants and as minus variants according to aims pursued by the breeder. Wide variation limits of the two components show existence of significant variations in the group of analyzed lines.

Lowest variability is recorded at neutral cellulose digestibility (NCGD) values. Reduced values of CV indicates a small fluctuation of these values around average. Therefore, we can say that there are no large differences in digestibility of cellulose between analyzed lines. This parameter is mainly used in fodder, for determination of formulas used for calculating of fodder energy values.

A moderate variability of studied parameters can also be noticed for neutral detergent fibers (NDF) represented by fractions of insoluble cell walls (hemicelluloses, celluloses, lignin, tannins etc.).

Generally, the two parameters, NDF and NCGD, are used to characterize food digestibility.

With higher protein content, lines in the HONEY group (TD 113 su and TD 114 su)

stand out, with 19.88% protein and 19.8% respectively, as can be seen in Figure 1. Also known is the reverse relationship of sugar and protein, which is confirmed by this study, as protein content is reduced when sugar content is increased, with deviations (Figure 1).

Between the two important chemical components of sugar and starch there is a well-established logical correlation even though it is not statistically assured in present case, probably because of lower number of analyzed cases.

Large sample dispersion around the regression line suggests that there is insignificant interaction between the two variables and that choosing forms with lower starch content is not necessarily associated with a significant increase in sugar. A significant deviation that strengthens this negative relationship can be noticed within lines of the Honey group within the circle in Figure 2.

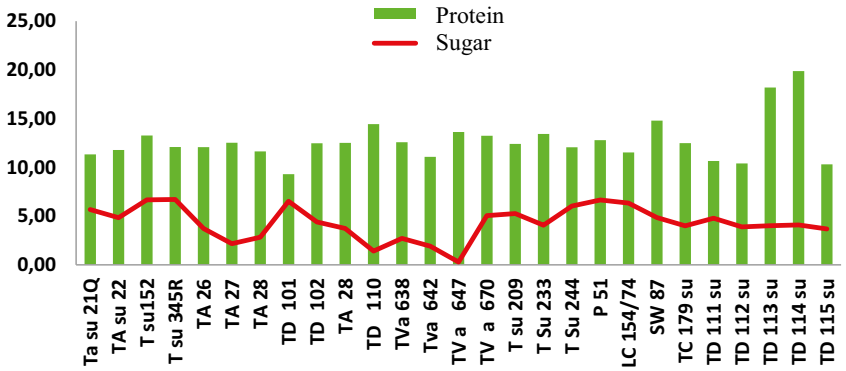

Figure 1. Protein and sugar content of 27 sweet corn lines (Turda, 2016)

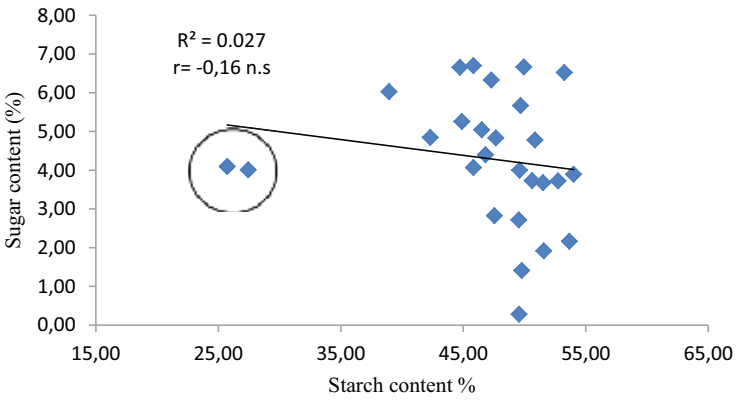

Figure 2. Correlation between sugar and starch content

Analyzing obtained data, between the analyzed lines there is an important variability of sugar content, values of sample F being very significant (Table 3). It can be stated with certainty that at the level of lines one can find some forms that included in hybridization programs can lead to the expression of a higher quality heterosis superior to parental forms. These lines are therefore of real use in improving the taste of sweet corn. Sugar content of sweet corn lines that directly influences their quality must be correlated with other factors such as cob production but also through other selection criteria.

Table 3. Analysis of the variance for sugar content in the 27 sweet corn lines

Variability source	SPA	GL	S^2	F
G	216.22	26	8.32	34.74***
R	7.03	2	3.52	
Error G	12.45	52	0.24	

Based on proposed calculation formulas from Singh (2014), genetic variation coefficients, environmental (residual) and phenotypic variation coefficients as well as broader heritability were calculated (after Nordby, 2008).

Analyzing data from Table 4, high values of genetic variation indicate the important contribution of genetic factor in obtaining sugar content. Obviously, phenotypic variance values are higher compared to those of genetic variance, suggesting major implications of the environment in accumulation of sugars in sweet corn. The coefficient of genetic variation indicates an important source of variability in sugar content that can be improved by amelioration, especially on the background of a high heritability index with high values of 0.57. Similar result were obtained by Haş and Haş in their study in 2009.

Table 4. Estimators of sugar content variability in analyzed sweet corn lines

Genetic Variance VG	Environment variance VM	Phenotypic variance VP	Coefficient of genetic variation CVG %	Coefficient of environment variation CVM%	Coefficient of phenotypic variation CVP%	H^2
Sugar content						
1.6	3.52	2.77	29.41	43.63	38.71	0.57

CONCLUSIONS

Use of sugary-1 (*su-1*) mutant gene for endosperm quality produces changes in the genetic determinism of carbohydrate synthesis.

Characters of sweet corn may be considered with complex determinism, both due to interactions between quality gene (*su-1*) of the endosperm and the polygenic complex on which it operates, as well as environmental conditions.

Higher heritability of transmitting sugar content makes recurrent selection during inbreeding very effective. Thus, improvement and fixation by individual selection of the quality character is very effective in the course of inbreeding.

Improving chemical composition of graines requires the combination of a set of procedures based on reciprocal recurrent selection, tandem selection with the requirement to control expression under various environmental conditions.

From the statistical analysis of the weight of the factors involved in the expression of the grain composition, contribution of genotype to environmental factors is predominant. Major share of genotype contribution in expressing grain quality suggests that sweet corn grain lines have relatively stable components in relation to environmental conditions.

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RESEARCH REGARDING THE INFLUENCE OF FERTILISER RATES ON MORPHOLOGICAL FEATURES IN COMMON BEAN (*PHASEOLUS VULGARIS* L. CONVAR. *VULGARIS*) PODS CULTIVATED IN SOLARIUM AT THE DIDACTIC AND RESEARCH BASE IN TIMIŞOARA, ROMANIA

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Abstract

Common bean or green bean (Phaseolus vulgaris L. - Family Papilionaceae syn. Fabaceae) is cultivated for its young pods before seed formation, used in some dishes and in the preserve industry. Trial was conducted in the solarium of the Didactic and Research Base in Timisoara, Romania, on 400 m^2. It was a poly-factorial trial with sub-divided plots and three replicates: Factor A (supplementary fertilisation) with 3 graduations (a_1 - Cropmax; a_2 - Lithovit; a_3 - Trainer); Factor B (basic fertilisation) with 3 graduations (b_1 - Orgevit; b_2 - Phenix; b_3 - Italpollina) and Factor C (cultivar) with 3 graduations (c_1 - Goldfield; c_2 - Ecaterina; c_3 - Aurie de Bacău). As far as pod length is concerned, there are distinctly significantly positive differences (1.54-1.70 cm) between the cultivar Ecaterina and the trial mean. As for pod width. there are very significantly positive differences (0.05-0.15 cm) between the three supplementary foliar fertilisers. From the perspective of pod diameter, there are very significantly positive differences (0.01-0.05 cm) also between the three supplementary foliar fertilisers.

Key words: *Phaseolus vulgaris* L. convar. *vulgaris, common bean, morphological characters, fertiliser rates, solarium type.*

INTRODUCTION

Common bean or **green bean** (*Phaseolus vulgaris* L. - Family *Papilionaceae* syn. *Fabaceae*) is cultivated for its young pods harvested before seed formation that are used in meals and in the can industry (Apahidean and Apahidean, 2000; Broughton et al., 2003).

The species originates in Central America and South America (Peru, Mexico), where Aztec tribes used to cultivate it in times immemorial. It was brought to Europe in the 16th century by the Spaniards and the Portuguese (Indrea et al., 2007). It spread from Spain and Portugal to the Netherlands, France, Germany and England (Ciofu et al., 2004).

Common bean is an annual. herbaceous. thermophilous plant. Two convarieties are cultivated: *nanus*, covering plants with determined growth, and *vulgaris*, covering plants with undetermined growth (Poşta, 2008).

The root system is superficial and it goes 30-40 cm deep in the soil. Some toots even reach 100 cm deep in the soil. The roots have nodosities containing nitrogen-fixing bacteria.

The stem is cylindrical-edged; it is covered by hairs. ramified in dwarf common bean cultivars, 25-30 cm tall, and voluble; it is less ramified in climbing common bean cultivars, and 4-5 m tall (Poşta, 2008).

Common bean cultivars with undetermined growth bloom 60-70 days after sprouting and continue to bloom.

The fruit is a dehiscent pod characteristic in size, shape and colour depending on the common bean cultivar.

As a plant originating from warm areas, it is heat demanding. Seed minimum germination temperature is 15°C, when sprouting occurs in 12-14 days. Optimum temperature is 20-27°C, when sprouting occurs in 4-8 days. During vegetation, optimum temperature is 20-25°C, while minimum temperature is 10-12°C (Konsens et al., 1991). Below 10°C growth stops and at -0.5°C, the plants die.

Common bean is a short-day plant. With long-day conditions, vegetative growth is intense. detrimental to fructification, which asks, in climbing common bean cultivars, the removal of leaves and branches once a week to favour

larger numbers of flowers and, implicitly, of pods. Critical water phases are when seeds germinate, at blooming and at pod formation (Beebe et al., 2013). Lack of soil water hinders blooming, while low air moisture causes flower abortion. Common bean responds well to phosphorus and potassium fertilisation. On poor soils, it is recommended to apply nitrogen fertilisers during the first vegetation phases (Araújo et al., 2004; Soratto et al., 2010; Turuko and Mohammed, 2014).

MATERIALS AND METHODS

Experiments were carried out at the Experimental Station of the Faculty of Horticulture and Forestry of Timisoara in a 400 m^2 solarium, built and equipped with environmental automated control systems and equipments.

Experiments carried out during 2016-2017 had a polyfactorial character; variants were set after the randomised block method with three replicates specific to experiments in forced protected areas of vegetable culture.

Factor A (supplementary fertilisation) with 3 graduations: a_1 - Cropmax; a_2 - Lithovit; a_3 - Trainer.

Factor B (basic fertilisation) with 3 graduations: b_1 - Orgevit; b_2 - Phenix; b_3 - Italpollina.

Factor C (cultivar) with 3 graduations: c_1 - Goldfield; c_2 - Ecaterina; c_3 - Aurie de Bacău.

The growth bio stimulator Cropmax (Holland Farming B.V., Holland) contains amino acids, macro- and micro-elements, vitamins and polysaccharides, being 100% organic. 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 solarium crops is 0.2%, every 7-10 days.

Foliar fertiliser Lithovit standard (zeovita GmbH. Roter) contains 75% $CaCO_3$, 4% $MgCO_3$, 0.25% Fe, 5.0% SiO_2, 0.1% K_2O, 0.015% N, 0.015% P_2O_5, 0.01% Mn, 0.002% Cu and 0.005% Zn. This ecological fertiliser is applied in concentrations of 0.5%, every 15 days.

Foliar organic fertiliser Trainer (Italpollina spa. Italy) contains organic nitrogen (5%), vegetal amino acids (26.3%) and organic matter (35.5%). Application concentration is 0.3-0.4%.

Certified ecological organic fertiliser Orgevit contains N (4.0%), P_2O_5 (2.5%), K_2O (2.3%), Ca (9.3%), MgO (1.1%), organic substances (65% guano) and microelements (Fe, Mn, Bo, Zn, Cu, Mo). In vegetables cultivated in greenhouses and solaria. application rate is 1.5-2.0 t/ha.

Phenix is an organic fertilizer containing N (6.0%), P_2O_5 (8.0%), K_2O (15.0%), MgO (3.0%), organic carbon (29.0%) and organic substances (50.0%). Application rate in greenhouses and solaria is 5.0 t/ha.

Natural organic fertiliser Italpollina (Italpollina spa. Italy) used to fertilise the soil contains 4.0% N, 4.0% P_2O_5, 4.0% K_2O, 0.5% MgO and 41% C (organic carbon). Application rate homologated in vegetables cultivated in greenhouses and solaria is 3-4 t/ha.

The biological material used in the trials consisted in nursery seedlings sown directly in 9 cm diameter pots; seedling age upon planting was 25-30 days. The planting scheme was on 90 cm equidistant rows, while planting distance per row was 35-40 cm.

During vegetation, we monitored the soil to determine the variability of the main soil morphological features.

Observations were made using the current technique of descriptors and evaluation grades specific to the species (Ciulca, 2002).

RESULTS AND DISCUSSIONS

Soils favourable to common bean are rich in humus and have a neutral to alkaline reaction (pH = 6.5-7.5) (Indrea et al., 2007).

Having a short vegetation period. climbing common bean extracts from the soil small amounts of easily assailable nutrients. Common bean responds well to fertilisation with phosphorus and potassium and. on poor soils, it is recommended to apply nitrogen fertilisers during the first vegetation phases, upon blooming and upon pod formation (Mourice and Tryphone, 2012). Specific nutrient consumption per ton of fresh produce is 7-9 kg

a.s. N, 2.5 kg a.s. P_2O_5, 6.5-7.0 kg a.s. K_2O, 10 kg CaO (Davidescu and Davidescu, 1992).

Assessing the influence of fertiliser rate on some morphological and yielding features in common bean was done from the perspective of the interdependence between basic and supplementary fertilisations and genetic factor (cultivar). Table 1 below show trial results regarding the unilateral influence of supplementary fertilisation on pod length.

Table 1. Influence of supplementary fertilisation on climbing common bean pod length (Didactic Base Timişoara, 2016-2017)

Supplementary fertilisation	Pod length (cm)	Relative values (%)	Difference/Significance
Cropmax	23.79	103.80	0.87**
Lithovit	22.36	97.60	-0.56^{0}
Trainer	22.60	98.60	-0.32
Control (average exp.)	22.92	100.00	0.00

$LSD_{5\%}$ = 0.39 cm; $LSD_{1\%}$ = 0.59 cm; $LSD_{0.1\%}$ = 0.94 cm.

Comparative analysis of the three products used in supplementary fertilisation shows distinctly significant positive differences of climbing common bean pod length (+0.87 cm) when applying the product Cropmax. This difference is due to the composition and concentration in macro- and micro-elements of the fertiliser Cropmax.

Our research also aimed at assessing the influence of basic fertilisation on climbing common bean pod length. Trial results are shown in Table 2 below.

Table 2. Influence of basic fertilisation on climbing common bean pod length (Didactic Base Timişoara, 2016-2017)

Basic fertilisation	Pod length (cm)	Relative values (%)	Difference/Significance
Orgevit	22.75	99.30	-0.17
Phenix	23.80	103.90	0.88***
Italpollina	22.20	96.90	-0.72^{000}
Control (average exp.)	22.92	100.00	0.00

$LSD_{5\%}$ = 0.36 cm; $LSD_{1\%}$ = 0.49 cm; $LSD_{0.1\%}$ = 0.67 cm.

When using the three basic fertilisers cultivated in solaria, there is a very significant positive difference in pod length (+0.88 cm) when applying the fertiliser Phenix.

The genetic factor (cultivar) used in the trial has a primordial influence on yield per area unit due to its biological and morphological features (Poşta and Berar, 2005). Trial data regarding the influence of soil on climbing common bean pod length are shown in Table 3 below.

Table 3. Influence of cultivar on climbing common bean pod length (Didactic Base Timişoara, 2016-2017)

Cultivar	Pod length (cm)	Relative values (%)	Difference/Significance
Goldfield	25.33	110.50	2.42***
Ecaterina	22.01	96.10	-0.90^{000}
Aurie de Bacău	21.40	93.40	-1.51^{000}
Control (average exp.)	22.92	100.00	0.00

$LSD_{5\%}$ = 0.32 cm; $LSD_{1\%}$ = 0.43 cm; $LSD_{0.1\%}$ = 0.56 cm.

As for the data shown in Table 3 above, there is a very significant positive difference in pod length (+2.42 cm) in the Goldfield climbing common bean. This significant change of the morphological feature is due to the biological improved cultivar feature (Madoşă, 2000; Nedelea and Madoşă, 2004).

For better assessment of the interdependence between trial factors we assessed the combination between basic fertilisation. supplementary fertilisation and cultivar. Trial results are shown in Table 4 below.

Table 4. Interdependence of the combination basic fertilisation x supplementary fertilisation x cultivar on climbing common bean pod length (Didactic Base Timişoara, 2016-2017)

Factor combination	Pod length (cm)	Relative values (%)	Difference/Significance
Orgevit x Cropmax x Goldfield	25.50	111.30	2.59***
Phenix x Cropmax x Goldfield	27.10	118.28	4.19***
Italpollina x Cropmax x Goldfield	26.30	114.79	3.39***
Orgevit x Cropmax x Ecaterina	22.42	97.86	-0.49^{00}
Phenix x Cropmax x Ecaterina	24.39	106.46	1.48**
Italpollina x Cropmax x Ecaterina	21.74	94.89	-1.17^{0}
Orgevit x Cropmax x Aurie de Bacău	22.94	100.13	0.03
Phenix x Cropmax x Aurie de Bacău	22.13	96.59	-0.78
Italpollina x Cropmax x Aurie de Bacău	21.59	94.23	-1.32^{0}
Orgevit x Lithovit x Goldfield	23.96	104.58	1.05*
Phenix x Lithovit x Goldfield	25.44	111.04	2.53***
Italpollina x Lithovit x Goldfield	24.45	106.72	1.54**
Orgevit x Lithovit x Ecaterina	21.07	91.96	-1.84^{000}
Phenix x Lithovit x Ecaterina	23.17	101.13	0.26
Italpollina x Lithovit x Ecaterina	20.21	88.21	-2.70^{000}
Orgevit x Lithovit x Aurie de Bacău	21.56	94.10	-1.35^{00}
Phenix x Lithovit x Aurie de Bacău	21.02	91.75	-1.89^{000}
Italpollina x Lithovit x Aurie de Bacău	20.07	87.60	-2.84^{000}
Orgevit x Trainer x Goldfield	24.22	105.71	1.31*
Phenix x Trainer x Goldfield	25.31	110.47	2.40***
Italpollina x Trainer x Goldfield	24.72	107.90	1.81***
Orgevit x Trainer x Ecaterina	21.28	92.88	-1.63^{00}
Phenix x Trainer x Ecaterina	23.41	102.18	0.50
Italpollina x Trainer x Ecaterina	20.43	89.17	-2.48^{000}
Orgevit x Trainer x Aurie de Bacău	21.79	95.11	-1.12^{0}
Phenix x Trainer x Aurie de Bacău	21.24	92.71	-1.67^{00}
Italpollina x Trainer x Aurie de Bacău	20.29	88.56	-2.62^{000}
Control (exp. average)	22.91	100.00	Mt

$LSD_{5\%}$ = 1.00 cm; $LSD_{1\%}$ = 1.35 cm; $LSD_{0.1\%}$ = 1.79 cm.

As far as trial results in Table 4 above are concerned. we need to note the very significant positive differences in the length of the pods (2.59-4.19 cm) in the Goldfield common bean cultivar treated with the foliar fertiliser Cropmax and the three basic fertilisers.

The second morphological feature analysed in this trial was common bean pod width with direct impact on yield quality and quantity. We made phonological observations and biometric measurements on pod width. Trial results are shown in Table 5 below.

Table 5. Influence of supplementary fertilisation on climbing common bean pod width (Didactic Base Timişoara, 2016-2017)

Supplementary fertilisation	Pod width (cm)	Relative values (%)	Difference / Significance
Cropmax	2.05	102.20	0.04***
Lithovit	1.97	98.40	-0.03^{000}
Trainer	1.99	99.40	-0.01^{0}
Control (average exp.)	2.00	100.00	0.00

$LSD_{5\%}$ = 0.01 cm; $LSD_{1\%}$ = 0.01 cm; $LSD_{0.1\%}$ = 0.02 cm.

Comparative analysis of the unilateral influence of supplementary fertilisation during vegetation on climbing common bean pod width shows a very significant positive difference (+0.04 cm) when using the product Cropmax.

Table 6 below shows trial results regarding the unilateral influence of basic (organic) fertilisation on climbing common bean pod width.

Table 6. Influence of basic fertilisation on climbing common bean pod width (Didactic Base Timişoara, 2016-2017)

Basic fertilisation	Pod width (cm)	Relative values (%)	Difference/Significance
Orgevit	1.95	97.50	-0.05^{000}
Phenix	2.04	101.90	0.04***
Italpollina	2.01	100.60	0.01**
Control (average exp.)	2.00	100.00	0.00

$LSD_{5\%}$ = 0.01 cm; $LSD_{1\%}$ = 0.01 cm; $LSD_{0.1\%}$ = 0.01 cm.

As for the influence of basic fertilisation on common bean pod width, there is a very significant positive difference (+0.04 cm) when applying the fertiliser Phenix on a soil treated with more potassium. The genetic factor (cultivar) is definitory in higher quality and quantity yields. We noted the unilateral influence of the common bean cultivar used in the trial on climbing common bean pod width in table 7 (Mercati et al., 2013).

Table 7. Influence of cultivar on climbing common bean pod width (Didactic Base Timişoara, 2016-2017)

Cultivar	Pod width (cm)	Relative values (%)	Difference / Significance
Goldfield	2.18	108.90	0.18***
Ecaterina	1.95	97.30	-0.05^{000}
Aurie de Bacău	1.88	93.80	-0.12^{000}
Control (average exp.)	2.00	100.00	0.00

$LSD_{5\%}$ = 0.01 cm; $LSD_{1\%}$ = 0.01 cm; $LSD_{0.1\%}$ = 0.01 cm.

In this case, the Goldfield climbing common bean cultivar is above the trial mean from the perspective of pod width (2.18 cm).

Trial data regarding the interdependence between fertiliser combinations and common bean cultivars are shown in Table 8 below.

Table 8. Interdependence of the combination basic fertilisation x supplementary fertilisation x cultivar on climbing common bean pod width (Didactic Base Timişoara, 2016-2017)

Factor combination	Pod width (cm)	Relative values (%)	Difference/Significance
Orgevit x Cropmax x Goldfield	2.29	114.50	0.29***
Phenix x Cropmax x Goldfield	2.21	110.50	0.21***
Italpollina x Cropmax x Goldfield	2.19	109.50	0.19***
Orgevit x Cropmax x Ecaterina	1.86	93.00	-0.14^{000}
Phenix x Cropmax x Ecaterina	2.02	101.00	0.02*
Italpollina x Cropmax x Ecaterina	1.97	98.50	-0.03^{00}
Orgevit x Cropmax x Aurie de Bacău	1.86	93.00	-0.14^{000}
Phenix x Cropmax x Aurie de Bacău	1.91	95.50	-0.09^{000}
Italpollina x Cropmax x Aurie de Bacău	1.99	99.50	-0.01
Orgevit x Lithovit x Goldfield	2.19	109.50	0.19***
Phenix x Lithovit x Goldfield	2.13	106.50	0.13***
Italpollina x Lithovit x Goldfield	2.12	106.00	0.12***
Orgevit x Lithovit x Ecaterina	1.78	89.00	-0.22^{000}
Phenix x Lithovit x Ecaterina	2.03	101.50	0.03**
Italpollina x Lithovit x Ecaterina	1.91	95.50	-0.09^{000}
Orgevit x Lithovit x Aurie de Bacău	1.78	89.00	-0.22^{000}
Phenix x Lithovit x Aurie de Bacău	1.83	91.50	-0.17^{000}
Italpollina x Lithovit x Aurie de Bacău	1.93	96.50	-0.07^{000}
Orgevit x Trainer x Goldfield	2.18	109.00	0.18***
Phenix x Trainer x Goldfield	2.14	107.00	0.14***
Italpollina x Trainer x Goldfield	2.14	107.00	0.14***
Orgevit x Trainer x Ecaterina	1.80	90.00	-0.20^{000}
Phenix x Trainer x Ecaterina	2.04	102.00	0.04**
Italpollina x Trainer x Ecaterina	1.93	96.50	-0.07^{000}
Orgevit x Trainer x Aurie de Bacău	1.80	90.00	-0.20^{000}
Phenix x Trainer x Aurie de Bacău	1.85	92.50	-0.15^{000}
Italpollina x Trainer x Aurie de Bacău	1.95	97.50	-0.05^{000}
Control (exp. average)	2.00	100.00	Mt

$LSD_{5\%}$ = 0.02 cm; $LSD_{1\%}$ = 0.03 cm; $LSD_{0.1\%}$ = 0.04 cm.

Based on comparative assessment of the combinations of trial factors we noted again very significant positive differences in common bean pod width (0.19-0.29 cm) in the Goldfield common bean cultivar fertilised supplementarily with Cropmax with the three basic fertilisations.

The quality of climbing common bean also depends on pod diameter.

From this perspective, we assessed the unilateral influence of supplementary fertilisation on common bean pod diameter. Trial results are shown in Table 9 below.

Table 9. Influence of supplementary fertilisation on climbing common bean pod diameter
(Didactic Base Timişoara, 2016-2017)

Supplementary fertilisation	Pod diameter (cm)	Relative values (%)	Difference / Significance
Cropmax	0.691	101.61	0.01***
Lithovit	0.670	98.52	-0.01^{000}
Trainer	0.677	99.55	-0.003^{000}
Control (average exp.)	0.680	100.00	0.00

$LSD_{5\%}$ = 0.001 cm; $LSD_{1\%}$ = 0.001 cm; $LSD_{0.1\%}$ = 0.001 cm.

Trial data shown in the table above show a very significant positive difference in pod diameter when using the foliar fertiliser Cropmax given its macro- and micro-element composition.

As in the morphological features analysed above, we assessed the unilateral influence of basic fertilisation on common bean pod diameter (Table 10).

Table 10. Influence of basic fertilisation on climbing common bean pod diameter
(Didactic Base Timişoara, 2016-2017)

Basic fertilisation	Pod diameter (cm)	Relative values (%)	Difference / Significance
Orgevit	0.671	98.82	-0.008^{000}
Phenix	0.684	100.73	0.005***
Italpollina	0.683	100.58	0.004***
Control (average exp.)	0.679	100.00	0.00

$LSD_{5\%}$ = 0.001 cm; $LSD_{1\%}$ = 0.001 cm; $LSD_{0.1\%}$ = 0.001 cm.

Comparing the three basic fertilisations of climbing common bean cultivated in solaria. we can note the very significant positive differences when using Phenix and Italpollina.

A synthesis of trial results regarding the unilateral influence of climbing common bean cultivar on pod diameter is shown in Table 11 below.

Table 11. Influence of cultivar on climbing common bean pod diameter
(Didactic Base Timişoara, 2016-2017)

Cultivar	Pod diameter (cm)	Relative values (%)	Difference/Significance
Goldfield	0.717	105.50	0.03***
Ecaterina	0.699	102.90	0.01***
Aurie de Bacău	0.622	91.50	-0.06^{000}
Control (average exp.)	0.680	100.00	0.00

$LSD_{5\%}$ = 0.01 cm; $LSD_{1\%}$ = 0.01 cm; $LSD_{0.1\%}$ = 0.01 cm.

From the perspective of pod diameter, we noted the very significant positive differences in the common bean cultivars Goldfield and Ecaterina.

Trial results regarding the interdependence between treated soil and common bean cultivar on the diameter of common bean pods are shown in Table 12 below.

Table 12. Interdependence of the combination basic fertilisation x supplementary fertilisation x cultivar on climbing common bean pod diameter (Didactic Base Timişoara, 2016-2017)

Factor combination	Pod diameter (cm)	Relative values (%)	Difference/Significance
Orgevit x Cropmax x Goldfield	0.709	103.65	0.025***
Phenix x Cropmax x Goldfield	0.724	105.84	0.040***
Italpollina x Cropmax x Goldfield	0.785	114.76	0.101***
Orgevit x Cropmax x Ecaterina	0.729	106.57	0.045***
Phenix x Cropmax x Ecaterina	0.735	107.45	0.051***
Italpollina x Cropmax x Ecaterina	0.695	101.60	0.011*
Orgevit x Cropmax x Aurie de Bacău	0.628	91.81	-0.056^{000}
Phenix x Cropmax x Aurie de Bacău	0.650	95.02	-0.034^{000}
Italpollina x Cropmax x Aurie de Bacău	0.641	93.71	-0.043^{000}
Orgevit x Lithovit x Goldfield	0.683	99.85	-0.001
Phenix x Lithovit x Goldfield	0.697	101.90	0.013*
Italpollina x Lithovit x Goldfield	0.749	109.50	0.065***
Orgevit x Lithovit x Ecaterina	0.712	104.09	0.028***
Phenix x Lithovit x Ecaterina	0.717	104.82	0.033***
Italpollina x Lithovit x Ecaterina	0.669	97.80	-0.015^{00}
Orgevit x Lithovit x Aurie de Bacău	0.610	89.18	-0.074^{000}
Phenix x Lithovit x Aurie de Bacău	0.629	91.95	-0.055^{000}
Italpollina x Lithovit x Aurie de Bacău	0.612	89.47	-0.072^{000}
Orgevit x Trainer x Goldfield	0.695	101.60	0.011
Phenix x Trainer x Goldfield	0.702	102.63	0.018***
Italpollina x Trainer x Goldfield	0.762	111.40	0.078***
Orgevit x Trainer x Ecaterina	0.716	104.67	0.032***
Phenix x Trainer x Ecaterina	0.712	104.09	0.028***
Italpollina x Trainer x Ecaterina	0.671	98.09	-0.013^{00}
Orgevit x Trainer x Aurie de Bacău	0.612	89.47	-0.072^{000}
Phenix x Trainer x Aurie de Bacău	0.630	92.10	-0.054^{000}
Italpollina x Trainer x Aurie de Bacău	0.616	90.05	-0.068^{000}
Control (average exp.)	0.684	100.00	Mt

$LSD_{5\%}$ = 0.01 cm; $LSD_{1\%}$ = 0.013 cm; $LSD_{0.1\%}$ = 0.015 cm.

As for the comparative analysis of the combination of trial factors, we can say here are very significant positive differences in pod diameter in the common bean cultivar Goldfield cultivated on a soil treated with Italpollina and treated with foliar fertilisers.

CONCLUSIONS

Based on trial results in the three climbing common bean cultivars cultivated in solaria with different basic and supplementary fertilisation, we can draw the following conclusions:

- Comparative assessment of the three climbing common bean cultivars from the perspective of the three morphological features points out the Goldfield common bean cultivar;
- Applying the product Phenix as basic fertiliser in climbing common bean cultivated in solaria has a very significant influence on the three morphological features under study;
- Common bean pod length and width are very significantly influenced by supplementary fertilisation with Cropmax on the three basic fertilisations;
- Climbing common bean pod diameter is very significantly influenced by the three foliar fertilisers (Cropmax, Trainer and Lithovit) on the soil with basic fertilisation (Italpollina);
- From the perspective of climbing common bean pod diameter, we noted the Ecaterina common bean cultivar with trial results close to those of Goldfield common bean cultivar;
- Ensuring optimum fertilisation rate with macro- and micro-elements during vegetation in climbing common bean cultivated in solaria influences morphological features and. Therefore, yielding capacity.

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THE EFFECTS OF DIFFERENT NITROGEN DOSES ON YIELD AND NUTRIENT UPTAKE OF ROCKET (*ERUCA SATİVA*) PLANT

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Abstract

The present study was conducted to investigate the effects of different nitrogen doses on yield and nutrient uptake of rocket plant. The study has been carried out with three repetitions according to the experimental pattern of randomized plots in the plastic pots with the capacity of 3 kg under the greenhouse conditions. Nitrogen doses; 0 mg N kg^{-1}, 50 mg N kg^{-1}, 100 mg N kg^{-1}, 150 mg N kg^{-1}, 200 mg N $kg^{-1}$and were applied as $CaNO_3.4H_2O$ form. The rocket plant was harvested after 60 days of sowing and various traits like; shoot dry weight, macro- and micro-elements concentrations were determined. The results of the study have shown that the highest shoot dry weight was determined in 200 mg N kg^{-1}application with 4.45 g pot^{-1}. In addition, the highest N concentration (5.80 % N) wa sobserved in 200 mg N kg^{-1}application, P concentration in 50 mg N kg^{-1}application (0.34% P), K concentration in 100 mg N kg^{-1} application (7.01% K), Mg and Ca concentrations in 150 mg N kg^{-1} application (0.92% Mg, 1.35% Ca) were determined. Considering all treatments together, it was observed that increasing nitrogen doses increased yieldand macro and microelements concentrations of rocket plant.

Key words*: rocket, nitrogen, yield, nutrient uptake.*

INTRODUCTION

Rocket (*Eruca sativa*) is an endemic species of *Brassicaceae* family and a single year herbaceous plant. This species is widely cultivated such Mediterranean countries as Italy, Greece and Turkey in particular (Aegean, Marmara and Mediterranean regions) (Morales and Janick, 2002; Barlas et al., 2011; Baser, 2016). Fresh leaves of rocket plant have long been used in salads of Mediterranean cuisine (Başer, 2016). With the current increase in the consumption of green vegetables which are beneficial to human health, the economic potential rocket plant has steadily increased recently (Eşiyok et al., 2006).

In order for being able to harvest quality yield in plentitude in vegetable cultivation (Stewart et al., 2005), plant nutrients are emphasized in recent years (Adediran et al., 2004; Naeem et al., 2006). Plants need at least 17 plant nutritional materials or elements to achieve and demonstrate optimal growth and development (White, 2006; Gardiner and Miller, 2008; Fageria, 2009; Bolat and Kara, 2017). Nitrogen (N) is one of these nutritional materials and it is necessary for the formation of new cells in the plants. The growth rate of the plants declines due to the nitrogen deficiency. Particularly the vegetative development of the plants is negatively affected.

Leave and root systems of the plants is relatively weakened. Similarly, development of roots and particularly the branching of the roots is weakened. Flowering and fruit production rates decrease and fruits are relatively small in size (Foth, 1984; Aktaş and Ateş, 1998; Kantarcı, 2000; Boşgelmez et al., 2001; Güzel et al., 2004; Fageria, 2009; Kacar and Katkat, 2010; Bolat and Kara, 2017).

Although nitrogen is the main nutritient element, which is the most absorbed nutrient in comparison to other elements, it is one of the most deficient nutrient elements. A great part of soils on the Earth is subject to nitrogen deficiency.

The main source of nitrogen in the soil is organic materials. Proteins, amino acids, nucleic acids, enzymes, chlorophylls, ATP, ADP are among significant organic materials

including nitrogen. As a result of the gradual decomposition of the organic materials, plants can make use of the nitrogen contained in these decomposed materials.

The soils of Turkey, which are particularly deficient in organic materials, are relatively poor in terms of nitrogen (Çepel, 1996; Aktaş and Ateş, 1998; Boşgelmez et al., 2001; Güzel et al., 2004; Gardiner and Miller, 2008; McCauley et al., 2009; Bolat and Kara, 2017). For this reason, chemical fertilizers which are commonly used for preventing nutritional imbalance in plants and satisfying needs of plants for nutritional elements are mainly composed of nitrogen content (Kacar and Katkat, 2010). Nitrogen has a significant effect on the plant quality.

The conducted research has demonstrated a direct correlation between nitrogen levels and quality of the plant yield. In accordance with the increase in the nitrogen dose applied to the plants, the N concentration in the plant grain and accordingly the grain quality also demonstrate increases (Marschner, 1997). However, in recent years, it has been detected that the remaining nitrogen in the soil from the high concentration nitrogen fertilizer applications results in environmental pollution (Zand-Parsa et al., 2006; Gollany et al., 2004; Beman et al., 2005) and leads to accumulation of materials in plants detrimental to human health (Ruiz and Romero, 1999).

For this reason, the management and programming of nitrogen in vegetable cultivation are of due significance (Adiloğlu et al., 2015).

The aim of the present study is determining the effects of administering nitrogen in varying quantities, which is a relatively significant nutrient element, on the productivity of and absorption of nutrient elements by rocket plant.

MATERIALS AND METHODS

This study was conducted at greenhouses of Plant and Animal Production Department of Cumhuriyet University Sivas Vocational Collage. Experiment was conducted in randomized plots design with 3 repetitions. Experimental soils were taken from 0-30 cm soil profile of experimental fields of the department. Soils were sieved through 2 mm sieve and 3 kg air-dried soils were placed in experimental pots.

Some physical and chemical characteristics of soil are provided in Table 1. Five levels of nitrogen; 0 mg N kg^{-1}, 50 mg N kg^{-1}, 100 mg N kg^{-1}, 150 mg N kg^{-1} and 200 mg N kg^{-1} (in $CaNO_3.4H_2O$ form) were applied. Before sowing, 100 mg kg^{-1} P and 125 mg kg^{-1} K (in the form of KH_2PO_4), 2.5 mg kg^{-1}Zn (in the form of $ZnSO_4.7H_2O$) and 2.5 mg kg^{-1} Fe (in the form of Fe-EDTA) were applied to each pot as basic fertilizers.

Plant analyses

Leaf samples were taken from the rocket plants 45 days after the sowing. Vegetative parts of the plants were washed through tap water, rinsed respectively through distilled water, 0.1 N HCl solution and twice though again distilled water. They were placed over coarse filter papers and excess water over them was removed.

Plant parts were then placed in separate paper bags and dried at 65°C until a constant weight. Following the measurement of dry weights, dry samples were ground in a plant mill. About 0.2 g of ground samples were wet digested in H_2O_2-HNO_3 acid mixture in a microwave oven. Resultant slurry was then completed to 20 ml with distilled water and filtered through blue-band filter paper. Samples were then subjected to P colorimetric K, Ca, Mg, Zn, Mn, Fe and Cu readings at 882 nm (Murphy and Riley, 1962) in an AAS (Atomic Absorption Spectrophotometer) (Shimadzu AA-7000) (Kaçar and İnal, 2008). N contents were determined with Kjeldahl distillation method (Bremner, 1965).

Data assessment

Experimental results were subjected to variance analyses (ANOVA) separately in accordance with randomized plots experimental design. SPSS 22.0 Windows software was used for statistical analyses. Means were compared with Tukey's test at $P<0.05$. Correlation analysis was performed to assess the relationships between the treatments.

Table 1. Some physical and chemical properties of soil

Soil Property	Depth (0-30cm)
pH	7.26
Lime (%)	17.0
Salt (%)	0.034
Organic matter (%)	1.38
Texture	SiCL
Total N (%)	0.087
Available P (kg ha^{-1})	47.3
Available K (kg ha^{-1})	744.8
Available Fe (mg kg^{-1})	3.11
Available Mn (mg kg^{-1})	1.04
Available Zn (mg kg^{-1})	0.41
Available Cu (mg kg^{-1})	1.22

RESULTS AND DISCUSSIONS

Effects of different nitrogen treatments on dry matter production of rocket plant were investigated and results are presented in Figure 1. The greatest dry matter production (4.45 g pot^{-1}) was obtained from 200 mg N kg^{-1} treatment (Figure 1).

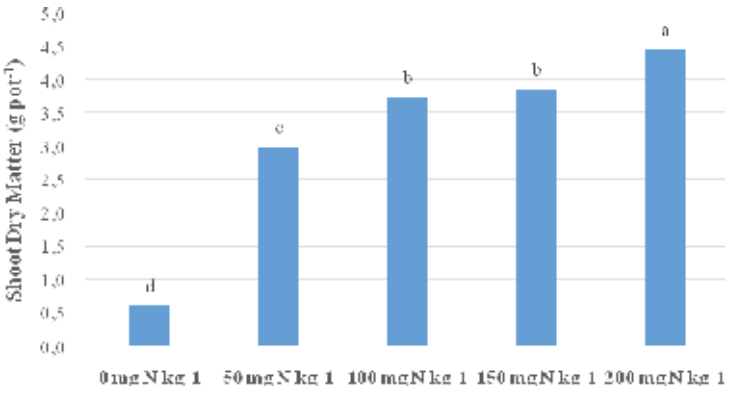

Figure 1. Effects of different nitrogen fertilizer on shoot dry matter of rocket plant

This treatment was followed by 100 mg N kg^{-1} and 150 mg N kg^{-1} treatments respectively with 3.86 g pot^{-1} and 3.75 g pot^{-1} dry matter productions which were placed in the same statistical group. The lowest dry matter production was observed in control treatment (0 mg N kg^{-1}) with 0.61 g pot^{-1}. Increasing dry matter production of rocket plants were observed with increasing N doses. Haag and Minami (1988) applied four different nitrogen doses (0, 100, 200, 300 kg ha^{-1}) to rocket plants and made harvests in June, July and August. It was reported that marketable product quantity increased from 4.4 ton ha^{-1} to 8.3 ton ha^{-1} (about doubled) with N treatments. Eşiyok et al. (1998) investigated the effects of different nitrogenous fertilizers on yield and nutrient uptake of rocket plants. Yield and nutrient uptakes varied with nitrogen doses and the greatest yield was obtained from 160 kg ha^{-1} nitrogen treatment. Researchers also reported decreasing leaf nitrogen, phosphorus,

potassium and other nutrient contents with the aging of the leaves as compared to young leaves harvested at early periods. Demiral et al. (2009) investigated the effects of N treatments on plant growth and nitrate (NO_3^-) accumulation of four different winter vegetables including chard (*Beta vulgaris* cv. Chard), rocket (*Eruca sativa*), lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*). Researchers applied three different N doses (110, 175 and 240 mg l^{-1}) to the plants in 4 replications and indicated improved plant growth with the experimental treatments. Hanaka et al. (2016) applied two different nitrate (0.3, 0.6 g N dm^{-3} of medium) and 3 different potassium (0.3, 0.6, 0.9 g K dm^{-3} of medium) doses to rocket plants and reported dry matter productions under experimental treatments as between 9.2-10.1 g.

Table 2. Effects of different nitrogen fertilizer on N, P and K concentrations of rocket plant (%)

Treatments	N	P (%)	K
0 mg N kg^{-1}	1.91 ±0.02d	0.15 ±0.01d	3.09 ±0.05e
50 mg N kg^{-1}	2.75 ±0.25c	0.34 ±0.02 a	4.40 ±0.02 d
100 mg N kg^{-1}	4.97 ±0.18 b	0.28 ±0.01 b	7.01 ±0.11 a
150 mg N kg^{-1}	5.16 ±0.04 b	0.28 ±0.03 b	6.71 ±0.04 b
200 mg N kg^{-1}	5.80 ±0.06 a	0.22 ±0.00 c	5.51 ±0.29c

P<0.05

Considering the effects of different nitrogen treatments on N concentration of the rocket plants, the greatest value (5.80% N) was obtained from the greatest nitrogen dose of 200 mg N kg^{-1} treatment.

Plant N concentration of the control (0 mg N kg^{-1}) treatment was 1.91% N. As it was in dry matter productions, increasing plant N concentrations were observed with increasing nitrogen doses. Similarly, Hanaka et al. (2016) also reported increasing leaf N concentrations of rocket plants with increasing N doses (especially when combined with S).

The greatest P concentration (0.34% P) was observed in 50 mg N kg^{-1} treatment and plant P concentrations decreased after this N dose.

The greatest K concentration (7.01% K) was obtained from 100 mg N kg^{-1} treatment. K concentrations decreased after this dose. All nitrogen doses significantly increased % N, % P and % K concentrations of rocket plants as compared to the control treatment. Nurzyńska-Wierdak (2015) applied different nitrogen and potassium doses to rocket plants and reported % P concentrations of the plants as between 0.51-0.61% P and % K concentrations as between 5.30-6.76% K. Barlas et al. (2011) in a survey study, collected rocket plants from 30 different fields and reported %N concentrations as between 2.94-5.23% N, % P concentrations as between 0.12-0.27% P and K concentrations as between 3.99-5.98% K.

Table 3. Effects of different nitrogen fertilizer on Ca and Mg concentrations of rocket plant (%)

Treatments	Ca (%)	Mg
0 mg N kg^{-1}	0.54 ±0.02 e	0.63 ±0.03 bc
50 mg N kg^{-1}	1.08 ±0.01 c	0.88 ±0.07 ab
100 mg N kg^{-1}	1.22 ±0.03 b	0.90 ±0.03 a
150 mg N kg^{-1}	1.35 ±0.02 a	0.92 ±0.03 a
200 mg N kg^{-1}	0.93 ±0.03 d	0.59 ±0.03 c

P<0.05

Considering the Ca concentrations of the rocket plants, the lowest value (0.54% Ca) was obtained from the control (0 mg N kg^{-1}) treatment and the greatest value (1.35% Ca) was obtained from 150 mg N kg^{-1} treatments (Table 3). Barlas et al. (2011) reported leaf Ca

concentrations of rocket plants as between 2.20-3.55% Ca. Bukhsh et al. (2007) reported the Ca concentration as 1900 µg g^{-1} Ca in seeds of rocket plants and as 700 µg g^{-1}. Ca in the leaves of rocket plants. Similar to Ca concentrations, the greatest Mg concentration (0.92% Mg) was obtained from 150 mg N kg^{-1} treatment. This treatments was followed by 100 mg N kg^{-1} treatment (0.90% Ca) which was placed in the same statistical group. In previous studies, Mg concentrations of rocket plants were reported as between 37-41 mg 100 g^{-1} (Haag and Manami, 1998) and as 46 mg 100 g^{-1} (Bianco, 1995). Eşiyok et al. (2006) reported Mg content of organic fertilizer-treated rocket plants as between 0.19-0.23% Mg.

Table 4. Effects of different nitrogen fertilizer on Fe, Zn, Mn and Cu concentrations of rocket plant

Treatments	Fe	Zn	Mn	Cu
	(mg kg^{-1})			
0 mg N kg^{-1}	73.4 ±1.31 d	10.9 ±0.48 c	26.8 ±1.05 d	4.4 ±0.04 c
50 mg N kg^{-1}	163.4 ±14.07 a	30.2 ±2.86 a	27.7 ±0.53 cd	5.5 ±0.32 b
100 mg N kg^{-1}	133.6 ±12.52 b	24.6 ±0.01 b	29.4 ±0.23 c	5.9 ±0.08 b
150 mg N kg^{-1}	151.3 ±5.23 a	24.6 ±0.10 b	35.7 ±0.21 b	6.3 ±0.16 ab
200 mg N kg^{-1}	97.6 ±1.39 c	12.1 ±0.07 c	42.9 ±2.18 a	7.1 ±0.71 a

P<0.05

Considering the micro element concentrations of rocket plants (Table 4), the greatest Fe concentration was obtained from 50 mg N kg^{-1} treatment (163.4 mg kg^{-1}). This treatment was followed by 150 mg N kg^{-1} (151.3 mg kg^{-1} Fe). As compared to control treatment, all nitrogen doses increased plant Fe concentrations. Zn concentrations of the rocket plants also increased with increasing nitrogen doses.

The lowest Zn concentration (10.9 mg kg^{-1} Zn) was obtained from 0 mg N kg^{-1} control treatment and the greatest Zn concentration (30.2 mg kg^{-1} Zn) was obtained from 50 mg N kg^{-1} treatment. As it was in P concentrations, Zn concentrations of rocket plants decreased after 50 mg N kg^{-1} treatments. Increasing nitrogen doses also increased Mn and Cu concentrations of the plant. The greatest Mn and Cu concentrations were obtained from 200 mg N kg^{-1} treatment. While the greatest Mn concentration was 42.9 mg kg^{-1}Mn, the greatest Cu concentration was observed as 7.1 mg kg^{-1} Cu. Bukhsh et al. (2007) reported Fe concentration of rocket plants as 60.62 µg g^{-1} Fe, Zn concentration as 56.1 µg g^{-1} Zn, Mn concentration as 19.0 µg g^{-1}Mn and Cu concentration as 32.0 µg g^{-1} Cu; reported leaf Fe concentration as 37.5 µg g^{-1} Fe, leaf Zn concentration as 1.12 µg g^{-1} Zn, leaf Mn concentration as 10.6 µg g^{-1}Mn and leaf Cu concentration as 21.0 µg g^{-1} Cu. In a survey study, Fe concentration of rocket plants was reported as 350.78 mg kg^{-1} Fe, Zn concentration as 64.86 mg kg^{-1} Zn, Mn concentration as 40.58 mg kg^{-1}Mn and Cu concentration as 5.37 mg kg^{-1} (Barlas et al., 2011).

Table 5. Correlation values of parameters evaluated in the study

Parameters	SDW***	N	P	K	Mg	Ca	Fe	Zn	Mn	Cu
SDW	1									
N	.907**	1								
P	.536*	.194	1							
K	.818**	.850**	.468	1						
Mg	.295	.197	.613*	.631*	1					
Ca	.767**	.640*	.780**	.882**	.781**	1				
Fe	.498	.188	.949**	.508	.768**	.834**	1			
Zn	.335	.029	.948**	.421	.730**	.755**	.948**	1		
Mn	.545*	.398	.138	-.015	-.468	.004	.007	-.148	1	
Cu	.913**	.891**	.293	.678**	.113	.586*	.282	.077	.661**	1

*Significant at P<0.05 **Significant at P<0.01 ***SDW=Shoot Dry Weight

Considering the correlations among investigated parameters, it was observed that SDW had significant positive correlations with N, K, Ca and Cu ($P<0.01$); there was significant positive correlation between P and Mn ($P<0.05$) (Table 5). There was significant positive correlations also between N and K, between Ca and Cu; P had positive correlations with Mg, Ca, Fe and Zn; there were positive correlations between K and Mg, between Ca and Cu; and Mg had significant positive correlations with Ca, Fe and Zn ($P<0.01$); Ca positively correlated with Fe, Zn and Cu; there were positive correlations between Fe and Zn and between Mn and Cu.

CONCLUSIONS

The present study was conducted to investigate the effects of different nitrogen doses on yield and nutrient uptake of the rocket plants.

Present findings revealed increasing dry matter productions, N, Mn and Cu concentrations of rocket plant with increasing nitrogen doses and 200 mg N kg^{-1} treatment was found to be prominent with regard to these parameters. On the other hand, 150 mg N kg^{-1} treatments were found to be prominent with regard to Ca, Mg and Fe. Considering the entire findings together, it was concluded that nitrogen treatments significantly increased dry matter yields and nutrient uptake of the rocket plant as compared to the control treatment.

Present findings revealed that nitrogenous fertilizers play a significant role in green vegetables like rockets with increasing consumptions.

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THE IMPACT OF DIFFERENT METHODS USED FOR IMPROVING FLOWER BIND UPON THE YIELD OF SOME TOMATO HYBRIDS CULTIVATED ON MINERAL WOOL SUBSTRATE IN INDUSTRIAL GREENHOUSES

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Abstract

Obtaining high and superior qualitative tomato yields, in conditions of forced culture system, being economically profitable, depends on modernizing the culture technology. In classical Venlo industrial greenhouses, modernization is possible till the technical limit allowed by their construction type. Among the technological links that can be modernized, and to which we refer in this study, are the following: cultivation of performant hybrids with high quantitative and qualitative potential (Noralee F1 and Marissa F1); fertilization during vegetation with modern chemicals, completely soluble (Yara and Haifa Chemicals), applied using drip irrigation system; using mineral wool as culture substrate (Grodan) and applying new and efficient methods for improving flower bind (pollination with bumblebees - Biobest or Natupol). This article presents the impact of all these modernized technological links upon the quantitative and qualitative tomatoes yields. Tomato-Stim determined large tomato yields, but the best results upon the yield quantity and quality were obtained from those tomato plants where natural pollination was improved by the use of bumblebees. Most of the results showed that the quality of the obtained yields (namely Extra+Ist quality) finds itself in a reversed proportionality with the production quantity per hectare. The influence of both graduation of the fertigation systems are substantiated in the very close quantitative production and from a statistic point of view were not covered, as there were no significant production differences. Results lead us to recommend the cultivation of Noralee F1 hybrid, the use of any of the fertilizers Yara and Haifa Chemicals via fertigation and the use of bumblebees (Biobest or Natupol) to improve the tomato flower binding and to obtain superior quality fruits.

Key words: *bumblebees fertigation, quality.*

INTRODUCTION

Modernizing the culture technology for vegetable cultivated in a protected or forced system, consists in improving some technological links (improving flower bind degree, using modern fertilizers, performant hybrids etc.) so that the effect would be and efficient yield, considering its productivity, quality and economic efficiency (Horgoş A. et al., 2002).

Today, tomatoes is widely cultivated throughout the world and adapted into many cultivars (Olmstead and Palmer, 1997).

The replacement of soil, as natural culture substrate, in greenhouses with an artificial culture substrate, in this case *mineral wool*, represents an important technological link in the modernization process as it determines the improvement of other technological links, which are irrigation and fertilization at the same time by using the drip-irrigation system (fertigation). (Horgoş A. et al., 2005).

Bumblebees are important pollinators for tomato crops. Foragers are capable of assessing the pollen reward of the flowers, the first flower visit is the most effective concerning pollen removal and, consequently, pollination, and small foragers are as efficient in pollinating tomato flowers as are big ones. In line with some earlier studies (Buchmann and Cane 1989; Harder, 1990; Shelly et al., 2000) provide clear evidence that bees are indeed

capable of perceiving the amount of pollen obtained while visiting a flower (Nunes-Silva Patricia et al., 2013).
The cultivated plant is autogamous. However, one of the features of the genus is the poricidal opening of its anthers, which requires the agitation of the flowers by wind and/or the presence of pollinators that vibrate their indirect flight muscles for the release of pollen grains, even in cultivated varieties of tomatoes and especially in the still air of greenhouses (Kevan et al., 1991; Morandin et al., 2001b). Teppner (2005), while conducting studies on tomato plants in central Europe, observed that bees, such as *Bombus* and *Lasioglossum*, can be good pollinators of the flowers by vibrating their anthers easily.
Pollination is an important component of crop production for many cultivated plants. Through pollination research focused on crop plants, agricultural practices become better designed to efficiently produce high quality crops (Morse A., 2009).
In conclusion, the combined influence of technological works listed above, in correlation with the micro-climate factors from industrial heated greenhouses; assure high yields, qualitatively superior, which determine the profitability of tomato cultures.

MATERIALS AND METHODS

Researches on the impact of different methods for improving flower binding in tomato hybrids culture with undetermined growth, on mineral wool, were developed in classic Venlo industrial greenhouses from Agro-Codlea Company (placed in the Western part of Romania, in Arad County). The experiment was developed upon two Dutch hybrids with undetermined growth: Noralee F1 (generative hybrid) new hybrid belonging to Enza Zaden company and Marissa F1, belonging to Royal Sluis company, which is vigorous and high performances regarding fructification and fruits' quality.
The hybrids were observed regarding their productive and qualitative potential under the impact of two fertigation systems (with two completely soluble modern chemical fertilizers-Yara and Haifa Chemicals) and also by using different methods for improving flower bind (fecundation).
The culture was established in spring, the beginning of March (the 2nd - 3rd of March 2015), when the seedling had 65 days and it was planted to density of 24,288 plants/ha. The experiment is considered to be poly-factorial, having the following factors:
Factor A - Hybrids with undetermined growth cultivated on mineral wool: a_1 - Noralee F_1; a_2 - Marissa F_1.
Factor B - Fertigation system: b_1 - Fertigation with completely soluble chemicals: Yara (Yara Mila Cropcare; Yara Mila Ferticare I-III; Yara Mila Folicare B; Folicare Zn; Folicare Cu; KNO_3 Krista; Ca $(NO_3)_2$, Calcinit; K_3PO_4);b_2 - Fertigation with completely soluble chemicals: Haifa-Chemicals (Multicote 4, Multicote 4 with microelements - ME, magnesium nitrate, complex soluble fertilizers N-P-K, mono-potassium phosphate - MPK).
Factor C - Improvement of flower bind (fecundation): c_1 - Control, no stimulants, natural pollination; c_2 - Sprayings with Tomato-Stim stimulant (artificial stimulation); c_3 - Mechanical methods (shaking plants, minting the plant supporting wires, producing air streams by using the pulverizer); c_4 - Using bumblebees (Biobest, Natupol) to improve natural pollination.
The culture technology was distinctive by three modern works: the use of artificial culture substrate (mineral wool used as "matress"), the drip irrigation system (Netafim-Israel installation type) and the fertilization done with completely soluble chemicals using the drip irrigation installation (fertigation process).
The researches were done in order to determine the efficacy of tomato culture when cultivating hybrids with undetermined growth, with high productive potential, cultivated on artificial substrate in conditions of modern technology, which is still limited by the Velno classical greenhouse.

RESULTS AND DISCUSSIONS

The experimental results presented in tables 1 and 2 and figure 1 are the expression of the interactions between the three experimental factors, materialized in the different production and quality levels, related to the variability of production elements (average number of fruits per plant and average weight of one tomato fruit). The number of fruits per plant is clearly

influenced by the method used for improving flower binding (factor C), the values being of 118.4% (c_3 - Mechanical methods) and 140.6% (c_2 - Sprayings with Tomato-Stim stimulant) compared to the control c_1 - No stimulants, natural pollination (100%). The same rule applies to the average weight of fruits, the maximum value being obtained under the impact of c_3 (Mechanical methods - plants' shaking, minting the plant supporting wires, producing air streams with the pulverizer) and c_4 (Using bumblebees for natural pollination), compared to the other two methods, c_1 (No stimulants, natural pollination) and c_2 (Sprayings with Tomato-Stim stimulant).

The hierarchy levels of production are in direct proportionality rule with the number of fruits per plant and the average weight of a fruit.

Production quality levels, in terms of its quantitative proportionality, do not follow the same route under the direct influence of all graduations of factor C (Method of improving the binding of flowers). There is a deviation under the impact of c_2 (Sprayings with Tomato-Stim stimulant - artificial stimulation), where at a production point almost equal to the one obtained under the impact of c_4 (Using bumblebees for natural pollination) the difference is not significant (-1.5 t/ha), while the extra and first quality production represent 72.5% of the total, which is with 13.8% less than under the impact of c_4 (Using bumblebees for natural pollination), where the share is 86.3%.

Under the impact of factor B's graduations (fertigation system), the production elements and the obtained quantities are almost equal (the differences varying between 0.8-2.8 t/ha, respectively 0.6-2.0%). Considering production's quality (extra and first quality), the difference between b_1 (Yara) and b_2 (Haifa Chemicals) is significant, in the limits of 2.8% (c_1) and 5.4% (c_2).

Factor A (the hybrid) contributes to the differentiation of production levels, at the same time quantitative and qualitative), in interaction with both factor B and C and their graduations. Hybrid Noralee F1 has a medium production level of 124.9 t/ha and a maximum of 145.2 t/ha in $a_1b_1c_4$ (Noralee F1-Yara-Bumblebee pollination), being followed by 140.5 t/ha in $a_1b_1c_2$ (Noralee F1-Yara-Tomato-Stim) and 139.8 t/ha in $a_1b_2c_4$ (Noralee F1-Haifa Chemicals - Bumblebee pollination). Considering its qualitative production, the extra and first quality yield shares of the total yield are relatively close, being 82.0% in a_1 - Noralee F_1 and 80.2% in a_2 - Marissa F_1.

Variations percentage share of extra and first quality production have values between 84.4% in a_1b_1 (Noralee F1-Yara), 79.5% in a_1b_2 (Noralee F1-Haifa Chemicals) and 82.4% in a_2b_1 (Marissa F1-Yara), 78.0% in a_2b_2 (Marissa F1-Haifa Chemicals).

Preceding with the analysis of the summary the 3rd table regarding the experimental result we arrive to the following:

-In all the graduations of factor C (method of flower binding improvement), the production achieved under the influence of b1-Yara is on an average higher than under the influence of b2-Haifa Chemicals, the difference being rather very small (1.6 t/ha, namely 1.3%); the highest difference being registered in C4 (the natural pollination via bumblebees), these being 5.4 t/ha, namely 4.0% (c4b1a1 = 145.2 t/ha→106.9% and c4b2a1 = 139.8 t/ha→102.9%);

-Compared to M_{X1} $[c_5 = (c_1 + \cdots + c_4)/4]$ = 120.0 t/ha→100.0%), the production from b_1 (Yara) is 120.8 t/ha→100.7%, and for b_2 (Haifa Chemicals) is 119.2 t/ha→99.3%, a difference of 1.6 t/ha→1.4% being very low; the difference is similar also in the case of M_{X2} $[c_6 = (c_5 - c_1)/3]$ →100.0%, meaning 1.7 t/ha →1.3%;

- The highest productions are obtained under the influence of graduation c_2 (Tomato-Stim), 134.3 t/ha → 153.3% compared to $c_1 - M_t$ and under c_4 (bumblebees pollination), 135.8 t/ha → 155.0% compared to c_1–M_t;

- The production quality of the tomatoes under the influence of c_2 (Tomato-Stim) is lower though, percentage wise down to 72.5% (97.4 t/ha E+Ist quality) from a total production of 134.3 t/ha, compared to the best quality of the same, namely the one from c_4 (bumblebees pollination), of 86.3% (117.2 t/ha E+Ist quality) of a total of 135.8 t/ha;

- The production quality obtained (Extra+Ist quality) finds itself in a reversed proportionality with the production quantity per hectare;

- Out of the four methods of flower binding, from a quantity point of view as well as quality of tomato production, the method of using Biobest or Natupol bumblebees takes first place (c_4), where the average production per hectare as well as the quality had maximum worth compared to the other graduations.

Table 1. Experimental results on cultivating undetermined growth tomato hybrids in industrial greenhouses on mineral wool substrate, Ist cycle - 2015

Factor C (Improvement of flower bind)	Factor B (Fertigation system)	Factor A (The hybrid)	Average no. of fruits/plant		Average weight/ fruit (g/piece)	Average production				
						kg/plant	t/ha	% than c_1	of which extra and Ist quality production	
			piece/ pl	%					t/ha	%
c_1- Control, no stimulants, natural pollination	b_1-Yara	a_1 - Noralee F_1	32.6	100.0	114.7	3.738	90.8	103.7	76.0	83,7
		a_2 - Marissa F_1	31.5	100.0	111.9	3.524	85.6	97.7	70.0	81,8
		Average a for c_1xb_1	*32.1*	*100.0*	*113.3*	*3.631*	*88.2*	*100.7*	*73.0*	*82,8*
	b_2-Haifa chemicals	a_1 - Noralee F_1	32.5	100.0	114.3	3.714	90.2	103.0	73.2	81,2
		a_2 - Marissa F_1	31.2	100.0	110.6	3.450	83.8	95.7	67.1	80,1
		Average a for c_1xb_2	*31.9*	*100.0*	*112.5*	*3.582*	*87.0*	*99.3*	*70.2*	*80,7*
	Average value of factor B for c_1		***32,0***	***100.0***	***112.9***	***3.607***	***87.6***	***100.0***	***71.6***	***81.7***
c_2- Sprayings with Tomato-Stim stimulant (artificial stimulation)	b_1-Yara	a_1 - Noralee F_1	46.9	143.9	123.3	5.785	140.5	104.6	106.9	76,1
		a_2 - Marissa F_1	43.7	138.7	121.4	5.307	128.9	95.8	95.6	74,2
		Average a for c_2xb_1	*45.3*	*141.1*	*122.4*	*5.546*	*134.7*	*100.3*	*101.3*	*75,2*
	b_2-Haifa chemicals	a_1 - Noralee F_1	45.9	141.2	122.7	5.632	136.8	101.9	97.3	70,9
		a_2 - Marissa F_1	43.5	139.4	124.0	5.394	131.0	97.5	90.0	68,7
		Average a for c_2xb_2	*44.7*	*140.1*	*123.4*	*5.513*	*133.9*	*99.7*	*93.5*	*69,8*
	Average value of factor B for c_2		***45,0***	***140.6***	***122.9***	***5.529***	***134.3***	***100.0***	***97.4***	***72.5***
c_3- Mechanical methods (shaking plants, minting the plant supporting wires, producing air streams)	b_1-Yara	a_1 - Noralee F_1	39.8	122.0	139.1	5.536	129.6	106.1	114.2	88,1
		a_2 - Marissa F_1	37.0	117.5	129.3	4.784	116.2	95.2	100.2	86,2
		Average a for c_3xb_1	*38.4*	*119.6*	*134.2*	*5.060*	*122.9*	*100.7*	*107.2*	*87,2*
	b_2-Haifa chemicals	a_1 - Noralee F_1	38.2	117.5	136.5	5.216	126.7	103.8	104.3	82,3
		a_2 - Marissa F_1	36.4	116.7	131.1	4.772	115.9	94.9	94.3	81,4
		Average a for c_3xb_2	*37.3*	*116.9*	*133.8*	*4.994*	*121.3*	*99.3*	*99.3*	*81,9*
	Average value of factor B for c_3		***37,9***	***118.4***	***134.0***	***5.027***	***122.1***	***100.0***	***103.3***	***84.6***
c_4- Natural pollination using bumblebees (Biobest)	b_1-Yara	a_1 - Noralee F_1	42.9	131.6	139.3	5.978	145.2	106.9	129.9	89,5
		a_2 - Marissa F_1	40.4	128.3	131.7	5.319	129.2	95.1	113.4	87,8
		Average a for c_4xb_1	*41.7*	*129.9*	*135.5*	*5.649*	*137.2*	*101.0*	*121.7*	*88,7*
	b_2-Haifa chemicals	a_1 - Noralee F_1	41.7	128.3	138.0	5.756	139.8	102.9	118.0	84,4
		a_2 - Marissa F_1	40.6	130.1	130.8	5.311	129.0	95.0	107.1	83,0
		Average a for c_4xb_2	*41.2*	*129.1*	*134.4*	*5.533*	*134.4*	*99.0*	*112.6*	*83,8*
	Average value of factor B for c_4		***41,5***	***129.7***	***135.0***	***5.591***	***135.8***	***100.0***	***117.2***	***86.3***

Culture density: 24,288 plants/ha

Table 2. Experimental results on cultivating undetermined growth tomato hybrids in industrial greenhouses on mineral wool substrate, Ist cycle - 2015

Factor A (The hybrid)	Factor B (Fertigation system)	Factor C (Improvement of flower bind)	Average no. of fruits/plant		Average weight/ fruit (g/piece)	Average production				
						kg/plant	t/ha	% than c_1	of which extra and Ist quality production	
			piece/ pl	%					t/ha	%
a_1 - Noralee F_1	b_1-Yara	c_1 - Control - natural pollination	32.6	81.5	114.7	3.738	90.8	100.0	76.0	83,7
		c_2 - Stimulation with Tomato-Stim	46.9	117.3	123.3	5.785	140.5	154.7	106.9	76,1
		c_3 - Mechanical pollination	39.8	99.5	139.1	5.536	129.6	142.7	114.2	88,1
		c_4 - Bumblebee pollination - Biobest	42.9	107.3	139.3	5.978	145.2	159.9	129.9	89,5
		Average of factor C for a_1xb_1	*40.5*	*101.3*	*129.1*	*5.209*	*126.5*	*139.3*	*106.8*	*84,4*
	b_2-Haifa chemicals	c_1 - Control - natural pollination	32.5	81.3	114.3	3.714	90.2	100.0	73.2	81,2
		c_2 - Stimulation with Tomato-Stim	45.9	114.8	122.7	5.632	136.8	151.7	97.0	70,9
		c_3 - Mechanical pollination	38.2	95.5	136.5	5.216	126.7	140.5	104.3	82,3
		c_4 - Bumblebee pollination - Biobest	41.7	104.3	138.0	5.756	139.8	155.0	118.0	84,4
		Average of factor C for a_1xb_2	*39.5*	*98.8*	*127.9*	*5.080*	*123.4*	*136.8*	*98.1*	*79,5*
	Average of factor B for a_1		***40.0***	***100.0***	***128.5***	***5.144***	***124.9***	*	***102.5***	***82.0***
a_2 - Marissa F_1	b_1-Yara	c_1 - Control - natural pollination	31.5	82.7	111.9	3.524	85.6	100.0	70.0	81,8
		c_2 - Stimulation with Tomato-Stim	43.7	114.7	121.4	5.307	128.9	150.6	95.6	74,2
		c_3 - Mechanical pollination	37.0	97.1	129.3	4.784	116.2	135.7	100.2	86,2
		c_4 - Bumblebee pollination - Biobest	40.4	106.0	131.7	5.319	129.2	150.9	113.4	87,8
		Average of factor C for a_2xb_1	*38.2*	*100.3*	*123.6*	*4.735*	*115.0*	*134.3*	*94.8*	*82,4*
	b_2-Haifa chemicals	c_1 - Control - natural pollination	31.2	81.9	110.6	3.450	83.8	100.0	67.1	*80,1*
		c_2 - Stimulation with Tomato-Stim	43.5	114.2	124.0	5.394	131.0	156.3	90.0	68,7
		c_3 - Mechanical pollination	36.4	95.5	131.1	4.772	115.9	138.3	94.3	81,4
		c_4 - Bumblebee pollination - Biobest	40.6	106.6	130.8	5.311	129.0	153.9	107.1	83,0
		Average of factor C for a_2xb_2	*37.9*	*99.5*	*124.1*	*4.731*	*114.9*	*137.1*	*89.6*	78,0
	Average of factor B for a_2		***38.1***	***100.0***	***123.9***	***4.733***	***114.9***	*	***92.2***	*80.2*

Culture density: 24,288 plants/ha.

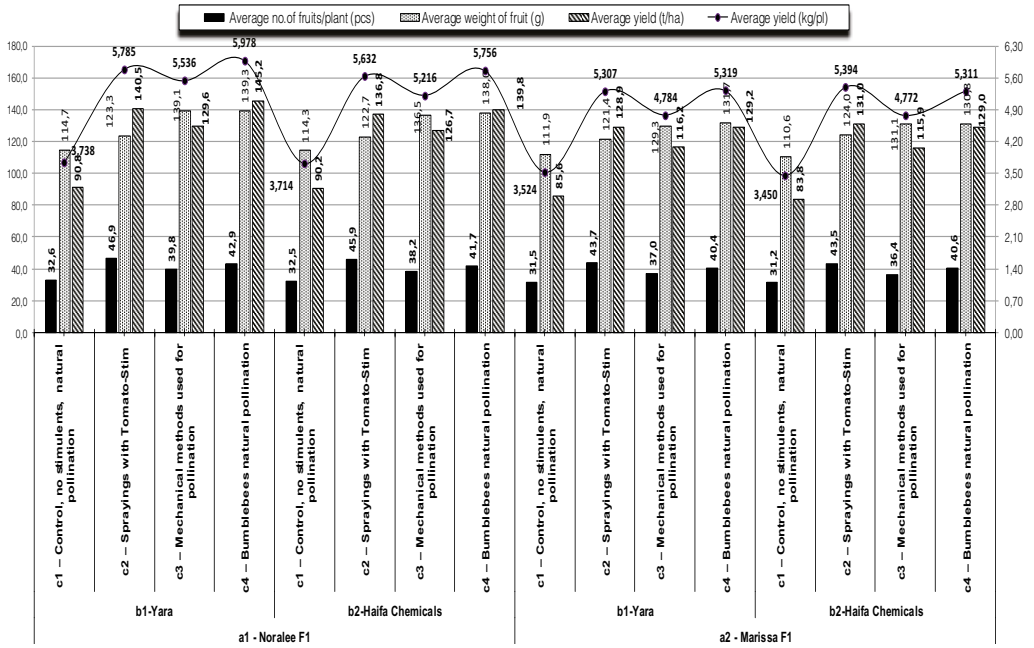

Figure 1. Experimental results on cultivating undetermined growth tomato hybrids in industrial greenhouses on mineral wool substrate, I[st] cycle - 2015

Table 3. Synthesis of experimental results on the non-determined growth tomato hybrids cultivated in warm greenhouses with modernized technological works

Factor experimental			Average production for:																	
			Factor A				Factor B							Factor C						
C	B	A	t/ha	% than c_{1-5}	E+I[st] quality		t/ha	% than $c_{1-5}b_1$	% $c_{1-5}b_{1-2}$ than Mx_1b_{1-2}	E+I[st] quality				t/ha	% than c_1	%c_{1-5} than Mx_1	E+I[st] quality				
					t/ha	%				t/ha	%	% than b_1	% than c_1b_{1-2}				t/ha	%	% than c_1	% than Mx_1	% than Mx_2
c_1		a_1	90.8	103.7	76.0	83.7														
	b_1	a_2	85.6	97.7	70.0	81.8	88.2	100.0	73.0	73.0	82.8	100.0	100.0							
		c_1xb_1	*88.2*	*100.7*	*73.0*	*82.8*														
		a_1	90.2	103.0	73.2	81.2														
	b_2	a_2	83.8	95.7	67.1	80.1	87.0	98.6	73.0	70.2	80.7	96.2	100.0	87.6	100.0	73.0	71.6	81.7	100.0	73.5	67.6
		c_1xb_2	*87.0*	*99.3*	*70.2*	*80.7*														
B average for a_1			***87.6***	***100.0***	***71.6***	***81.7***	***87.6***	***99.3***	***73.0***	***71.6***	***81.7***	***98.1***	***100.0***								
c_2		a_1	140.5	104.6	106.9	76.1														
	b_1	a_2	128.9	95.8	95.6	74.2	134.7	100.0	111.5	101.3	75.2	100.0	138.8							
		c_2xb_1	*134.7*	*100.3*	*101.3*	*75.2*														
		a_1	136.8	101.9	97.0	70.9														
	b_2	a_2	131.0	97.5	90.0	68.7	133.9	99.4	112.3	93.5	69.8	92.3	133.2	134.3	153.3	111.9	97.4	72.5	136.0	100.0	92.0
		c_2xb_2	*133.9*	*99.7*	*93.5*	*69.8*														
B average for a_2			***134.3***	***100.0***	***97.4***	***72.5***	***134.3***	***99.7***	***111.9***	***97.4***	***72.5***	***96.2***	***136.0***								
c_3		a_1	129.6	106.1	114.2	88.1														
	b_1	a_2	116.2	95.2	100.2	86.2	122.9	100.0	101.7	107.2	87.2	100.0	146.8							
		c_3xb_1	*122.9*	*100.7*	*107.2*	*87.2*														
		a_1	126.7	103.8	104.3	82.3														
	b_2	a_2	115.9	94.9	94.3	81.4	121.3	98.7	101.8	99.3	81.9	92.6	141.5	122.1	139.4	101.8	103.3	84.6	144.3	106.1	97.5
		c_3xb_2	*121.3*	*99.3*	*99.3*	*81.9*														

Factor experimental			Average production for:																		
			Factor A				Factor B							Factor C							
C	B	A	t/ha	% than c_{1-5}	E+Ist quality		t/ha	% than $c_{1-5}b_1$	% $c_{1-5}b_{1-2}$ than Mx_1b_{1-2}	E+Ist quality				t/ha	% than c_1	%c_{1-5} than Mx_1	E+Ist quality				
					t/ha	%				t/ha	%	% than b_1	% than c_1b_{1-2}				t/ha	%	% than c_1	% than Mx_1	% than Mx_2
B average for a_3			***122.1***	***100.0***	***103.3***	***84.6***	***122.1***	***99.3***	***101.7***	***103.3***	***84.6***	***96.4***	***144.3***								
c_4	b_1	a_1	145.2	106.9	129.9	89.5	137.2	100.0	113.6	121.7	88.7	100.0	166.7	135.8	155.0	113.2	117.2	86.3	163.7	120.3	110.7
		a_2	129.2	95.1	113.4	87.8															
		c_4xb_1	*137.2*	*101.0*	*121.7*	*88.7*															
	b_2	a_1	139.8	102.9	118.0	84.4	134.4	97.9	112.8	112.6	83.8	92.5	160.4								
		a_2	129.0	95.0	107.1	83.0															
		c_4xb_2	*134.4*	*99.0*	*112.6*	*83.8*															
B average for a_4			***135.8***	***100.0***	***117.2***	***86.3***	***135.8***	***99.0***	***113.2***	***117.2***	***86.6***	***96.3***	***163.7***								
c_5 (Mx1)	***b_1***	a_1	126.5	105.4	106.8	84.4	120.8	100.0	100.0	100.8	81.1	100.0	138.1	120.0	137.0	100.0	97.4	81.2	136.0	100.0	91.8
		a_2	115.0	95.8	94.8	82.4															
	b_1		120.8	100.7	100.8	83.4															
	b_2	a_1	123.4	102.8	98.1	79.5	119.2	98.7	100.0	93.9	79.6	93.2	133.8								
		a_2	114.9	95.7	89.6	78.0															
	b_2		119.2	99.3	93.9	78.8															
	a_1		125.0	104.2	102.5	82.0	*	*	*	*	*	*	*								
	a_2		115.0	95.8	92.2	80.2	*	*	*	*	*	*	*								
Mx_1			***120.0***	***100.0***	***97.4***	***81.2***	120.0	99.3	100.0	96.5	80.4	95.7	134.8								
c_6 (Mx2	***b_1***	*	*	*	*	*	131.6	100.0	100.7	110.1	83.7	100.0	150.8	130.7	149.2	108.9	105.9	81.0	147.9	108.7	100.0
	b_2	*	*	*	*	*	129.9	98.7	99.4	101.8	78.4	92.5	145.0								
	*	a_1	136.4	104.4	111.7	81.9	*	*	*	*	*	*	*								
		a_2	125.0	95.6	100.1	80.1	*	*	*	*	*	*	*								
Mx_2			***130,7***	***100,0***	***105.9***	***81.0***	***130.7***	***99.3***	***100.0***	***105.9***	***81.0***	***96.2***	***150.0***								

In table 4 there are presented the statistical results of production differences significances under the impact of the interaction between the experimental factors.

Table 4. Unilateral and interaction impact of experimental factors upon the production of some non-determined growth tomato hybrids, cultivated on mineral substrate in industrial greenhouses

Variant	Average production (t/ha)		Relative production (%)	Difference (±t/ha)	Significance
1. Unilateral impact of the hybrid upon tomato production					
a2-a1	114.95	124.95	91.99	-10.00	000
DL 5% = 1.60		DL 1% = 2.42		DL 0.1%= 3.89	
2. Unilateral impact of the fertigation system upon tomato production					
b2-b1	119.15	120.75	98.68	-1.60	-
DL 5% = 2.69		DL 1% = 3.71		DL 0.1% = 5.11	
3. Unilateral impact of the flower bind improvement method upon tomato production					
c2-c1	134.30	87.60	153.31	46.70	***
c3-c1	122.10	87.60	139.38	34.50	***
c4-c1	135.80	87.60	155.02	48.20	***
c5-c1	119.96	87.60	136.94	32.36	***
c3-c2	122.10	134.30	90.92	-12.20	000
c4-c2	135.80	134.30	101.12	1.50	-
c5-c2	119.96	134.30	89.32	-14.34	000
c4-c2	135.80	134.30	101.12	1.50	-
c5-c3	119.96	122.10	98.25	-2.14	-
c5-c4	119.96	135.80	88.33	-15.84	000
DL 5% = 2.60		DL 1% = 3.52		DL 0.1% = 4.71	

Variant	Average production (t/ha)		Relative production (%)	Difference (±t/ha)	Significance
4. Interaction impact between different hybrids and the same or different fertigation systems upon tomato production					
a2b1-a1b1	114.97	126.53	90.87	-11.55	000
a2b2-a1b2	114.93	123.38	93.15	-8.45	000
a2b2-a1b1	114.93	126.53	90.83	-11.60	000
DL 5% = 3.13			DL 1% = 4.41	DL 0.1% = 6.32	
5. Interaction impact between the same hybrid and different fertigation systems upon tomato production					
a1b2- a1b1	123.38	126.53	97.51	-3.15	-
a2b2- a2b1	114.93	114.97	99.96	-0.05	-
DL 5% = 3.81			DL 1% = 5.24	DL 0.1% = 7.22	
6. Interaction impact between the same hybrid and different flower bind improvement methods upon tomato production					
a1c2- a1c1	138.65	90.50	153.20	48.15	***
a1c3- a1c1	128.15	90.50	141.60	37.65	***
a1c4- a1c1	142.50	90.50	157.46	52.00	***
a1c5- a1c1	124.97	90.50	138.08	34.47	***
a1c3- a1c2	128.15	138.65	92.43	-10.50	000
a1c4- a1c2	142.50	138.65	102.78	3.85	*
a1c5- a1c2	124.97	138.65	90.13	-13.68	000
a1c4- a1c3	142.50	128.15	111.20	14.35	***
a1c5- a1c3	124.97	128.15	97.52	-3.18	-
a1c5- a1c4	124.97	142.50	87.70	-17.53	000
a2c2- a2c1	129.95	84.70	153.42	45.25	***
a2c3- a2c1	116.05	84.70	137.01	31.35	***
a2c4- a2c1	129.10	84.70	152.42	44.40	***
a2c5- a2c1	114.95	84.70	135.71	30.25	***
a2c3- a2c2	116.05	129.95	89.30	-13.90	000
a2c4- a2c2	129.10	129.95	99.35	-0.85	-
a2c5- a2c2	114.95	129.95	88.46	-15.00	000
a2c4- a2c3	129.10	116.05	111.25	13.05	***
a2c5- a2c3	114.95	116.05	99.05	-1.10	-
a2c5- a2c4	114.95	129.10	89.04	-14.15	000
DL 5% = 3.68			DL 1% = 4.98	DL 0.1% = 6.66	
7. Interaction impact between the same fertigation system and different flower bind improvement methods upon tomato production					
b1c2- b1c1	134.70	88.20	152.72	46.50	***
b1c3- b1c1	122.90	88.20	139.34	34.70	***
b1c4- b1c1	137.20	88.20	155.56	49.00	***
b1c5- b1c1	120.75	88.20	136.90	32.55	***
b1c3- b1c2	122.90	134.70	91.24	-11.80	000
b1c4- b1c2	137.20	134.70	101.86	2.50	-
b1c5- b1c2	120.75	134.70	89.64	-13.95	000
b1c4- b1c3	137.20	122.90	111.64	14.30	***
b1c5- b1c3	120.75	122.90	98.25	-2.15	-
b1c5- b1c4	120.75	137.20	88.01	-16.45	000
b2c2- b2c1	133.90	87.00	153.91	46.90	***
b2c3- b2c1	121.30	87.00	139.43	34.30	***
b2c4- b2c1	134.40	87.00	154.48	47.40	***
b2c5- b2c1	119.17	87.00	136.97	32.17	***
b2c3- b2c2	121.30	133.90	90.59	-12.60	000
b2c4- b2c2	134.40	133.90	100.37	0.50	-
b2c5- b2c2	119.17	133.90	89.00	-14.73	00
b2c4- b2c3	134.40	121.30	110.80	13.10	***
b2c5- b2c3	119.17	121.30	98.24	-2.13	-

Variant	Average production (t/ha)		Relative production (%)	Difference (±t/ha)	Significance
b2c5- b2c4	119.17	134.40	88.67	-15.23	000
DL 5% = 3.68			DL 1% = 4.98	DL 0.1% = 6.66	
8. Interaction impact between different fertigation systems and the same or different flower bind improvement methods upon tomato production					
b2c1- b1c1	87.00	88.20	98.64	-1.20	-
b2c2- b1c2	133.90	134.70	99.41	-0.80	-
b2c3- b1c3	121.30	122.90	98.70	-1.60	-
b2c4- b1c4	134.40	137.20	97.96	-2.80	-
b2c5- b1c5	119.17	120.75	98.69	-1.58	-
b2c2- b1c1	133.90	88.20	151.81	45.70	***
DL 5% = 4.25			DL 1% = 5.80	DL 0.1% = 7.84	
9. Interaction impact between different hybrids and the same or different flower bind improvement methods upon tomato production					
a2c1- a1c1	84.70	90.50	93.59	-5.80	00
a2c2- a1c2	129.95	138.65	93.73	-8.70	000
a2c3- a1c3	116.05	128.15	90.56	-12.10	000
a2c4- a1c4	129.10	142.50	90.60	-13.40	000
a2c5- a1c5	114.95	124.97	91.98	-10.02	000
a2c2- a1c1	129.95	90.50	143.59	39.45	***
DL 5% = 3.65			DL 1% = 5.04	DL 0.1% = 6.99	
10. Interaction impact between the same hybrid and the same fertigation system and different flower bind improvement methods upon tomato production					
a1b1c2- a1b1c1	140.50	90.80	154.74	49.70	***
a1b1c3- a1b1c1	129.60	90.80	142.73	38.80	***
a1b1c4- a1b1c1	145.20	90.80	159.91	54.40	***
a1b1c5- a1b1c1	126.53	90.80	139.35	35.73	***
a1b1c3- a1b1c2	129.60	140.50	92.24	-10.90	000
a1b1c4- a1b1c2	145.20	140.50	103.35	4.70	-
a1b1c5- a1b1c2	126.53	140.50	90.06	-13.97	000
a1b1c4- a1b1c3	145.20	129.60	112.04	15.60	***
a1b1c5- a1b1c3	126.53	129.60	97.63	-3.07	-
a1b1c5- a1b1c4	126.53	145.20	87.14	-18.67	000
a2b2c2- a2b2c1	131.00	83.80	156.32	47.20	***
a2b2c3- a2b2c1	115.90	83.80	138.31	32.10	***
a2b2c4- a2b2c1	129.00	83.80	153.94	45.20	***
a2b2c5- a2b2c1	114.93	83.80	137.15	31.13	***
a2b2c3- a2b2c2	115.90	131.00	88.47	-15.10	000
a2b2c4- a2b2c2	129.00	131.00	98.47	-2.00	-
a2b2c5- a2b2c2	114.93	131.00	87.74	-16.07	000
a2b2c4- a2b2c3	129.00	115.90	111.30	13.10	***
a2b2c5- a2b2c3	114.93	115.90	99.17	-0.97	-
a2b2c5- a2b2c4	114.93	129.00	89.10	-14.07	000
DL 5% = 5.20			DL 1% = 7.05	DL 0.1% = 9.42	
11. Interaction impact between the same hybrid and different fertigation systems and the same flower bind improvement method upon tomato production					
a1b2c1- a1b1c1	90.20	90.80	99.34	-0.60	-
a1b2c5- a1b1c5	123.40	126.53	97.52	-3.13	-
a2b2c2- a2b1c2	131.00	128.90	101.63	2.10	-
a2b2c5- a2b1c5	114.93	114.97	99.97	-0.03	-
DL 5% = 6.01			DL 1% = 8.20	DL 0.1% = 11.09	
12. Interaction impact between different hybrids and the same fertigation system and the same flower bind improvement method upon tomato production					
a2b1c1- a1b1c1	85.60	90.80	94.27	-5.20	-
a2b1c2- a1b1c2	128.90	140.50	91.74	-11.60	000

Variant	Average production (t/ha)		Relative production (%)	Difference (±t/ha)	Significance
a2b1c3- a1b1c3	116.20	129.60	89.66	-13.40	000
a2b1c4- a1b1c4	129.20	145.20	88.98	-16.00	000
a2b1c5- a1b1c5	114.97	126.53	90.86	-11.57	000
a2b2c1- a1b2c1	83.80	90.20	92.90	-6.40	0
a2b2c2- a1b2c2	131.00	136.80	95.76	-5.80	0
a2b2c3- a1b2c3	115.90	126.70	91.48	-10.80	000
a2b2c4- a1b2c4	129.00	139.80	92.27	-10.80	000
a2b2c5- a1b2c5	114.93	123.40	93.14	-8.47	00
DL 5% = 5.60			DL 1% = 7.68	DL 0.1% = 10.50	

Analysing the significance of the production differences from the table the following conclusions emerge:

Point 1 - the unilateral influence of the hybrid on the production - shows that the obtained production from the two hybrids have a statistical assurance, the significance of the production differences between Marissa F1 (a_2) and Noralee F1 (a_1) being considerably negative, meaning that the Noralee F1 (a_1) hybrid has superior production qualities compared to Marissa F1 (a_2), 125 t/ha and respectively 115 t/ha, but quality as well, as the data from table 3 shows;

Point 2 - the unilateral influence of the fertigation system on the production - shows that the productions obtained under the influence of the two fertigation systems do not have a statistical assurance, having no significant production differences, which proves that both fertigation systems can be used because the difference between the two is very low in relation to the average production per hectare (119.2 t/ha respectively 120.8 t/ha);

Point 3 - the unilateral influence of the improved flower binding method on the production - shows that the production obtained under the influence of method c_2 (stimulation with Tomato-Stim), c_3 (mechanical pollination) and c4 (natural pollination via bumble-bees) have statistical assurance, the significance of the production differences compared to c1 (unstimulated and non-pollinated Mt) being essentially positive in all cases, which shows that all used methods are beneficial for the improvement of flower binding compared to the controlled variant; also significance of the difference between c3 (mechanical pollination) and c_2 (stimulation with Tomato-Stim) is essentially negative, which proves that the production has statistical assurance, namely under the influence of c_2 (stimulation with Tomato-Stim) being quantity wise superior but not quality wise (table 3);

Analysing points 4-12 from table 4 we see that based on the bi- or tri-factorial interactions, there is a very big diversity in the significance of the differences in production, that include all degrees of assessment (very significantly positive or negative, distinctively significant positive or negative and significant positive or negative). This proves the intensity of the unilateral influences or the interactions of the experimental factors on the productions obtained from a quantitative angle.

CONCLUSIONS

The productions following the influences of some experimental factors, such as the fertigation system with different types of modern chemical instant fertilizers, administered interacting bi-factorial with the cultivated hybrid, have proved the complexity of the interactions (tri-factorial) with the improved flower binding methods by differentiating them quantitatively and qualitatively in a tomato culture performed on a substrate of mineral cotton.

The diversity of factor C graduation (Improved flower binding method) over the tomatoes production, have determined influences over the quantity as well as quality types, namely the ones which through their natural influence have contributed to a better pollination and fertilization, thus a better flower binding. The same as for Carlos de Melo e Silva Neto et al. (2013) results, our results show that native bees buzz-pollinate tomato flowers, increasing the pollen load on their stigma and consequently fruit production and quality.

Due to the influence of c4 graduation (bumblebees natural pollination) and c2 (Tomato-Stim) the biggest average tomato production within the experiment was of 135.8 t/ha and respectively 134.3 t/ha, but not the best quality in both graduations, as Extra+Ist quality production. This is due to the fact that the pollen dose added to the stigmas of tomato flowers (with the help of bumblebees) lead to a good pollination (meaning a good production increase) and also, if we are to consider the other aspects, an increase in the number of fertilized eggs, which also mean an increase in the production of seeds in the fruits. Related to this, studies developed by Tanksley (2004) and Paran et al. (2007) have shown that the number of seeds in development in tomato fruits influences the activity of the fw 2.2 gene, which is responsible for the production of stimuli for the ovary walls growth and fruit formation (quoted by de Carlos de Melo e Silva Neto et al., 2013).

It has been observed that under the c_2 graduation (Tomato-Stim) the production on second place in quantity size is on the last place regarding the quality Extra+Ist quality production.

The Extra+Ist quality production, from a percentage aspect at its highest was obtained under the c4 graduation (bumblebees natural pollination) - 86.3%, followed chronologically by c_3 (mechanical pollination) - 84.6%. According to Carlos de Melo e Silva Neto et al., 2013, it has been shown that in greenhouses *Melipona quadrifasciata* bees, gains in fruit production reached 15% (Bispo dos Santos et al., 2009), while with *B. impatiens*, gains reached 50% in fruit mass and up to the double in the number of seeds (Morandin et al., 2001a).

The classification from a quality point of view of the production on the first two places, productions under the influence of c_4 (bumblebees natural pollination) and c_3 (mechanical pollination), is based on the natural pollination phenomenon which determines the growth and development on the plant of non-parthenocarpic fruits, with outstanding physical (size, weight and color etc.), chemical and organoleptic (flavor, smell etc.) characteristics.

The influence of both graduation of the fertigation systems (Yara and Haifa Chemicals) are substantiated in the very close quantitative production and from a statistic point of view were not covered, because there were no significant production differences.

Noralee F1 and Marissa F1 hybrids are valuable both due to the quantitative level of production as well as the value of Extra+Ist quality production, these ranging without taking into account c_1 - Mt, throughout the following intervals:

- Noralee F1: 126.7-145.2 t/ha, of which 97.3-129.9 t/ha Extra+Ist quality production, meaning 70.9-89.5%;
- Marissa F1: 115.9-131.0 t/ha, of which 90.0-113.4 t/ha Extra+Ist quality production, meaning 68.7-87.8%.

Based on the conclusions following the performed research, the recommendation is:

- The cultivation of Noralee F1 hybrid;
- The use of both fertilizers Yara and Haifa Chemicals via fertigation;
- The use of mechanical means or bumblebees (Biobest or Natupol) to improve the tomato flower binding and to obtain superior quality fruits.

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RESEARCH ON THE EFFECT OF CLIMATE CHANGE ON THE PRODUCTION CAPACITY OF SOME SWEET POTATOES VARIETIES (*IPOMOEA BATATAS*) CULTIVATED ON PSAMOSOILS IN THE SOUTHERN AREA OF ROMANIA

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Abstract

Between 2015 and 2017, at the Research and Development Station for Plant Culture on Sands Dăbuleni, were studied three varieties of sweet potato to determine the influence of environmental factors on some plant metabolic processes and, consequently, on the production achieved. The varieties studied were Yulmi, Juhwangmi and KSP 1. The results revealed the influence of the variety, the planting season and the climatic conditions on the development of physiological processes, their intensity influencing the quantity and quality of production. The best results on the production were recorded at the variants cultivated under the climatic conditions of the 2016 year, detaching the variant cultivated with the Juhwangmi variety, with an average production of 43.6 tons. At Yulmi and KSP 1 varieties it was obtained the highest production at variants grown in the first epoch of 2016 year (May 10-15), respectively 34.3 tons at Yulmi variety and 31.7 tons at KSP 1 variety.

Key words*: climate change, foliar transpiration, photosynthesis, production, sweet potato.*

INTRUDUCTION

Sweet potato (*Ipomoea batatas* L.), the seventh most important food crop after wheat, rice, maize, potato, barley and cassava, is a staple food in many developing countries of the tropics and sub-tropics, and also serves as animal feed and raw material for several food and feed based industries (Ray and Ravi, 2005).

This New World crop has high biological efficiency of converting solar energy into edible energy (152 MJ/ha/day) in the form of tuberous (storage) roots and could

be the food for the ever growing human population in future (Ray and Tomlins, 2010).

Asia leads in area (60.75%) and production (86.89%) of sweet potato in the world. The world average sweet potato root yield was 13,729 kg/ha. However, the highest productivity of 19,634 kg/ha was found in Asia (FAOSTAT, 2008).

Sweet potato was first described by Linnaeus as *Convolvulus batatas* in 1753. However, in 1791 Lamarck classified this species within the genus *Ipomoea* on the basis of the stigma shape and the surface of the pollen grains. Therefore, the name was changed to *Ipomoea batatas* (L.) Lam.

Sweet potato was originally a herbaceous perennial but was domesticated as an annual and grows best in moderately warm temperature of 21-26^{o}C. It requires light textured soil with the optimum pH of 5.5-6.5 for good growth of the crop. At low pH sweet potato suffers aluminum toxicity.

Sweet potato is sensitive to alkaline and saline conditions (Dasgupta et al., 2006; Mukherjee et al., 2006).

A well distributed rainfall of 75-150 cm is favourable for its cultivation. It can tolerate drought to some extent but cannot withstand water logging. It requires plenty of sun shine, whereas shade causes reduction in yield. However, sweet potato is grown as intercrop under plantation/orchard crops with the motto of profit maximization and crop intensification (Nedunchezhiyan et al., 2007).

Excess of rainfall and cloudy conditions encourage vine growth and reduce storage root

yield. Dry season storage root yields were higher than rainy season yields (Nedunchezhiyan and Byju, 2005).
In Romania was introduced in 1954, and the result of the experiments performed by Maier I. was further amplified (Florescu, Maxim, Dragotă, Ciofu, Popescu) and allowed the acclimatization of this new species, as well as the establishment of sequences on the way of multiplication, planting periods, crop areas. In the current context of climate change, the importance of sweet potato cultivation lies both in the possibility of expanding culture in the areas where the potato is degenerating (Ruxandra Ciofu et al., 2003) and the need to diversify the vegetable variety with less known species but with a nutritional value which would make efficient use of the pedoclimatic conditions in southern Oltenia.

MATERIALS AND METHODS

At RDSPCS Dabuleni, 3 potato varieties were studied between 2015-2017 to determine the influence of environmental factors on plant metabolic processes and, consequently, on the production achieved.
The varieties studied were Yulmi, Juhwangmi and KSP 1. The experimental variants were cultivated according to the technology, namely in field with ridges, protected with transparent polyethylene mulch, in three planting epochs, at an interval of 10-15 days between epochs (Table 1), and water was provided by irrigation using the drip irrigation method.

Table 1. Experimental variants

Experimental variants			
Variety	Yulmi	Planting epoch	I (10-15 May)
	Juhwangmi		II (20-25 May)
	KSP 1		III (01-10 June)

For the analysis of the climatic conditions which influenced the sweet potato plants in the experimental field, were used the meteorological data recorded at the RDSPCS Dabuleni meteorological station. With the LC PRO + portable device were determined the diurnal variation of photosynthesis and foliar transpiration rate in different vegetation phenophases. Determinations have also been made on the commercial production obtained during the three years of study, depending on the variety and planting epoch.

RESULTS AND DISCUSSIONS

From the data presented in Table 2 it can be estimated that the three years under study (2015-2017) are different from the climatic point of view.
Analyzing the air temperature values, during the sweet potato growing season (May-September), there is a tendency to increase the average monthly temperature over the three years of study. It is also noted that the monthly average temperature of each year was higher than the average multiannual temperature. On the other hand, the maximum temperatures also increased steadily, in 2017 values exceeding 40^{o}C in all summer months (June-August).
Heat temperatures have blocked physiological processes in sweet potato plants, negatively influencing the development of vegetation phenophases and the accumulation of starch in tuberous roots.
The atmospheric precipitations, as well as their relationship with foliar transpiration values, are of particular importance for the southern Oltenia sweet potato culture.
The precipitation recorded between May and September showed values between 227.8 mm in 2016 and 330.8 mm in 2015, unevenly distributed over the vegetation period and with variations from one month to the next. Throughout the analyzed period there is a significant reduction in the amount of precipitation in July, August and September. Although sweet potato is a drought-resistant species, heat temperatures correlated with a major lack of rainfall have led to droughts and have made it absolutely necessary the irrigation of crop.

Table 2. The variation of the main climatic elements during the growing season of sweet potatoes in the period 2015-2017

Year	Climatic element	Month				
		May	June	July	August	September
2015	Medium temperature (°C)	19.2	20.5	24.8	24.34	20.08
	Minimum temperature (°C)	8.6	10.2	12.5	12.9	7.8
	Maximum temperature (°C)	30.2	36.1	39.2	37.6	37.3
	Precipitations (mm)	52.4	134.2	11	48.4	84.8
	Atmospheric relative humidity (%)	73.04	73.77	62.92	68.21	77.30
2016	Medium temperature (°C)	16.8	23.6	24.8	23.5	20.4
	Minimum temperature (°C)	5.5	11	11.4	16.1	5.1
	Maximum temperature (°C)	32.9	37.3	38	38	34.1
	Precipitations (mm)	104.4	53.2	31.6	1	37.6
	Atmospheric relative humidity (%)	82.22	72.68	65	68.3	71
2017	Medium temperature (°C)	17.8	24	24.8	24.8	20.21
	Minimum temperature (°C)	4.7	12.9	13.3	11	6.7
	Maximum temperature (°C)	31.4	41.2	40.8	40.4	36.9
	Precipitations (mm)	78.6	17.4	120.8	28,8	18.2
	Atmospheric relative humidity (%)	77	67.05	65.01	63	66
Multiannual medium temperature (1956-2016)		16.8	21.6	23.1	22.4	17.8
Precipitations, multiannual total (1956-2016)		62.12	69.30	53.15	37.28	47.83

In the context of climate change, the results regarding physiological processes on sweet potato plants grown on sandy soils in southern Oltenia highlighted the following aspects:

Both the rate of photosynthesis and the foliar transpiration rate varied depending on the variety, the vegetation phenophase analyzed, the planting epoch and the climatic conditions present at the time of the measurements. From the data presented in Table 3 it can be noticed that in the phase of intense growth of the strains, there were no photosynthetic differences between the three studied varieties, the average values of this physiological process being between 20.29 μmol $CO_2/m^2/s$ at the Yulmi variety, respectively 22.55 μmol $CO_2/m^2/s$ at the KSP 1 variety. As regards the influence of the planting epoch on the photosynthesis in this vegetation phenophase, the results are easily differentiated according to the variety as follows: Yulmi variety has presented the best results at cultivation in first epoch (21.66 μmol $CO_2/m^2/s$); the Junhwangmi and KSP 1 varieties showed the highest photosynthetic yields at cultivation in the third epoch (21.8 μmol CO_2/m^2 /s, respectively 23.52 μmol $CO_2/m^2/s$). This can be explained by the fact that at the plants cultivated in the first epoch this vegetation phenophase coincides with the harshest period from the climatic point of wiew, high photosynthetic yields beeing presented only the drought-resistant varieties (in the present case the Yulmi variety).

Table 3. Variation of photosynthesis at sweet potato in the phenophase of intense growth of strains according to variety, planting epoch and the culture year

Variety	Year	Photosynthesis rate (μmol $CO_2/m^2/s$)			Average / Variety
		The first epoch May 10-15	The second epoch May 20-25	The third epoch June 01-10	
Yulmi	2015	22.32	16.90	16.02	18.41
	2016	18.33	13.26	12.72	14.77
	2017	24.34	29.42	29.33	27.69
Average/Epoch		**21.66**	**19.86**	**19.35**	**20.29**
Juhwangmi	2015	19.32	19.34	15.77	18.14
	2016	18.08	16.59	17.8	17.49
	2017	21.34	25.29	31.83	26.15
Average/Epoch		**19.58**	**20.40**	**21.80**	**20.59**
KSP 1	2015	22.20	15.95	25.43	21.19
	2016	15.28	20.82	17.82	17.97
	2017	28.96	29.18	27.33	28.49
Average/Epoch		**22.14**	**21.98**	**23.52**	**22.55**

The intensity of foliar transpiration is directly related to the temperature and hydrological deficit of atmospheric air. By decreasing the degree of saturation of air in water vapor, the water deficit and the intensity of foliar transpiration increase. Table 4 presents the variation of this physiological process in the phenophase of intense growth of strains according to variety, planting epoch and culture year. The most intensely has sweated the KSP

1 variety, with an average value over the three-year study of 5.47 mmol $H_2O/m^2/s$, but evaporated water was efficiently used because this variety also recorded the highest average value of photosynthesis. Regarding the influence of the planting epoch on the intensity of foliar transpiration (Table 4), it is observed that the highest values were recorded in the plants grown in the third epoch, indifferent to the variety. If we analyze the year's influence on transpiration, it is noticed that the highest values were recorded in the climatic conditions of 2017, this year being one of the droughts.

Table 4. Variation of foliar transpiration at sweet potato in the phenophase of intense growth of strains according to variety, planting epoch and the culture year

Variety	Year	Foliar transpiration rate (mmol $H_2O/m^2/s$)			Average / Year
		The first epoch May 10-15	The second epoch May 20-25	The third epoch June 01-10	
Yulmi	2015	6.56	3.90	4.67	5.04
	2016	3.65	3.64	2.45	3.24
	2017	3.46	5.72	9.67	6.28
Average/Epoch		**4.55**	**4.42**	**5.59**	**4.85**
Juhwangmi	2015	6.11	3.18	5.25	4,84
	2016	4.46	4.21	3.25	3.97
	2017	5.13	6.32	8.55	6.66
Average/Epoch		**5.23**	**4.57**	**5.68**	**5.16**
KSP 1	2015	6.30	3.75	6.46	5.50
	2016	3.44	5.57	3.14	4.05
	2017	6.01	6.15	8.46	6.87
Average/Epoch		**5.25**	**5.15**	**6.02**	**5.47**

In the root tuberosis phenophase, both physiological processes studied have reduced their intensity as a result of the maturation process of the plants (Table 5).

Remarkable are the results obtained in experimental year 2017, in this vegetation phenophase the values of photosynthesis and foliar transpiration beeing unusually large (comprised in the range 22.28-26.53 μmol $CO_2/m^2/s$, respectively 4.89-5.28 mmol $H_2O/m^2/s$).

Heat days, with temperatures exceeding 40°C in all the summer months (June to August), determined an increase in the physiological processes of sweet potato plants towards the end of the vegetation period when the climatic conditions became optimal.

This fact also had repercussions on the production, especially on the plants grown in the first epoch.

Table 5. Variation of photosynthesis and foliar transpiration at sweet potato in the root tuberosis phenophase according to variety, planting epoch and the culture year

Variety	Year	Photosynthesis rate (μmol $CO_2/m^2/s$)			Average / Year
		The first epoch May 10-15	The second epoch May 20-25	The third epoch June 01-10	
Yulmi	2015	10.17	10.6	13.89	11.55
	2016	17.77	14.76	10.4	14.31
	2017	15.68	24.09	27.08	22.28
Average/Epoch		14.54	16.48	17.12	**16.04**
Juhwangmi	2015	5.36	8.87	12.89	9.04
	2016	21.18	15.69	13.91	16.92
	2017	25.48	21.05	33.07	26.53
Average/Epoch		17.34	15.20	19.95	**17.5**
KSP 1	2015	6.64	8.91	11.2	8.91
	2016	19.19	12.71	14.75	15.55
	2017	22.93	19.44	27.48	23.28
Average/Epoch		16.25	13.68	17.81	**15.91**
Variety	Year	Foliar transpiration rate (mmol $H_2O/m^2/s$)			Average / Year
		The first epoch May 10-15	The second epoch May 20-25	The third epoch June 01-10	
Yulmi	2015	1.40	2.35	2.21	1.98
	2016	3.83	4.06	2.41	3.43
	2017	3.12	3.91	7.65	4.89
Average/Epoch		**2.78**	**3.44**	**4.09**	**3.43**
Juhwangmi	2015	1.34	2.13	1.76	1.74
	2016	4,33	4.24	2.63	3.73
	2017	4.27	5.04	6.53	5.28
Average/Epoch		**3.31**	**3.80**	**3.64**	**3.58**
KSP 1	2015	1.35	1.33	2.01	1.56
	2016	4.49	2.43	1.81	2.91
	2017	4.55	5.42	5.69	5.22
Average/Epoch		**3.46**	**3.06**	**3.17**	**3.23**

Analyzing the production of commercial tubers obtained at 120 days after the sweet potato planting in the experimental field, it note the importance of the variety, of planting epoch and the climatic conditions of the three years of study on the results obtained (Table 6). The best results on the production were recorded at the variants cultivated under the climatic conditions of the 2016 year, detaching the variant cultivated with the Juhwangmi variety, with an average production of 43.6 tons.

At Yulmi and KSP1 varieties it was obtained the highest production at variants grown in the first epoch of 2016 year (May 10-15), respectively 34.3 tons at Yulmi variety and 31.7 tons at KSP 1 variety. Analyzing the influence of the planting epoch on the production obtained, the best results were obtained when the sweet potato planting was done during May 20-25, with an average of 33.3 tons per hectare.

Table 6. Production results obtained at 120 days after planting of sweet potatoes, depending on variety, year and planting epoch

Variety	Year	Epoch I 10-15 May	Epoch II 20-25 May	Epoch III 10-15 June	Variety average kg/ha
Yulmi	2015	32200	26425	18550	25725
	2016	34300	33250	11550	26366.67
	2017	20800	25333.33	33400	26511,11
Epoch average/variety		**29100**	**28336.11**	**21166.67**	**26200.92**
Juhwangmi	2015	40250	41300	30800	37450
	2016	48066.67	56000	26845	43637.22
	2017	24933.33	44000	43000	37311.11
Epoch average/variety		**37750**	**47100**	**33548.33**	**39466.11**
KSP 1	2015	28233	22400	23800	24811
	2016	31733.33	25200	12810	23247.78
	2017	17333.33	26093	14933.33	19453.22
Epoch average/variety		**25766.55**	**24564.33**	**17181.11**	**22504**
Epoch average/ 3 years		**30872.18**	**33333.48**	**23965.37**	

CONCLUSIONS

The results obtained showed that sweet potato cultivated in drip irrigation conditions, successfully tolerates the thermo-hydric stress characteristic of the sandy soils area, the second planting epoch (May 20-25th) offering optimal conditions for the development of physiological processes in plants, regardless of variety. Very high temperatures during the vegetation period (which at the leaves level are with a few degrees higher than in the air), strong solar radiation, very low air humidity, have acted as dehydrating forces of the foliar apparatus, increasing water losses through foliar transpiration. Evaporated water was, however, used efficiently, as varieties with large water losses through foliar transpiration also recorded the highest CO_2 accumulations in the photosynthesis process.

The high photosynthetic yield places sweet potato among the most productive vegetable species grown on the sandy soils at Dabuleni. Although the climatic conditions were different, notable commercial productions were obtained in all the studied years, quantitatively detaching the Juhwangmi variety. Regarding the planting epoch, the best results were obtained at plants grown between May 20-25 (2^{nd} epoch), with an average of 33.3 tons per hectare.

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THE INFLUENCE OF FOLIAR BIOACTIV TREATMENTS ON TOMATOES SEEDLINGS

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Abstract

Tomato (Lycopersicon esculentum), the world's most important vegetable species, are known for its sensitivity regarding different stress factors such as heat, drought, lack of nutrients. Therefore, is very important to obtain tomatoes seedlings with balanced growth and development, but especially with a strong root system and increased capacity of adaptation to different conditions of stress. This paper presents the results of some researches related to foliar bioactive substance treatment of tomatoes seedling, Rio Grande cultivar, with Spraygard 1%, Razormin 0.1%, BAC Foliar Spray 0.3% and Bio Roots 0.2%. The treatments were performed in two distinct stages of development: at one, respectively two weeks after the seedlings transplantation. Analysis of recorded data indicated that all variants treated with bioactive substances are superiors comparing with the untreated control variant but Razormin 0.1%, followed closely by Bio Roots 0.2% treatments showed the best results in obtaining of tomatoes seedlings with a strong roots systems and a good development. These results are supported by an increased physiological activity.

Key words*: growth, photosynthesis, root, transpiration.*

INTRODUCTION

Abiotic stresses such as extremes temperature, drought and salinity can reduce the yield of major crops and limit agricultural production worldwide (Wang et al., 2003; Sharma et al., 2012; Hasanuzzaman et al., 2012; Hasanuzzaman et al., 2013).

Different factors of abiotic and biotic stress determine in plants an avalanche of physiological and biochemical changes, which begins with the perception of stress by the specialized receptors and ends with the resulting biomorphological expression as a result of this action. Therefore, the metabolism of the plants and the activity of some enzymes is modified, known as the fact that all the chemical processes are catalyzed by enzymes. In plants, stress triggers a wide range of responses, from gene expression and cell metabolism to changes in growth and production, but when stress is severe, the plant dies (Sharma et al., 2012). The response of plants to stress is determined by a number of factors, such as those that characterize the plant (their genotype and stage of development) and those that characterize stress (intensity, duration, repeatability, and the various synergistic effects of the interaction of several factors when it is multiple stress (Pallavi et al., 2012; Hasanuzzaman et al., 2013).

A biostimulator is an organic material that, when applied in small quantities, enhances plant growth and development such that the response of the plant cannot be attributed to the application of traditional nutrients (Shekhar Sharma et al., 2014)

Different studies have shown that seaweed extracts protect plants against a number of biotic and abiotic stresses and offers potential for field application. Further, seaweed extracts are considered an organic farming out as they are environmentally safe for the health of animals and humans (Khan et al., 2009). Recent research showed that the use of red macroalgal on crop plants can generate multiple benefits. It has been reported effects including enhanced rooting, enhanced photosynthetic activity, freezing, drought and salt tolerance, resistance to fungi, bacteria and virus, and higher crop and fruit yields (Shekhar Sharma et al., 2014). Also, in recent years, a number of exogenous protectants, such as proline, glycinebetaine, nitric oxide, silicon, selenium, salicylic acid, and polyamines have been tested and found to be beneficial in

protecting plants against damage from temperature extremes (Hasanuzzaman et al., 2012).

At this time, agricultural biostimulators suppose different compounds with diverse formulations of substances and other products, such as microorganisms, trace elements, enzymes, plant growth regulators and algal extracts that are applied to plants or soils to regulate and enhance the crop's physiological processes, in a very efficient manner (Shekhar Sharma et al., 2014).

In our country, similar research have been made on tomato and pepper seedlings that have benefited from biofertilization with Cropmax, Razormin, or of treatment with Spraygard (Chilom et al., 2000; Bălan et al., 2014; Dobrin et al., 2014). The research on eggplant seedlings reported that used of a different growth regulators (Razormin, Spraygard, BAC Foliar spray, Bio Roots) as foliar treatments, induces biochemical, physiological and metabolic changes that have led to the improvement of seed growth and development but also to the improvement of the oxidative protection system of plant and attenuate the negative impact of heat stress (Bălan et al., 2018).

The present research reported were performed on tomatoes (*Lycopersicum esculentum* Mill.), a main specie of vegetables grown in the field, in the summer-autumn crop established by seedlings. Tomato seedlings are grown in protected areas during April-May, when heat is often installed, with maximum daily and nocturnal temperatures well above optimum vegetation. It is imperative to apply a series of technological measures in order to accustom seedlings with provided variable nonfatal stress that could exist in the place of cultivation and to allow formation of "memory stress": the correct and careful directing of the vegetation factors and the application of special treatments helping not only to form this memory of stress but also supporting the growth and development of plants (Sekara et al., 2012; Mangrich and Saltveit, 2000; Knight et al., 1996; Jennings and Saltveit, 1996). It were used different growth regulators as foliar treatments on tomatoes seedlings and the comparative results were studied.

MATERIALS AND METHODS

The experiment was installed into an experimental greenhouse of the Hortinvest Research Centre - USAMV Bucharest in April-May, aimed to test the action of biofertilizers Razormin, BAC Foliar Spray and Bio Roots and of the universal adjuvant Spraygard on growth and development of tomatoes seedlings, in order to be recommended to the seedlings producers as supportive treatment of the growth rate and to improve metabolism seedlings. This could lead to shortening their age with implications for reduction of production cost.

Spraygard is a complex product that acts as safener, penetrant, dispersant, creates adhesion of the treatment solutions on the leaves. Spraygard adjuvant has an unique formula in a single coating based on the synthetic resin that is "environmentally friendly" and the polymer di-1-p-menthene and ethoxylatedalcohol by applying it on the plant and on its leaves forms a pellicle that persists 2 days up to 2 weeks, having as a side effect the reduction of perspiration and, therefore, a better water management within the plant. This fact causes the physiological chain reactions whose results are being expressed by increasing the plant resistance to stress factors such as the drought and the cold. The effect of reducing transpiration recommends to apply the product strictly on the leaves.

Razormin is an environmentally friendly bio-stimulating product, which determines a rooting effect. Their chemical composition is complex and balanced, so that induces mainly a root system development, than the development of vegetative part through cell division. It contains free amino acids and polysaccharides, which stimulate the nutrients absorption, leading to the further development of plant.

BAC Foliar is a foliar organic nutrient which stimulates chlorophyll production in the leaves.

Bio Roots is a natural fertilizer that stimulates root growth, helping plants to form healthy and vibrant root systems. It contains vitamins, enzymes, organic and humic acids that stimulate the growth of roots. It activates the growth of microorganisms in the root area and improves its development. This allows the development of a strong root system and a better adaptation of the plant to its

environment. At the same time it helps the plant to cope with diseases and pathogenic molds.

We established a monofactorial experiment with 5 variants, considering application of bioactives substances Sparygard 1%, Razormin 0.1%, BAC Foliar Spray 0.3% and Bio Roots 0.2% on tomatoes seedlings (Rio Grande) in two distinct stages: at one, respectively two weeks after the seedlings transplantation (April 23 and 30).

The experimental variants were: V1-untreated seedlings; V2-seedlings treated with 1% Spraygard; V3-seedlings trated with 0.1% Razormin; V4-seedlings treated with BAC Foliar Spray 0.3%; V5-seedlings treated with Bio Roots 0.2%.

Sowing was made on April and because heat and water were optimal provided, mass emergence of seedlings occurred after 6 days (April 11). The transplantation was done after 5 days into alveolar pallets (alveolar ø = 6 cm), in a professionally nutrient substrate KEKKILABP 75% +25% perlite. During the growth period specific agrotechnics for seedling production was applied: daily ventilation, watering, weeding. The temperature was kept at 24-26^{o}C to 30^{o}C at day and 22-24^{o}C at night. A treatment with CE Bravo 0.2% was made in order to prevent seedlings fall and also to avoid a *downy mildew* attack.

Observations and measurements of plant growth were made during the development of experiments in different stages: a two and respectively three weeks after transplantation (at the end of the experiment), after 27 days to the emergence, when most of seedlings have reached the optimum for a succesfull planting.

Observations and measurements were made on seedlings growth, as follow:

- *biometric parameters of seedlings*: plant height; the number of true leaves; weight of aerial vegetative unit; seedlings total weight; root weight and volume;
- *measurements of the main physiological processes intensity* (photosynthesis, transpiration, stomatal conductance) at the end of the experiment. We used the LC pro+ photosynthesis system. The measurements were performed on the active leaves located in the middle third part of the plant.

RESULTS AND DISCUSSIONS

The results of the analysis of the first stage (one week after the first treatment) are shown in Table 1.

Since then tomato seedlings presented important nuances regarding growth as a result of the application of different treatments in the sense that, in the non-fertilized V1 variant, the plants have recorded biometric parameters and indicators far inferior to fertilized variants. The plant height recorded an amplitude of 3.2 cm, respectively, between 14.2 cm to V1 and 17.4 cm to V4. The number of leaves varied significantly, from 4.2 leaves formed at V1 to 5.2 leaves formed at V4. The leaf frequency is relatively constant, of 0.3 leaf/cm PA to V1, V3 and V4 and 0.31 leaf/cm PA to V2.

Table 1. Growth of tomatoes seedlings 7 days after the first treatment

Variant	Plants height HPA (cm)	No. of leaves	Leaves frequency (no./cm HPA)
V_1	14.2	4.2	0.30
V_2	15.4	4.8	**0.31**
V_3	16.2	4.9	0.30
V_4	**17.4**	5.2	0.30
V_5	16.0	4.8	0.30

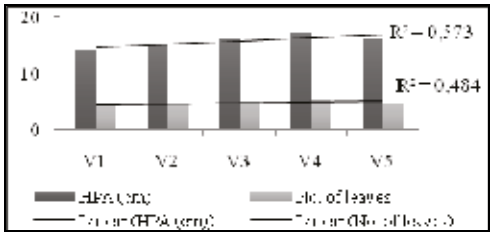

Figure 1. Influence of applied treatment on growth of tomatoes seedlings 7 days after first treatments

Context analysis highlights V4 (treated with BAC Foliar Spray 0.3%) as the variant where seedlings had the most balanced growth and the fact that the treatment applied since then has exerted a decisive influence on the growth of tomato seedlings, especially in terms of plant height ($R^2 = 0.573$) (Figure 1).

In order to determine the overall effect and influence of the treatments program applied on the tomatoes seedlings were made observations and measurements also one week after application of the second treatment. The results obtained are shown in Tables 2 and 3, respectively in Figures 2, 3 and 4.

Table 2. Growth of tomatoes seedlings at one week after the second treatment

Variant	No. of leaves	Plants height HPA (cm)	Roots length HR (cm)	Plants total lenght HT(cm)	Leaves frequency (no./cm HPA)
V_1	6.2	34.6	21.2	55.8	0.18
V_2	6.8	33.6	15.8	49.4	0.20
V_3	8.4	32.4	18.4	50.8	0.26
V_4	7.6	32.6	15.6	48.2	0.23
V_5	8.2	32.4	16.4	48.8	0.25

Applied treatment program determined differences regarding on the growth of tomatoes seedlings. It is noteworthy that all variants where have been applied different treatments with bioactive substances have achieved a shorter plant heights and roots lengths, compared to the untreated control variant.

Analysis of the results on the growth of seedlings showed that the best option working was V3-Razormin 0.1%. In this variant plants have achieved the best and balanced growth, all indicators analyzed had very good values (8.4 leaves formed, 32.4 cm plant height, 18.4 cm root length, total length 50.8 cm plant; 0.26 frequency leaves). In contrast, untreated V1 seedlings, although recorded the highest plant height (34.6 cm) and the largest length of the roots (21.2 cm), achieved the lowest number of leaves (6.2 leaves) and respectively the lowest frequency of leaves, 0.18. This context, for V1, shows an unbalanced growth that is based on an elongation phenomenon due to the presence of stress factors.

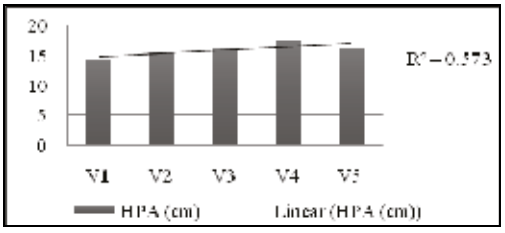

Figure 2. Influence of applied treatment on tomatoes seedlings growth one week after the second treatment

As can be seen from Figures 2 and 3, schedule treatments with bioactive substances exert a distinctive influence on the general growth of tomatoes seedlings, respectively, on the frequency leaves (R^2=0.639) and on the plant height (R^2 = 0.573).

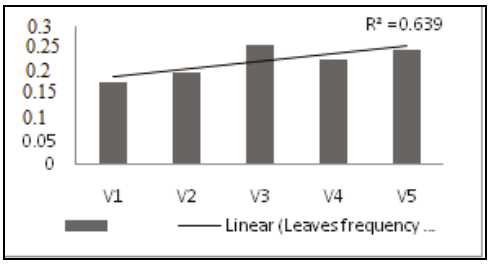

Figure 3. Influence of applied treatment on the frequency leaves of the tomatoes seedlings one week after the second treatment

Regarding on plants growth, taken together the results obtained for tomatoes seedlings morphometry, we estimate that the most balanced variant is V3 (fertilized with Razormin 0.1%) followed by V5 (Bio Roots 0.2%).

Developing of tomatoes seedlings one week after the second treatments was quantified by various indicators of mass and volume and by diameter of collet. The obtained results (Table 3; Figures 4 and 5) regarding tomatoes mass ratio highlights two situations:

1. At V3 variant all indicators recorded the highest values compare to the other variants (aerial part mass 16 g; mass root 4.5 g; 23.5 g total mass, volume roots 8.5 cm^3; 7 mm collet diameter). Good results have also recorded the seedlings treated with Bio Roots and BAC Foliar Spray.
2. In contrast, V1 variant recorded, to all most indicators, the lowest values (aerial part mass 11.5 g; 19 g total mass; volume roots 2.5 cm^3; 3.5 mm collet diameter), excluding the roots mass (7.5 g) and roots volume (8 cm^3).

Table 3. Developing of tomatoes seedlings at one week after the second treatments

Variant	Aerial part mass MPA (g)	Roots mass MR (g)	Total mass TM (g)	Roots volume VR (cm^3)	Ø collet (mm)
V_1	11.5	7.5	19.0	8.0	5.8
V_2	12.0	7.0	19.0	8.0	6.0
V_3	16.0	7.5	23.5	8.5	7.0
V_4	15.5	6.0	21.5	6.5	7.0
V_5	15.8	7.5	23.3	8.5	7.0

Also, was noticed a strong influence of the applied treatment on the aerial part mass and of the collet diameter (Figure 4).

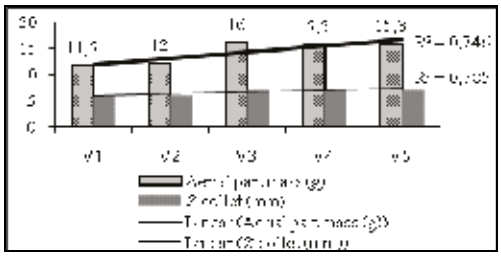

Figure 4 Influence of applied treatment on the aerial part mass and collet diameter at the second moment

The results of the physiological measurements performed on the experimental variants are shown in Table 4. As can be seen, the leaf temperature was relatively constant (27.2-28.3°C) and light intensity (Q) registered the value of 1280-1360 mmol/m^2/s.

Table 4. Physiology of the tomatoes seedlings one week after the second treatment

Var.	A [µmol/ m^2/s]	E [µmol/ m^2/s]	A/E	Leaf temp.T [^{0}C]	Q [µmoli/ m^2/s]
V_1	11.35	3.11	3.65	28.3	1280
V_2	12.13	1.60*	7.58	28.3	1280
V_3	13.39	1.74	7.69	27.2	1360
V_4	12.39	1.87	6.62	27.2	1360
V_5	12.96	1.78	7.28	27.2	1360

The results analysis revealed that V3 recorded the highest values of Photosynthesis rate A = 13.39µmol/m^2/s and efficiency (A/E = 7.69) on the backgroundof a low Transpiration rate E = 1.74 µmol/m^2/s. Also, V5 recorded high values for the physiologic indicators. These results confirm the good growth and development of seedlings from these two variants.

V1 untreated recorded a very good Photosynthesis rate (A = 11.35 µmol/m^2/s) but very intense Transpiration rate (E = 3.11 µmol/m^2/s) determined a low efficiency of photosynthesis (A/E = 3.65). The intensify of physiological processes without translocation and accumulation of photoassimilated substances is, in fact, the response of plants to the action of stressors whose intensity action does not endanger the life of plants. On the overall results can be noted V4 (BAC Foliar spray) as the most balanced variant regarding physiological activities.

CONCLUSIONS

In the last few years, amid the development of integrated and biologically horticultural concepts, it has been necessary to reconsider fertilizers used in horticultural practice by introducing into the technological stream new complex substances such as phytohormones or biofertilizers, natural and non-polluting.

Treatments with bioactive substance are applied with good results in vegetable practice to accelerate or inhibit the growth of seedlings or to support plants life in various stress situations.

Tomato seedlings from the very first moment of analysis showed important nuances as a result of the application of bioactive substances treatments in sense that, in the untreated V1 variant, the plants have recorded biometric indicators far inferior to those of the treated variants and the context analysis highlights V4-fertilized with BAC Foliar Spray 0.3% as the seedlings had the most balanced growth and development.

Analysis of the results of the growth and development of tomatoes seedlings at the end of experimental period showed that the best option working was V3-Razormin 0.1%. In this variant plants have achieved the best and balanced growth and development, all indicators analyzed had very good values. This is followed by V5-Bio Roots 0.2%

Physiologic activities revealed that V3 recorded the highest values of Photosynthesis rate and efficiency on the background of a low Transpiration rate. Also, V5 recorded high values for the physiologic indicators.

These results confirm the good growth and development of seedlings in the two mentioned variants (Razormin 0.1 % and Bio Roots 0.2 %) which we recommended as support treatment to improving quality of tomatoes seedlings.

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INTER-SPECIFIC (*CAPSICUM CHACOENSE* HUNZ. AND *CAPSICUM ANNUUM* L.) INHERITANCE OF FRUIT DETACHMENT FORCE TRAIT IN HOT PEPPER

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Abstract

In red pepper cultivation for spice production, harvesting the fruits is time consuming and high cost requiring process. Also all the cultivated pepper (Capsicum spp.) fruits for processing have to be separated from the pedicel after or before drying. The pedicel is tightly attached with the calyx to the fruit pod in most pepper cultivar. For this reason fruit detachment force (FDF) is an important trait to improve pepper cultivars suitable for mechanical harvesting. Gene action for FDF was calculated using inter-specific crosses between four C. annuum L. (3.860 to 7.340 N FDF) genotypes and C. chacoense Hunz. (0.199 N FDF), C. annuum variety 'Totolapa' (0.173 N FDF). FDF values obtained from parents and F1 generation indicated that gene effects for this trait were mostly displayed dominance and additive. No differences were calculated with respect to heterosis both Capsicum species related FDF. Parents could generate hybrids with high degree of negative heterosis varying between 86.56% and 94.50% related with FDF. Except K7 x CC hybrid easily detached from the fruit with 0.153 N, all the hybrids have fruit detachment force over 'Totolapa'.

Key words: *Fruit detachment force, heterosis, gene effect, pepper.*

INTRODUCTION

Chilies (dried red pepper) is one of the most important crops in South East Anatolian Region where produced 95% of 228,531 tons total chili production of Turkey.

Harvesting of chilies requires high labor and cost due to pick the fruits by hand. Harvest machines for dried chilies have been developed but cultivars suitable for mechanically harvesting needs some improvements (Palau and Torregrosa, 1996; Akay et al., 2009).

Fruit detachment force (FDF) is the most important trait related with either hand or mechanically harvesting.

Inheritance of detachment trait firstly reported with single dominant gene (Smith, 1951) and softening of fruit considered another gene controlling to soft fruited trait. Gersch et al. (1998) correlated FDF with cell size and cell wall thickness in fruit and calyx.

Finally Rao and Paran (2003) detachment or deciduous fruit and soft fruited traits were revealed by pleiotropic effect of endo-polygalacturonase (PG) gene. With this article the gene action of FDF was calculated with line × tester parental design using interspecific crossing in pepper.

MATERIALS AND METHODS

Capsicum annuum (Totolapa) and *Capsicum chacoense* (C. cha.) exhibited easy fruit detaching traits were used as male and *Capsicum annuum* (Dila, Sena, K7 and K8) exhibited hard genotypes were used as female parents. FDF was measured at red mature stage using with two different interval (0.001-5 and 0.1-500 N) digital push and pull force gauge as Newton (Geratech Inc. SH-5 and SH-50) (Figure 1). Variance of general and specific combining ability effects and heterosis were calculated by TNAUSTAT-Statistical package (Manivannan, 2014) with line × tester mating design (Singh and Chaudhry, 1985) using testers as male (Totolapa and C. cha.) and lines female (Dila, Sena, K7 and K8). Experiment was designed completely randomized blocks with 15 replicates. Mean differences among hybrids and parents related with FDF were compared LS Means Differences Tukey HSD. Two hybrid population of *Capsicum chacoense*

and *Capsicum annuum* species matched by paired sample t-test. JMP 5.0.1 software was used for statistical analysis.

RESULTS AND DISCUSSIONS

The fruit detachment trait which is wild characteristic (Paran and Knapp, 2009; Mao and Motsenbocker, 2001) decrease in population with the domestication of pepper. Fruits of many pepper cultivars using fresh or processed tightly adhere to the calyx (Motsenbocker, 1996). Due to time consuming and great effort requirements both harvesting and post-harvest process detachment trait come into prominence for harvest technology so that to eliminate the pedicel from the pepper fruit.

Table 1. Analysis of variance for Line × Tester and Combining ability analysis for FDF traits

Line × Tester (Including parents)				
SOURCE	DF	SS	MSS	F ratio
Replicates	14	2.40	0.1715	0.24
Genotypes	13	1443.06	111.0049	157.85
Cross	7	0.19	0.0278	0.03
Line (c)	3	0.09	0.0320	0.04
Test (c)	1	0.04	0.0488	0.06
L x T (c)	3	0.04	0.0165	0.02
Parent	5	705.15	141.0307	200.55
Line (p)	3	98.82	32.9429	46.84
Test (p)	1	0.92	0.9289	1.32
L (p) vt (p)	1	605.39	605.3961	860.89
CrovsPAR	1	737.71	737.7152	1049.05
Error	182	127.98	0.7032	
Total	209	1573.44		
Combining ability analysis				
Replicates	14	0.10	0.0075	2.01
Cross	7	0.19	0.0278	7.43
Line (c)	3	0.09	0.0320	8.57
Test (c)	1	0.04	0.0488	13.07
L x T (c)	3	0.04	0.0165	4.42
Error	98	0.36	0.0037	
Total	119	0.66		
Proportional contribution of lines, testers and their interactions to total variance				
Contribution of lines				49.39
Contribution of testers				25.10
Contribution of L × T				25.51

According to analysis of variance for line × tester analysis cross and lines significantly ($p<0.05$) important contribution of FDF traits. Percentage of contribution related with FDF 49.39, 25.10 and 25.51 for lines, testers and lines × testers, respectively (Table 1).

Figure 1.Measuring of FDF using push-pull digital gauge

Variance of general combining (σ^2_{GCA}) and of lines (σ^2_{SCA}) related crosses in respect to FDF were shown in Table 2. General combining ability of Dila and Sena varieties was found non-significant on account of FDF while K7 and K8 has significant ($p<0.01$) general combining ability among the lines using as female plants. GCA effects of both testers were significant for FDF trait. Variance of specific combining ability (σ^2_{SCA}) was non-significant for all of the crosses. Variance of general combining ability was found greater than that of specific combination ability. This situation indicated that additive genes could be effect on FDF traits. Werner and Honma (1980) explained fruit detachment at the fruit receptacle controlling by additive gene effects and pointed out no differences between reciprocal crosses. However mid, low, better parent and standard heterosis percentage of hybrids for FDF were found significantly and negatively. The lowest FDF values are important for easy pick by hand and harvest for the fruits (Werner and Honma, 1980; Motsenbocker, 1996; Gersch et al., 1998).

Therefore negative heterosis is expected from the hybrids for this trait. The highest negative mid parent heterosis percentage was observed K7 x Totolapa with -95.12%. Low parent heterosis was -97.03% at Dila x C. cha. and better and standard heterosis percentage same at this hybrid because of using Dila variety as standard to calculate standard heterosis. But the highest negative standard heterosis percentage was obtained from K7 x Totolapa hybrid with-97.82% (Table 2). All of the hybrids have deciduous fruit and soft fruit flesh. For this reason deciduous fruit trait was expressed along with soft fruit flesh dominantly. Nevertheless fruit detachment force associated with deciduous trait was impressed on additive gene effects combination with dominance. Rao and Paran (2003) determined deciduous fruit and soft fruited trait under control pleiotropic effect of PG gene.

This study exhibited that expression of PG gene could be diversified of phenotype. Gersch et al. (1998) emphasized that FDF related with genotype, maturity and plant growth regulator and also was affected by changing environment such as greenhouse and field.

Table 2. General and specific combining ability effects and heterosis percentage of lines testers and crosses for FDF

Lines	σ^2_{GCA}	Cross	σ^2_{SCA}	Heterosis (%)			
				Mid Parent	Low Parent	Better Parent	Standard
Dila	0.01 ns	Dila x C. cha.	-0.02ns	-94.50**	-97.03**	-97.03**	-97.03**
Sena	0.01 ns	Sena x C. cha.	0.02ns	-92.12**	-95.96**	-95.96**	-95.96**
K7	0.04**	K7 x C. cha.	0.03ns	-93.83**	-96.69**	-96.69**	-96.39**
K8	-0.04**	K8 x C. cha.	-0.03ns	-93.89**	-96.87**	-96.87**	-96.58**
Testers		Dila x Totolapa	0.01ns	-88.32**	-93.38**	-93.38**	-96.09**
Totolapa	-0.02*	Sena x Totolapa	-0.01ns	-86.56**	-92.98**	-92.98**	-95.86**
C.chacoense	0.02*	K7 x Totolapa	-0.02ns	-95.12**	-97.33**	-97.33**	-97.82**
		K8 x Totolapa	0.02ns	-91.74**	-95.74**	-95.74**	-96.52**

*Significant differences at p<0.05 ** p<0.01 and ns: non-significant

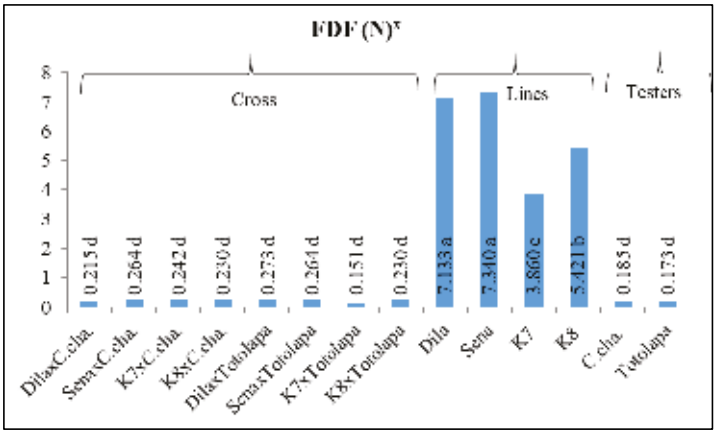

Figure 2. Fruit Detachment Force (FDF) of lines tester and crosses.
[x]Mean separation within the columns by LSMeans Differences Tukey HSD multiple range test at p≤ 0.05

Fruit detachment force of lines, testers and crosses were demonstrated in Figure 2. The highest FDF was 7.340 N at Sena variety. K7 was the less adhere to the receptacle genotypes in the lines with 3.860 N FDF. The testers Totolapa and C. cha. displayed 0.173 and 0.185 N FDF, respectively. K7 x Totolapa hybrid exhibited the lowest FDF among all the lines, testers and crosses. However all of the crosses and testers placed same statistically group (Figure 2.). Motsenbocker (1996) determined the lowest FDF value with 2.5 N at red mature stage "McIlhenny Select" tabasco (*Capsicum frutescens*) pepper variety in field study while it was 0.6 N in greenhouse condition. Mao and Motsenbocker (2002) observed 2.9 N FDF

from McIlhenny Select and no differences find between ethephon applied and control treatment on account of FDF. With this study two F1 populations of *Capsicum chacoense* and *Capsicum annuum* species were compared. No differences observed on these two population concerning FDF according to t-test. The mean of *Capsicum chacoense* population was 0.237 N and Totolapa (*C. annuum*) was 0.229 N (data not shown).

CONCLUSIONS

The fruit detachment force of pepper fruits from peduncle is under control additive gene effect with dominance. No differences inter or intra specific crossing were found for FDF traits. Fruit flesh softness and deciduous fruit trait were complete dominant and inherited jointly. Nevertheless the FDF can be affected by female genotypes adhered calyx tightly and differentiated phenotypically.

High percentage of mid, low, better parent and standard heterosis was calculated for FDF traits. Quite low FDF was observed on crosses from two different Capsicum species. These FDF values are promising to improve pepper varieties for mechanical harvesting.

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ASSESSING TOMATO GERMPLASM FROM V.R.D.S. BUZAU TO IDENTIFY GENOTYPES WITH DISTINCT FEATURES

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Abstract

V.R.D.S. Buzau is well known for tomato breeding, over time here were obtained valuable varieties appreciated by both consumers and growers. Currently, V.R.D.S. Buzau has an important germplasm collection consisting of over 1500 genotypes in different breeding phases. Since 1996, research on breeding this species were intensively undertaken, been obtained for the first time in our country hybrids with a certain destination. The research carried out so far has mainly focused on the germplasm evaluation accumulated to identify genotypes with distinct features for the breeding process, as well as availability and genetic stability in the progeny. The study aimed the observation of shape, size and fruit color to identify distinct features. The stable identified genotypes were organized into two groups: cherry type varieties and large fruits of different shapes like pear, bell peppers, long pepper, lemon, banana varieties, with different colors like white, various shades of yellow, red, pink, brown, black, burgundy varieties. Two of these genotypes have been proposed for patenting and are to be expanded on a large scale in production.

Key words*: biodiversity, breeding, Estera, Hera*, *specific destination.*

INTRODUCTION

Tomatoes were brought to Europe quite late, in the 16th century and more lately in Romania, 19th century. Although they were brought recently in Romania, this species is the most cultivated among vegetables. Edible tomato has its origin in the small and yellow wild tomato. Both plant and fruits sepals of the wild tomato are covered by big aggressive spines. Cultivars with red edible fruits of different sizes were obtained by selection and breeding methods and nowadays are cultivated on large scale. Domestication is often controlled by a limited number of chromosomal regions with major phenotypic effect In tomato, edible fruits, attractive red colour and fruit size increase are characterizing this process (Purugganan et al, 2009).

Following the selection and modern breeding methods, nowadays were obtained numerous genotypes of this species with distinct phenotypic traits. Also the consumers and producers demands imposed new breeding directions to obtain cultivars with precise use.

From first domestication to modern breeding, the tomato has been continually subjected to human selection for a wide array of applications in both science and commerce. Current efforts in tomato breeding are focused on discovering and exploiting genes for the most important traits in tomato germplasm. In the future, breeders will design cultivars by a process named 'breeding by design' based on the combination of science and technologies from the genomic era as well as their practical skills (Yuling Bai, 2007). Beside the recent breeding aims, crop yield and quality remain the main objectives The major goals of tomato breeders (higher productivity, better tolerance to biotic and abiotic stresses and increased sensory and health value of the fruit) require a good comprehension and management of tomato genetic resources diversity (Guillaume Bauchet, 2012). V.R.D.S. Buzau was interested in breeding this species since its establishment in 1957. There were obtained well known and appreciated varieties by both consumers and growers. Since 1996 the research was focused on enriching the Official Crop Plants List of Romania with new tomato varieties.

MATERIALS AND METHODS

Research started with collecting and assessing the tomato germplasm and breeding the

valuable genotypes. Now the germplasm collection contains over 1500 genotypes in different breeding phases. The genotypes assessing and organizing was made on the following criteria: type of growth and progeny genetic stability (Figure 1).

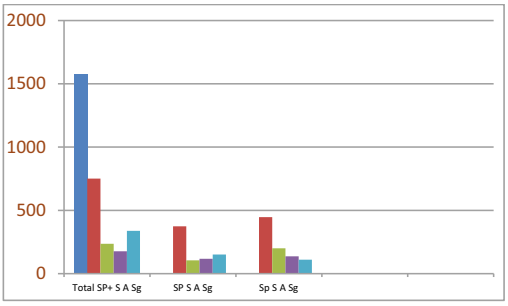

Figure 1. Tomato germplasm grouped on: SP+ (indeterminate) - 751 from which: S (Stable) 236, A (advanced) - 177, Sg (segregant) - 338; SP (semi-determinate) - 374 from which: S 105, A 118, Sg-151; Sp-(determinate) - 447 from which: S 200, A - 137 and Sg 110

Due to the large number of collected and assessed genotypes, new selection and breeding methods were implemented.

Our study was focused on individual repeated selection. Beside this, there were used hybridization, segregation, mutations and controlled genetic drift.

RESULTS AND DISCUSSIONS

Following the germplasm assessing there were retained 12 accessions that correspond to the main breeding proposed objectives (Table 1). Concerning the main plant features, accession (A) 532 has semi-determinate type of growth and the rest of the accessions have indeterminate growth.

Concerning the plant height, A 80 reached the highest value of 291 cm. A 631 has the richest foliage, this feature protects the fruits against solar burns.

Maximum number of trusses per plant was scored by A 709 with 11 trusses.

Concerning the fruits features, there were selected accessions with distinct traits which demonstrates the diversity of this species.

Thereby A 631 presents yellow lemon shaped fruits, productive plant and the fruit weights 83.3 g on the average. A 312 has cherry striped fruits, dark brown with red stripes and dark red pulp. The fruit weights 26.3 g on the average and has good firmness. A 524 has bell pepper shaped fruits, red coloured at maturity that weights 233.6 g. The fruits have large interior spaces and can be used to prepare some dishes like stuffed peppers.

A 522 has light yellow big fruits with red pulp, with 252.3 g average weight. A 308 has red with yellow stripes bell pepper fruit shaped and very productive plants. A 306 has very tasty cherry black fruits that weights 18.4 g on the average. A 532 has banana intense yellow shaped fruits that lengths 7.2 cm and A 709 is the most tasty accession due to sugar content of 12-16%. A 724 is a different accession with dark black fruits and uniparous raceme.

The pulp is dark red coloured. A 2000 has low productive plants but very big tasty heart shaped fruits. This accession comes from the Ox heart heirloom (Table 2) (Figure 2)

From these accessions, A 80, A 28 and A 2000 were selected as the most interesting and genetic stable genotypes that correspond to the consumers and growers requirements.

These accessions were registered for patenting since 2016 at ISTIS Bucharest.

Table 1. The main plant features

Studied feature / Accesion	631	312	524	522	28	308	306	532	709	80	724	2000
Growth type	SP+	SP+	SP+	SP+	SP+	SP+	SP+	SP	SP+	SP+	SP+	SP+
Plant height (cm)	271	281	268	272	263	288	268	165	258	291	263	250
Lateral shoots	12	13	11	13	13	10	11	9	12	14	13	18
Leaves/plant	36	30	27	21	23	31	26	17	32	26	28	35
Leaf length (cm)	36	35	52	38	40	42	40	33	32	43	36	49
Trusses/plant	9	8	6	5	5	5	8	5	11	10	7	5
Distance between trusses (cm)	22	24	34	27	28	31	36	28	35	20	21	28

Table 2. The main fruit features

The main studied feature	Fruit weight (g)	Fruit height (cm)	Fruit diameter (cm)	Seminal lodges	Pulp thickness (mm)	Main distinct feature	Sugar content (%)
A 631	83,3	6,7	4,6	3	50	Lemon shaped fruit	5.3
A 312	26.3	3.7	3.2	2	50	Cherry striped fruit	11.8
A 524	233.6	8.9	7.4	4	70	Bell pepper fruit	6
A 522	252.3	5.5	9	16	50	Pineapple coloured fruit	4.3
A 28	111.9	12	3.5	2	50	Long pepper shaped	5.7
A 308	178.9	5.5	8.5	5	90	Bell pepper striped fruit	3.8
A 306	18.4	2.5	2.5	3	30	Black cherry fruit	7
A 532	75.6	7.2	3.9	3	50	Banana shaped fruit	7
A 709	11,1	4	2	2	30	Pear shaped fruit	12-16
A 80	12.1	3.6	2.1	2	40	Ovoid cherry fruit	11.2
A 724	42,8	4,7	3,9	3	70	Black fruits	5.8
A 2000	560	9,5	10,3	20	70	heart shaped big tasty fruits	5.4

A 522 A 308 A 709

A 631 A 524 A 724

Figure 2. Studied accesions

Accession 80 presents indeterminate growth and can be cultivated both for fresh consumption and processing, can be cultivated both in open field and greenhouse, in trailing system. The accession has ovoid fruits that weights 16-20 g and a reduced number of seeds, < 20 seeds/fruit. The fruits have a high content of sugar, 11.2%.

The main feature of this accession is its distinct taste and flavor. The fruits tastings showed that this accession is superior in what concerns taste quality in comparison with the other accessions from the germplasm collection (Figure 3).

A 28 has indeterminate growth and can be cultivated both in open field and greenhouses, in trailing system, both for fresh consumption and for processing.

Figure 3. Crop detail of A 80

The accession has big fruits, long pepper shaped ranged in 100-150 g weight with high content of dry matter.

The main feature of this accession is the shape of the fruit. These are big in length slightly pointed (Figure 4).

Figure 4. Crop detail of A 28

A 2000 has indeterminate growth and can be cultivated in trailing system, in open field.

The genetic material comes from the Ox heart heirloom.

This accession has big fruits that ranged 300-950 g weight/fruit, heart shaped with green shoulder at the physiological maturity.

The accession is different due to specific taste and flavour of traditional Romanian tomato (Figure 5).

Figure 5. Crop detail of A 2000

CONCLUSIONS

The research undertaken until now ended with collection and assessing the germplasm collection and organizing it according to genetic stability and type of growth.

We obtained 12 genotypes with distinct features which correspond to the main breeding aims.

A 80 patented under the name of Estera, A 28 patented as Hera and A 2000, Bizon, were registered for approval and patenting.

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NEW EGGPLANT GENOTYPES WITH DISTINCT PHENOTYPIC EXPRESSIVITY OBTAINED AT V.R.D.S BUZĂU

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Abstract

V.R.D.S. Buzău has a great tradition in the process of breeding for this species. Here were maintained by conservative selection the first valuable Romanian eggplants like 'Danubiana', 'Bucurestene', 'Pana Corbului' along with 'Zaraza' and 'Dragaica'. Recently, the first hybrid of eggplants obtained by the Breeding Laboratory was patented, and registered in the Romanian Official Catalogue for Crop Plants under the name of 'Rebeca F1'. As a result of the researches made over time the Laboratory gathered an important germplasm collection and numerous informations regarding the activity of breeding this species. At this time, the germplasm collection is being compound of over 60 valuable genotypes with distinct phenotypic expressivity for their shape, size and colour. Stable genotypes that are a very important part of this paper were obtained. These have large white fruits, red coloured, brindled, green etc. Of these, the accession A 10 (Camelia), with large white fruits is in the final testing stage for patenting and will be extended on a large scale in production.

Key words*: aubergine, breeding, Camelia, germplasm collection, Solanum melongena.*

INTRODUCTION

„The eggplant arrived in Europe around 1300, and the eggplant fruits were used as food after the sixteenth century" (Cakir et al., 2017).
In Romania, the eggplant crop production was introduced after the 19th century. In the early times, foreign varieties were used in order to establish the crops, and the mainly varieties used for this purpose were: 'Delicates', 'Odesa', 'Lungi violete'. The first Romanian creations in this species were achieved after the Research Stations were founded, and the earliest and most used were 'Danubiana', 'Bucurestene' and 'Pana Corbului'. VRDS Buzau gave a special attention for breeding eggplants since April 1957, this being the year of it's founding, and managed to create two very valuable varieties that are still being used and appreciated: 'Dragaica' and 'Zaraza'. „Now there are an increasing number of F1hybrid varieties bred by seed companies and the seed production of egg-plant is shifting from farmer's hands to seed companies" (Chen et al., 2001). Recently, the first eggplant hybrid creation was patented and registered in the Official Catalogue for Romanian Crop Plants under the name of 'Rebeca F1'. If in the past the requirements of processors and consumers were limited at cultivars with traditional fruits, black or violet, of ovoid or cylindrical shape, now, their requirements have diversified a lot. 'Vegetables from imports are more varied than those derived from domestic production (Soare et al., 2016).
Therefore since 1996, in establishing the breeding objectives the buyer's actual requirements were taken into consideration. Modern breeding methods and a varied genetic material were implemented allowing obtaining a great number of genotypes with distinct phenotypic characteristics included in this paperwork.

MATERIALS AND METHODS

Researches debuted by achieving the germplasm collection. The main focus was on valorisation the autochthonous genetic potential consisting of varieties and local populations. The majority of the genotypes within the

collection belong to the *Solanum melongena* species, but among these are also included varieties from *S. macrocarpon* and *S. ethiopicum.* Therefore, the total collection for the eggplant complex collection is of 286 accessions.

After establishing the eggplant collection, researches were continued by evaluating the genetic stability in lineage and by identifying the useful characteristics for the breeding process.

The main breeding methods used were inter and intraspecific hybridization, the segregating phenomenon and also by breeding the valuable genotypes thru repeated individual selection. The main breeding objectives were channelled towards productivity, earliness and phenotypic distinctiveness.

A special emphasis was placed on breeding the lines that present a special colour variation from white, purple, green, with stripes, patches, and in various shape and size, small, cylindrical, globular, ovate, pear shaped, club shaped, ellipsoid, cylindrical, ribbed.

Simultaneously, genetic stability was monitored. Researches were made in protected areas (greenhouses without a heating system). Sowing was made in alveolar trays with 70 orifices, using as a substrate peat, in the first decade of March.

The seedlings were planted in the second decade of April, using the distance for planting of 70 cm between rows and 40 cm between plants per row. It was kept a distance of 120 cm between the planting strips.

The cultivation technology applied was the one specific to this species, and the special works consisted of trellising and pruning. The selected accessions are: A10, A20, A21B, A22A, A23A, A26, A29A, A30C, A51A, A58 (Figure 1).

A10 presents the greatest interest for the present work has large white fruits, very productive with a very pleasant commercial appearance.

A20 has very small fruits, similar in shape and size with an egg, this being the most attractive feature of it. Also it has a great number of fruits, over 40 allowing the harvesting for the entire cycle of production.

A21B has white fruits with purple stripes. These are of a medium size with a good yield production.

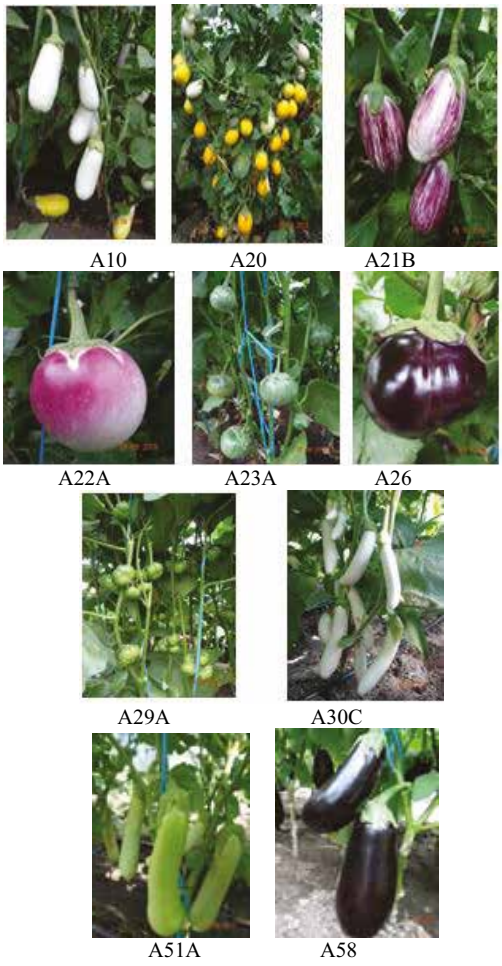

Figure 1. Aspects of the selected genotypes

A23A has greenish rounded and ribbed fruits that are small in size and have a good store resistance.

A26 has medium large fruits, round and a very pleasant appearance due to its purple skin coloration.

A29A was selected for the plant vigour and green fruits that when are fully ripen turn orange-red, their appearance being very similar to a small pumpkin.

A30C has white cylindrical small fruits that produce a great number of fruits with a good storage capacity.

A51A has typical purple ellipsoid fruits. It is a very productive accession with a good resistance to pests and diseases. The fruits are weighing between 700 g and 1100 g, depending on the technology used.

A58 has very interesting fruits due to their green coloration and club shaped appearance.

RESULTS AND DISCUSSIONS

Researches started with the achievement of a rich germplasm collection followed by its evaluation and acknowledgement regarding its genetic stability and also inventing the useful characters for the breeding process.

After establishing and evaluating the germplasm collection, this was structured on 3 groups according to their genetic stability. Of 286 accessions studied, a number of 62 accessions showed genetic stability in lineage, 86 accessions were included in the genetically advanced genotypes group these being the genotypes that proved a small variation regarding the variation expressiveness of the main characteristics.

A number of 138 genotypes form the group of segregating genotypes.

These ones present a great variability regarding the expressiveness of the main characters, the majority originating from the segregation of hybrid combinations made during the breeding program.

From the group of stable genotypes 10 accessions with distinct phenotypic expressivity regarding colour, shape and fruit size were selected. The main plant characteristics are shown in table 1.

Table1. Main plant characteristics-mean values

Plant features / Genotype	**A10**	**A20**	**A21B**	**A22A**	**A23A**
Plant height (cm)	162	155	200	100	156
Stem length(cm)	8	8	13	6	19
No. of side shoots	3	3	2	3	3
Leaf length(cm)	24	15	25	18	22.5
Leaf width(cm)	17	10	15	11	13
Plant features / Genotype	**A26**	**A29A**	**A30C**	**A51A**	**A58**
Plant height (cm)	99	140	114	165	110
Stem length(cm)	17	12	11	14	16
No. of side shoots	2	2	3	2	2
Leaf length(cm)	16	21	26	24	25.5
Leaf width(cm)	7	13	14	18	14

As shown in table no. 1, the accessions studied present different heights, A21B being the one with the most luxurious growth, reaching in mean values 200 cm length, and on the other side A26 has the smallest height of 99 cm-mean value.

Stem length varies from 6 cm to 19 cm with no correlation between plant height and stem length. A10 has a medium height value of 162 cm but is very well developed due to the number of side shoots (3) which gives it an aspect of fullness. These accessions present different leaf sizes and shapes correlated with the plant vigour.

Table2. Fruit characteristics-mean values

Plant features / Genotype	**A10**	**A20**	**A21B**	**A22A**	**A23A**
Peduncle and sepals colour	Green	Green	Green	Green	Green
Peduncle length (cm)	9	3.08	5.1	4.4	3.82
Presence of spines	On sepals	Absent	On sepals	Absent	Absent
Fruit colour at consumption maturity	White	White	White lined with purple	White	White lined with green
Fruit colour at physiological maturity	Yellow	Yellow	Yellow lined with brown	Yellow	Yellow
Fruit length (cm)	18	5.1	11	13	4.83
Median fruit diameter (cm)	9.5	3.71	7	6.2	6.30
Plant features / Genotype	**A26**	**A29A**	**A30C**	**A51A**	**A58**
Peduncle and sepals colour	Purple	Green	Green	Green	Green
Peduncle length (cm)	3.9	1.74	4.52	9	4.5
Presence of spines	On sepals	Absent	On sepals	On sepals	On sepals
Fruit colour at consumption maturity	Purple	Green	White	Purple	Green
Fruit colour at physiological maturity	Brownish	Red	Yellow	Brownish	Yellow
Fruit length (cm)	9.2	3.25	11.13	22	23
Median fruit diameter (cm)	7.8	5.10	3.69	12.1	4.05

According to the data registered in Table 2, accession A10 differs from the other white eggplants presented, through the size of the fruit, having larger fruit than the others, as we can see accession A10 has 18 cm fruit length and the others have fruits of 13 cm and even 5.1 cm. Also the proportion between fruit length and diameter (18 cm/9.5 cm) suggests the good commercial ellipsoid aspect that makes it very attractive for the production market.

Table 3. Fruit harvest-mean values

Plant features / Genotype	A10	A20	A21B	A22A	A23A	A26	A29A	A30C	A51A	A58
Fruit weight (g)	615	31	420	290	125	220.4	32	94	880	164
No. of fruits/plant	11	42	13	19	27	22	45	26	9	21
STAS **I** production (g)	4305	985	4120	3920	2896	3274	1115	2096	5842	3094
STAS **II** production (g)	1830	264	986	1260	345	964	284	279	1111	283
Substandard	630	53	354	330	134	610.8	41	69	967	67
Total production /plant (g)	6765	1302	5460	5510	3375	4848.8	1440	2444	7920	3444

As shown in Table 3, the most productive accession is A51A that presents typical large purple-black fruits followed with a difference of 1155g by A10, which is our point of interest with a production of 6765 g/plant. For A10, the substandard fruit production represents 9.313% of total fruit production/plant, while the STAS I production is 63.637% and STAS II is 27.051% of fruit production/plant. The lowest production was registered at A20, with the specification that this genotype has small fruits that are very attractive for culinary uses, being a great replacement for mushrooms.

After a further evaluation regarding the commercial quality of the fruits the first accession is A51A with a medium STAS I production of fruits/plant of 5842 g, followed by A10 with a medium STAS I production of fruits/plant of 4305. The harvest with the commercial aspect that was slightly depreciated was directed to STAS II; the fruit were slightly deformed or were having an extended blossom point.

All the small fruits, highly depreciated that were not proper for commercial purposes were directed to the substandard group. We need to specify that there were no interventions on the technological flow with fertilizers or additional technological works. For the future researches will continue with the implementation of modern fertilizing plans and pollen stimulation factors.

Applying these new factors, certainly the productivity and quality of the harvest will increase. In the applied experiences, the classical culture technology for eggplants was used in order to evaluate the real genetic potential.

CONCLUSIONS

Researches finalized with the establishment of a valuable germplasm collection, followed by its evaluation according their genetic stability and directions of use.

Ten new genotypes were identified with pronounced traits of distinctiveness especially in matter of shape, size and fruit colour. Of these, A10 accession was registered to ISTIS Bucharest for patenting under the name of *Camelia*. In the present it is in the 2nd year for further testing, and the feedback collected from the partner farmers from the main vegetable holdings are positive. This new achieved genotype has large white fruits, with few seeds in the fruit and a pleasant commercial appearance. It has a specific flavour and taste, a buttery pulp with the specification that the organoleptic qualities don't alter during processing. It can be cultivated both in protected areas and in open field in all the areas favourable for this culture. The research has been completed with the rehabilitation and reduction of the main character variables in cultivar 51 A, a valuable local bio-creation that has been neglected in culture for a long period of time. Also for this new variety the documentation for patenting was prepared and sent to ISTIS Bucharest. The identified and studied cultivars open new directions for use in culinary preparations.

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STUDY OF THE EFFECT OF ULTRASOUND ON VEGETABLE CROPS IN DIFFERENT EXPOSURES

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Abstract

The paper examines the impact of ultrasound on seeds of vegetable crops of the Apiaceae family in different exposition. The results of these studies show that seed treatment with ultrasound influences seed quality sowing. Ultrasonic treatment of the appropriate duration increases not only germination energy but also laboratory germination. It should be borne in mind that ultrasound stimulation with a certain duration, depending on the culture, may also have a negative effect.

Key words*: ultrasound, vegetable crops, quality sowing.*

INTRODUCTION

Vegetable production is one of the main sub-sectors of agriculture and an important share of plant growing. Root vegetables - carrot, parsley and celery are crops of great economic importance due to their high nutritional and biological value.

Carrots (*Daucus carota*), which are the main root crop, are used both for fresh and canned consumption. Besides food, carrots are used in pharmacy and perfumery industry. For rhythmic supply to the market and canning industry, carrots can be sown from early spring to early summer and early winter. Traditional sowing times are the end of February - early March, the first half of June and the first half of December.

Parsley and celery are used as a spice and are important for improving the palatability and digestibility of the food. This is due to the essential oils contained in them. With the content of nutrients and especially of essential oils, celery takes one of the first places among vegetable cultures used as seasonings. With the content of nutrients and especially of essential oils, celery takes one of the first places among vegetable cultures used as seasonings.

Celery is the only vegetable crop that grows through seedlings. Due to its long vegetation period, the nursery cultivation method and the large requirements for agrotechnical factors, the production of celery is considerably more labor intensive than other root crops (Cholakov, 2009). The areas occupied with celery in 2014 are 5 ha and the production is 57 t.

The purpose of this study is to investigate the possibility of pre-treatment of seeds with ultrasound to increase germination and seed germination. Carrot, parsley and celery are two-year-old vegetable crops of the *Apiaceae* family. Carrot refers to the genus *Daucus*, parsley to the genus *Petroselinum*, and celery to the genus *Apium*.

Various methods of pre-seed treatment have been developed, such as pre-soaking the seeds in water, rooting, heat treatment, impact with physical agents, etc., aiming at improving their seed qualities (Esfandiar F. et al., 2012).

In the system of organic farming, the use of certain physical factors on the biological development of crops is a modern method of achieving high yields. The use of physical factors is preferable to chemical treatments that can lead to plant contamination (Kwiatkowska B. et al., 2011).

The positive influence of ultrasound on seed sowing properties has been found in other crops such as rice (Liu Y. et al., 2003), wheat (Yaldagard M. et al., 2007), dill (Esfandiar Fateh, 2012; Zhao Yan et al., 2012), sunflower (Machikowa et al., 2013) and others.

The first scientific studies on the influence of ultrasound on plant development date back to

the 30-40 years of the last century. Experiments conducted in the Soviet Union show that ultrasonic oscillations have a beneficial effect on germination and subsequent plant development (Istomina O., Ostrovskiy E., 1936; Davidov G.K., 1940).
The use of ultrasound to accelerate germination of seeds is effective only when they are placed in a liquid medium, since this method of treatment allows the absorption of water from the seed that causes germination (Gordon, 1963; Yaldagard et al., 2008).

MATERIALS AND METHODS

The survey was conducted in the period 2016-2017 years in the Department of Mechanization and Department of Horticulture at the University of Plovdiv.
The subject of the study was the carrot, parsley and celery seeds. An ultrasonic bath is used to perform the study (Figure 1).

Figure 1. BRAND CT-405

The bathtub uses the principle of intense sound pressure, created by an ultrasound source, was born in a limited volume of liquid - a plurality of microscopic bubbles are formed that shrink and expand in sync with the frequency at which they hit the surface of the objects placed in the liquid.
Three exposures of ultrasound treatment were tested at 3, 6 and 12 minutes at a frequency of 43-45 kHz. To perform the ultrasound treatment, the seeds were placed in distilled water.

For the control untreated seeds of the crops set to germinate in Petri dishes. Depending on the duration of seed treatment, the following options were tested:
Carrot:
1. Control;
2. Exposure 3 min.;
3. Exposure 6 min.;
4. Exposure 12 min.;
Parsley:
1. Control;
2. Exposure 3 min.;
3. Exposure 6 min.;
4. Exposure 12 min.;
Celery:
1. Control;
2. Exposure 3 min.;
3. Exposure 6 min.;
4. Exposure 12 min.;
The experiment was set in 3 replicates and the number of seeded counts in each iteration was 100. After the treatment, the seeds were placed in a thermostat at 24°C, with the number of seed germinated daily. The counting continued until the 14th day for carrot, 21 for celery and 28 for parsley.
Two parameters were determined to determine the viability of seeds and their germination, namely laboratory germination (%) and germination (%). As a result of day-to-day readings, the speed and cooperativity of seed germination were determined.
Laboratory germination, showing normally germinated pure seeds under optimum conditions for a specified period of time, is determined by counting the number of germinated seeds according to the accepted BDS standards (601 84) as follows:
1) On the 14th day for carrot;
2) On the 28th day for parsley;
3) On the 21st day for celery.
The germinating energy, expressing the percentage of normally germinated seeds under optimum conditions, but for a shorter period of time, was reported to:
1) 7th day for carrots (under BDS);
2) 10th for parsley (under BDS);
3) 10th for celery (under USSR).

RESULTS AND DISCUSSIONS

1. Germination energy (%)

Research results show that seed treatment with ultrasound has a positive effect on the germination energy indicator and is best shown in carrots.

From the results presented in Figure 2, it is seen that as the exposure increases, the percentage of germination energy also increases. Depending on the duration of the ultrasound seed treatment, the germination energy increased from 30% as measured by the control to 43% in the 3 minutes version. Prolonged treatment has a stimulating effect since the value of the test item increases regularly and reaches 65% in the 6 minutes variant and up to 100% in the 12 minutes exposure. The excess over the control was 116.7% and 233.3%, respectively.

Under the influence of ultrasound stimulation, the terminable energy recorded in parsley (Figure 3) increased, although slightly, from 20% (control readings) to 26% in the 6 minutes exposure scenario, and increased to 28% in the exposure duration variant 3 min. The excess over the control in these variants is 30% and 40%, respectively.

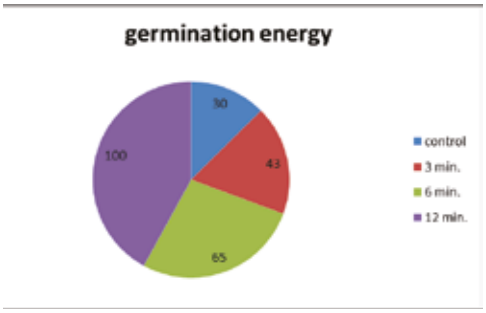

Figure 2. Germinating energy (%) of carrot seed, average for the period 2016-2017

Treatment of 12 minutes ultrasound parsley seeds has a rather negative effect as no germinating seed is reported at this exposure, and germinating energy is 0%, respectively.

The treatment of celery seeds with ultrasound (Figure 4) affects the germination energy only when its duration is 12 minutes. The reported sparkling energy in this variant is 14%. For all other variants, including the control, the germinating energy reading is 0%.

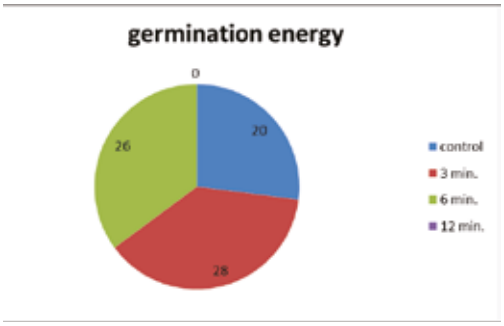

Figure 3. Germination energy (%) of parsley seeds averaged over the period 2016-2017

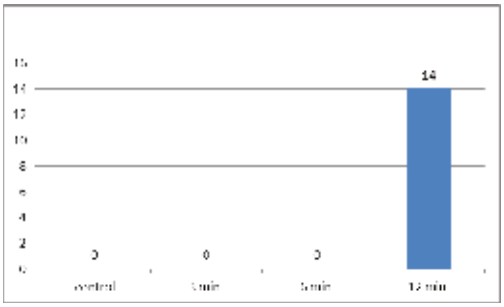

Figure 4. Germination energy (%) of celery seeds, averaged over the period 2016-2017

2. Cultivation (%)

Germination is a very important indicator of a biological aspect that provides information on the viability of the seeds, their suitability and the determination of the seed norm (Murtazov, 1984). And with this main indicator, the trend established in carrots to enhance the effect of seed treatment with increasing duration is retained (Figure 5).

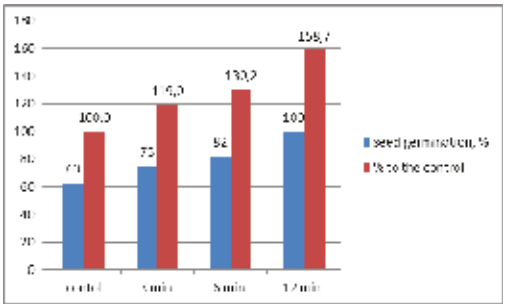

Figure 5. Laboratory germination (%) of carrot seed, averaged over the period 2016-2017

One hundred percent germination was reported in the 12 minutes exposure scenario, and it

should be noted that these values were recorded as early as the 7th day when germinating energy was determined. The excess over the control for this variant is 58.7%.
In the two other exposures, the reported values were 75% for variant 2 (3 minutes) at 19% over the control and 82% germination in variant 3 (6 minutes) with the control over 30.2%. The germination rate at the control was 63% lower than the other variants.
Attention is paid to the results presented in Figure 6 providing information on the speed and cope of seed germination in carrots.

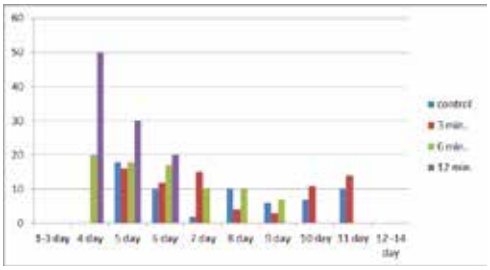

Figure 6. Germination of carrot seeds by day

It is seen that under the influence of ultrasound treatment with a duration of 6 and 12 minutes seed germination begins on the 4th day after the treatment, which is a day earlier than the control.
Attention is paid to the results reported in the 12 minutes variant, in which seed germination is most common. Within 3 consecutive days, the number of germinated seeds in this variation reaches 100.
By comparison, the control for the same period of time is the number of sprouts 30. In the 6 minutes variant, seed germination ends on day 9, and for the other two options, including the control - on the 11th day.

In Figure 7 shows the results for laboratory germination of parsley seeds. The highest percentage of laboratory germination was reported in a 6 min. exposition, with a 33.3% increase over the control.

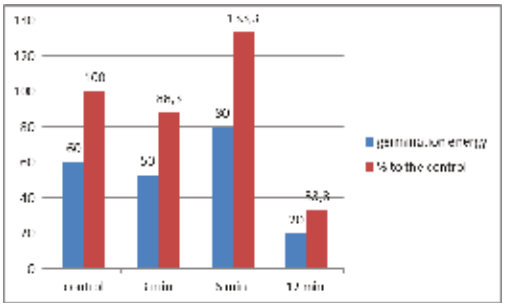

Figure 7. Laboratory germination (%) of parsley seeds averaged over the period 2016-2017

Laboratory germination reported at the control was 60%. For the other two exposures, the reported test value is lower than that of the control. While in the 3 minutes variant the difference is only 7% in favour of the control, in the 12 minutes seed treatment variant, the difference in untreated seed (control) is drastic and reaches 40%.
Obviously, this indicator can be said to be negatively affected by the prolonged treatment of the seeds with ultrasound - 12 min.
The results presented in Figure 8 are quite different, so it is difficult to establish any tendency or regularity. However, seed germination may vary from day 7 to day 11, with the 3 minutes exposure being at its earliest, and at 12 minutes exposure at the latest. The control sprout starts on the 9th and ends on the 14th. For all other variations, this process ends until the 19th day.

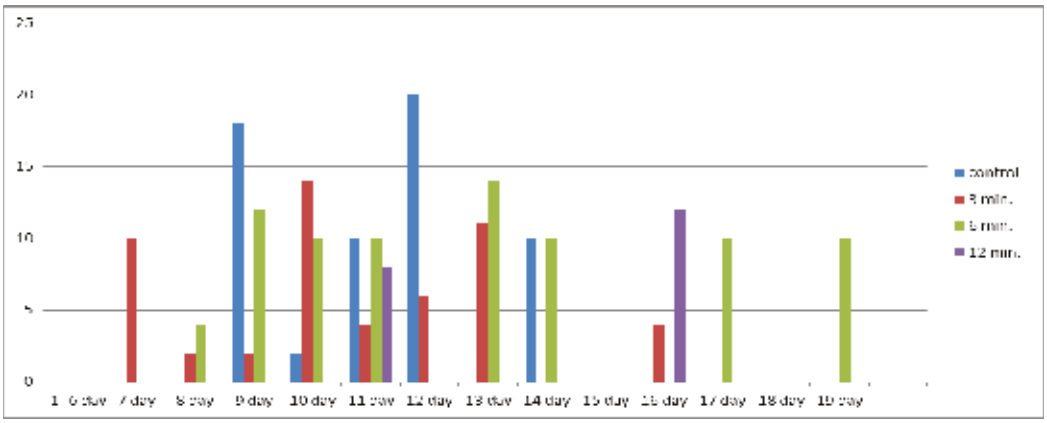

Figure 8. Sprouting seeds of parsley by day for the whole period.

In Figure 9 the results for laboratory germination of celery are presented. Laboratory germination was only reported for 6 minutes and 12 minutes exposures. Under the influence of 6 minutes treatment seed germination reaches 60% at 0% for the control. Significantly lower and at the same time higher than that of the control is laboratory germination in the variant with 12 min. duration - 20%.

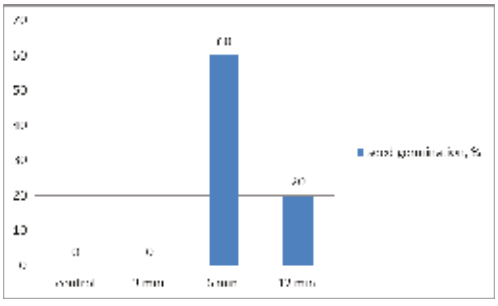

Figure 9. Laboratory germination (%) of celery seeds, average for the period 2016-2017

The germination process begins at day 9 in the variant (Figure 9) with 6 minutes exposure and at day 12 in variant 4 (12 minutes). In the same two variants seed germination ends on day 17 on day one and on day 18 in the second variation. There is also a difference between the variants in the seed germination rate. While in the 6 minutes version the seeds germinate at regular intervals of days (daytime), in variant 4 only two of the readings are found to have sprouted seeds, with the difference between the first and the second being 6 days.

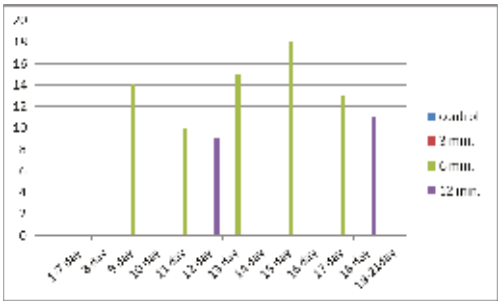

Figure 10. Germination of celery seeds by day for the whole period

CONCLUSIONS

Seed treatment with ultrasound influences seed sowing.

The optimum duration of ultrasound exposure in carrots lasts for 12 minutes, and for parsley and celery - 6 minutes.

This method of seed treatment increases the carrots energy of carrots by up to 233.3% and of parsley by up to 40%. Celery has 14% germinated energy at 0% for the control.

Ultrasonic treatment of the appropriate duration increases not only germinating energy but also laboratory germination. In carrots it reaches 100%, in parsley 80%, and in celery - up to 60%. The excess over the control with 58.7% for carrots and 33.3% for parsley. In celery reported laboratory germination is 0%

It should be borne in mind that ultrasound stimulation with a certain duration, depending on the culture, may also have a negative effect.

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DETERMINATION OF CHLORINE CONCENTRATION AND PRODUCTIVITY IN SOME BEAN GENOTYPES, FROM NORTH-EAST OF ROMANIA, UNDER SALT STRESS

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Abstract

From the environmental stress factors, salinity is one of the most important causes which affects the security of mankind. Impact of soils with excessive salinity on the productivity of different cultures is sometimes disastrous, which determines the identification and the creation of new genotypes of plants tolerant to osmotic stress conditions. From the viewpoint of stress concept, chlorine (Cl^-) is viewed as being biologically aggressive osmolite, based on its small ionic diameter and its strong tendencies to attract water (high hydration capacity). High concentrations of these ions in apoplast lead to imbalances in hydric and ionic relationships. For these reasons, stress caused by salinity is also a dehydration stress and ionic stress. In present study, it was pursued the influence of saline solutions of 100 mM and 200 mM NaCl on Cl^- concentration and productivity, to 7 local populations of common beans (Phaseolus vulgaris L.) collected from areas with saline soils, in North-East of Romania and grown under greenhouse conditions, at the pots, analyzing the largest/lowest amount of chlorine in the leaves and the correlations between the chlorine content and the average number of pods/plants, the average number of grains/pods, the average weight of the grains/pods and the average yield/plant, indicators that define productivity.

Key words*: correlations, NaCl, Phaseolus vulgaris L., productivity.*

INTRODUCTION

Excess salinity in the soil affects total growth and yield and the impact extent depends on the degree of salinity (Flowers and Yeo, 1995; Munns, 2002). Crop plants will not grow in high concentrations of salt: only halophytes grow in concentrations of sodium chloride higher than about 400 mM. The physiological and molecular mechanisms of tolerance to osmotic and ionic components of salinity stress are reviewed at the cellular, organ, and whole-plant level. Salinity is considered one of the major factors affecting the agricultural productivity worldwide. In the arid and semiarid regions, soil salinization may be caused by poor irrigation water which contains considerable amounts of salts, salt accumulation in the soil surface layer due to over-irrigation, proximity to the sea and/or the capillarity rise of salts from underground water, into the root zone, due to the excessive evaporation (Gama et al., 2007). Salinity reduces the ability of plants to utilize water, causes a reduction in the growth and yield, and changes in the plant metabolic processes (Munns, 1993, 2002).

The chloride ion is present in abundance almost everywhere in the world. It is required as a micronutrient for optimal plant growth, at a rate of only 0.3-1 mg/g dry matter in most plants (Marchner, 1986). The influence of the chloride ion on plant growth depends on the plant variety (Tottingham, 1919). The dependence of modern agriculture on irrigation and chemical fertilization causes more concern about the toxicity of chloride than about Cl^- deficiency (Marschner, 1986).

Generally, most non woody crops tolerate excessive levels of Cl^-, whereas many woody plants species and beans are susceptible to Cl^- toxicity (Maas, 1986).

Chloride toxicity in plants is often hard to diagnose, for two reasons: it is difficult to separate the effects of chloride from those of any accompanying cations, commonly sodium;

and it is difficult to distinguish between the specific toxic effects of ions and the cellular dehydration caused by their excessive external concentrations (Bar et al., 1997).
Chlorine is involved in photosynthesis to remove harmful oxidants from photochemical systems and stimulate electron transport. It has a role in regulating the osmotic potential by maintaining the hydric cell level, ensuring the opening of the stomata. On the other hand, it stimulates enzymatic activity and is antitranspirant (Davidescu, 1988). In the chloroplast, the Cl^- concentration remains relatively constant regardless of whether the plant growth medium is characterized by deficienty or excess.
Excess of chlorine negatively affects plant growth, by degradation of carbohydrate metabolism. Species resistant to excess chlorine are: beans, potatoes, tomatoes etc. (Şumălan, 2004). Chlorine deficiency reduces foliar growth, followed by wasting, chlorosis, brunification, and ultimately necrosis; at the same time, the fruit decreases in number and size, because beans are a sensitive plant from this point of view (Toma et Jităreanu, 2007).
Phaseolus vulgaris L. is a salt - sensitive species. For this reason, the purpose of the present paper was to determine the effect of excess NaCl on production as an indicator of salt stress tolerance.

MATERIALS AND METHODS

The experience was done under greenhouse conditions and the research took place in the Plant Physiology Laboratory, USAMV Iaşi. The biological material was represented by seven local bean populations (Blăgeşti 1, Blăgeşti 2, Blăgeşti 3, Blăgeşti 4, Moşna, Săveni, Truşeşti 2) collected from saline soils from the North-Eastern region of Romania, known as the region of Moldova (Iaşi, Vaslui and Botoşani).
The bifactorial experience was set up in 12 liter seed pots, in randomized blocks with three repetitions. They were exposed to saline stress for a period of 30 days, being constantly watered with concentrations of 100 mM NaCl and 200 mM NaCl.
The concentration of chlorine was determined by potentiometric titration with silver ions, using the Analytical Chloride Titrator, as described by Slabu et al. (2009), and expressed as mg/100 g DW.
The results were statistically interpreted using the Microsoft Excel- Data Analysis application, determining the correlation coefficient and magnitude of the effect in the linear correlation r (Pearson).

RESULTS AND DISCUSSIONS

Analysis of the Cl^- content of bean leaves subjected to saline stress, over a period of 30 days reflects the fact that in the case of the control variant, values ranged from 0.15 to 0.48 mg/100 mg DW. For the variant treated with 100 mM NaCl, the values were higher compared to the control variant, so the maximum was recorded at the Moşna genotype (6.60 mg/100 mg DW) and the minimum value at the Blăgeşti 2 (5.25 mg/100 mg DW).
The values are significantly higher than the control variant and for the 200 mM NaCl stress, genotypes with a maximum of 12.10 mg/100 mg DW to the local population Blăgeşti 1, which denotes the resistance of this genotype to saline stress (Table 1).

Table 1. The content of Cl^- (mg/100 mg DW) of bean leaves under salinity stress for a period of 30 days

Population	Control	100 mM NaCl	200 mM NaCl
Blăgeşti 1	0.23	6.35	12.10
Blăgeşti 2	0.48	5.25	5.45
Blăgeşti 3	0.22	6.40	10.10
Blăgeşti 4	0.29	6.60	7.15
Moşna	0.34	6.95	9.75
Săveni	0.15	5.40	7.95
Truşeşti 2	0.19	5.50	5.75

As a result of the T-test, it is noted that there are insignificant statistical differences between the control variant and the variant subjected to 100 mM NaCl; between the control variant and the one with a NaCl concentration of 200 mM statistical differences are very significant, and statistically significant values were recorded between variants treated with the two saline solutions (Table 2).

Table 2. Statistical differences between control (I) and variants treated with saline solutions: 100 mM (II) and 200 mM (III) in terms of Cl^- content, expressed as mg/100 mg DW

Comparative variants	t-stat	P two-tail	Signification
Control -100 mM NaCl (I)	-22.5725	2.474255	**Ns**
Control - 200 mM NaCl (II)	-8.58179	0.000006	***
100 mM NaCl - 200 mM NaCl (III)	-2.87781	0.014069	*

-Test Paired Two Sample for Means: Ns-statistically insignificant differences ($p \geq 0.05$) between variants; *Significant statistical differences ($p \leq 0.05$) between variants; **Significant distinct statistical differences ($p \leq 0.01$) between variants; ***very significant statistical differences ($p \leq 0.001$) between variants.

Assessing the influence of saline stress on plant production is a very important aspect in determining how salinity affects productivity, and that is why the fructification process has been studied on the basis of determinations of different indices: average number of pods/plant, average number of grains/pods, average weight of grains/pods and average production/plant.

Analyzing the average number of pods/plants in saline-stressed bean plants over a period of 30 days, it is noted that, in comparison to the control variant, for all genotypes treated with 100 mM NaCl and 200 mM NaCl solutions the average number of pods/plant was low.

From table 3 it can be observed that the highest genotype of the analyzed character is the number of pods/plant is Blăgești 4, which records a number of 25.33 pods/plant in the control variant, 24 pods/plant for the variant 100 mM NaCl and 23.66 for the 200 mM NaCl variant. The lowest number of pods/plants was recorded in the Moșna: 3.33 pods/plant for the control variant, 1.66 pods/plant for the 100 mM NaCl (Table 3) .

Table 3. Effect of saline stress on the average number of pods/plant after 30 days exposure to saline stress

Population	**Control**	**100 mM NaCl**	**200 mM NaCl**
Blăgești 1	15.33	11.33	8.00
Blăgești 2	25.00	18.66	12.00
Blăgești 3	7.00	4.00	3.00
Blăgești 4	25.33	24.00	23.66
Moșna	3.33	1.66	0.00
Săveni	3.33	4.00	1.00
Trușești 2	15.33	9.00	6.00

The correlation between Cl^- content and average number of pods/plants after 30 days of exposure to saline stress was found to be acceptable in accordance with the rules established by Colton (1974). Chlorine concentration at leaf level had a negative influence on the average number of grains/pods, in this respect the excess of chlorine having a toxic effect (Figure 1).

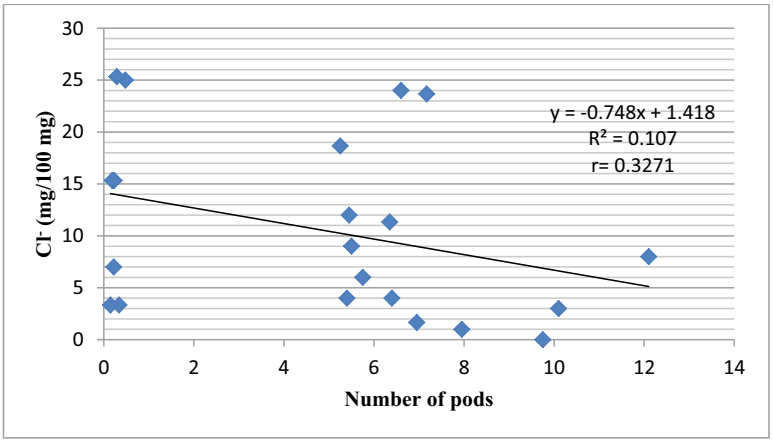

Figure 1. Correlation between Cl^- (mg/100 mg DW) and average number of pods/plant and after 30 days exposure to saline stress

Regarding the determination of the average number of beans/pods it is noted that compared to the control variant, all local populations treated with 100 mM NaCl and 200 mM NaCl solutions determined a decrease of the analyzed parameter.

Thus, the Blăgeşti 4 genotype, which had a number of 6.03 number of grains/pod in the control variant, 5.61 number of grains/pod for the 100 mM variant and 4.06 grains/pod for the 200 mM NaCl variant, was particularly highlighted.

The lowest value of this analyzed parameter was recorded in the Mosna genotype: 1.72 number of grains/pod for the control variant, 1.25 number of grains/pod for the 100 mM NaCl variant and number of pod/plant for the 200 mM NaCl treated variant (Table 4).

Table 4.Effect of saline stress on the average number of grains/pod after 30 days of exposure to saline stress

Population	Control	100 mM NaCl	200 mM NaCl
Blăgeşti 1	3.56	3.16	2.72
Blăgeşti 2	4.42	3.19	2.72
Blăgeşti 3	2.72	2.26	2.16
Blăgeşti 4	6.03	5.61	4.06
Moşna	1.72	1.25	0.00
Săveni	3.31	3.00	1.33
Truşeşti 2	4.20	3.54	2.40

The correlation between Cl^- content and the average number of grains/pod after 30 days of exposure to saline stress has shown an acceptable degree of association, indicating that the chlorine concentration recorded at the leaf level negatively influenced the average number of grains/pod, from this point of view the excess of chlorine having a toxic effect (Figure 2).

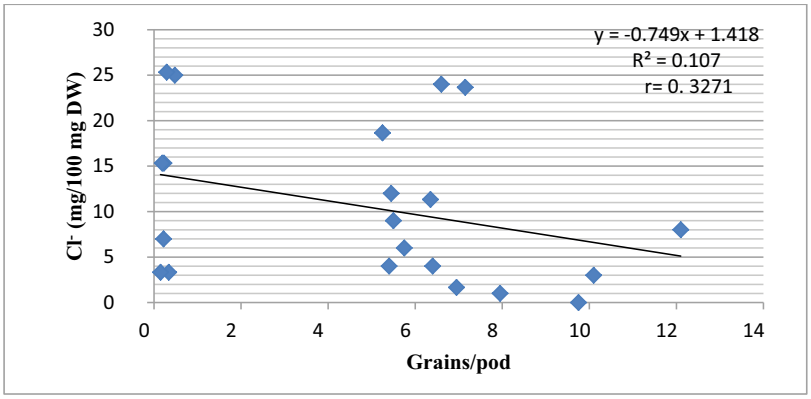

Figure 2. Correlation between Cl^- (mg/100 mg DW) content and average number of grains/pod after 30 days exposure to saline stress

Determinations by the influence of saline stress on weight grains/pod after 30 days of saline stress, in the case of the 200 mM NaCl variant, four genotypes recorded lower values, except the Blăgești 3 genotype at which the average weight of the grains/pod was the same, as in the 100 mM NaCl treated variant. And this time the Moșna genotype was noted for lack of productivity (Table 5).

Table 5. Effect of saline stress on average weight of grains/pod after 30 days exposure to saline stress

Population	Control	100 mM NaCl	200 mM NaCl
Blăgești 1	0.82	0.56	0.37
Blăgești 2	1.10	0.96	0.73
Blăgești 3	0.56	0.32	0.32
Blăgești 4	1.85	1.70	1.30
Moșna	0.39	0.31	0.00
Săveni	0.63	0.54	0.33
Trușești 2	1.36	1.22	0.74

The correlation between Cl^- content and average weight of grains/pod after 30 days of exposure to saline stress also indicates an acceptable degree of association (Figure 3).

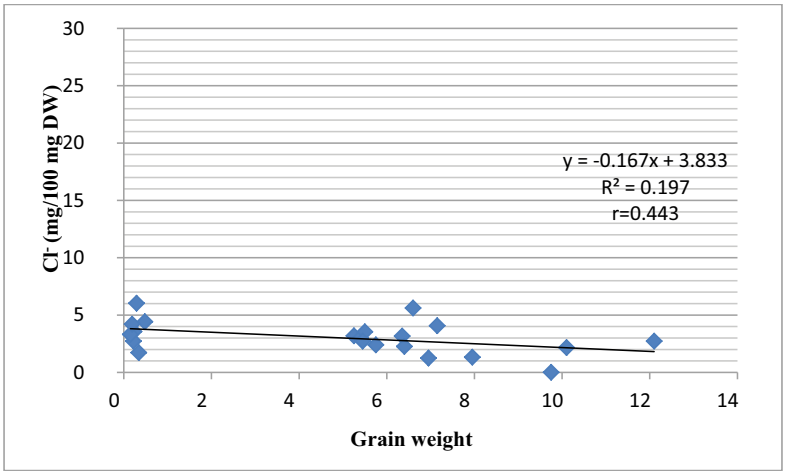

Figure 3. Correlation between Cl^- content and average weight of grains/pod after 30 days exposure to saline stress

Concerning in the mean production per plant compared to the control variant, it is observed a decrease with the application of saline treatments to all seven studied.genotypes.
It was found that the highest production of the Blăgeşti 4 genotype, both for the control variant (46.86 g) and for the saline-treated variants (40.80 g and 30.75 g).
The smallest production was recorded in the Moşna genotype, from the 100 and 200 mM NaCl variants, namely 0.51 and 0 grains/plant (Table 6).

Table 6. Effect of saline stress on average production/plant after 30 days exposure to saline stress

Population	Control	100 mM NaCl	200 mM NaCl
Blăgeşti 1	12.57	6.34	2.92
Blăgeşti 2	27.50	17.91	8.76
Blăgeşti 3	3.92	1.28	1.28
Blăgeşti 4	46.86	40.80	30.75
Moşna	1.29	0.51	0.00
Săveni	2.09	2.16	0.33
Truşeşti 2	20.84	10.98	4.94

Regarding the correlation between Cl^- content and average production/plant after 30 days of exposure to saline stress also indicates an acceptable degree of association (Figure 4).

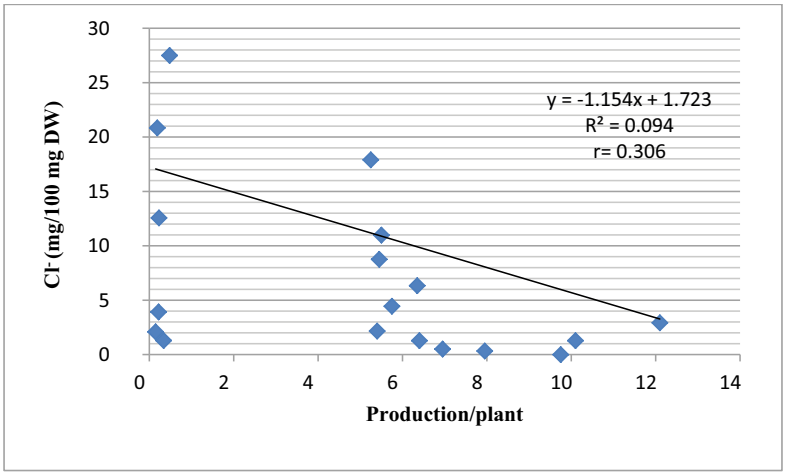

Figure 4. Correlation between Cl^- (mg/100 mg DW) content and average production/plant after 30 days exposure to saline stress

Excess chlorine accumulated in plants following NaCl treatments at 100 mM and 200 mM, respectively, has a negative influence on the average number of pods/plants, the average number of grains/pods, the average weight of the grains/pods and the average yield/plant, in which case chlorine becomes toxic to plants, inhibiting the fructification process. The differences in susceptibility to toxicity between the bean genotypes studied are correlated with the ability to reduce chlorine transport in stems and leaves.
This character is genetically determined and can be used to improve resistance to toxicity.
The results obtained are in full agreement with those presented in the literature, according to which the high concentrations of Cl^- negatively influence the production (Singh et al., 2012).

CONCLUSIONS

Cl^- content values were higher than control variant for genotypes subjected to a 200 mM NaCl. The maximum value reached was 12.1 mg/100 mg DW in the Blăgești 1, and the minimum value was 5.75 mg/100 mg DW at Truşeşti 2. The Blăgeşti 4 genotype recorded the highest production in all four indices analyzed, and the lowest values were registered on Moşna local population, with the specification that the variant treated with 200 mM NaCl did not have any production.

The fructification process decreased compared to the control variant of all treated genotypes with 100 mM NaCl and 200 mM NaCl. The correlation between the Cl^- content and the average number of pods/plant, the average number of grains/pods, the average weight of grains/pods and the average yield/plant in bean plants exposed to saline stress for a period of 30 days from exposure to saline stress was found to be acceptable in accordance with the rules established by Colton (1974).

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STUDY OF THE INFLUENCE OF A BIOSTIMULATOR USED IN THE TREATMENT SEED OF FAMILY *APIACEAE* BY ULTRASOUND

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Abstract

Root vegetables - carrot, parsley and celery are crops of great economic importance due to their high nutritional and biological value. The article explores the effect of biostimulator and the different duration of ultrasound treatment on the sowing quality of carrot, parsley and celery seeds. From the studies done, it was found that the biostimulator effect differs for the three cultures and depends on the duration of the ultrasound treatment.

Key words: *biostimulator, rood vegetables, seeds, ultrasound.*

INTRODUCTION

Root vegetables - carrot, parsley and celery are very important crops for economic and nutritional reason.

Carrots (*Daucus carota*), which are the main root crop, are used both for fresh and canned consumption (Cholakov, 2009). They refer to polyvitamins vegetables containing vitamins B_1, B_2 and C. The content of carotene is between 5-15 mg per cent. Besides food, carrots are used in pharmacy and perfumery industry.

The harvest of early production occurs during the spring and summer months and is consumed exclusively in a fresh state. The late Polish production, which is essential for our country, has the task of satisfying the needs of the canning industry and the population during the winter months.

With the content of nutrients and especially of essential oils, celery takes one of the first places among vegetable cultures used as seasonings. The importance of celery stands out particularly well given the fact that root crops are excellent and can be used fresh during the winter and spring months. (Liu et al., 2003)

The aim is investigating the impact of the biostimulator used in seed treatment by ultrasound (Istomina et al., 1936; Esfandiar et al., 2012; Gordon et al., 1963) on the seed qualities of carrot, parsley and celery.

MATERIALS AND METHODS

The survey was conducted in the period 2016-2017 in the Department of Mechanization and in the Department of Gardening at the Agricultural University of Plovdiv. The subject of the study was the carrot, parsley and celery seeds. An ultrasonic bath is used to perform the study (Figure 1)..

Figure 1. BRAND CT-405

The bathtub works on the principle of intense sound pressure, created by an ultrasound source, filled in a limited volume of liquid - a number of microscopic bubbles are formed that shrink and expand in sync with the frequency at which they hit the surface of the objects placed in the liquid.

Two exposures of ultrasound treatment were tested at 6 and 12 minutes at a frequency of 43-45 kHz. Two different liquids were used to

perform the ultrasound treatment. The first one was distilled water, and the second in the distilled water was added a Winner biostimulator at a concentration of 1: 1000.

The biostimulator Winner has the following composition: nitrogen 125 g/l, phosphorus 75.2g / l, potassium 54.5 g/l, calcium 0.5 g/l, magnesium 7.5 g/l, iron 0, 5 g/l. It also contains manganese, copper, zinc, sulphur, pine and molybdenum. It is rich in organic substances - amino acids, hormones, vitamins - 57.2%. It is applied to foliage and soil, but it also serves to treat seeds in different concentrations depending on the species.

Non-treated seeds of the indicated crops planted for germination in petri dishes were used for the control. Depending on the duration of the seed treatment and the used liquid, the following variants were tested when loading the ultrasonic bath:

Carrot:

1. Control;
2. Exposure 6 min. + Winner;
3. Exposure 12 min. + Winner;

Parsley:

1. Control;
2. Exposure 6 min. + Winner;
3. Exposure 12 min. + Winner;

Celery:

1. Control;
2. Exposure 6 min. + Winner;
3. Exposure 12 min. + Winner.

The experiment was set in 3 replicates and the number of seeded counts in each iteraction was 100. After the treatment, the seeds were placed in a thermostat at 24°C, with the number of seed germinated daily. The counting continued until the 14th day for carrot, 21 for celery and 28 for parsley.

Two parameters were tested - laboratory germination (%) and germinating energy (%). As a result of day-to-day readings, the speed and cooperativity of seed germination were determined.

Laboratory germination, showing % normal germinated pure seeds under optimum conditions for a specified period of time, was determined by counting the number of germinated seeds according to the accepted BDS standards (601 84) as follows:

1) On the 14th day for carrot;
2) On the 28th day for parsley;
3) On the 21st day for celery.

The germinating energy, expressing the percentage of normally germinated seeds under optimal conditions, but for a shorter period of time, was reported to:

1) 7th day for carrots (under BDS);
2) 10th for parsley (under BDS);
3) 10th for celery (under BDS).

RESULTS AND DISCUSSIONS

1. Germination energy (%)

The results of the performed studies show an increase in exposure and with the addition of a biostimulator, the percentage of germinating energy increases.

Highest test values were recorded in the 12 minutes seed treatment scenarios where the germinating energy reaches 100% and the excess over the control was 233.3%. At 6 minutes exposure, the germinating energy increased to 85%.

The lowest values were recorded in the control - 30% sparying energy (Figure 2).

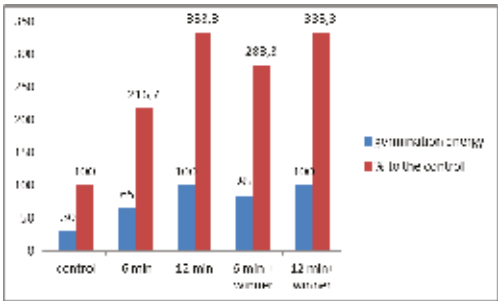

Figure 2. Germinating energy (%) of carrot seed, average for the period 2016-2017

While the germinating energy increases in carrots with the addition of biostimulants, the effect of the studied effects is less pronounced in parsley and celery.

The germinating energy recorded in parsley (Figure 3) increases, although slightly less than 20% (seen in the control) and reaches 30% when the biostimulator is added.

Exceedance of the control for this option is 50%.

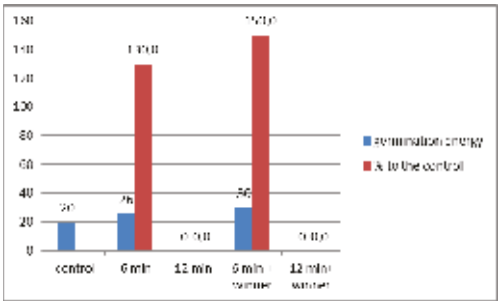

Figure 3. Germination energy (%) of parsley seeds averaged over the period 2016-2017

The treatment of celery seeds with ultrasound (Figure 4) affects germination energy only when its duration is 12 minutes.
Within this exposure, the measured values of the test item do not differ significantly between each other and the difference between the two tested media is only 5%, with the biostimulator variant being 19%.
In the other variants, including the control, 0% of germinating energy was reported.

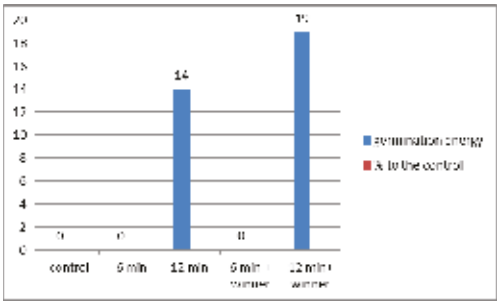

Figure 4. Germination energy (%) of celery seeds, averaged over the period 2016-2017

2. Laboratory germination (%)

Germination is a very important indicator of a biological aspect that provides information on the viability of the seeds, their suitability and the determination of the seed norm (Murtazov, 1984).

And with this main indicator, the trend found in carrots to enhance the effect of adding a biostimulator is retained (Figure 5).
The reported germination exceeds that of the control by 58.7%. Within 6 minutes exposure, germination reached 95%, and the over-control was 50.8%. Significantly lower than the other variants is germination, reported in the control - 63%.

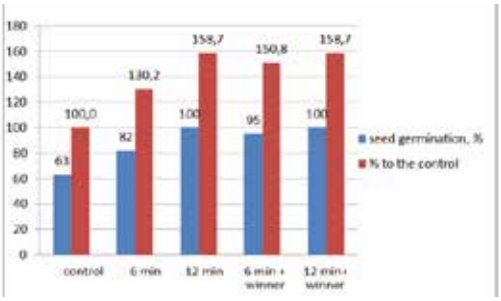

Figure 5. Laboratory germination (%) of carrot seed, averaged over the period 2016-2017

Attention is paid to the results presented in Figure 6, providing information on the speed and suitability of seed germination in carrots. It can be seen from the figure that, with or without biostimulator, in the first 3 days after this phase occurs, the number of germinating seeds depending on the variant is between 55 and 100. For comparison, in the control for the same period of time the number of sprouts is 30.
In the biostimulator variant on Day 4, 60 germinations were reported and the remaining 40 on the 5th day. The biostimulator effect in the 6 minutes exposition was also well-known, whereas on day 6 (3 days after start of germination) the number of sprouted seeds recorded is 27.3% higher than the other variant with the same treatment duration.

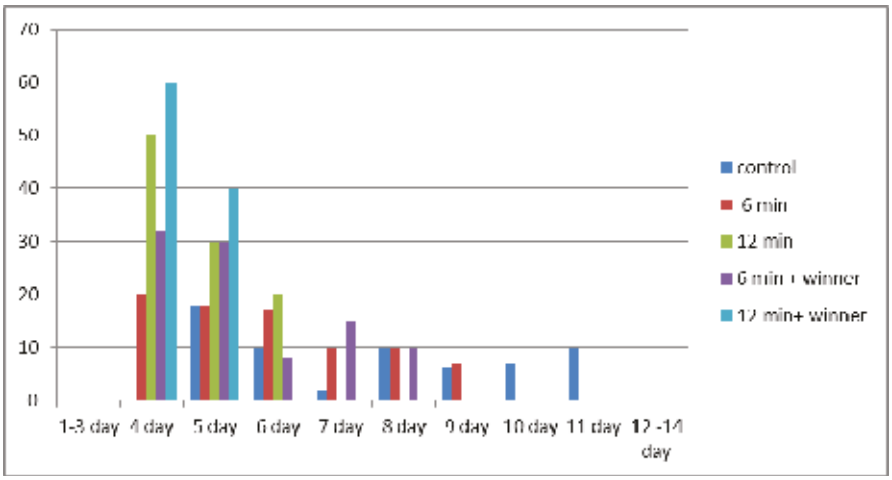

Figure 6. Germination of carrot seeds by day

The most cumbersome and fastest germinating seeds are at 12 minutes exposures, with 60 germination on day 4 and the remaining 40 on day 5.

The biostimulator effect is also well-known in 6 minutes exposure, on the 6th day (3 days after germination) the number of germinated seeds recorded was 27.3% higher than the other variant with the same treatment.

In Figure 7 shows the data on laboratory germination of parsley seeds.

The highest percentage of laboratory germination was reported in the 6 minutes exposition test, with the biostimulator reading being 90%.

The difference between the two 6 minutes exposure variants is 10%. In the control, the germination rate was 60%.

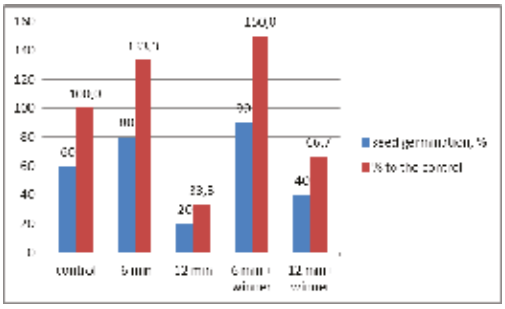

Figure 7. Laboratory germination (%) of parsley seeds averaged over the period 2016-2017

The results of Figure 8 it is noted that seed germination begins from day 6 until day 11, at the earliest 6 minutes biostimulator exposition and at 12 minutes exposure at the latest. The control sprout starts on the 9th and ends on the 14th.

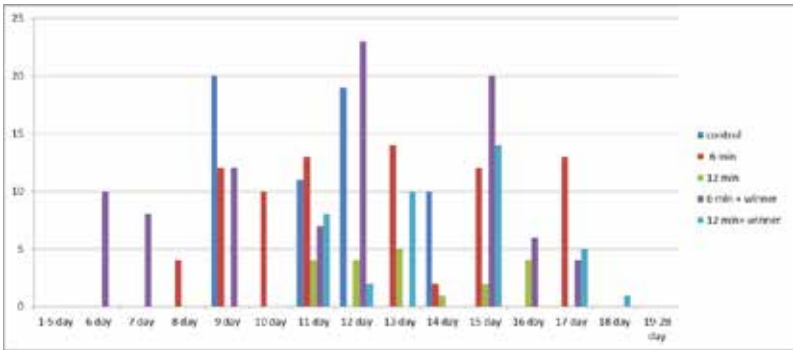

Figure 8. Sprouting seeds of parsley by day for the whole period

In Figure 9 the results for laboratory germination of celery are presented. Under the influence of ultrasound stimulation germination of seeds strongly increases.
At 0% of the control, laboratory germination increases with increasing treatment duration and reaches 70% in the 12 minutes exposure scenario. 6% lower is the germination reported in option 2 (6 minutes).

As far as the biostimulator is concerned, it has a negative effect, because in both embodiments involving this additive there are reported significantly lower results compared to the distilled water variants.
It is noteworthy that even here the tendency to increase germination with an increase in ultrasonic processing is maintained. Although 9% of the benefit is for the 12 minutes option.

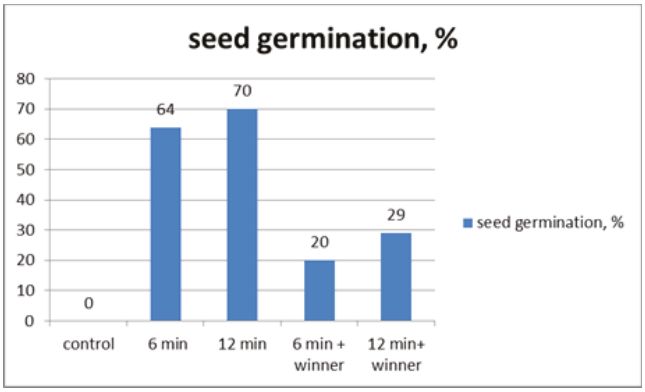

Figure 9. Laboratory germination (%) of celery seeds, average for the period 2016-2017

The germination process (Figure 10) starts at the earliest with 12 minutes exposures - 8th day for variant 3 (12 min.) and 9th day for variant 5 (12 min. + Winner). In the same two variants seed germination ends on day 17 on day one and on day 18 in the second variation.
The first germinated seeds in the 6-minute exposure variants were counted on the 13th day after treatment, with no new germinated seeds being reported for the biostimulator addition until 19th day. Interestingly, the results are reported for option 2. It is clear from the graph that, despite the later onset of germination (on day 13), the number of sprouts at each subsequent counting increases and reaches it's maximum on the 17th day - 35 pieces.
This option is the shortest and the start-end period of germination - 5 days, for which 64 sprouted seeds have been reported. When compared to option 3 (12 minutes), where the highest germination rate was recorded, it is seen that the period is 10 days. Apparently 6 minutes seed treatment with ultrasound helps to seed the seeds more comfortably, and this is essential for obtaining quality and even plants.

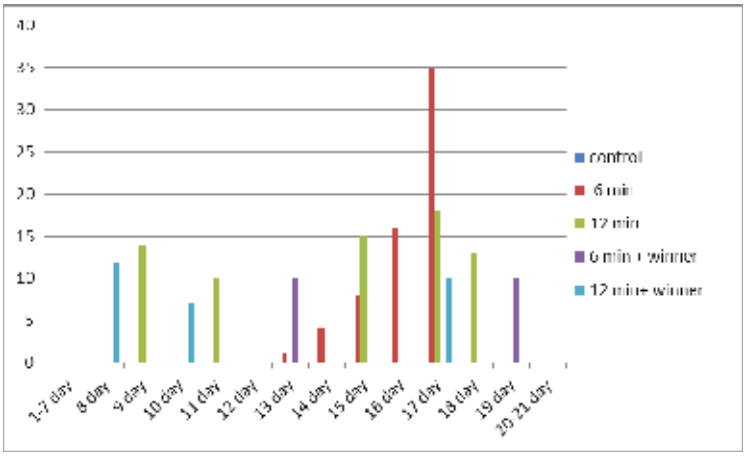

Figure 10. Germination of celery seeds by day for the whole period

CONCLUSIONS

The biostimulator effect is different for the three cultures and depends on the duration of the ultrasound treatment.

In carrots and parsley, the addition of biostimulator increases germination by up to 13% in carrots and by up to 20% in parsley.

It should be borne in mind that adding a biostimulator can also have a negative effect.

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EFFECT OF FOLIAR BIOACTIVE TREATMENTS ON THE OXIDATIVE STRESS TOLERANCE IN TOMATO SEEDLINGS

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Abstract

Under the current climate changes, enhancing temperature is now considered to be one of the major abiotic stresses inducing negative effects on plant growth as results of increased production of toxic reactive oxygen species. The tolerance of stress is correlated with higher activities of antioxidant defense enzymes which are activated to prevent oxidative damage. On the other hand, the use of biofertilizers appears to be involved in increase of tolerance to various abiotic stresses, besides their important role in regulating plant growth, development and fruiting. Influence of environmental conditions and of some growth regulators treatments (Spraygard 1%, Razormin 0.1%, BAC Foliar spray 0.3% and BIO Roots 0.2%) on the chlorophylls and carotenoids content and on the activity of peroxidase and catalase have been investigated in leaves of tomato seedlings. Analysis of the obtained data emphasize the potential of growth regulators in enhancing the resistance to abiotic stresses by protecting the photosynthetic apparatus and improving the activity of antioxidant enzymes system.

Key words*: carotenoids, catalase, chlorophylls, heat stress, peroxidase.*

INTRODUCTION

Tomato (*Solanum lycopersicum*) is considered one of the most important vegetable whose production is constantly growing since its fruits are widely consumed either fresh or processed. Beside the high nutritional value, the ripe tomato fruits are a valuable source of vitamin C, lycopene, carotenoids and minerals such as iron and phosphorous that are daily required for a healthy diet (Nour et al., 2013). At present, the tomatoes acquired great popularity among consumers, being considered a protective food since the discovery that lycopene has anti-oxidative and anti-cancer properties (Gajowik, 2014; Raiola et al., 2014).

Although tomato plants can grow under a wide range of climatic conditions, they are extremely sensitive to growing conditions: high temperature (both day and night), humidity, rainfall and light intensity are the limiting factors of tomato production (Ahmad, 2002).

Temperature stress induces negative effects on plant growth and metabolism, so high temperature is now considered to be one of the major abiotic stresses causing yield reduction in crops (Hasanuzzaman et al., 2012).

Recently some countries practice tomato growing even at high temperature through application of plant growth regulators. Several authors reported that application of substances like auxin, gibberellic acid, synthetic auxin 4-CPA (4-chloro phenoxy acetic acid) have resulted in good tomato production under adverse environmental conditions (Sasaki et al., 2005; Gemici et al., 2006; Khan et al., 2006; Poliquit et al., 2007; Gelmesa et al., 2012). Also good results in improving freezing resistance by growth regulators as Ruter AA, Terra Sorb and Razormin were noticed in experiment with winter wheat cultures (Gaveliene et al., 2016).

Besides their important role in improvement of nutritional quality of food crops and their efficiency to regulate plant growth, development, fruiting and senescence (El-Rokiek et al., 2012), it seems to be involved in induction of tolerance to various abiotic stresses (Salehi et al., 2011; Gaveliene et al., 2016).

Plants exposed to extreme temperatures activated the self-defense mechanisms including several non-enzymatic and enzymatic antioxidants (α-tocopherol, carotenoids, chlorophylls, glutathione, ascorbic acid,

oxidases such as catalase, peroxidase, polyphenol peroxidase, superoxide dismutase) to prevent oxidative damage (Vranova et al., 2002; Torres-Barceló et al., 2013). Some scientific works reported that higher activities of antioxidant defense enzymes are correlated with higher stress tolerance (Almeselmani et al., 2006; Babu et al., 2008; Almeselmani et al., 2009).

This study was aimed to investigate the effect of growth regulators containing free amino acids, macro- and micro-elements (Razormin, Spraygard, BAC Foliar spray, Bio Roots) used as foliar treatments on tomato seedlings and the comparative results were studied. The effect of the bioregulators on antioxidant enzymes and photosynthetic pigments in tomato plant is less known, so that the influence of environmental conditions and of some foliar treatments on biochemical parameters (chlorophylls and carotenoids content, activity of peroxidase and catalase) has been investigated in leaves of tomato seedlings.

MATERIALS AND METHODS

The experiment was installed into an experimental greenhouse of the Hortinvest Research Centre - USAMV Bucharest. The main objective was testing the effects of applications of simple growth regulators Razormin 0.1%, Spraygard 1%, BAC Foliar spray 0.3% and BIO Roots 0.2% on heat stressed and unstressed tomato seedlings in two distinct stages: one week, respectively two weeks after transplant operation.

Razormin is a mixture of growth factors (amino acids, polysaccharides, macro and micro-nutrients), which induces development of the root system, stimulate the nutrients absorption, increases vegetative mass and quality of production.

Spraygard is a complex product that acts as safener, penetrant, dispersant, creates adhesion of the treatment solutions on the leaves.

BAC Foliar is a foliar organic nutrient which stimulates chlorophyll production in the leaves.

Bio Roots is a natural root growth supplement which contains vitamins, enzymes, organic and humic acids that helps plants establish healthy and vibrant roots.

Experimental variants were noted:

T0 - unstressed control;

T1 - tomato seedlings exposed at heat stress in absence of growth regulators treatment;

T2 - tomato seedlings exposed at heat stress and treated with Spraygard 1%;

T3 - tomato seedlings exposed at heat stress and treated with Razormin 0.1%;

T4 - tomato seedlings exposed at heat stress and treated with BAC Foliar spray 0.3%;

T5 - tomato seedlings exposed at heat stress and treated with BIO Roots 0.2%.

Specific agrotechnics for transplant nursery was applied during the growth period: daily ventilation, watering, weeding. The unstressed control variant was maintained at the temperature of 22-26^{o}C at day and 18-20^{o}C at night. For the heat stressed variants, the temperature was not controlled and registered the following variations:

- the maximum effective temperature average for April in the greenhouse was 31.8^{o}C and the minimum effective temperature average at 24.6^{o}C;
- the maximum effective temperature average for May in the greenhouse was 37.4^{o}C and the minimum effective temperature average at 25.2^{o}C.

The biochemical determinations in the active leaves were performed at the end of the experiment (after 27 days), when most of seedlings have reached the optimum for a succesfull planting.

In order to estimate the oxidative stress occurred on cell level, characteristic parameters were analyzed, such as proteins content, specific activities of catalase as well as peroxidase, assimilatory pigments content using appropriate methods of analysis.

- ***The proteins content*** was determined by Lowry method, which is based on the reactivity of the peptide nitrogen with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteu phosphomolybdic-phosphotungstic acid to heteropoly-molybdenum blue by the copper-catalyzed oxidation of aromatic aminoacids (Lowry et al., 1951). The results were expressed in g/100 g fresh weight.
- ***The activity of peroxidase*** was determined by spectrophotometric measuring of the speed of colour achievement at 436 nm

and 25°C in the decomposition reaction of hydrogen peroxide with guaiacol as hydrogen donor, which is catalyzed by peroxidase (Bergmayer, 1974).

$$4\ \text{guaiacol} + 4\ H_2O_2 \xrightarrow{\text{peroxidase}} \text{tetraguaiacol} + 8H_2O$$

The enzymatic unit: the amount of enzyme which catalyzed the transformation of one micromole of hydrogen peroxide/minute at 25°C.

- ***The activity of catalase*** was determined with method essentially described by Beers and Sizer (1952), in which the disappearance of peroxide is followed spectrophotometrically at 240 nm.

The enzymatic unit: one unit decomposes one micromole of H_2O_2 per minute at 25°C and pH 7.0 under the specified conditions.

- ***Determinations of the assimilatory pigments content*** in the active leaves**:** c*hlorophyll* and *carotenoid pigments* were extracted in 80% acetone and the absorbance of the extract was measured at three wavelengths (663 nm, 647 nm and 480 nm) with an UV/Visible ThermoSpectronic Helios spectrophotometer. The results were calculated using the extinction coefficients and equations described by Schopfer (1989) and were expressed in mg/100 g fresh weight (FW).

RESULTS AND DISCUSSIONS

Temperature is a major factor affecting the rate of plant development. Warmer temperatures expected with climate change will affect the physiological processes in plants, therefore the plant productivity. Some adaptation strategies are available to manage with temperature extremes depending on the plant species, being genetically determinate. Beside this, application of some bioregulators is expected to increase tolerance to high temperature stress. So analyze of assimilatory pigments and peroxidase and catalase activity as oxidative stress markers was performed.

Determination of assimilatory pigments content

Chlorophylls **a** ***and*** **b** represent the major photosynthetic pigments in plants, playing an important role in the photochemical reactions involved in photosynthesis (Taiz and Zeiger, 2009), while carotenoids are considered as accessory components in the photosynthetic complex by providing photoprotection and stability of proteins present in the photosystem (Torres-Netto et al., 2005; Simkin et al. 2008).

Photosynthesis, one of the most heat sensitive processes, can be completely inhibited by high temperature possibly as a result of structural and functional disruptions of chloroplasts and reduction of chlorophyll accumulation under high temperature stress (Camejo et al., 2001; Dekov et al., 2000).

The determinations performed on the tomato seedlings showed that high temperature affected both chlorophylls *a* and *b*, therefore the total chlorophyll content. A decrease of about 40% was registered in the untreated plants exposed to heat stress (T1) compared to the unexposed control plants (T0) (Figure 1).

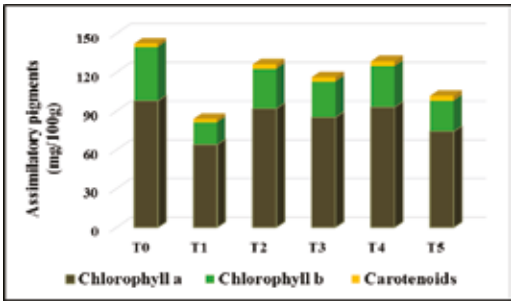

Figure 1. Assimilatory pigments content in the experimental variants

The effect of high temperature exposure on chlorophyll content has been extensively studied and similar results were reported at some tomato cultivars (Berova et al., 2009) and also in other plants as *Triticum aestivum* (Tewari et al., 1998; Almeselmani et al., 2012), *Festuca arudinacea* (Cui et al., 2006), *Solanum* ssp. (Aien et al., 2011). Other authors have found an increase in chlorophyll *a* content in lemon stressed plants with high temperatures (Martin et al., 1995).

A higher reducing in chlorophyll *b* content compared to chlorophyll *a* content (Table 1) was registered, so the chlorophyll *a*/*b* ratio registered an increase in the stressed plants compared with control plants, in according to the data reported by other authors (Cui et al., 2006; Zhu et al., 2011).

Table 1. Values and ratio of assimilatory pigments in the experimental variants

Variants	Chlorophyll (a+b) (mg/100 g FW)	Carotenoids (mg/100 g FW)	Chlorophyll (a+b)/ carotenoids	Chlorophyll a/b
T0	139.66	2.52	55.42	2.34
T1	81.25	2.66	30.55	3.70
T2	122.67	3.45	35.56	2.96
T3	112.68	3.57	31.56	3.08
T4	124.67	3.77	33.07	2.92
T5	98.06	3.71	26.43	3.12

Scientific studies reported that a 10-15^{o}C increase over normal growth temperature results in degradation of chlorophyll and thus affecting photosynthesis process. The reasons for decreasing in photosynthetic pigments under high temperature may be attributed to the inhibition of biosynthesis, changes in ultrastructure of chloroplast and photo-deterioration (Tewari et al., 1998; Reda et al., 2011).

Observations and measurements performed on the experimental variants under growth regulators treatment showed a smaller diminution of total chlorophyll content in stressed plants: about 11% in the plants treated with BAC Foliar (T4) and Spraygard (T2) compared to the unexposed control plants (T0). Generally, it seems that the growth regulators treatment induced a better accumulation of chlorophylls in the tomato leaves.

Carotenoids are an important class of antioxidants which play a major role in the protection of plants against photo-oxidative processes (Stahl et al., 2003; Gramza-Michalowska et al., 2010). Therefore, maintaining a higher or invariable level of total carotenoids during stressful conditions may induces some stress tolerance of the plants (Loggini, 1999; Logan et al., 1996; Ruban, 1999). Carotenoids destruction through oxidation may reduce efficiency of the antioxidant defense system (Chedea et al., 2013).

A slight increase of the carotenoids content was registered as result of heat exposure of the tomato seedlings (Table 1): 2.66 mg/100 g FW carotenoids determined in stressed control in absence of treatment (T1) compared to 2.52 mg/100 g FW carotenoids in unstressed control. Also a decrease of *chlorophyll (a+b)/carotenoids* ratio was registered in the stressed plants (Table 1). Previous studies reported lower *chlorophyll (a+b)/carotenoid* ratio in two heat stressed cultivars of *Festuca arudinacea* in relation to the control plants (Cui et al., 2006) and carotenoids amounts increased in *Populus cathayana* cuttings exposed under moderate stress conditions (Xiao et al., 2008).

However, the increase of carotenoids amounts was more pronounced in the stressed tomato plants under growth regulators treatment (between 3.45 mg/100 g FW in Spraygard treated variant and 3.77 mg/100 g FW in BAC Foliar treated variant) compared with untreated control (2.66 mg/100 g FW at T1). Similar observations of bioregulators treatment increasing carotenoids content were reported in some species of pepper plants (*Capsicum annuum* var. *grossum*, *Capsicum annuum* var. *accuminatum)*, in eggplant seedlings (Balan et al., 2018), while higher bioregulator concentrations impacted negatively on carotenoid content in *Capsicum chinense* plants (Olaiya et al., 2013), also in *Triticum aestivum* plants (Sahu et al., 2011). Total carotenoid content did not change with stress conditions in tomato cv. Amalia, while increased in Nagcarlang cv. (Camejo, 2001). This results suggests that the response to treatments with the bioregulators is probably genetically determined.

It is well documented that carotenoids act as antioxidant compounds involved in protection of photosynthetic systems, therefore a higher level of total carotenoids support the plant to tolerate the stressful condition. These results are in agreement with previous studies related to plant acclimation to stress (Loggini et al., 1999; Ruban et al., 1999).

Determination of content in proteins

Proteins content analyzed in the leaves of tomato seedlings showed that heat exposure induced an increase, so that higher values of this parameter were registered in the stressed

plants (1.37 g %) in absence of bioregulators treatment compared with the unstressed control (1.25 g %) (Figure 2). However, the increase of protein content was more pronounced in the leaves of the tomato treated with growth regulators (between 1.57-2.15 g %) in comparison to untreated stressed control (1.37 g %), BAC Foliar treated variant (T4) reaching the highest value of proteins amount (2.15 g %). Previous studies noticed that an increase in protein content might suggest a change in the gene expression that would be associated with a possible thermotolerance and acclimatization to stress condition (Camejo et al., 2001).

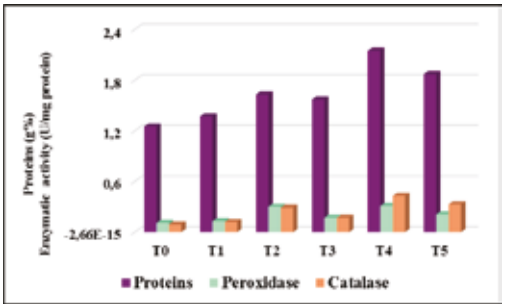

Figure 2. Enzymatic activity and proteins content in the experimental variants

Determination of enzymatic activity in the seedlings leaves

There are numerous previous studies which indicate that the tolerance to temperature stress in plants may be positively correlated with an increase in antioxidants content (Babu et al., 2008; Almeselmani et al., 2009; Hasanuzzaman et al., 2013).

A slight increased activity of ***peroxidase*** was registered in the untreated tomato seedlings under heat stress (0.13 U/mg protein) compared with control (0.11 U/mg protein), but response to heat stress was amplified in the tomato seedlings under growth regulators treatment by enhancing the peroxidase activity (between 0.17 U/mg protein in Razormin variant and 0.31 U/mg in BAC Foliar variant).

Catalase activity follows the same dynamics as peroxidase: 0.09 U/mg protein in control tomato seedlings, which increased at 0.12 U/mg protein in absence of bioregulators. Also the catalasic activity increased in the stressed plants treated with growth regulators. Higher catalase activities were registered in the tomato under treatment with roots stimulator BIO Roots (0.33 U/mg protein) and with BAC Foliar (0.43 U/mg protein).

Other authors also reported that some treatments of plants with plant growth regulators showed positive effects on oxidases activities. For example, methyl jasmonate-treated raspberries, strawberries and blueberries showed higher activities of peroxidase and superoxide dismutase (Chanjirakul et al., 2006).

Previous studies documented that the activation of protein synthesis in plants in combination with increase of oxidases activity under stress conditions may be the result of metabolism conversion in order to obtaining a good tolerance of plant to stressful conditions. (Tucic et al., 2007; Chkhubianishvili et al., 2011; Wu et al., 2014).

CONCLUSIONS

The researches performed on the tomato seedlings showed that high temperature affected both chlorophylls *a* and *b*, therefore the total chlorophyll content. At the same time it seems that the growth regulators induced a better accumulation of chlorophylls in the tomato leaves since a smaller diminution of total chlorophyll content in stressed tomato plant was noticed under growth regulators treatment.

A slight increase of the carotenoids content in the control plant occured as result of heat exposure but higher values of this parameter were determined in the stressed tomato plants under influence of growth regulators treatment.

Also the protein content and the oxidases activity were enhanced under high temperature conditions, mostly in the tomato seedlings treated with growth regulators.

An overview of the researches performed on the tomato seedlings allows us to conclude that growth regulators treatments determined an increased plant capacity to face the effects of heat stress by protecting the photosynthetic apparatus and enhancing antioxidant enzyme systems. Good results in this sense were obtained mainly with BAC Foliar spray and Razormin, which proved the best ameliorative effect under heat stress conditions.

However, further studies should be considered in order to asses possible combined treatments with several growth regulators to achieve optimum effects in improving the plants growth and productivity under environmental stress in the conditions of global climate change.

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DETERMINATION OF THE RESPONSES OF DIFFERENT TOMATO SPECIES TO *TUTA ABSOLUTA*

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Abstract

In this study, the harmful effect of Tuta absoluta on different tomato species was investigated. Six tomato genotypes which are Solanum chmielewskii (LA1028), Solanum neorickii (LA0247), Solanum hirsutum (LA777), Solanum pimpinellifolium (LA0722), and Solanum lycopersicum cv. Cuatomate and Solanum lycopersicum cv. Ailsa Craig (LA2838A) species were used as plant material in the study. To determine the incidence of Tuta absoluta on the plants, the egg and larvae were counted weekly by taking 5 leaves from the species. According to eggs and larvae counts, the plants were exposed to 5 types of severe damage while Solanum lycopersicum cv. Cuatomate was found to be later and less effective than the others. These results indicate that this species exhibits antixenosis properties against Tuta absoluta pests and that the other species that have been exposed to earlier and more severe damage lack such property.

Key words*: Tuta absoluta, Solanum species, antixenosis.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important food crops in the world in terms of human consumption, while total global production exceeds 5 million hectares worldwide with an average annual production of more than 170 million metric tons (FAO, 2017). However, declining agricultural areas and increasing pests and diseases are affecting yield and tomato fruit quality negatively. Chemical control methods have been trusted to control this insect, but the feeding habits of the larvae, the increasing number of resistant strains of this pest, together with the negative impact of the chemical into the environment makes the chemical control method not sustainable (Moreno, 2011; Cocco et al., 2012). Thus, the use of nature-friendly biological control methods and tolerance varieties has been preferred in recent years. Therefore, the implementation of environmentally safe measures that reduce the use of chemicals will contribute to the sustainability of productions. There are many insects, diseases and weeds which cause significant damage to tomato production. The most widespread of these are the whitefly (*Bemisia tabaci*), leaf miner (*Liriomyza trifolii*), and red spider (*Tetranychus urticae*) (Uygun et al., 1998). The tomato leaf miner is one of the major devastating pests of processing and fresh tomatoes, both in greenhouse and open field condition. *Tuta absoluta* larvae can absolutely destroy the tomato canopy by excavating the leaves, stems and buds; and burrows into fruits causing the quality decline of fresh tomato and yield loss (Cocco et al., 2012). It is an oligophagous insect that feeds on at least 39 species belonging to ten families, including *Amaranthaceae*, *Asteraceae*, *Chenopodiaceae* and *Poaceae* (Siqueira et al., 2000).

Other cultivated *Solanaceous* species host-plants are *Solanum tuberosum*, *S. melongena* and *Nicotiana tabacum* are cited (García and Espul, 1982).

Tuta absoluta was detected in Europe for the first time in the northern part of eastern Spain at the end of 2006. Since then, it has rapidly invaded other European countries, including Norway and threatening tomato cultivation (Sundheim, 2017), and spread throughout the Mediterranean area, including parts of North Africa and the Middle East where it immediately reached damaging levels and fruits (Desneux et al., 2010). At present, *T. absoluta* is considered to be one of the most devastating pests for tomato crops (*Solanum lycopersicum* L.) and a serious threat in all of these newly infested areas. It is still one of the major pests

in many areas of Turkey. As a result, it has damaged the tomato production, both in open field and greenhouse areas. *Tuta absoluta* larvae can absolutely destroy the tomato canopy by excavating the leaves, stems, and buds and burrows into fruits causing the quality decline of fresh tomato and yield loss that range from 50% to 100% (Cocco et al., 2012). According to literature, against *T. absoluta* many control strategies have been used such as cultural hygiene, pheromone, biological control and resistant genotypes. Nowadays, most of the European countries producers fulfil the GLOBALGAP requirements, which also implies more environmentally friendly technologies. One of the most important ways of sustainable nature-friendly growth is the development of disease-tolerant plants. The plants have shown 3 basic mechanisms against insects. One of them is antixenosis. According to this defensive mechanism, the plants cannot be damaged by insects in terms of the plant's characteristics and cannot feed and lay eggs on the plants. The second mechanism is antibiosis. This feature is a deterioration of the biology of the insect with respect to the characteristics of the plants. The third mechanism is tolerant plants. This attribute is different from the first two, in which case there is no relationship between plant and insect. As an example of this feature; wild relatives of tomato have been used as sources of insect resistance (Oliveira et al., 2009). Insect resistance is generally associated with the presence of trichome, of different types and densities (McDowell et al., 2011; Tissier, 2012; Glas et al., 2012). Antibiosis and antixenosis are the two resistance mechanisms reported in *S. hirsutum* (Weston et al., 1989; Channarayappa et al., 1992; Eigenbrode et al., 1993; Eigenbrode et al., 1994; Kumar et al., 1995). Sesquiterpenes present on leaf glandular trichomes of *S. hirsutum* f. typical, mainly type IV and VI, are commonly reported in the literature as the chemical causes of insect resistance in this genotype (Snyder and Hyatt, 1984; Lin et al., 1987; Carter et al., 1989; Eigenbrode et al., 1993; Eigenbrode et al., 1996; Snyder et al., 1998). Although several studies have shown that the accession LA1777 of *L. hirsutum* f. typicum constitutes a resistance source to tomato insect-pests, its resistance to *T. absoluta* and the variability of pest resistance among plants of this accession, especially as related to plant chemical composition, are unknown. Host plant resistance can be an important component of IPM programs. Resistant plants can help maintain pest populations below economic injury levels and are usually compatible with other control methods (Smith, 2005). Sources of resistance to several insect pests have been identified in wild tomato species (Oliveira et al., 2012; Bottega et al., 2015).

In this study, we investigated interactions among some different tomato genotypes and *Tuta absoluta* by assessing on number of larvae and eggs reared.

MATERIALS AND METHODS

In the experiment, *Solanum chmielewskii (*LA1028), *Solanum neorickii* (LA0247), *Solanum hirsutum* (LA1777) *S. pimpinellifolium* (LA0722), *Solanum lycopersicum* cv. Cuatomate, and *Solanum lycopersicum* cv. Ailsa Craig (LA2838A) were used as plant material. Seeds were germinated in a mixture of peat: perlite: vermiculite in 7:2:1 ratio. After 15 days, tomato seedlings at the second-true leaf stage were transferred to 8 L plastic pods included peat: perlite (2:1) mixture. The experiment was carried out in a glass greenhouse, with day temperature 28-30^{o}C, night temperature 20-22^{o}C and 60-70% relative humidity in summer time (June-August). Plants were grown up until 8-10 real leaf stages for *Tuta absulta* application in the greenhouse equipped with automatically-controlled side walls, ventilation fans and wet-pads for humidity control and in order to maintain a maximum temperature of 30°C. The seedling, irrigated with a nutrient solution of EC: 2.2-2.5 and pH: 5.5-6.0 were planted to 8 L pods after 7 days. The concentrations of the following elements were determined for the nutrient solution: (M): 3.0x/10-3 $Ca(NO_3)_2$; 0.9x/10-3 K_2SO_4; 1.0x/10-3 $MgSO_4$; 0.2x/10-3 KH_2PO_4; 1.0/10-5 H_3BO_3; 1.0x10-6 $MnSO_4$; 1.0x/10-7 $CuSO_4$; 1.0x/10-8 $(NH_4)_6Mo_7O_{24}$; 1.0x10-6 ZnSO4; 1.0x/10-4 Fe EDTA.

When the tomato plants reached 8-10 true leaf stage, were taken in a 2000 lux illuminated climate chamber with *T. absoluta* production

temperature of 25 ± 1°C and 60 ± 5% relative humidity conditions.

The plants were left in this room for 7 days with *T. absoluta*. After this period, the plants were moved into the insect-proof climate chamber under 25°C, 65% relative humidity, and 2000 lux illumination, where the phenotype experiments were performed for six weeks.

Six different genotypes were arranged in a randomized complete design with five replications. *T. absoluta* was regenerated one times a week and maintained by feeding the clean tomato plant, *S. lycopersicum* cv. Ailsa Craig. Each week 5 leaves were taken from each tomato genotype and eggs and larvae were counted at intervals of 7 days. Larvae and eggs were counted under a Leica® S6D stereo microscope. Other pictures were taken by Canon® D600 digital camera (Figure 1).

All analyses were performed with SPSS software package Version 18.0 (SPSS Inc., Chicago, IL) for Windows by General Linear Model univariate test. Normality of variance was checked before analysis, and the, mean was compared using an LSD test.

Egg Larvae Adult

Figure 1. Larvae, egg, and adult of *Tuta absoluta*

RESULTS AND DISCUSSIONS

According to results, LA1028, LA0247, LA1777, LA0722, and LA2838A were determined more sensitive than *Solanum lycopersicum* cv. Cuatomate to *Tuta absoluta* (Figure 2).

Figure 2. *S. lycopersicum* cv. Cuatomate and other used genotypes in the study at the 2nd week

However, in a previous study, the accession line LA1777 (*S. hirsutum*) was found more tolerant genotype to *Tuta absulta* (Ecole et al., 1999). To examine the survival ability of the insect the number of the eggs was determined for all genotypes. In the first-week egg counts, *S. chmielewskii*, S. *pimpinellifolium, S. lycopersicum* cv. Cuatomate, *S. neorickii*, *S. lyocpersicum* and *S. hirsutum* genotypes were found to have average 4.4, 3.0, 3.0, 3.4, 3.2 and 3.2 eggs respectively on the leaves. In the second week 0.8, 0.8, 0.8, 0.4, 1.2 and 1.2 eggs were observed respectively (Table 1).

After the second week only *S. lycopersicum* cv. Cuatomate survived, all other genotypes of the green canopy being completely destroyed by *Tuta absoluta*. For this reason, subsequent counts continued with *S. lycopersicum* cv. Cuatomate. The number of eggs during 6 weeks was 3.0, 0.8, 0.2, 0.4, and 0.0, respectively. It was observed that this resistant genotype died after the 5th week as well (Table 1).

Table 1. Number of the eggs according to tomato varieties during the six weeks (Sc: *S. chmielewskii,* Sp: *S. pimpinellifolium*, SlC: *S. lycopersicum* cv. Cuatomate, Sn: *S. neorickii*, Sl: *S. lyocpersicum,* Sh: *S. hirsutum*)

	Genotypes					
	Sc	Sp	SlC	Sn	Sl	Sh
1. Week	4.4	3.0	3.0	3.4	3.2	3.2
2. Week	0.8	0.8	0.8	0.4	1.2	1.2
3. Week	0.0	0.0	0.8	0.0	0.0	0.0
4. Week	0.0	0.0	0.2	0.0	0.0	0.0
5. Week	0.0	0.0	0.4	0.0	0.0	0.0
6. Week	0.0	0.0	0.0	0.0	0.0	0.0

In the first week of larval counts, *S. chmielewskii, S. pimpinellifolium, S. lycopersicum* cv. Cuatomate, *S. neorickii, S. lyocpersicum, S. hirsutum* genotypes were observed to have average 6.4, 3.6, 3.6, 2.8, 3.4 and 3.4 larvae, respectively. In the second week counts, mean 11.6, 6.2, 6.2, 4.4, 5.8 and 5.8 larvae were determined, respectively. After two weeks, only one tomato genotype (*S. lycopersicum* cv. Cuatomate) survived while other genotypes were found to be completely dead. On this genotype, the counts in the third, fourth and fifth weeks, the average number of larvae was found as 5.4, 5.8 and 7.4, respectively (Table 2).

As a result of both counting of eggs and larvae, 5 species were severely damaged by the *T. absoluta* attack, while *S. lycopersicum* cv. Cuatomate has been found to be more tolerant. These results indicate that S. *lycopersicum* cv. Cuatomate genotype could be more tolerant to *T. absoluta* then others.

Table 2. Number of the larvae according to tomato varieties during the six weeks (Sc: *S. chmielewskii,* Sp: *S. pimpinellifolium*, SlC: *S. lycopersicum* cv. Cuatomate, Sn: *S. neorickii*, Sl: *S. lyocpersicum,* Sh: *S. hirsutum*)

	Genotypes					
	Sc	Sp	SlC	Sn	Sl	Sh
1. Week	6.4	3.6	3.6	2.8	3.4	3.4
2. Week	11.6	6.2	6.2	4.4	5.8	5.8
3. Week	0.0	0.0	5.4	0.0	0.0	0.0
4. Week	0.0	0.0	5.8	0.0	0.0	0.0
5. Week	0.0	0.0	7.4	0.0	0.0	0.0
6. Week	0.0	0.0	0.0	0.0	0.0	0.0

It is known that besides the reactions of the wild lines tomato against the tomato leaf miner, different kinds of reactions are given within themselves in some commercial varieties as well. Indeed, in a study by Çekin and Yaşar (2015) they determined the life schedule parameters of *T. absoluta* on comercial tomato varieties of Newton, Caracas, Torry, and Simsek.

The oviposition rate of *T. absoluta* were significantly different among varieties. The lowest oviposition rate was found in Simsek tomato genotype. As a result, it was seen that tomato was less preferred than other tomato varieties.

As shown in Table 3, egg counts were found to be close to each other as a result of the first two weeks of counting and there was no statistical difference between them (P> 0.05).

Table 3. Mean the number of larvae and eggs of *T. absoluta* after 2 weeks (mean ± SE). Different letters indicate significant differences at LSD, p<0.05

	Eggs/per leaf	Larvea /per leaf
S. chmielewskii	2,60±0,670 **a**	9,00±1,170 **a**
S. pimpinellifolium	1,90±0,482 **a**	4,90±1,040 **bc**
S. lyc. cv. Cuatomate	2,20±0,772 **a**	1,60±0,221 **c**
S. neorickii	1,90±0,567 **a**	3,60±0,581 **bc**
S. lycopersicum	2,20±0,593 **a**	4,60±0,968 **bc**
S. hirsutum	2,90±0,888 **a**	6,00±0,943 **ab**
	***P= 0.883 > 0,05**	****P=0.000 < 0,05**

However, in the larva counts, low amount of larvae were determined on *S. lycopersicum* cv. Cuatomate and it was determined that the difference between the other species was statistically significant (P <0.05).

According to other studies, sources of resistance to several insect pests have been identified in wild tomato species (Baldin et al., 2005; Oliveira et al., 2012; Bottega et al., 2015), and the corresponding genes have been introgressed into commercial cultivars.

For example, tomato plants possess glandular trichomes that accumulate metabolites toxic to herbivorous insects (Weinhold and Balwin, 2011).

CONCLUSIONS

S. lycopersicum cv. Cuatomate tomato variety was determined to be more resistant to *T. absoluta* than other varieties. On the other hand, *S. chmielewskii*, *S. neorickii*, *S. hirsutum*, *S. pimpinellifolium* and S. *lycopersicum* tomato varieties had a high number of total eggs per female on the first two weeks (Table 3). We saw that the larvae did not choose the *S. lycopersicum* cv. Cuatomate when compared to others. For this reason, it is thought that this variety can be used by breeders for its resistance to *Tuta absoluta*.

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RESPONSES ABOUT SEED FORMATION AND SEED PRODUCTION IN DIFFERENT GENOTYPES OF CAPE GOOSEBERRY (*PHYSALIS PERUVIANA* L.)

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Abstract

The main goal of the present study was to be established the responses in seed formation and seed production in several genotypes of cape gooseberry (Physalis peruviana L.) with a target to predict the seed yield and its realization. The experiments were carried out in Agricultural University of Plovdiv, Bulgaria with six genotypes of the different origin. The number of seed per fruit, the percentage of normally developed seed, the weight of 1000 seeds, germination energy, germination, mean germination time, uniformity of germination, length of embryo root and hypocotyls and fresh weight and deviation in seedlings were established. The weight of seeds per kilogram fruit, the yield of fruit and seed also have been determinated. Significant variation in the numbers of seed per fruit was observed. High germination was an account of each genotype. The differences in seed production and sowing quality between genotypes were registered. These results can be applied for the prediction of seed productivity and also in the determination of the price of seed depending on the genotyping insemination characteristics.

Key words: *germination, seedlings, fruit, yield, germination time.*

INTRODUCTION

According to Geleta et al. (2005), the genetic diversity is very important in relation to increase the peculiarities of plant material in both scopes, to improve the production as well as use in breeding. The authors emphasized that the presence of more genotypes is considered as one of the criteria for the selection and for the reaching of the opportunities of the breeding programs. The importance and availability of rich genetic material of various origins, which are suitable to be included in selection activities are reported also from Martirosyan and Sargsyan (2014).

Many researches established strong genotypic response, studied a wide range of varieties in various vegetable crops (Haytova and Babricov, 2006; Haytova and Gergova, 2011; Todorova 2011; Todorova et al., 2011; Todorova and Pevicharova, 2012)

The genus *Physalis* covers on significant number of annual and perennial species, according to various authors between 70 (Christov, 2010) and 110 (Skvorcova, 1997). The most common and practical applications are three main species, namely *Physalis peruviana* L., *Physalis pruinosa* L., *Physalis ixocarpa* Brat. and *Physalis pubescens* L. (Moriconi et al., 1990; Crawford, 2004), the cape gooseberry (*Physalis peruviana* L.) being the most widely used. Christov (2010) is of the opinion that production and trade with cape gooseberry have increased over the last decades. Its cultivation in the countries of Europe has become more perspective, particularly appropriate for warmer climates and for small-scale farms (Paksi et al., 2007; Popova et al., 2010). According to this author (Christov, 2010), varieties as a product of targeted selection are practically absent and local forms and ecotypes are being grown massively, therefore it is necessary to investigate different genotypes and ecotypes under different conditions.

Experiments with a large number of genotypes of cape gooseberry outdoors and under controlled conditions are conducted by Abak et al (1994) in Chukurova, Turkey. Panayotov (2010) reports on the selection of the first variety of cape gooseberry in Bulgaria, called Plovdiv.

This researcher examines the adaptability of genotypes of *Physalis peruviana* L. with different origins, with a goal directed to identifying the most suitable for cultivation in

the region of Southern Bulgaria (Panayotov, 2016). McCain (1993) and Crawford (2004) reported that cape gooseberry is propagated most commonly by seeds. Chernok (1997) confirms the thesis that this crop is grown by seeds, pointing out that its cultivation very often takes place by direct sowing on the open fields. This requires to be provided a sufficient quantity of high-quality seeds.

Therefore the scientific researches into the process of seed production are necessary and must be carried out. In terms of seed production of the cape gooseberry, the published studies in the world literature are insufficient and very few. Lawrence (1993) points out that seed production of cape gooseberry is related to genotypic belonging, on the other hand, depends on the size of plants, which affects the quantity and vitality of the pollen and hence the seed productivity.

In the larger plants, sometimes the amount of pollen is reduced, but because they develop more flowers, their number of seeds is higher, in opposite to smaller ones. In order to increase seed production, the author recommends additionally applying manual pollination to support the natural ones.

The effect of this manipulation is much higher in genotypes that develop weaker plants, as they also rely on a small number of flowers. Thomson and Wit (1987) investigated the optimal temperature regime for both the growth and the germination of the seeds of two types of physalis - *Physalis angulata* L. and *Physalis virginiana* L. var. *underglared*. They establish a strong species and genotype response, both in seed set up and seed development, as well as the temperature regime for maximum sprouting.

The main goal of the present study was to achieve a comparative evaluation of developmental features, productivity and vitality status of the seeds of different genotypes of cape gooseberry (*Physalis peruviana* L.) in relation to predicting of the seed yield and the need for a specific approach for its realization.

MATERIALS AND METHODS

The experiments were carried out in 2015-2017 years in the Scientific field and laboratories of Department of Horticulture at the Agricultural University of Plovdiv, Bulgaria with several genotypes cape gooseberry (*Physalis peruviana* L.) with different origin:

1. Variety Plovdiv - origin from Bulgaria;
2. Genotype Obrazec 1 - origin from the USA;
3. Genotype Obrazec 2 - origin from the USA;
4. Genotype Obrazec 3 - origin from the USA;
5. Genotype 08-2010 - origin from Turkey;
6. Genotype 11-2012 - origin from Germany.

The plants were grown by unpricked seedlings, produced in a plastic unheated greenhouse with sowing on March 15th. The seed rate per one hectare was 80-100 g, and per square meter was sown at 1.5 g. The planting took place between May 20-25 on furrows, on the scheme 70 x 50 cm (Panayotov and Tcorlianis, 2000). All necessary agro-technological practices were applied during vegetation. The experiments took place in four replicates with 25 plants in each and the size of the experimental plot was 9 m^2.

At the stage of full botanical maturity, all the ripe fruits were harvested and their yield was determined. The seed was extracted and the seed yield was established. The seed yield from one kilogram of fruit was studied. Separately on 5 fruits from each replicates all seeds were extracted.

The following indicators: number of seeds per fruit - determined by counting the seeds of five fruits of each replicates; percentage of fully developed seeds in one fruit (insemination) - by counting of the normally developed seeds and calculating their percentage to the total number of seeds per fruit were registered.

The linear seed sizes - length, width and thickness of seed, measured on 15 seeds with electronic calliper; weight of one seed - set on 15 seeds, weight of 1000 seeds (ISTA, 2013), in 4 replicates; germinating energy (ISTA, 2013), in 4 replicates; germination (ISTA, 2013) in 4 replicates; uniformity of germination (according to Strona, 1966); mean germination time (MGT) - in four replicates, each of 100 seeds, calculated according to the equation given below were determinated.

$$M.G.T. = \frac{\Sigma (G \times T)}{F}$$, where:

T - day in which the seed is germinated;
G - numbers of seeds that germinated in this;
F - final number of germinated seeds (Battle and Whittington, 1969).

On the day of counting of germination in, the four replicates: the fresh mass of a seedlings, by measuring all developed seedlings; length of the embryo root and of the hypocotyl - by measuring 10 seedlings from each replicates; deviations from the normal structure of the seedlings by Welington (1970) - short embryo root, lack of branches in embryo root, lack of hairs on the embryo root, undeveloped cotyledons, unopened cotyledons, lack of hypocotyl were established.

Data of the study were subjected to analysis of variance, and the least significant differences between means were calculated by the Fisher test at p = 0.05. A method for ANOVA and a method for dispersion are described in Fowel and Cohen (1992). Due to the one-way trend of the results of all vegetation, the data presented are average values in the course of three years.

RESULTS AND DISCUSSIONS

In the number of seeds setting up in one fruit the genotypic differences were established (Table 1). The variation on this index is significant, as results ranging from 61.5 for Obrazec 1 to 145.7 for genotype 11-2012. Relatively smaller numbers of seed there were developed in variety Plovdiv (73.0) and in selecting line 08-2010 (88.5) numbers. The average numbers of seeds per fruit for all tested genotypes is 90.7. The variation against this mean value between genotypes with the lowest number of seeds and the highest one is between -29.2 and +35. The difference between the highest and the lowest, established result, for the number of seeds in the experimental genotypes is even greater, almost double (64.2) numbers. The differences between the variants are of statistical significance with exception of those between Obrazec 2 and Obrazec 3 and between Ovbrazec 3 and line 08-2010.

Besides inseminating of the fruit, the quantity of fully developed seeds is of particular importance. In almost all tested cape gooseberry genotypes, most seeds are fully developed. For Plovdiv variety and Obrazec 3, they reached up to 100.0%.

Despite the highest number of seeds found in the fruit of line 11-2012, they demonstrated the least development (93.6%) of the total seed set up, reached to the stage of full formation. Both the setup and development of seed are directly related to the pollination and fertilization processes. Similarly, opinion has Lawrence (1993), who found differences in seed production of the cape gooseberry that associated with both genotypic belonging and plant development and pollination.

The weight of 1000 seeds is one of the important indicators for determining their sowing qualities. The average value for all included in this experimental genotypes was 1.06 g, and the variations in this indicator were relatively low. With the highest mass of 1000 air dry seeds is characterized Plovdiv variety (1.14 g), followed by Obrazec 3 (1.11 g). The lowest, although with a small difference was the weight of 1000 seeds of line 11-2012 (1.0 g), where was mentioned above that the highest number of seeds in one fruit was reported. There were also shown to be of a low value in the seed of Obrazec 1 (1.03 g).

Table 1. Cape gooseberry seed formation

№	Genotype	Seed number/fruit	% of normal developed seeds	Weight of 1000 seeds (g)
1	Plovdiv	73.0	100.0	1.14
2	Obrazec 1	61.5	97.7	1.03
3	Obrazec 2	99.8	97.8	1.05
4	Obrazec 3	95.9	100.0	1.11
5	08-2010	88.5	95.9	1.04
6	11-2012	125.7	93.6	1.00
	LSD p=0.05%	12.2		0.4

Besides, as a seed material, according to Zhang and Wen (1996), the cape gooseberry seeds can be used as an indicator for taxonomic identification between species and genotypes of the genus *Physalis*. In the experiments conducted, however, significant differences in seed morphology were not established (Table 2). The weight of one seed was changing to a narrow range from 1.15 mg (Obrazec 2) to 1.28 mg (Obrazec 1). A similar tendency was also observed for the width of the seed from 1.0 mm to 1.4 mm, as well as the thickness from 0.45 mm to 0.5 mm. Significant, though slight, differences are observed at the length of the seeds. On average, for the explored sample of genotypes, the length of one seed is 1.92 mm. Deviations from this mean value are from -0.21 for Obrazec 3 to + 0.23 for genotype 08-2010.

Table 2. Morphology behaviours of cape gooseberry one seeds

№	Genotype	Weight (mg)	Length (mm)	Width (mm)	Thickness (mm)
1	Plovdiv	1.23	1.99	1.2	0.5
2	Obrazec 1	1.28	1.84	1.4	0.5
3	Obrazec 2	1.15	1.86	1.2	0.45
4	Obrazec 3	1.25	1.71	1.1	0.45
5	08-2010	1.21	2.15	1.3	0.45
6	11-2012	1.17	1.99	1.0	0.45
	LSD p=0.05%	0.2	0.31	0.2	0.18

The most important indicator for sowing qualities and for seed development is their vitality (Table 3). From genotypes included in the experiment, it can be pointed out that they all have very high germinating energy between 74.0% for genotypes 11-2012 to 98.7% for Plovdiv variety. On this background, the germinating energy for Obrazec 2 and Obrazec 3 was also relatively high 95.3% and 96.7%, respectively. As Balck et al. (2008) and Copeland and McDonalds (2001) emphasized the seeds that have demonstrated higher germinating energy are with better vital performance because they have germinated for a shorter period.

Thomson and Wit (1987) reported that the seed of the cape gooseberry is characterized by high germination, and yet at the eighth week after flowering, it reaches over 90%. In this experiments the germination does not differ significantly from the values of the germination energy, indicating that most seeds are with high vitality. An exception was observed for the seeds of line 11-2012, where the germination exceeds more the data for energy with 12.7%. The highest germination was recorded for the seeds of the Plovdiv variety and for Obrazec 1 (98.7%), and the lowest one at 11-2012 (86.7%). Mathematical proof of differences is established. The statistical significance between Obrazec 1 and Obrazec 2 was not observed.

In connection with the more prices study of the seed qualities, the indicator mean germination time and uniformity of germination (Table 4) are of great importance. The mean germination time was the highest in Plovdiv variety (4.38 days), followed by the seed on genotype 08-2010 (4.44 days). The seeds of Obrazec 2 germinated most slowly, for 5.52 days. This indicates that the mean germination time is relatively high. The uniformity of germination range from 23.69% for line 08-2010 to 32.89% at 11-2012. High uniformity was established in the Plovdiv variety and also in Obrazec 2 over 29%. This data once again confirm the opinion that the cape gooseberry seeds has a good vitality and ability to germinate and that their high uniformity, as Panayotov (2015) and Black et al. (2008) maintains, it will allow them to better overcome the resistance of soil during sprouting.

The morphological characteristics of the seedling (Table 5) contribute to a more detailed clarification of the seed vitality status. In the fresh weight of the seedling of one seed, the differences are more significant.

Table 3. Germination behaviours of cape gooseberry seeds

№	Genotype	Germination energy (%)	Germination (%)
1	Plovdiv	98.7	98.7
2	Obrazec 1	93.3	96.0
3	Obrazec 2	95.3	96.0
4	Obrazec 3	96.7	98.7
5	08-2010	93.3	96.7
6	11-2012	74.0	86.7
	LSD p=0.05%	2.6	2.2

Table 4. Sowing parameters of cape gooseberry seeds

№	Genotype	MGT (days)	Uniformity (%)
1	Plovdiv	4.38	29.78
2	Obrazec 1	4.85	24.0
3	Obrazec 2	5.52	29.33
4	Obrazec 3	4.66	24.67
5	08-2010	4.44	23.69
6	11-2012	4.64	32.89
	LSD p=0.05%	1.1	6.2

The weight of the seedling indicates the possibilities of seed to germinate more easily and is very often used to determine their vigour (Copeland and Mc Donalds, 2001; Panayotov, 2015).

The values range from 14.8 mg for Obrazec 1 and line 08-2010 to 18.3 mg for Obrazec 2.

The length of the embryo root for the seed of line 11-2012 is the highest and it's reaches to 3.92 cm. Next one is those of Obrazec 3 with 3.41 cm.

Slightly developed was the embryo root of the line 08-2010 (2.89 cm).

The seedlings of Obrazec 2, Obrazec 1 and 08-2010 are characterized by the highest length of the hypocotyl 3.91 cm, 3.84 cm, and 3.78 cm, respectively.

The smallest one was in line 11-2012 (3.4 cm).

These results unambiguously show that the variation between individual genotypes is weak.

Table 5. Morphological characteristics of cape gooseberry seedling

№	Genotype	Fresh weight (mg)	Length of hypocotyl (cm)	Length of embryo root (cm)
1	Plovdiv	17.6	3.69	3.15
2	Obrazec 1	16.9	3.84	3.31
3	Obrazec 2	18.3	3.91	3.34
4	Obrazec 3	14.8	3.47	3.41
5	08-2010	14.8	3.78	2.89
6	11-2012	15.2	3.4	3.92
	LSD p=0.05%	4.2	2.6	1.9

It is normally some of the seedlings to have different deviations from their normal morphology (Table 6). The percentage of abnormally seedling grown was the highest in Obrazec 2 (15.6%), followed by line 11-2012 (12.3%). The lowest values were recorded for the Plovdiv variety. The most common types of deviation are the lack of root hair, from 22.3% for 11-2012 to 52.0% for Plovdiv, calculated towards all not well-developed seedlings which were accepted for 100%. The lack of branching in the root, take the next place, most often observed in line 08-2010 to (46.6%) and in the Plovdiv variety (36.6%). Minor deviations are associated with lack of hair on the hypocotyl, unopened cotyledons, and short embryo root.

Table 6. Deviation of normal developed seedlings (%)

Genotypes	%	Short embryo root	Lack of branches on embryo root	Lack of heirs on embryo root	Undeveloped cotyledons	Unopened cotyledons	Lack of hairs on hypocotyl
Plovdiv	10.5	0.0	36.6	52.0	0.0	11.4	0.0
Obrazec 1	13.2	17.3	24.8	31.4	0.0	15.2	11.3
Obrazec 2	15.6	9.66	15.5	36.6	22.0	15.6	0.0
Obrazec 3	11.2	9.30	10.5	30.3	3.30	46.6	0.0
08-2010	10.8	0.0	46.6	33.0	7.66	12.3	0.0
11-2012	12.3	0.0	35.0	22.3	4.20	38.5	0.0

For agronomic and economic evaluation in relation to prediction of the expected seed yield the seed quantity in one kilogram of fruits in botanical maturity was determined, and it with an average of 30.69 g (Table 7) for the studied genotypes. Most of the seeds of one kilogram of fruit were obtained in Obrazec 3 (37.95 g), and in the Plovdiv variety (34.85 g). Relatively high results were observed for line 08-2010 (31.52 g). The least one in their quantity in one kilogram of the fruit was of Obrazec 1 (20.66 g). The statistical significance of the differences between the tested genotypes is established with exception of that between Plovdiv and Obrazec 3.

The ratio between the quantity of fruit and the seeds extracted from them contributes to a complete assessment of the insemination and indicates how many fruits are necessary to be extracted in order to be obtained an adequate quantity of seeds. A high genotypic response has been observed since the values of this marker are in a very large range from 26.35 for Obrazec 3 to 48.40 for Obrazec 1, or an average for the tested population is 33.85.

The seed yield of the cape gooseberry is directly related to the yield of fruit (Table 8). The highest yield was harvested in the Plovdiv variety, it is 2245.5 kg ha^{-1}, followed by Obrazec 2 (2055.0 kg ha^{-1}). For other genotypes it is between 1581.8 kg ha^{-1} to 1668.3 kg ha^{-1} for Obrazec 3 and for Obrazec 1, respectively.

The genotypic features of cape gooseberry also occur with respect to the obtained seeds. Most seeds are obtained from Plovdiv variety with 78.2 kg ha^{-1}, followed by Obrazec 2 with 63.2 kg ha^{-1}. This mainly could be due to the higher yield of fruits and on the other hand to the relatively low ratio fruits: seeds. The least yield, 34.4 kg ha^{-1}, was recorded for Obrazec 1, the genotype with the lowest fruit productivity and a quite high ratio of fruit: seed weight. The average seed yield of the tested genotype of cape gooseberry was 55.51 kg ha^{-1}. The differences according to seed productivity between Plovdiv and Obrazec 1 and Obrazec 2 and also between Obrazec 1, and Obrazec 2 and Obrazec 3 are mathematically proven.

These results can be used to predict seed productivity and, at the same time, to determine the selling price of seeds, depending on genotypic characteristics. For samples with a lower seed yield and a higher ratio between the quantities of fruits and the seeds obtained therefrom the price is appropriate to be higher, because the input costs for fruit production are almost the same between separate genotypes. Furthermore, for those with low seed yields, there are additional costs associated with extraction of more fruits to be obtained the corresponding amount of seed.

Table 7. Productivity behaviours of cape gooseberry genotypes

№	Genotype	Seed yield/ 1 kg fruits (g)	Ratio fruit : seeds
1	Plovdiv	34.85	28.69
2	Obrazec 1	20.66	48.40
3	Obrazec 2	30.79	32.47
4	Obrazec 3	37.95	26.35
5	08-2010	31.52	31.72
6	11-2012	28.37	35.24
	LSD = 0.05%	4.4	

Table 8. Productivity of cape gooseberry

№	Genotype	Yiled of fruit (kg $ha^{-1)}$	Yiled of seed (kg ha^{-1})
1	Plovdiv	2245.5	78.2
2	Obrazec 1	1668.3	34.4
3	Obrazec 2	2055.0	63.2
4	Obrazec 3	1581.8	59.9
5	08-2010	1642.2	51.8
6	11-2012	1599.1	45.6
	LSD p=0.05%	120.1	14.0

CONCLUSIONS

Genotypic differences were found in the number of seeds in one fruit of cape gooseberry.

The percentage of fully developed seeds for all cape gooseberry lines is high. Slight differences are noted with respect to the morphological features of the seed.

The seeds of the cape gooseberry are characterized by high vitality.

More significant are the differences in the fresh weigh of the one seedling. The type of deviations from the normal development of seedling structure most often they included the lack of hairs or branchings of the embryo root.

Genotypic differences also exist in insemination. The fruit-to-seed ratio also changes in a wide range.

The average seed yield between tested genotypes of cape gooseberry is 55.51 kg ha^{-1}, and variations between individual genotypes being are significant.

The obtained results can be used on one hand to predict the expected yield of cape gooseberry seeds and on the other hand to precise the realization price, necessary for future economic analyzes and to be increased the efficiency of the seed production, depending on the genotyping insemination characteristics.

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NEW BRED CULTIVARS OF BELL PEPPER OBTAINED AT V.R.D.S. BUZĂU

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Abstract

Among the vegetables grown in Romania, bell peppers occupies a priority place due to favourable pedo-climate conditions, especially in the southern area of the country, as well as of high demand from consumers and food-processor. In Romania, peppers has traditionally been field-grown and harvested at mature-green stage. Lately, as a result of worldwide producers, the expressiveness of colour and shape had greatly diversified and the demand for high-quality coloured peppers has led farmers to look at quality-pepper cultivars grown in greenhouse. Starting from these premises, the Breeding Laboratory at Vegetable Research-Development Station Buzău approach a new theme aimed to obtain new cultivars with high yield potential and distinct phenotypic characteristics. Besides evaluation of yield potential, a particular emphasis has been focused on obtaining varieties that has different shapes and colours. Research has been completed by far with the achievement of new cultivars with colour ful fruits like orange, chocolate and indigo.

Key words*: breeding, Capsicum annuum L., Romania, coloured pepper.*

INTRODUCTION

In the past, in Romania, farmers limited themselves and had cultivated only traditional varieties of peppers, but now, as a result of imported seeds and imports of different kind of peppers, the expressiveness of colour and shape has greatly diversified.

Pepper (*Capsicum annuum* L.) is an important vegetable crop worldwide and can be consumed in many colours green, red, yellow, orange, or brown when ripe (Ilić et al., 2014). Improvements made in pepper breeding sector have allowed a diverse and large number of varieties into the market with distinct expressivity in accordance with direction of use. Peppers can be used fresh in salads, cooked in different dishes, used as a hot or sweet spice, and also in the industry, for pickles and different kind of preserves. They are fast gaining popularity, not only for their attractive colours, characteristic taste and aroma, but also for their health-promoting properties.

Peppers exhibit great genetic diversity in terms of colour, size, shape and chemical composition and therefore vary greatly in their antioxidant properties, vitamins and other phytochemicals (Chuah et al., 2008). According to a study, the colour of bell peppers is the major factor associated with the consumer purchasing decisions (Frank et al., 2001). Starting from these premises, Breeding Laboratory from Vegetable Research-Development Station Buzau, had approached a new theme aimed to obtain new genotypes with high yield potential and distinct phenotypic characteristic. So far, V.R.D.S. Buzau has a valuable collection of germplasm of peppers. Acquaah (2012) states that germplasm needs to be periodically rejuvenated and multiplied, because it represents the life of plant breeding and without it, this activity could not be carried out. Thus, it is used a genetic material for breeding and can itself be improved in order to increase the performance of the biological material obtained.

Any breeding process begins by collecting the genetically diversified germplasm that must incorporate a sum of features useful for the objectives pursued. The results obtained are determined by a large extent and abundance of genetic sources, by the combination of useful genes of which accumulation is sought in the organisms created. (Leonte C.N., 2011).

In this study, a special emphasis was placed on obtaining new genotypes of bell peppers with different shapes and colours, compared to the known ones, while evaluating their yield potential.
One important part in the breeding sector is the evaluation of the germplasm collection and the comparison between a few superior candidate genotypes to select one or more to be released as cultivars. The goal of this research was to evaluate four ascensions and to study their potential to identify the most promising one.

MATERIALS AND METHODS

Researchers who are working in the breeding sector have to choose between hundreds of plants in a segregation population to select only a small fraction of promising plant to be used in the advanced program. So far, V.R.D.S. Buzau has ended with the achievement of a valuable germplasm collection and has a number of 214 Capsicum genotypes, structured in three groups regarding genetic stability: segregating, advanced and stabile.
From a number of 42 varieties of *Capsicum annuum* var. *grossum* L., there have been selected 4 ascensions (A 60, A 62, A 66 and A 68) with distinct phenotypic characteristics. Afterwards they have been genetically stabilized by specific, long term breeding work.
On 7th March the seeds were sown in seedling trays with 70 cells, in a substrate with white peat and added nutrients. In the 3rd of May they were transplanted in a greenhouse.
The specific care work was respecting the crop technology and it was added the special techniques that consisted in handling the bell pepper shoots and the removal of the first floral button.
For reducing the damage caused by high temperatures and sunburn, starting from 15th of June, a shading net had been mounted on the top of the greenhouse. This had led to a decrease of damage caused by flower abortion and sunburn fruits. In this study, there were analyzed one hundred plants for each experimental variant.
During the vegetation period, phenological and biometric observations were made according to IPGRI standards for *Capsicum* spp.

RESULTS AND DISCUSSIONS

Sowing was made on 7th March 2017 and after 12 days A 60 was the first one who sprout, with a germination percentage of 88%, it was followed by A 68 with the highest germination rate, 95%, afterwards A 62 had the lowest germination percentage only 81% and the last one who sprout was after 17 days A 66 with a germination rate of 91%.
Laboratory germination was also made and in Figure 1 can be observed the difference between the two of them.

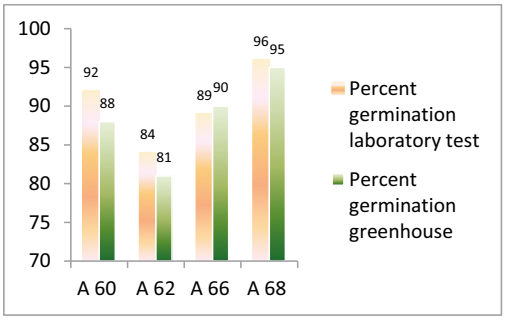

Figure 1. Germination results

Overall, at the beginning, the seeds germinated more slowly in the greenhouse than the lab. The highest difference was recorded by A 60, with a decrease of 4% towards laboratory germination value.
The ascensions A 62, A68 were also followed by a decrease towards laboratory germination value. The germination conditions in the lab are very controlled compared to the greenhouse; this may explain why there were some differences between germination results.
Only A 66 has recorded a 1% higher than laboratory germination analyses, but it has sprout at last. The peculiarities of climatic zoning for Buzau in 2017, was that in the second decade of April, there have been snow and ice frosts, for why vegetable crops had delayed till 15 days.
Planting was made in greenhouse on 3rd May 2017, when the seedling has reached the optimum planting age and the climatic conditions were favourable.
Plant growth habit on **Ascension 60** (A 60, Figure 2) was intermediate and has recorded an average height of 85 cm (stage 4 in IPGRI descriptors).

Figure 2. Ascension 60, plant view

Bell pepper fruit set percent was high, even on high temperature during summer. The imamture fruit is dark, indigo-black, crispy, aromatic and can be consumed fresh or cooked. At physiological stage fruits are red. When fruits turn red they have a sweet and strong aromatic flavour. More details about fruit characteristics can be found in Table 1. The root system is medium developed and shows medium resistance to pests (*Meloidogyn*e spp.).

Table 1. Fruits characteristics

Studied character	A 60	A 62	A 66	A 68
Fruit colour at intermediate stage	Indigo-black	Dark green	Intense indigo-black	Green
Fruit colour at mature stage	Red	Orange	Dark red	Brown
Fruit average length (cm)	10.62	8.71	7.99	11.68
Fruit average width (cm)	10.12	8.53	9.23	7.83
Fruit average wall thickness (mm)	9.83	6.93	6.10	6.45
Fruit average weight (g)	158.1	142.1	133.5	130.03
Fruit average pulp weight (g)	124.8	142.1	114.9	119.73
Fruit average receptacle weight (g)	33.3	23.2	18.6	10.3

Ascension 62 (A 62, Figure 3) has a intermediate plant growth habit, with an average height over 92 cm (stage 5 in IPGRI descriptors).

Figure 3. A 62, plant view

Fruit set was medium during hot summer. Unripe fruit has a dark-green colour and orange colour when ripe. As an important notice, it has been found that fruit tend to lose their flavour on their ripening process. The root system has a medium vigour and a medium to weak resistance to pest. There was a slight attack of nematodes on the roots (*Meloidogyne* spp.), but did not influence in a significant percentage the yield.

Ascension 66 (A 66, Figure 4) has recorded the lowest average height from studied ascension and fits to the lower limit of IPGRI descriptors (75 cm).

Figure 4. A 66, plant view

Observations made have shown that this ascension grows well and fruit set in vary conditions. It has recorded very good results on high temperature during summer and also on low temperature during autumn (October). Intermediate fruits are darker in colour than A 60 and at physiological stage turn dark red. The fruit taste is good, full of flavour in both ripening stages. The root system is vigorous and shows medium resistance to pest (*Meloidogyne* spp.).

Plant growth of **Ascension 68** (A 68, Figure 5) was intermediate, with a average height over 85 cm, according to IPGRI descriptor in 5th stage.

Figure 5. A 68, plant view

Fruit set is medium during high temperature in the summer. On intermediate fruit stage, the fruits are green and, as they ripen, they turn brown. Fruits have gone pretty fast to brown colour. The fruits had been remarkable by its taste and aroma. Root system is medium developed and has a medium pest resistance (*Meloidogyne* spp.).
In figure 6 are presented fruits in different ripening stages.

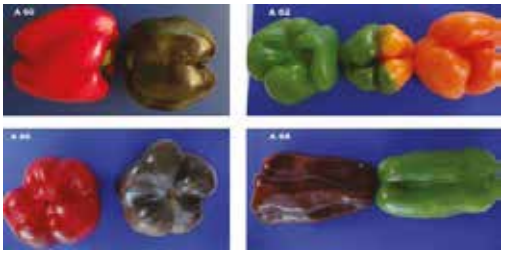

Figure 6. Fruits in different ripening stages

Regarding yield potential, an assessment of quality and quantity of yield was made for each studied genotype. In relation to yield quality, the fruits were separated in: first, second and third class. Records of obtained yields of all ascension are found in Table 2.

Tabel 2. Obtained yield

Ascensions	A 60	A 62	A 66	A 68
Fruit average weight, 1st class (g)	224.4	186.4	180.7	179.1
No fruits 1st class	8	9	12	10
Average Yield of 1st class/plant (g)	1779.2	1677.6	2168.4	1791
Fruit average weight 2nd class (g)	150	140	120	120
No fruits 2nd class	6	7	8	8
Average Yield of 2nd class/plant (g)	900	980	960	960
Fruit average weight 3rd class (g)	< 100	< 100	< 100	< 100
No fruits 3rd class	6	5	12	7
Average Yield of 3rd class/plant (g)	599.4	499.5	1198.8	699.3
Yield/plant (g)	3278.6	3157.1	4327.2	3450.3
Yield/plant (kg)	3.28	3.16	4.32	3.45

First-class fruits had the organoleptic characteristics of the variety, were uniform in colour, taste, appearance, texture and size. Ascension 66, has recorded a medium yield potential with a value of 2.168 kg/plant, and the smallest one was registered by Ascension 62 with 1.677 kg/plant. Fruits of smaller size and with minor flaws had been included in the second class and the registered average yield varies from genotype to genotype. The highest yield was recorded by A 62, with a value of 0.98 kg/plant and the lowest yield was reported by A 60 with a average yield of 0.9 kg/plant.
As a result of long vegetation period of bell pepper in Romania, it was found that all studied ascensions have a high yield potential, but some of them do not reach full ripening. In this study it has been framed as a 3rd class yield, a yield that cannot be marketed due to unripening of the fruits and their smaller size. For all ascensions, fruits bellow 100g were included on the 3rd class. The highest yield for the 3rd class was registered by A 66 with a value of 1.19 kg/plant and the lowest yield was recorded by L 62 with only 0,499 kg/plant. More details about yields registered by other ascensions can be found in Table 2. Recording a large number of fruits from the 3rd class has led to the conclusion that the varieties can also be grown in a prolonged cycle in a heated greenhouse and in this case, the yield of first and second class will have a significant increase. Regarding total yield potential of ascensions the record was owned by A 66 with a value of 4.32 kg/plant and the lowest yield was reported by A 62 with a 3.16 kg/plant.

CONCLUSIONS

The absence of national cultivars of bell pepper with different colour adapted to Romanian pedo-climate conditions motivated the beginning of this research at V.R.D.S. Buzau. In the study, four ascensions were examined with distinct phenotypic characteristics which will be enrolled and tested at ISTIS to be patented. Ascension 68 had distinguished by a special colour with a distinctive flavour and a potential yield plant of 3.45 kg/plant. It is well-known that bell pepper fruit set is very sensitive to environmental condition, in particular, to low or high temperatures that can affect pollen development and another dehiscence. From all studied genotypes, Ascension 66 has recorded the highest fruit set in extreme conditions and the highest yield, 4.32 kg/plant. Ascesion 60 was noted with earlier fruits and a yield of 3.28 kg/plant. The

lowest yield potential was recorded by Ascension 62, with only 3.16 kg/plant.

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PRELIMINARY STUDY RELATED ON YIELD AND QUALITY POTENTIAL OF TWO NEW SWEET PEPPERS VARIETIES OBTAINED AT V.R.D.S BUZĂU

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Abstract

Sweet pepper is an important vegetable used in Romania and it holds a significant area crop. At present, there are a lot of new varieties of sweet peppers in Romania, but, unfortunately, most of them comes from worldwide seed producers. Being demand for autochthonous competitive varieties, suitable for growing in greenhouse, Vegetable Research-Development Station Buzău has been preoccupied with obtaining of new varieties of sweet peppers. This study was conducted to investigate the qualitative and quantitative potential of two new varieties, 'Ideal' and 'Carmin'. As the study shown, 'Ideal' variety has a higher yield potential, while 'Carmin' variety has a higher content of vitamin C. Dry matter content and soluble substances it has higher values in 'Ideal' variety. New bred varieties have demonstrated downstream genetic stability in the phenotypic expressiveness of the main characters and have also recorded high-quality yield production, thereby contribute to the enrichment of the inland assortment suitable to be cultivated in greenhouses.

Key words*: breeding, Capsicum annuum L., Carmin, Ideal, Romania.*

INTRODUCTION

Capsicum annuum L. var. *grossum* Sendt also known as bell pepper, sweet pepper and green pepper is one of the well known and productive species from *Solanaceae* family and holds significant crop areas in Romania. An important part of Romanian varieties of *Capsicum* spp. are not suitable for growing in the greenhouse, that is why one of the main objectives of Vegetable Research-Development Station (V.R.D.S.) Buzău, was to obtain valuable genotypes. V.R.D.S. Buzău owns a diverse germplasm collection of different kind of peppers. Recently, two varieties of peppers, 'Ideal' and 'Carmin', have been patented and the aim of this study was to research their quantity and quality potential. Among the characteristics of the bell pepper fruits, the length and the diameter stand out and these characteristics have been considered extremely important for commercialization (Blat et al., 2007). These characteristics are still important in defining the shape of these fruits and it's fundamental to determine the group to which they belong whether conical, rectangular or square (Ramos et al., 2017). 'Carmin' fruits belong to the square group and 'Ideal' has fruit with conical shape both of them are from *Capsicum annuum* var. *grossum*. It is well known that pepper fruits are very used and appreciated by their high content of ascorbic acid (AsA) and antioxidant activities (carotene and flavonoids) (Burzo, 2015). AsA, the anti-scorbutic factor, is a required human nutrient, and its biological functions are centred on its antioxidant properties in biological systems, preventing common degenerative processes (Padayatty et al., 2003). Of course, the values are influenced by variety, crop technology and harvest time. That is why, in this study, for biochemical analysis fruits were harvested in two different times: green stage and red stage. Their attractive red colour is due to the profuse

synthesis of various carotenoid pigments during ripening. These include oxygenated carotenoids such as capsanthin, capsorubin and crypto-capsin, which are exclusive to this genus and have been shown to be effective free radical scavengers (Matsufuji, Nakamuro, Chino, Takeda, 1998). Also results of Kim et al. (2016) shows that intake of sweet pepper might be helpful for lowering the risk of diseases caused by oxidative stress. The aim of this study was to evaluate the yield and quality potential of those two new *Capsicum* varieties, ˈIdealˈ and ˈCarminˈ.

MATERIALS AND METHODS

To assess the morphological characteristics, agronomic performance and biochemical compounds, varieties were grown under protected environment within V.R.D.S. Buzau site during spring-autumn of 2017. During summer, from June to end of August, the greenhouse was covered with shading net with a shading degree of 40%.

The seeds were sown on 07.03.2017 and transplanted in the greenhouse at the beginning of May, on 03.05.2017. Planting was made in strips in the following formula 70 cm x 35 cm and 1.2 m between the strips.

The experiment was organized in randomized blocks, in two rehearsals and the observations were made on 100 plants for each variety. Both varieties have received standard cultural practices, a special care was made to handle the bell pepper shoots and to remove of the first floral button.

During vegetation period, there have been recorded some agronomic traits like: earliness, number of fruits/plant, weight of fruits, weight of receptacle, weight of fruit pulp, the thickness of pulp.

To highlight the variability of the two varieties, a morphological characterization was performed using the DUS test, according to European Union - Community Plant Variety Office (2007) (CPVO-TP/076/2).

The characteristics of fruits analyzed were as follows: unripe colour fruit, cross-section fruit, number of locules, fruit shape at blossom end, fruit shape at pedicel attachment and thickness of the pulp.

Physico-chemical analysis

Researches have been carried out in the Research Center for Studies of Food Quality and Agricultural Products from University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania. Physico-chemical analyses were performed on unripe fruit (green stage) at the beginning of August and on ripe fruits (red) in September.

For each variety were harvested an increase number of sampling so that the confidence interval of the sample to get wider. Each analysis was an average sample of several fruits.

Ascorbic acid content was determined with HPLC - Aqilent Technologies 1200 Series equipment. Ascorbic acid was determined by using methods described by Jones and Hughes (1983), Deepa et al. (2006), Topuz et al. (2007), Ghasemnezhad et al. (2011) with slight modification. From average sample of fresh fruits were taken 1 g and mixed with a pinch of quartz sand.

The mixed sample was homogenized with 10 mL of metaphosphoric acid. The samples were kept in the dark for 45 minutes at 4°C. The extract has been centrifuged for 1 minute at 1000 rotation/minute.

The supernatant was filtered through a filter Agilent RC 0.2 μm and then it was injected in HPLC. The samples were analysed in duplicate for each varieties and were expressed in mg/100 g.

Total soluble solids (TSS) and titratable acidity (TA). TSS is an index of soluble sugar content in fruit. Soluble solids were determined from bell pepper juice using refractometer Kruss (% Brix).

Titratable acidity (TA) was determined using 10 g of ground bell pepper diluted in 50 ml of distilled water. The titratable acidity of peppers was determined by titration with 0.1N NaOH to pH 8.1 (Sadler and Murphy, 2010; Serrano et. al, 2010).

For titration was used automatic titrator TitroLine easy. The results were expressed in milligrams citric acid/100g.

The ***dry matter*** was determined by drying stove for 15 hours at 105°C, until the samples reach constant weight.

RESULTS AND DISCUSSIONS

Vegetable Research-Development Station Buzău had undertaken research under the breeding program which aimed primarily in obtaining new varieties of bell pepper suitable for greenhouse growing, adapted to pedo-climate conditions of Romania and meet the requirements of farmers and consumers.
The new varieties obtained, 'Ideal' and 'Carmin', were morphologically analyzed according to DUS test and the result obtained are shown in the Tabel 1.

Tabel 1. Main fruit characteristics

Variety	'Ideal'	'Carmin'
Fruit: colour before maturity	3 (green)	3 (green)
Fruit: colour at maturity	8 (red)	8 (red)
Fruit: shape in cross section (at level of placenta)	3 (circular)	3 (circular)
Fruit: number of locules	2 (predominantly two)	4 (equally three and four)
Fruit: depth of stalk cavity	5 (medium)	7 (deep)
Fruit: shape of apex	2 (moderately acute)	4 (moderately depressed)
Fruit: thickness of flesh (mm)	7 (thick)	6 (medium)

'Ideal' variety had been characterized by earliness, 12 days earlier than 'Carmin' variety and with a high average yield of 3.5 kg/plant compared to 'Carmin' variety 2.59 kg/plant average yield. Although 'Carmin' has less fruit, the fruit average weight is higher.
'Ideal' variety (Figure 1) has a vigorous plant growth habit, with a height over 1 m, strongly branched, with a high percentage of fruit set even at high temperatures during hot days in the summer.

Figure 1. Crop view 'Ideal' variety

The average number of fruits per plant has been 26 fruits. Fruits are slightly conical with two locules, light green before maturity and red when ripe.
The average weight of the fruits is 135.2 g and the thickness of the flesh is on average 6.8 mm. The average weight of the receptacle has been 19.3 g and the average pulp weight 115.9 g.
The low temperature during summer nights causes the fruits to form a pointed end at the apex of the fruit.
'Carmin' variety (Figure 2) has a compact bush, with a height over 90 cm, very well branched. The fruit set percentage is medium in the hot summer days and high during autumn, September-October period.

Figure 2. Crop view 'Carmin' variety

There has been, in average, a number of 16 fruits/plant. Fruits have a pleasant commercial appearance, slightly waxed, with three of four lobes. The fruits have light green colour when unripe and red when they reach physiological maturity. The average of fruit weight has a value of 152.3 g, and the average of pulp thickness has a value of 5.78 mm. The average of receptacle has weighted 22.4 g and the pulp had 129.9 g.
In Figure 3 can be seen fruits of 'Ideal' and 'Carmin' variety in different colours during ripening period.

Figure 3. Different colour of fruits during ripening

In this study, the fruit quality of the sweet peppers variety was assessed by: ascorbic acid

content, dry matter, total soluble solids and titratable acidity. The quality indicators were different in both ripening stages: green and red.
The amount of ascorbic acid was higher in 'Carmin' variety, in both stages green and red, as seen in Figure 4, values vary from 164.31 mg/100 g when green stage and 189.27 mg/100 g when ripe.
'Ideal' variety records a value of 79.29 mg/100 g when fruits are unripe and green and an increased value of 122.5 mg/ 100 g when fruits have reached physiological maturity.

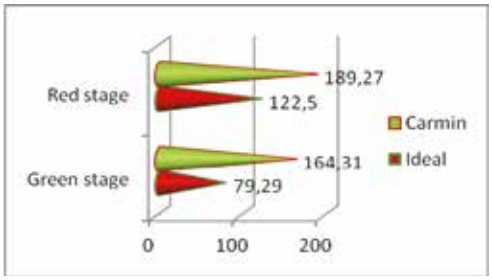

Figure 4. Variation of ascorbic acid during ripening period

As fruit ripens, an increase in ascorbic acid content was observed, these results have been in assent with previous studies (Howard et al, 1994; Deepa et al., 2006; Osuna et al., 1998; Marin et al., 2004). Smirnoff and Wheeler (2000) states that the role of ascorbate content as a photoprotector has been reported for leaves acclimated to high light, which have a higher ascorbate concentration than leaves grown at low intensity. The amount and intensity of light during the growing season have also been described to have a definite influence increasing the ascorbic acid formed. The light exposure could explain the increase in ascorbic acid content of red fruits as compared to the green ones (Lee, 2000).
To provide antioxidant protection, a Recommended Dietary Allowance (RDA) of vitamin C is 90 mg/day for adult men and 75 mg/day (Bendich A., 2001), individuals who smoke require 35 mg/day more. According to the results obtained for 'Carmin' and 'Ideal' variety, it was learned that it can provide the recommended dietary allowance and even more with just 100 g/day.

Physico-chemical analyses of total soluble solids (TSS), titratable acidity (TA) and dry matter values are presented in Table 2.

Table 2. Physico-chemical analyses

Stage	Analyse	'Ideal'	'Carmin'
Green	TA (citric acid) %	0.23 mg/100g	0.17 mg/100 g
	TSS °Brix	4.87	4.52
	Dry matter (g)	7.99	6.23
Red	TA (citric acid) %	0.28 mg/100g	0.29 mg/100 g
	TSS °Brix	7.25	6.75
	Dry matter (g)	9.43	9.01

As seen in Table 2, the quantity of titratable acidity increased with ripening of varieties. While the fruit ripens, the metabolic reactions increase, increasing the concentration of organic acids involved in the Krebs cycle. Apart from this, these acids make up the energetic reserves and the metabolic reactions that involve the synthesis of pigments, enzymes and other materials and the degradation of pectins and celluloses, which are essential for the ripening process. The organic acids are active substances during ripening in these alterations (Chitarra and Chitarra, 1990). For the totally green bell peppers, these organic acids are present in small quantities, as the ripening process has not yet started, presenting differences in relation to the other stages of ripening. The same behaviour was observed by Molinari et al. (1999) and by Ghasemnezhad et al. (2011).
Total soluble solids it is also increasing during ripening period on both sweet pepper varieties. As fruits starts ripening there is an increased of total soluble substance due to the degradation or biosynthesis of the polysaccharides and the accumulation of sugars. The metabolic processes related to the advance of ripening are directly influence by the level of TSS, where fruits in advance stages of ripening present the highest levels of TSS. These increases in soluble sugars and acidity have also been found during physiological ripening in many cultivars of both hot and sweet pepper, being correlated to colour changes (Lyon et al., 1992; Ghasemnezhad et al., 2011; Behera et al., 2004; Orowski et al., 2004; Tadesse et al., 2002, Serrano et al., 2010).
The amount of dry matter is an important quality attribute of bell pepper. High values of this parameter induce a better flavour and taste. It has been registered an increase in green stage on 'Ideal' variety from 4.87 g to 7.25 g in the

red stage and referring to 'Carmin' variety, the registered differences oscillated between 4.52 g to 6.75 g. Concluding, 'Ideal' variety has a stronger flavour and a more pleasant taste than 'Carmin' variety. Fruits who have reached physiological maturity have a higher amount of dry matter, which is probably due to high temperature during summer, which allows an increase nutrition and plant metabolism. Similar results were recorded by Amalfitano et al. (2017), in Naples, Italy.

CONCLUSIONS

The two new varieties obtained at Vegetable Research-Development Station Buzău, 'Ideal' and 'Carmin', have shown genetic stability in terms of phenotypic expressiveness of the main characters and have also recorded high quality yield, contributing to the enrichment of the local assortment suitable for greenhouse growing.

'Carmin' variety was highlighted by its high content of vitamin C, and the 'Ideal' variety by a higher yield and a high content of dry matter and total soluble substance.

This study can help promote and stimulate growing in the greenhouse of those two new varieties.

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INFLUENCE OF PLANT MANAGEMENT SYSTEMS ON GROWTH AND FRUCTIFICATION OF TOMATO PLANTS IN PROTECTED CULTURE

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Abstract

Tomatoes have a high capacity to form shoots on the stem which is why plants can be managed with multiple stems, thus influencing the increase in production and decrease in the number of plants per hectare. Research was conducted in the teaching field of the Faculty of Horticulture in Bucharest, within a prolonged cycle in solarium, during 2016-2017. Two varieties were used, Belmonte and Canestrino, and two hybrids, Cinto F1 and Clarabella F1, managed with one and a double stem. Stem management was performed through eliminating the portion of the plant from above the cotyledonal leaves, during the seedling phase, after the formation of the first pair of leaves, the percentage of stem formation being over 90 %. The planting was made at a distance of 0.8 m/0.4 m for the plants with one stem, resulting in 3.1 plants/m^2, and at 1 m/0.4 m for the plants with a double stem, resulting in 2.5 plants/m^2. The reduction in the number of plants was of 20% for the ones with double stem. The largest production of fruit per plant was obtained for the tomatoes with a double stem: 7.2 kg for the Belmonte variety and 7.2 kg for Canestrino, 6.9 kg for the hybrid Clarabella F1 and 6.4 kg for Cinto F1. The plants with one stem recorded a production of 2.6-2.9 kg/plant. The production per square meter was directly influenced by the production obtained per plant; thus, the plants with double stem recorded values of 16-18 kg, while the tomatoes with one stem produced 8.06-8.70 kg.

Key words: *double stems, pinching off, production, seedling.*

INTRODUCTION

Tomatoes (*Lycopersicon esculentum* Mill) have their origins in South America and, compared to other vegetable species, have the largest cultivation area in the world, being very appreciated by consumers. The fruits are rich in vitamins, minerals, amino acids and pigments (Dinu et al., 2017; Soare et al., 2015) and poor in calories, being considered very healthy for the human body. The biochemical composition of the tomato fruit is influenced, amongst other factors, by the position of the fruit within the inflorescence. The fruit in the middle has the highest content of polyphenols. The sugar content increases simultaneously for the fruits ranging from the base to the top of the inflorescence while fruit size decreases (Coyago-Cruz, 2017). Researches performed on tomatoes are diverse and attention is currently on improving the culture technology. From a biological point of view, tomatoes are herbaceous annual plants, with a very good capacity to form shoots, which has led to numerous researches regarding the increase of the production per plant and per unit of area, without affecting the production quality. In a culture tomatoes are usually managed with one stem; however numerous researches have been performed in which plants have been managed with 2, 3 and 4 stems, using only cultivars with undetermined growth. Obtaining several stems can be achieved through pinching off the plant above the cotyledonal leaves (Hoza and Stanciu, 2012; Mouaro et al., 2014), pinching off above the first pair of real leaves (Mouaro et al., 2014), pinching off above the second inflorescence (Franco et al., 2009) or through removing the growth peak of the seedling at planting and using the first 2 shoots at the base

of the plant (Hoza et al., 2011; Hoza, 2013). The research conducted by Mouaro et al. (2014) proved that plants managed with 2 stems generate larger productions (26.5 kg m^{-2}) compared to plants managed with 3 and 4 stems, for which the production was smaller (19.5 kg m^{-2}). Comparing the production for the double stem of the same plant, the differences were not significant. According to Rahmatian et al., 2014, grafted tomatoes managed with 2 stems in a hydroponic system formed more fruits per plant (46.04) than the ones with only one stem (27.43); the production per plant was larger (4.45 kg) compared to plants with one stem (3 kg), the fruits were firmer and of a higher quality, but the average fruit weight was lower by 12 %. In addition, grafted plants with a double stem accumulated a larger quantity of dry substance in various plant organs. Tomatoes with 2 and 3 stems formed from shoots led to an increase in the number of fruits (Hoza and Stanciu, 2012) and in the commercial production compared to plants with one stem. However their fruits were slightly smaller and the plants did not registered significant differences regarding quality and number of stems; plants conducted with 3 stems also extend the harvesting period (Mbonihankuye et al., 2013). For cherry tomatoes, plant management with 2 stems is a current practice in southern Spain, stem planning being made after the second inflorescence forms. According to Franco et al., 2009, for cherry tomatoes cultivated in greenhouse, managed with 2 stems on which are kept 2 side-shoots with 2 inflorescences on each, the production and number of fruits increase per unit of area, without significantly affecting the average fruit weight. By analyzing the correlation between the number of stems and the tomato production, it has been observed that plants managed with 2 stems have had a higher production and number of fruits, but the average fruit weight and the commercial production have been slightly lower for the plants with 2 stems compared to the ones with on stem (Ece and Darakci, 2007). Plant management with more stems in association with different doses of nitrogen demonstrated that the largest production has been registered at plants with 2 stems and 256 kg N ha^{-1} (Hossain, 2007).

MATERIALS AND METHODS

The experiment was conducted in the teaching field of the Faculty of Horticulture Bucharest, in solarium, during 2016-2017. The main purpose of the experiment was to determine the influence of the number of stems and of the cultivar on plant growth and fructification, in the climatic conditions of the reference area, and the introduction of this particular plant management system into the tomato culture management. Two varieties were used, Canestrino and Belmonte, highly perishable, with superior characteristics regarding the taste and large fruits and two F1 hybrids, Clarabella and Cinto, very productive, with large, uniform, firm fruits with high resistance to various pathogens. Plants were managed with one and a double stem and 6 inflorescences were kept on each stem. Double stems were obtained through eliminating the portion of the plant from above the cotyledonal leaves when the tomato seedlings formed the first pair of real leaves. The buds at the base of the cotyledons formed 2 shoots that later became double stems. Specific maintenance works were applied for the tomato seedlings up to the planting moment.

Field preparation was made through soil loosening and crumbling works and fertilization with Orgevit 2 t/ha incorporated into the soil before planting.

The culture was established with seedling produced in warm greenhouse, of 55 days of age. During seedling production period, plants managed with one stem were kept in colder spaces (16-18°C) compared to the ones with a double stem, in order to reduce the growth difference and to ensure the planting could be made at the same time. This aspect is aimed be a subject to further research themes. Planting was made during the first decade of April for both experimental years, at a distance of 0.8 m/0.4 m for plants with one stem, resulting in 3.1 pl./mp, and at a distance of 1 m/0.4 m for plants with a double stem, resulting in 2.5 pl./mp and a reduction in the number of plants of 20%. Plants with a double stem were led as a V-shape towards the spaces between rows in order to allow light to penetrate through more efficiently. During the vegetation period, all lateral shoots were eliminated, plants were supported on

strings and optimal growth conditions were ensured: temperature, humidity and light according to the phenophase requirements. Phased foliar fertilizations were applied to ensure the nutrition necessary for the optimal growth and fructification of the tomato plants using: Codicevo at planting and Energevo at fruit formation. During growth and fruit maturation Finalevo and Rezistevo were applied. Fertilizer composition was as follows:

- Codicevo 19:19:19+ME 0.2%, with a content of NPK of 19:19:19, S 1.4%, MgO 1.8%, B 0.1%, Fe 0.07%, Mn 0.03%, Zn 0.03%, Cu 0.006% and Mo 0.002%, that favors a rapid and improved absorption of the soil nutrients;
- Energevo 9:53:9 0.2 %, with a content of N 9%, P_2O_5 53%, K_2O 9%, MgO 2.5%, Fe 0.1%, S 1.0%, B 0.1%, Mn 0.05%, Zn 0.1%, Cu 0.05% and Mo 0.005%, that increases plant resistance to various stress factors and accelerates the ongoing of vegetation phases;
- Finalevo 5:14:42 0.3 %, that contains N 5%, P_2O_5 14%, K_2O 42%, MgO 2.5%, S 6%, B 0.1%, Fe 0.1%, Mn 0.05%, Zn 0.1%, Cu 0.05% and Mo 0.005%, and improves the quantity and quality of the harvest;
- Rezistevo 0.3 %, that has N 14%, CaO 18%, K_2O 1.7%, MgO 4.2%, Mn 0.05%, B 0.2%, Zn 0.01% and Mo 0.005%, applied at an interval of 15 days to diminish the effects of calcium deficit and increase the fruit resistance to cracking, transport and storage.

The experiment was bifactorial, organized in random blocks, with three repetitions. The following elements were used: four cultivars with large fruits and undetermined growth and two plant management systems - S1 with one stem and S2 with a double stem.

Measurements were made on five plants from each repetition, selected at the beginning of the experiment; average fruit weight was determined through harvesting fruit per variant, counting and weighting the fruits at each harvest and calculating the average weight.

Measurements were made also regarding plant growth, determined by the height at the end of the vegetation period, distance from the soil for the first inflorescence and average distance between inflorescences. During the fructification period, the number of fruits per plant, number of fruits per square meter, average fruit weight and the production per plant and per square meter were measured, according to the type of cultivar and plant management system used. The primary data was processed statistically by the variant analysis method.

RESULTS AND DISCUSSIONS

Regarding the vegetative growth of the tomato plants it was observed that between the plants with one stem and the ones with a double stem small differences were recorded, but the cultivar had a stronger influence on some indicators (Table 1). Regarding the distance from the soil for the first inflorescence, it had higher values for the varieties Camestrino and Belmonte, compared to the F1 hybrids Clarabella and Cinto. The average distance between inflorescences was slightly larger for the plants with two stems, except for the variety Canestrino, for which the average distance between inflorescences was smaller for the plants with double stem. Average plant height was smaller for the varieties Canestrino and Belmonte with a double stem, while Clarabella and Cinto had lower values for the plants with one stem; on average, plants with double stems slightly surpassed the average height of the ones with just one.

Table 1. Synthesis of the results regarding the growth of tomato plants with one and a double stem

Cultivar	Plant management system	Distance from soil for first inflorescence (cm)	Average distance between inflorescences (cm)	Plant height (cm)
Canestrino	S1	36.2	36.9	221.4
	S2	35.7	35.3	214.1
Belmonte	S1	32.6	26.5	175.0
	S2	34.2	28.9	173.6
Clarabella F1	S1	24.4	31.5	187.2
	S2	24.9	33.5	192.5
Cinto F1	S1	23.6	31.2	185.8
	S2	22.2	32.9	196.1
Average (mt)	S1	29.2	31.5	192.4
	S2	29.3	32.7	194.1

The interaction between the cultivar and number of stems was obvious in the potential of the fructification capacity, the plants with double stems producing more fruits than the ones with only one stem (Table 2).

The number of fruit per plant recorded an average increase amongst the studied varieties of 80%; for the variety Canestrino the increase was more than double compared to the other varieties, respectively 119 %, while for the two hybrids it was of 64%.

The number of fruits per plant with a double stem was between 40 and 45.5, being close to the data recorded in the professional literature (Ece and Darakci, 2007; Hoza and Stanciu, 2012; Rahmatian et al., 2014.).

The number of fruits obtained per square meter depended on the number of fruits per plant and the number of cultivated plants per square meter, the average increase of fruit varying between 32% for the hybrids and 77% for he varieties, having an average value of 45%. The number of fruits per square meter was between 100 fruits for Clarabella F1 and 113.8 for Belmonte, values slightly lower than the ones obtained by Mouaro et al., 2014, respectively 121.5 and 125 fruits per m^{-2}, dependant on the stem planning.

The cultivar's influence on the fruit production was poorly highlighted, both regarding the number of fruits per plant and the number of fruits per square meter.

The average fruit weight (Table 2) was lower for all cultivars with a double stem compared to the ones with one stem, fact proved by the professional literature (Ece and Darakci, 2007; Hoza and Stanciu, 2012; Rahmatian et al., 2014).

Table 2. Number of fruits per plant and per m^2 depending on the cultivar and number of stems

Cultivar	S1(one stem)			S2 (double stem)			Difference between S1 and S2, %	
	Average fruit weight, g	No of fruits $plant^{-1}$	No of fruits m^{-2}	Average fruit weight, g	No of $fruit/pl^{-1}$	No of $fruits/m^{-2}$	$plant^{-1}$	m^{-2}
Canestrino	133.7 ***	20.2 ^{o}	62.6^{ooo}	132.6***	44.3 *	110.8 N	+119	+77
Belmonte	111.2^{ooo}	25.0 N	77.5 **	109.9^{ooo}	45.5***	113.8 **	+82	+47
Clarabella F1	117.8^{oo}	24.4 N	75.6 N	116.6^{ooo}	40.0^{ooo}	100.0^{ooo}	+64	+32
Cinto F1	126.3 **	27.0 N	83.7 ***	124.7***	44.2 *	110.5 N	+64	+32
Media (mt)	122.5	24.2	74.9	121.0	43.5	108.8	+80	+45
DL 5%	2.20	3,41	1,83	1.13	0.73	2.45	-	-
DL 1%	3,33	5.16	2,77	1.72	1.11	3.72	-	-
DL 0.1%	5.31	8.23	4,41	2.74	1.77	5.92	-	-

The productive capacity of the tomato plants was influenced by the cultivar, but especially by the number of stems (Table 3). For the plants managed with a double stem, the production was superior compared to one stem plants as shown also by Hossain (2007) and Mouaro et al. (2014). For this experiment, the largest production per plant was recorded for the variety Canestrino with a double stem, 5.9 kg $plant^{-1}$, the production increase being of +3.2 kg $plant^{-1}$ compared to the one stem plants. Conversely, the smallest was obtained from the hybrid Clarabella, 4.6 kg $plant^{-1}$, the production increase being of +1.7 kg $plant^{-1}$ for two stem plants. Analyzing the average production per plant for the used cultivars, it was noted that plants with one stem formed 3 kg $plant^{-1}$, while the ones with double stems formed 5.3 kg $plant^{-1}$. Franco et al., 2009, showed that for cherry tomatoes cultivated in greenhouses, with a double stem and with two shoots on each stem, the production per unit of area increased.

The present experiment confirms this fact, the production for the plants with two stems being larger than for the tomatoes with one stem, with values varying from 11.6 kg m^{-2} for Cinto F1 and 14.7 kg m^{-2} for Canestrino. The average production of the cultivars with one stem was of 9.1 kg m^{-2}, while for the ones with double stems was of 13.2 kg m^{-2}.

Table 3. Tomato production per plant and m^2 depending on the cultivar and number of stems

Cultivar	S1 (one stem)		S2 (double stem)		Difference between S1 and S2	
	Production		Production			
	kg plant^{-1}	kg m^{-2}	kg plant^{-1}	kg m^{-2}	kg plant^{-1}	kg m^{-2}
Canestrino	2.7 N	8.4^{oo}	5.9 **	14.7 ***	+3.2	+6.3
Belmonte	2.8 N	8.6^{o}	5.0 N	12.5^{oo}	+2.2	+3.9
Clarabella F1	2.9 N	8.9 N	4.6^{oo}	11.6^{ooo}	+1.7	+2.7
Cinto F1	3.4 *	10.6 ***	5.5 N	13.8 **	+2.1	+3.2
Average (mt)	3.0	9.1	5.3	13.2	+2.3	+4.1
DL 5%	0.34	0.38	0.34	0.39	-	-
DL 1%	0.51	0.57	0.51	0.59	-	-
DL 0.1%	0.82	0.92	0.82	0.95	-	-

CONCLUSIONS

The research conducted with four tomato cultivars and two plant management systems, one with one stem and one with a double stem, showed that tomato plants react very well when they have multiple stems.

From a vegetative point of view, no significant differences were recorded between plants with one stem and plants with a double stem. However, on average, plants with a double stem had a slightly higher growth (194.1 cm) compared to plants with just one (192.4).

The fructification process was influenced by the plant management system, plants with double stems forming by 80% more fruits per plant and by 45% more fruits per square meter than the single stem plants.

The largest average production per plant (5.3 kg) and the largest production per square meter (13.2 kg) were obtained also from the double stem plants, result justified by the presence of a double locus of fructification on the plant.

Average fruit weight was slightly smaller for the double stem plants (121.0 g) compared to the plants with one stem (122.5 g), however this result cannot be used as an argument that plants with one stem ensure an obvious increment of larger fruits.

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EFFECT OF GRAFTING ON YIELD AND FRUIT QUALITY OF PEPPER (*CAPSICUM ANNUUM* L.) GROWN UNDER OPEN FIELD CONDITIONS

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Abstract

The aim of the study, determining of grafting could improve the agronomic behaviour of pepper (Capsicum annuum L.), an open field experiment was carried out to determine growth, yield and fruit quality of long type pepper hybrid cultivar ('Efil'). As a scion plant material was used 'Efil F1' and rootstock 'Guclu F1'. 'Efil/Efil' ('Scion/Scion'), 'Efil/Guclu' ('Scion/Rootstock') and non-grafted 'Efil' ('Sicon') were used as the grafted combination. According to experiment, grafted plants were taller than control (non-grafted). Total yield, fruit number, fruit flesh firmness, fruit weight and stem diameter were influenced by rootstock and grafting. Grafted pepper produced 16% more yield than control plants for 'Efil/Guclu'. A similar trend was also observed on 'Efil/Efil'. The lowest yield recorded on 'Efil' (non-grafted).

Key words*: grafting vegetable, Capsicum annuum, scion, rootstock.*

INTRODUCTION

Pepper (*Capsicum annuum* L.) is a crop of high economic importance in Turkey. The area of pepper grown in Turkey is 1.141.216 da with 3.414.852 million tons of pepper production (TUIK, 2016). Pepper growing is getting more difficult day by day, because of crop damage due to the specific pathogens, *Phytophthora capsici*, *Verticillium dahliae* and *Meloidogyne* spp. in soil (Morra and Bilotto, 2006; Myung et al., 2006).

One of the major goals in pepper breeding is the development of a cultivar completely resistant to soil-borne diseases. However, that is very difficult to achieve and requires much time and effort.

For the alleviation of soil-borne diseases, cultural practices such as crop rotation and sanitation are recommended, but pesticide is generally applied to control the diseases (Kim et al., 2010; Semi et al., 2010; Tran and Kim, 2010; Yeon et al., 2008). Grafting is an environment-friendly alternative method for disease control (Oka et al., 2004; Rivard and Louws, 2008). Grafting scions onto resistant rootstocks makes it possible to control soil-borne diseases and increase yield of the susceptible cultivar (Lee and Oda, 2003). Recently, the cultivated area of grafted *Solanaceae* and *Cucurbitaceae* have increased tremendously (Lee et al., 2010). At present, grafting is mainly used in order to reduce infections by soil-borne pathogens and to enhance the tolerance against abiotic stresses (King et al., 2008; Louws et al., 2010; Rivero et al., 2003).

One way of avoiding or reducing losses in production caused by pathogens in high-yielding genotypes would be to graft them onto rootstocks capable of reducing soil-borne diseases and increasing yield and fruit quality (Lee, 1994). In order to prevent soil-borne diseases in continuous cropping, peppers are generally grafted onto the rootstocks that are of the same species as scions (*C. annuum* L.) that have resistance to *Phytophthora blight* (King et al., 2010). It was reported that grafting of peppers also improved tolerance to high salt conditions (Chung and Choi, 2002) and low temperature (Jang et al., 2008).

The aim of this study was to investigate and evaluate the agronomic performance of hybrid pepper (scion) under open field conditions following grafting on pepper rootstocks, in comparison with un-grafted and scion self-grafted (scion/scion) plants.

MATERIALS AND METHODS

The F1 hybrid, long type 'Efil' (Asgen, Turkey) was grafted on commercial rootstocks,

'Guclu' (Graines Voltz, Türkiye). Un-grafted 'Efil'cultivar and itself grafting 'Efil/Efil' were also used as control.

The cleft grafting was realized when rootstocks and grafts showed six and two true leaves, respectively. Grafted and un-grafted pepper plants were transplanted on 10 April 2016 in open field condition on the Experimental Farm of Suleyman Demirel University.

The experimental soil was loamy (Bouyoucos, 1962) having pH 7.9 (1:2.5 soil to water ratio), 9.5 % $CaCO_3$, 1.1% organic matter (Jackson, 1962), 15.9 mg kg^{-1} $NaHCO_3$ extractable P (Olsen et al., 1954), 125, 266, 375 mg kg^{-1} 1 N NH_4OAC exchangeable K and Ca and Mg (Knudsen et al., 1982). DTPA extractable Fe, Cu, Zn and Mn concentrations (Lindsay and Norwell, 1978) were 2.9, 0.55, 0.89 and 11.9 mg kg^{-1}, respectively.

Treatments were arranged in a randomized complete-block design with three replications, each consisting of 30 plants. Plants were grown in single rows (1.0 m × 0.50 m) at a plant density of 2.5 plants m^{-2}.

Irrigation was applied by drip-irrigation and was scheduled using tension meters to ensure that water was non-limiting (the high and low tension set points were –30 kPa and –1 kPa, respectively).

The irrigation frequency was arranged every morning. The Following nutrient content; (mg/L): N: 100; K: 50; P: 60; Mg: 30; Ca: 30; Fe: 3.0, Mn: 2.0; Zn: 0.25; B: 0.70; Mo: 0.05 was used during the experiment.

During the experiment, pH 6-7 and EC 1.2-1.8 were set.

Plants were kept free from weeds, insects, and diseases using standard growing management. The experiment was terminated after 120 days from transplanting, plant height was recorded on eight plants per plot. Mature green fruits were harvested regularly every week.

Fresh weight of marketable fruits, fruit number, length, and diameter were recorded on 20 plants per plot. Fruits that were deformed or badly misshapen were considered unmarketable. Fruit shape index (SI), defined as the ratio between width and length was also calculated. Three representative marketable fruits of each plot were analyzed for fruit quality parameters.

All data were statistically analyzed by ANOVA using the MINITAB software package.

RESULTS AND DISCUSSIONS

Plant height was not significantly influenced by grafting combination while plant diameter affected significantly by grafting (Table 1). Grafted plants were 9 and 15% thinner scion diameter than control for 'Efil/Efil' and 'Efil/Guclu', respectively.

Among the combination, the greatest plant diameter was recorded on non-grafted plant 'Efil'. The plant height was slightly influenced by grafted (Table 1).

Table 1. Effect of grafting combination on plant diameter (mm) and plant height (cm)

	Plant diameter (mm)			Plant height (cm)		
Efil	12.83 A	±	0.14	58.55	±	0.82
Efil/Efil	11.65 B	±	0.15	60.82	±	0.77
Efil/Güçlü	10.87 C	±	0.20	59.23	±	0.97

There was no differences determinate plant fresh and dry weight between grafted and non - grafted plants (Table 2).

Table 2. Effect of grafting combination on plant fresh and dry weight (g)

	Plant fresh weight (g)			Plant dry weight (g)		
Efil	190.7	±	6.86	40.59	±	1.39
Efil/Efil	190.6	±	5.75	40.58	±	1.70
Efil/Güçlü	179.2	±	6.66	36.23	±	1.22

Generally, it has been reported that grafting promotes vegetative growth at different levels dependent on rootstock (Colla et al., 2008). Promoted vegetative growth (plant height) was explained by the vigorous root system of rootstocks, which are often capable of absorbing water and plant nutrients more efficiently than scion roots and serve as a good supplier of endogenous hormones (Kato and Lou, 1989).

But, in this study, we could not determinate highly differenceson vegetative grown such as plant height and fresh weight etc., and between grafted and non-grafted plants.

This situation can be explained by the fact that there is no good combination between the scion and the rootstock.

Table 3. Effect of grafting combination on fruit length (cm) and fruit stem length (cm)

	Fruit length (cm)			Fruit stem length (cm)		
Efil	19.12 A	±	0.19	2.117	±	0.05
Efil/Efil	18.38 B	±	0.17	2.183	±	0.05
Efil/Güçlü	17.58C	±	0.24	2.075	±	0.04

Fruit length was significantly influenced by rootstock; whereas no significant difference was observed on fruit stem length, fruit length (Table 3) fruit diameter and fruit flesh diameter (Table 4).

Table 4. Effect of grafting combination on fruit diameter (mm) and dry fruit flesh diameter (g)

	Fruit diameter (mm)			Fruit flesh diameter (mm)		
Efil	23.475	±	0.33	2.457	±	0.04
Efil/Efil	23.168	±	0.38	2.462	±	0.04
Efil/Güçlü	22.339	±	0.48	2.426	±	0.06

Total yield was influenced by rootstock, whereas fruit weight was not affected by grafting. The highest yield was obtained by the combination 'Efil/Guclu', while the lowest value was recorded on the control (non-grafted) and 'Efil/Efil' (grafted itself) (Table 5).
Grafted rootstock ('Efil/Guclu') produced around 12% more yield than control plant (Table 5).

Table 5. Effect of grafting combination on fruit weight (g) and total yield (kg/plant)

	Fruit weight (g)			Total Yield (kg/plant)		
Efil	32.98	±	0.70	0.66	±	0.09
Efil/Efil	33.22	±	0.70	0.66	±	0.04
Efil/Güçlü	31.12	±	1.18	0.75	±	0.10

It was demonstrated that grafting per se directly affects plant yield (Nielsen and Kappel, 1996). Its influence can be exerted by the interaction of some or all of the following processes: increase of water and nutrient uptake due to the rootstocks vigorous root system (Lee, 1994), enhanced production of endogenous-hormones (Zijlstra et al., 1994), and enhancement of scion vigor (Leoni et al., 1990).
The joint action of some or all of these processes could explain the higher yield in pepper from grafted plants. There are some reports that certain rootstocks may cause deterioration in fruit quality (Lee, 1994).

CONCLUSIONS

In the present study, some of qualities such as fruit length, fruit stem length, fruit diameter, fruit flesh diameter were not affected by grafted combination. Therefore, the use of grafted pepper plants under open field conditions would represent a potential strategy for an increase in total yield and some of the soil diseases without having remarkable deterioration in the taste of the peppers.

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RESEARCH ON THE EFFECT OF VERMICOMPOST FERTILIZATION ON EGGPLANTS SEEDLINGS (*SOLANUM MELONGENA* L.)

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Abstract

Although worldwide, the use of vermicomposts in horticulture is a well-known technique, in Romania, this is at pioneering level. Therefore, the present study presents the results of an experiment that aimed at determining the influence of the application of vermicompost in the substrate in different proportions and the foliar application in different concentrations of the vermicompost filtered macerate on the seedlings of eggplants (Solanum melongena L.). The analysis of the results revealed that, compared to the blank variant, to which no vermicompost was applied, all the other variants have recorded superior results in the growth and development of seedlings. The best results were obtained in substrate fertilizer variants, 20% and 10%. Favorable results were also recorded in the variant where the vermicompost was applied foliar as filtered macerate at a concentration of 30%.

Key words*: Solanum melongena L, vermicompost, growth, eggplants.*

INTRODUCTION

The massive use of chemical fertilizers in horticulture has already produced negative effects on the quality of production, the environment and human health over the last three decades, with the concept of sustainability becoming a key issue for the future (Lichtfouse et al., 2009). Lately, amid the development of concepts such as integrated and biological horticulture, it has been necessary to reconsider the fertilizers used in horticultural practice. An alternative may be the use of vermicomposts, which, compared to conventional composts, are the product of accelerated biooxidation of organic matter by the use of high densities of earthworms without the thermophilic phase (Dominiguez et al., 1997).

Vermicompost is rich in nutrients, easily soluble in plants (Chaoui et al., 2003). It also contains growth hormones, enzymes and beneficial microorganisms and does not contain pathogens and toxic chemicals (Canellas et al., 2002). The use of vermicomposts leads to the stimulation of plant immunity. Edwards et al. (2004) established that the vermicompost has reduced some fungal diseases such as *Phytium* in cucumbers, *Rhizoctonia* in radishes, *Verticillium* in strawberries and *Phomoposis* and *Sphaerotheca fulginea* in field grapes.

Several studies have evaluated the impact of the application of vermicomposts in substrate mixtures for the production of seedlings, in terms of plant growth and development.

The best responses usually occurred when the vermicompost had a relatively small proportion (20-40%), higher amounts of vermicompost, did not always improve plant growth (Atiyeh et al., 2000; Hashemimajd et al., 2004; Prasanna et al., 2010).

Another possibility of using vermicomposts is foliar application, as a filtered macerate (vermicompost tea), which can provide a rich and active and alive bacterial flora, organic acids, growth regulators and mineral nutrients that are soluble in aerial organs of plants (Edwards et al., 2006).

The present experiment aimed at testing the fertilizing action of the vermicompost obtained at VRDS Buzau over the seedlings of the *Solanum melongena* L. eggplants in order to recommend it to the Romanian sapling producers as a fertilizing support of the growth rate and development of the plants, which would lead to a better utilization of organic residues and waste, with implications for reducing the production costs of seedlings and a significant reduction of environmental pollution.

MATERIALS AND METHODS

The experiment was carried out in the spring of 2017 within the vegetable growing sector of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, and it assumes the installation of a monofactorial experimental scheme with the following variants: V1 - unfertilized (100% peat); V2 - seedlings fertilized in the substrate with 10% vermicompost (90% peat); V3 - seedlings fertilized in the substrate with 20% vermicompost (80% peat); V4 - foliar fertilized seedlings with filter macerated vermicompost in 20% concentration and V5 - foliar fertilized seedlings with 30% filtered macerated vermicompost. In order to produce the seedlings were made directly in the box in the production department of the seedlings in the Hortinvest greenhouses on the 14th of February 2017, the boxes being filled with the Klasman TS3 peat. Mass uprising occurred very quickly, 9 days after the date of sowing, February 23, 2017. Sowing was done on March 2nd in 330 cc plastic pot filled with Klasman TS3 ± vermicompost 10% and 20% (V2 and V3) peat, according to the experimental protocol. Care work has been applied uniformly to ensure optimal growth and development conditions, automated, computer-assisted: Temperature T^{o} = 20-28^{o}C; substrate humidity 55-65%; ventilation of the space and maintaining the correct lighting regime.

In order to evaluate the influence of vermicompost on the growth and development of seedlings, observations and measurements were made at one moment only, at the end of the experimentation period, when the seedlings were good for planting, April 10, 2017, the seedlings age being of 52 days.

By direct observations and measurements we determined: plant height (HPA); root length (HR); the number of true formed leaves (NFZ); leaf frequency (NFZ / HPA); collet diameter (collet Ø); mass of aerial vegetative apparatus (MPA); mass (MR); the volume of roots (VR); and the total mass of seedlings (Mtot).

The vermicompost used in the experience was produced at the Vegetable Research-Development Station Buzau, using the system of continuously fed beds. Beds are elongated, in a rick style, with a height of 50 cm. The order of the operations involved first placement of the bed, followed by inoculation with earthworms (*Eisenia foetida*) and then repeatedly covering with thin layers of 10-15 cm of compost.

In time, a stratified stratum is created, with the finished product at the bottom (vermicompost), partially consumed in the middle and fresh topped food.

RESULTS AND DISCUSSIONS

The analysis of the seed growth results is presented in Table 1.

It is highlighted that the plants achieved the best and balanced growth in V3, where all the analyzed indicators recorded the highest values: 6.2 formed leaves; 28.2 cm plant height; 22.8 cm root length and 0.21 leaf frequency, followed closely by V5 and V4 that had balanced growths. At the opposite end, V1, unfertilized, achieved the weakest growth, all the indicators analyzed being lower than the other variants.

Table 1. Growth of eggplant seedlings at the end of the experimentation period

Var.	NFZ	HPA (cm)	HR (cm)	NFZ/HPA (no./cm)
V_1	5.8	22.6	13.6	0.25
V_2	6.0	25.8	18.2	**0.23**
V_3	**6.2**	28.2	**22.8**	0.21
V_4	5.6	25.6	21.8	0.21
V_5	5.8	**29.2**	16.8	0.19

As can be seen from Figures 1 and 2, vermicompost fertilization has influenced at least significantly the height of the aerial part (R^2HPA = 0.639) and the seed growth balance, respectively the leaf frequency (R^2 = 0.9423).

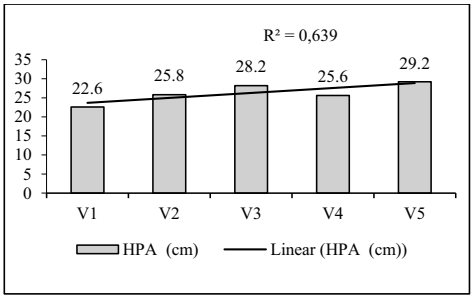

Figure 1. Influence of fertilization with vermicompost on the growth of eggplant

Results on the development of eggplants, evaluated by means of mass, volume and diameter indicators, are presented in Table 2 and Figure 3.

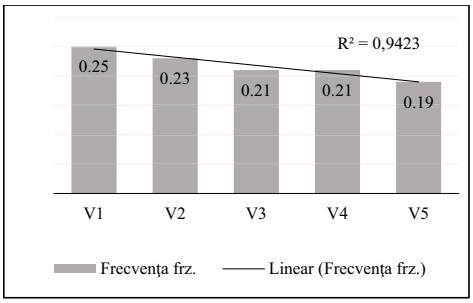

Figure 2. Influence of fertilization with vermicompost on the frequency of leaves in eggplants

Results on the development of eggplants, evaluated by means of mass, volume and diameter indicators, are presented in Table 2 and Figure 3.

Analysis of the results again reveals V3 as the most balanced; In general, the indicators have recorded superior values (10.4 g aerial parts weight, 9.8 cm^3 root volume and 6.2 mm collet diameter), or equal to the other variants: root mass 9.3 g and total mass 19.6 g. This is followed by V2 and V4, variants that have values close to V3 and superior to the other variants. V1 has been shown to be the weakest variant in terms of accumulation compared to other variants.

Table 2. Development of seedlings at the end of the experimentation period

Var.	MPA (g)	MR (g)	Mtot (g)	VR (cm^3)	Ø collet (mm)
V1	7.2	5.2	12.4	5.2	5
V2	8.4	11.8	**20.2**	**9.8**	5
V3	**10.4**	9.2	19.6	**9.8**	**6.2**
V4	7.2	**12.4**	19.6	9	5
V5	9.4	9.2	18.6	9	5.2

From Figure 3 it can be seen that fertilization with vermicompost has a decisive and complex influence on the total mass accumulations of plants ($R^2 = 0.83$).

The indicator showing the weakest differences is the volume of the root system, where for V2 and V3 it recorded 9.8 cm^3, and for V4 and V5 9 cm^3. The other indicators showed much stronger nuances.

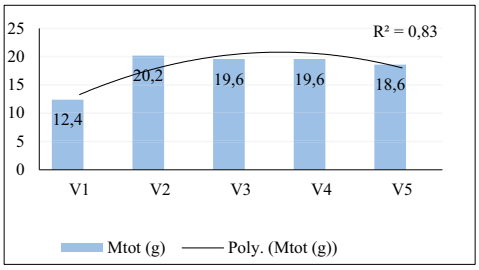

Figure 3. Influence of vermicompost fertilization on the total weight of eggplant seedlings

The indicator showing the weakest difference is the volume of the root system where, for V2 and V3, it recorded 9.8 cm^3 and for V4 and V5 9 cm^3. The other indicators showed much stronger tones, presented and discussed throughout the paper.

Looking at the overall experience, from the point of view of the growth and development of the eggplants, V3 is highlighted, fertilized in substrate with 20% vermicompost, having the best and balanced growth and development of seedlings. Diametrically opposed, the least favourable variant is the unfertilized control variant, which recorded the most unbalanced growth and development of seedlings.

CONCLUSIONS

In carefully analysing the results and interpretations made, we can say that substrate fertilization with 20% vermicompost, determined the obtining of the best seedlings. Very good results in the quality of seedlings have been obtained in the case of V2 and V5. We note that fertilization in the substrate has provided a better growth and development of eggplants seedlings, compared to the foliar application of filtered macerate vermicompost.

As a result of the vermicompost fertilization program of eggplants, their age has decreased from 75 - 80 days (Ciofu et al., 2003; Dobrin 2016), to 52 days, which makes it possible to achieve important economies with the warming of the seedlings production areas, because the sowing can be done almost 1 month later.

Therefore, we recommend to the seedlings producers, using the three fertilization formulas, respectively 20% and 10% vermicompost in the substrate and foliar fertilization with 30% filter maceration vermicompost for the production of eggplants.

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EFFECT OF THE USE OF NEW METHODS FOR THE REMEDIATION OF OIL POLLUTED SOIL

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Abstract

The present study performed the investigation of new environmental friendly methods to remediate polluted soil with crude-oil. The experiment was carried out in greenhouse, in controlled condition using polluted soil and perlite, in different percent. Also, we used five organic fertilizers (Vermiplant, Amalgerol, Poco, Iguana and Formulex). Based on the data obtained under controlled conditions, the best results were obtained in variants where the percentage of perlite used in the soil mix was 25%. The 50% and 75% perlite mix with polluted soil quantities used in the blend did not bring significant differences in plant growth. The research on the influence of remedial measures of the polluted soil on main growing parameters revealed significant differences on physical and chemical properties of the soil, on plant growth, biomass production and plant biochemical composition before and after the treatment. The proposed technology effectively recovered soil properties and plant growth. Microbial populations included 6-15 bacterial strains belonging to Pseudomonas, Bacillaceae and actinomycetes and 5-10 fungal species. Treatments, especially when applied on soil diluted with perlite stimulated species diversity increasing and pathogen inhibition.

Key words*: bean, bioremediation, microbial populations, pollution, perlite.*

INTRODUCTION

The technology for remediation of soil contaminated with petroleum residues or residual salts, which is the subject of this scientific work, is concerned with the use of expanded perlite as a constitutive element of soil quality improvement recipe.

The expanded perlite is a granular, light and porous product based on chemically stable (SiO_2, Al_2O_3, K_2O, Na_2O, Fe_2O_3, CaO, MgO, TiO_2) with density ranging from 40 to 250 kg/m^3. The raw material is the perlitic rock, of volcanic origin, generated by the lava solidified in water. Through this process, the molecular structure of the rock remained chemically bound water. The presence of water molecules causes the explosion of rock granules brought to temperatures of 800-1000^{o}C. Due to the semiplastic state, where the granules of rock are found at the temperature of the furnace, there is the phenomenon of expansion and breaking of the granules. A granular material is obtained very easily and porous, with various granules, from 0 to 6 mm.

Plants germinate and grow in environments where water, mineral elements and air are available. Oil pollution is a major risk for the biodiversity of the land due to the fact that both crop and bacterial microflora are affected.

Oil reduces soil fertility so nutrients become inaccessible for plants. Oil has a higher density than water and displaces air and water between soil particles, making it difficult for mineral absorption and triggering anaerobic metabolism. Soil properties determine the rate of absorption and plant growth, directly affecting crop production.

Bioremediation and natural rehabilitation occur over a long time, and as such in the rehabilitation of these soils, the scientific community is involved in finding optimal and

rapid solutions (Malschi, 2015; Baek et al., 2004). Aromatic hydrocarbons found in petroleum are large complex high-persistence molecules that typically require powerful reagents to annihilate their action.
Compared with biological methods, physical and chemical methods may produce secondary pollution to reclaim oil contaminated soil (Zhan et al., 2017).

MATERIALS AND METHODS

Experiments were performed in the greenhouse conditions. We used the cultivar Unidor of bean characterized through determined hight, vegetative period of 79 days and resistences at anthracnose, *Colletotrichum lindemuthianum*; BCMV-VMT and PSP-*Pseudomonas syringae* pv. *phaseolicola*.
The polluted soil was collected from Icoana commune, Olt district, situated in the SV part of Romania.
We performed soil mixtures with perlite in varying proportions, and fertilization was performed with Amalgerol, Vermiplant, Poco, Iguana and Formulex in a 2%. The experimental variants are presented in Table 1.

Table 1.The experimental variants

Variant	V1 100% polluted soil	V2 75% polluted soil +25% perlite	V3 50% polluted soil +50% perlite	V4 25% polluted soil +75% perlite
Control	V1/1	V2/1	V3/1	V4/1
Amalgerol	V1/2	V2/2	V3/2	V4/2
Vermiplant	V1/3	V2/3	V3/3	V4/3
Poco	V1/4	V2/4	V3/4	V4/4
Iguana	V1/5	V2/5	V3/5	V4/5
Formulex	V1/6	V2/6	V3/6	V4/6

We determined: the amount of water required to soak up the soil; the amount of water consumed by plants (throughout the experiment); growth dynamics of plants; the total vegetative mass resulting; distribution of the root system (at the end of growth).
The caracteristic of fertilizers used in experiment. **Amalgerol** is a product with that contain natural oils with benefic effects on the plant regarding increases the root mass of plants and the absorption of nutrients.
VermiPLANT is a totally natural product obtained from earthworms that contains microelements such as barium, zinc, iron, manganese and amino acids. **POCO** is a totally natural product with pH: 8.5-9.5 that contained: Calcium 0.04-0.05%; Iodine: 6.30-12.70 mg/l; Magnesium 0,50-0,80 mg/l; Nitrogen (N): 0.025-0.038 mg/l; Potassium: 0.50-0.64%; Sodium (Na): 0.088-0.120%; Sulfur 0.028-0.050%. **Iguana** is a organic product with 4% nitrogen, 3% phosphorus, 6% potassium. **Formulex** is also an organic product that contains: N 2.40%; P_2O_5 0.85%; K_2O 3.36%; CaO 1.85%; B 0.0108% and microelements.

RESULTS AND DISCUSSIONS

Data obtained from bean plants grown under controlled conditions showed that the best results were obtained in variants where the percentage of perlite used in the soil mix was 25%. The 50% or 75% perlite quantities used in the blend did not bring significant differences in plant growth (Figure 1).

Figure 1. Experimental variants

In the case of polluted soil (V1), the biomass of bean plants was higher in the variant that we administered the Vermiplant product (176.25 g V1/3). This was with 16.75 g higher than the control variant (159.5 g V1/1)). The variant we improved the soil with 25% perlite and fertilized with Vermiplant produced an average plant biomass of 201.67 g, with 32.67 g more than the control variant (Figure 2).

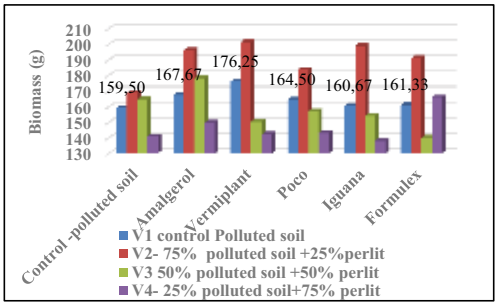

Figure 2. Influence of substrate on plant biomass

Statistically analyzing the obtained data, we found that, in the case of 25% perlite soil improvement and organic fertilizers, we obtained increases in the mass of plants, in all experimental variants, the differences being very positive at V2 (Table 2).

Table 2. Total biomass of bean plants

Variants	V1	V2	V3	V4
Control	159.50	169.00***	164.67**	141.00 ^{ooo}
Differences	100.00	105.96	103.24	88.40
	DL5%= 2.690 DL1% = 4.080 DL0.1%= 6.50	DL5% in % = 1.6865 DL1% in % = 2.5580 DL0.1% in %= 4.0752		
Amalgerol	167.67	196.33***	178.00***	150.00 ^{ooo}
Differences	100.00	117.09	106.16	89.46
	DL5% = 2.050 DL1% = 3.100 DL0.1% =4.950	DL5% in % = 1.2226; DL1% in % = 1.8489; DL0.1% in %= 2.9522		
Vermiplant	176.25	201.67***	150.67 ^{ooo}	142.50 ^{ooo}
Differences	100.00	114.42	85.49	80.85
	DL5% = 0.110 DL1%= 0.170; DL0.1% = 0.280	DL5% in % = 0.0624; DL1% in % = 0.0965; DL0.1% in %= 0.1589		
Poco	164.50	184.00***	157.33 ^{oo}	143.25 ^{ooo}
Differences	100.00	111.85	95.64	87.08
	DL5% = 4.230 DL1% = 6.400 DL0.1% = 10.190	DL5% in % = 2.5714 DL1% in % = 3.8906 DL0.1% in %= 6.1945		
Iguana	147.56	199.00	154.20	138.17
Differences	100.00	134.86 **	104.50	93.64
	DL5% = 21.350 DL1% = 32.300	DL5% in % = 14.4690; DL1% in % = 21.8899		
Formulex	161.33	191.25	140.00	166.00
Differences	100.00	118.55***	86.78 ^{ooo}	102.89 **
	DL5% =1.970 DL1% = 2.980 DL0.1% = 4.760	DL5% in % = 1.2211; DL1% in % = 1.8471; DL0.1% in %= 2.9505		

V1 - contaminated soil
V2 - contaminated soil 75%+25% perlite
V3 - contaminated soil 50%+50% perlite
V4 - contaminated soil 25%+75% perlite

In the case of applying a higher amount of perlite to the soil by applying the fertilizer dose, we obtained statistically significant negative results in most of the variants except for the variant to which the Formulex product was applied.

The correlations between the applied product and the perlite-polluted soil mixture, in varying percentages (Figures 3-8), led to the conclusion that there was a significant correlation with the Vermiplant product (R^2=0.5386) (Figure 5). For Formulex, the relationship was insignificant (R^2 = 0.0522) (Figure 8).

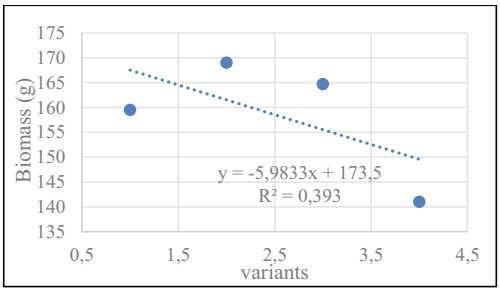

Figure 3 Influence of soil improvement with perlite at the control variant on plant biomass

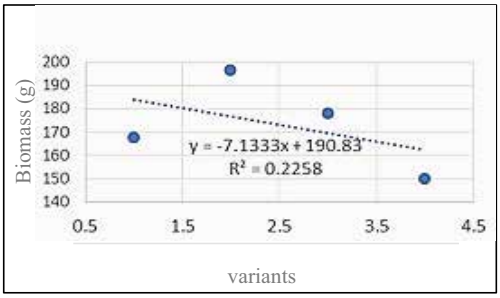

Figure 4. Influence of soil improvement with perlite at variant fertilized with Amalgerol on plant biomass

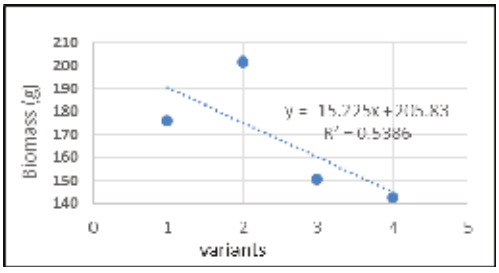

Figure 5. Influence of soil improvement with perlite at variant fertilized with Vermiplant on plant biomass

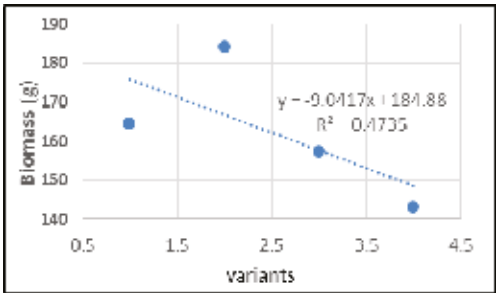

Figure 6. Influence of soil improvement with perlite at variant fertilized with Poco on plant biomass

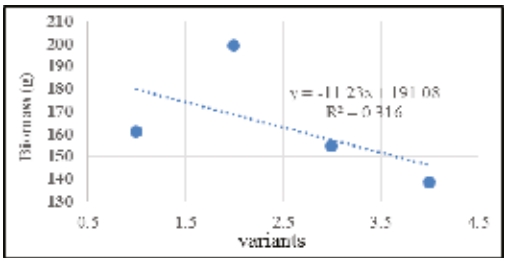

Figure 7. Influence of soil improvement with perlite at variant fertilized with Iguana on plant biomass

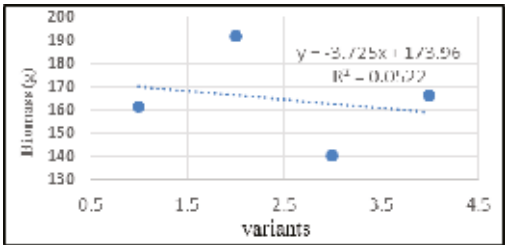

Figure 8. Influence of soil improvement with perlite at variant fertilized with Formulex on plant biomass

Analyzing the influence of the above mentioned fertilizers on the perlite-soil combinations (Figures 9-12), we estimate that for the control variant (polluted soil), the influence of fertilization application showed insignificant relationship (R^2=0.0405) (Figure 9).

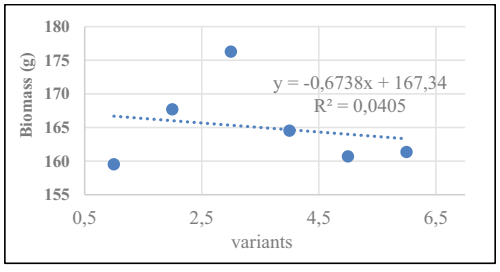

Figure 9. Influence of fertilizers on polluted soil

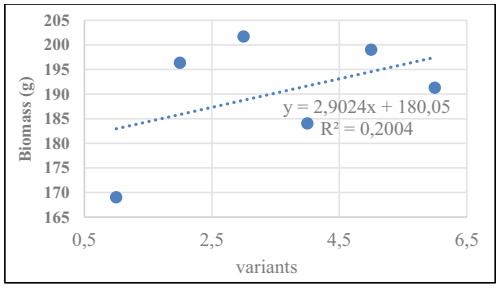

Figure 10. Influence of fertilizers on polluted soil ameliorated with perlite in percent of 25%

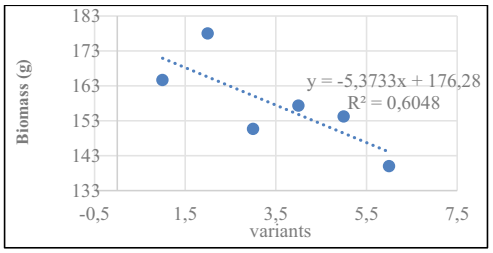

Figure 11. Influence of fertilizers on polluted soil ameliorated with perlite in percent of 50%

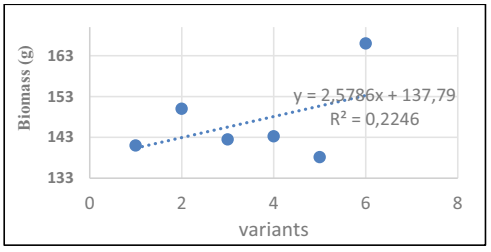

Figure 12. Influence of fertilizers on polluted soil ameliorated with perlite in percent of 75%

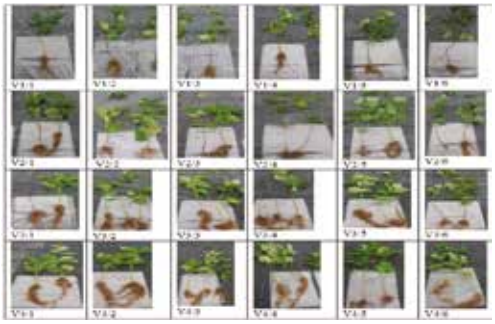

Figure 13 Aspect of bean plants in experiment

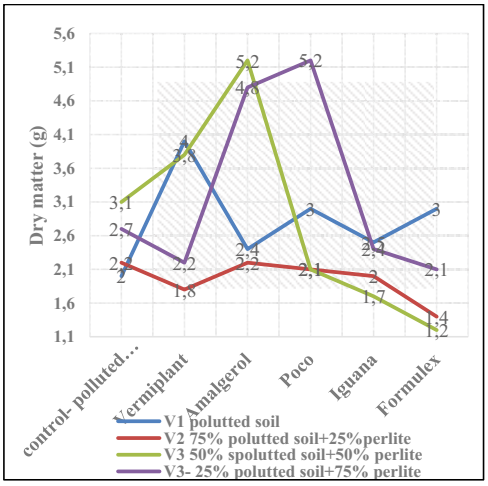

Figure 14. Dry matter content of the roots

Analyzing the aspect of plants (Figure13) and dry matter content of the roots, we found that the highest values were recorded in soil variants improved by 50% perlite, and fertilized with Vermiplant (5.2 g) and the variant improved with 75% perlite and treated with Poco (Figure 14).
The highest dry matter content of the leaves was recorded in the 25% perlite-enhanced soil variant, and fertilized with Iguana (Figure15).

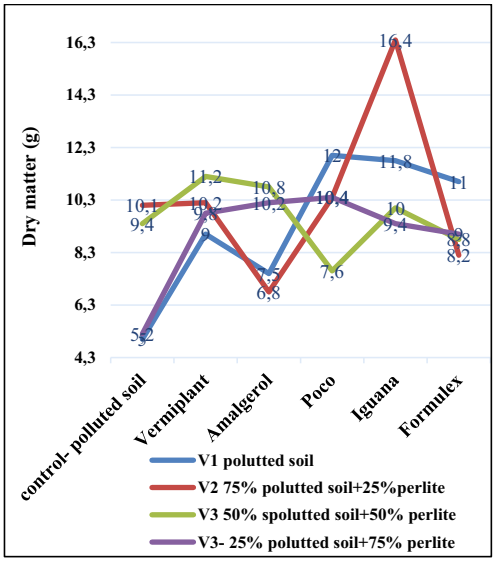

Figure 15. Dry matter content of the leaves

Microbial populations in experimental variants were represented by 6 to15 bacterial species including actinomycetes belonging to 1-4 Series. The stimulation of species diversity was noticed and ecologic ameliorative treatments were added to soil, especially in variants mixed with various proportions of perlite.
In many variants, *Pseudomonas fluorescens* was dominant, followed by different species of *Bacillaceae* (Figure 16). Fungal microflora included communities of 5 to maximum 10 species with variation as a function of experimental factors (substrate type and the nature of stimulating treatment applied). An inhibition of *Fusarium* pathogenic species as compared with control was evidenced in polluted soil under the influence of various stimulate treatments and the dominance of cellulolytic and hydrocarbon degrading species *Cunninghamella elegans*. Species of genus *Aspergillus*, accompanied by antagonists *Trichoderma, Paecilomyces* and other species with high ability for enzymatic degradation of various organic substrates were identified with high frequency in soil with perlite and stimulating treatments (Figure 17).

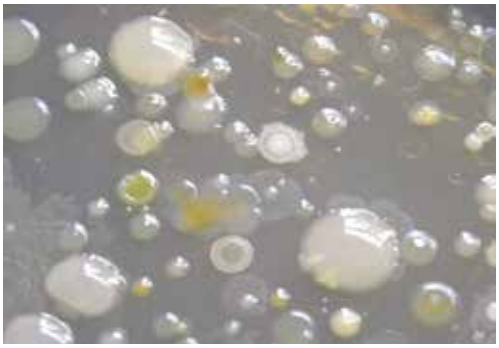

Figure16. Aspects of the bacteria developed in 75% polluted soil + 25% perlite and Iguana (V2/5)

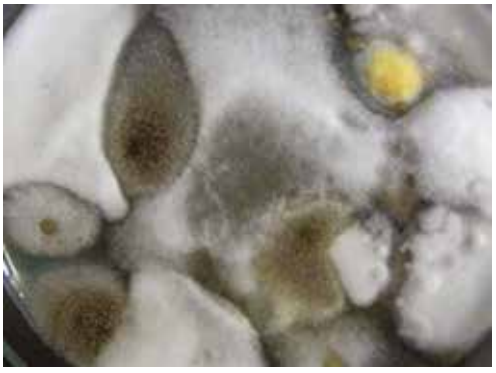

Figure17. Aspects of the fungi developed in 25% polluted soil + 75% perlite (V4/2) and Amalgerol

Results of the present study confirm our previous research that showed the involvement of pseudomonads (Matei et al., 2007; Scarlat et al., 2015) and *Aspergillus* species in hydrocarbon degradative processes.
Other authors reported beneficial effect of bioremediation technologies including bacterial and fungal biodegraders with stimulating treatments for soil conditions improvement and plant growth and enhancing (Potin et al., 2004; Verma et al., 2006).

CONCLUSIONS

Application of organic fertilizers has led to the increase of plant mass in all variants. When

Vermiplant was applied (V2/3), the highest plant mass was 201.67 g compared with the rest of the variants.

There were direct relationships between the polluted soil mixture (75% polluted soil and 25% perlite) and the Vermiplant product (V2/3).

Regarding the dry matter content of the roots, it was result that the variants cultivated on a mixture of 50% polluted soil mixed with 50% perlite with application of Amalgerol (V3/2) and also in the 25% polluted soil mixed with 75% perlite with application of Poco products (V4/4), the dry matter content was the highest (5.2 g for both variants).

The highest dry matter content at the leaves was recorded in the fertilized variant with the Iguana product, plants grown on the substrate with polluted soil (75%) and perlite (25%) (V2/5).

Microbial populations included 6-15 bacterial species dominated by *Pseudomonas, Bacillaceae* and actinomycetes and 5-10 fungal species.

Treatments, especially when applied on soil diluted with perlite stimulated species diversity increasing and pathogen inhibition.

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THE INFLUENCE OF ALTERNATIVE TECHNOLOGICAL SEQUENCES ON THE QUALITY OF MELON PRODUCTION

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Abstract

At present, there is a growing consumer interest in high value-added vegetable products. It is therefore necessary to limit the use of fertilizers and synthetic chemical pesticides because of the harmful effect on the environment. In this regard, a melon culture was established in the polyethylene tunnels to which Lignohumat biostimulator product was applied in vegetation as an alternative for chemical foliar fertilization. It is a humic product, granulated with microelements and with the effect of growth and protection against stress factors. Three treatments were applied in three doses of fertilization. The specific of variants was: V1 (Control)-unfertilized; V2-fertilized with 100 g/ha; V3-fertilized with 150 g/ha; V4-fertilized with 200 g/ha. The determinations focused on the quality of the production. The 200 g/ha Lignohumat dose influenced the quality attributes and the dose of 100 g/ha Lignohumat the antioxidant activity of fruit. Thus, was recorded a SUS content of 8.05%, SUT of 12.29%, reducing sugars of 3.73%, vitamin C of 29.4 mg/100 g, TP of 42.03 mg GAE/100 g, carotene of 20.98 mg/100 g f.w. and antioxidant activity of 184.44 μMTE/100 g.

Key words: *Cucumis melo, carotene, organic fertilization.*

INTRODUCTION

In Romania, the watermelon (*Cucumis melo* L.) is highly appreciated and annually cultivated especially in South and South-East Romania, and in recent years there has been an increase in protected crops. Melon fruits are consumed at physiological maturity, fresh or mixed with other fruits, such as dessert salads and also processed in the form of jams, etc. Cantaloupe is a very good source of vitamins A, C and β-carotene and can be used as juice fruit (Mohamed and Maha-Mohamed, 2016). Also, the melon seeds are a good source of natural antioxidants with significant biological functions and may serve as food ingredients (Zeb., 2015). By its composition, melon can be a rich source of many nutrients related to human health (Lester, 2008).

It is very important to find the optimal fertilization dose as well as the appropriate combinations of fertilizersfor plant health, high productivity and fruit quality (Dinu et al., 2017; Bouzo and Cortez, 2015). Potassium is an important macronutrient and the most abundant cation of the superior plants. Also, K^+ is essential for enzyme activation, protein synthesis, for photosynthesis and mediates osmoregulation during cell expansion and for stomatal movements and tropisms (Silva et al., 2007). Nutritional imbalances, particularly inadequate K supply, often contribute significantly to yields and low crop quality (Jifon, 2011).

In recent years, bio-fertilizers based on humic acids are increasingly used, preparations that reduce the negative effects of chemical fertilizers on the environment. Fertilizers with humic substances can be used on different types of soil, as well as in technologies for improving degraded or contaminated soils and are efficient for many cultures (Sîrbu et al., 2016). Humic acids replace the requirements of other fertilizers, increase productivity, improve soil drainage and establish a favorable environment for the development of soil microorganisms (Salman et al., 2005). In addition, they can induce changes in plant primary and secondary metabolism related to abiotic stress tolerance which collectively modulate plant growth (Canellas et al., 2015). The application of humic acids has been

reported by many authors with a positive effect on tomato plant growth and production (Dinu et al., 2015), the growth of pepper production (Karakurt et al., 2009), germination of tomato seeds (Dinu et al., 2013), growth and development of *Spathiphyllum wallisii* (Manda et al., 2014). Thus, the application of biostimulators can be considered a good strategy of vegetative and generative growth of plants, a high nutritional value of production and low impact on the environment (Becherescu et al., 2016; Dobrin et al., 2016; Naidu et al., 2016; Parađiković et al., 2011).

This study aims to investigate the effect of Lignohumat doses, biological fertilizer based on humic acids, on the quality of melon fruit.

MATERIALS AND METHODS

The experience was placed in the polyethylene tunnels of the Faculty of Horticulture and Faculty of Agriculture, University of Craiova, Romania, in randomized blocks, in three repetitions, in 2015-2016. The biological material was Ananas cultivar (*Cucumis melo* var. *reticulatus*) oval fruits with a pineapple scent and orange skin with reticulated appearance. Date of planting to the polyethylene tunnels was in the first decade of May while plant density was 3.2 plants m/2. The specific of the variants was: V1 (Control) - unfertilized; V2- fertilized with 100 g/ha Lignohumate; V3-fertilized with 150 g/ha Lignohumate; V4- fertilized with 200 g/ha Lignohumate. This is a foliar humic biostimulator, containing microelements in the form of chelates having the highest dose of Potassium Humate 850 g/kg.

Lignohumat organic fertilizer was applied foliarly in three treatments at 10 days interval and the culture technology was that specific to melons grown in polyethylene tunnels. During the growing season, it also pursued the application of some products to combat weeds, diseases and pests with low environmental impact products for sustainable agriculture. Thus, at the basic fertilization was applied 20 t/ha compost.

Samples preparation

A quantity of100g of each sample was broken up in blenduire to mix. 1 gram of homogenized sample (pulp) was mixed with 10 mL 80% methanol, intensely agitated for 10 minutes using avortex. The extraction of phenolic compounds was carried out by keeping the mixture in an ultrasonic bath for 70 minutes. The mixture was filtered, and to the residue solid was added 5 mL of 80% methanol and the extraction procedure repeated. Obtained the two extracts were combined and analyzed using protocols specific work to determine the total polyphenols and antioxidant capacity.

Dry matter content was determined gravimetrically at 105°C to constant weight (ISO751: 2000) (%) and the soluble solids content (SSC) (%) was determined using a digital refractometer (KrussOptronic DR 301-95) at 20°C.

The acidity was determined by titration with 0.1 N sodium hydroxide (NaOH) and expressed as (%) citric acid and reducing sugars (%) were extracted in distilled water (1:50 w/V) and assayed colorimetric with 3.5-dinitrosalicylic acid using glucose as standard.

The determination of vitamin C content

A sample of 5-10 g of tomatoes, previously ground with quartz sand has been put into a 100 ml balloon by using a solution of 2% hydrochloric acid. It has been stirred and after sedimenting it has been filtered into a dry glass. A 10 ml aliquot has been passed into a Berzelius glass, to which 30 ml of distilled water; 5 ml of 1% potassium iodate and 1 ml solution of starch have been added. It has been then titrated with potassium iodate N/250 stirred until becoming bluish (Chowdhury, 2004).

The determination of total carotenoids

The weighed samples, having been put separately in 95% in acetone (50 ml for each gram), were homogenized with Braun MR 404 Plus for one minute. The homogenate was filtered and was centrifuged using the Hettich Universal 320/320R centrifuge at 2500 rpm for ten minutes. The supernatant was separated and the absorbances were read at 400-700 nm on Cary 50 spectrophotometer.

The total phenols (TP) in melon fruit was estimated by the method proposed by Mallick and Singh (1980).

The capacity of extracts to reduce the radical 2.2-diphenyl-1-picrylhydrazyl was assessed colorimetric. 2 mL of 0.075 mM DPPH solution in ethanol was mixed with 0.1 mL

extract and vortexed thoroughly. The absorbance of the remaining DPPH radicals was measured at 517 nm. The results were expressed as μMTrolox equivalents (TE)/100 g. The ABTS radical cation scavenging activity of the methanolic extract was assessed colorimetric. 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 and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. 0.1 ml of sample extract was mixed with 2.9 ml of diluted ABTS radical cation solution. After reaction at room temperature for 6 min., the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of ABTS radical cation scavenging activity. The final results were expressed as μMTrolox equivalents (TE)/100 g fw (Soare et al., 2016).

Statistical analysis

The data recorded were statistically processed by using the analysis of the variance (ANOVA) with a significance level of $p<0.05$ by Duncan's multiple range test. Also, were carried correlations between the analysed characters (Pearson's correlation analysis).

RESULTS AND DISCUSSIONS

Inappropriate fertilizations with N, P and K cause nutritional imbalances, also inducing deficiencies of calcium and boron, forming fruits with deformations, abnormal color and low storage capacity (Silva et al., 2007). Precise estimates of quantities of nutrients for crops as well as application in critical times are essential for increasing production and quality while protecting the environment.

Fruit quality is determined by complex networks of metabolic pathways developed during fruit ripening. melon (*Cucumis melo* L.) comprises a broad array of genotypes, in which the fruit accumulate soluble sugars, organic acids, secondary metabolites of pigmentation and aroma volatiles to varying levels. In addition, *Cucumis melo* includes fruit with different biochemical accumulations in ripening physiology, levels of ascorbic acid, additional secondary metabolites and other components of quality (Katzir et al., 2008).

In the present paper, the total dry substance (TDS) of melon fruit recorded upward values, along with increasing the fertilization dose, from 7.59% at the control variant to 12.29% at the fertilized variant with Lignohumat 200 g/ha. Regarding the soluble solids content (SSC), it also recorded upward values with the fertilization dose (Table 1). The values found are similar to those reported by Salman et al. (2005), on the growth of total dry substance and the soluble solids content with the increase in humate acid potassium, in watermelon, Aswan hybrid. Some authors have concluded that in cucumber plants treated with nitrogen-based biofertilizer at higher doses, it resulted total soluble solids higher compared to lower doses (AbdAlla et al., 2009; Oliveira et al., 2003)

The effect of the culture system on titratable acidity may actually be an important factor in the quality fruit. The titratable acidity varied in the four studied variants depending on the fertilization dose. The highest values were recorded in variant which had the highest fertilization dose, Lignohumat 200 g/ha. Different concentrations of humic acid-based products can influence the acidity level of edible cabbage (Soare et al., 2017) or from pepper (Aminifard et al., 2012).

Table 1. Analysis of some quality attributes of melon fruits

Variant	TDS (%)	(SSC) (%ºBrix)	Acidity (citric acid %)	Reducing sugars (%)	Vitamin C (mg/100g f.w.)
V1(Control)- unfertilized	7.59b	5.55c	0.123ab	3.15b	21.2b
V2- fertilized with Lignohumat 100 g/ha	7.94b	6.25bc	0.112bc	1.81c	29.4a
V3- fertilized with Lignohumat 150 g/ha	7.86b	6.95b	0.074c	2.84b	17.9c
V4- fertilized with Lignohumat 200 g/ha	12.29a	8.05a	0.156a	3.73a	22.8b
LSD≤0.05	0.82	0.69	0.04	0.50	1.85

Different letters within the same row indicated significant differences ($P < 0.05$) among variants

Reducing sugars, an important parameter for determining fruit quality of melon, recorded fluctuating values depending on the variant. The content of reducing sugars in fruits treated with Lignohumat 200 g/ha was signicantly higher (3.73%) in comparison with fruits from the control variant. The increase of reducing sugars under the influence of products based on humic acids was also recorded in autumn white cabbage (Soare et al., 2017). Lester (2008) claims that the accumulation of sucrose in melon depends on several factors and is maximal in the final stages of fruit maturation.

The antioxidant potential was evaluated by total polyphenols and vitamin C contents, carotenes, and antioxidant capacity. The presence of phenolic compounds, such as flavonoids, phenolic acids and anthocyanins, besides the vitamins C, E and carotenoids, contribute to the beneficial effects on human health (Podsedek, 2007).

Concerning the vitamin C, in the present study, it was registered a variation amplitude of 17.9 mg/100g f.w. (Lignohumat 150 g/ha) to 29.4 mg/100g f.w. (Lignohumat 100 g/ha). In the variant fertilized with Lignohumat 200 g/ha, vitamin C was 22.8 mg/100g f.w. There is a high variability of ascorbic acid (active form of vitamin C) influenced by Lignohumat concentration. Similar results have been reported Salandanan et al. (2009) in a study regarding the antioxidant properties and quality attributes or 10 melon (*Cucumis melo* L.) cultivars grown under conventional and organic conditions, recorded a variation from 16.2 to 38.1 mg/100 g fresh weight C vitamin. According to some authors, the vitamin C content of vegetables can vary depending on the intensity of light, temperature, humidity, pollution (Dinu et al., 2016).

Total polyphenols in the present study recorded significant differences in the variant treated with 100 g/ha of Lignohumat, 42.03 mg GAE/100 g f.w., after which it decreased with the increase of the fertilization dose. Salandanan et al. (2009) noted a high variability of the total polyphenols in some melon varieties, influenced by yearly environmental effects and production system (conventional and organic), from 40.5 to 71.7 mg GAE/100 g f.w. and Selale et al. (2012) in their paper, at 42 melon lines and cultivars of different types, reported that total phenolic content in the melon ranged from 118.5 to 357.8 mg GAE/kg^{-1}.

The highest value of antioxidant activity was also recorded in the fertilized variants compared to the control. Astfel, the ABTS value ranging between 88.76 μMTE/100 g (unfertilized) and 150.72 μMTE/100 g (fertilized with Lignohumat 100 g/ha). Increasing doses of Lignohumat did not influence the antioxidant capacity, probably due to the higher content of Potassium Humate and microelements. The results are similar to those reproted by Salandanan et al. (2009) at the organically and conventionally fertilized melon cultivars values of antioxidant capacity from 49 to 220.5 μmol TEAC/100 g f.w.

Table 2. Biochemical determinations of melon fruit

Variant	Total phenolics (mg GAE/100 g f.w.)	Total carotene (mg/100 g f.w.)	Antioxidant activity	
			ABTS μMTE/100 g	DPPH μMTE/100 g
V1(Control)- unfertilized	30.1b	17.38d	88.76c	91.46d
V2- fertilized with Lignohumat 100 g/ha	42.03a	20.98c	150.72a	188.44a
V3- fertilized with Lignohumat 150 g/ha	23.91c	26.28a	113.29b	136.39b
V4- fertilized with Lignohumat 200 g/ha	23.24c	25.1b	89.89c	113.13c
LSD⩽0.05	3.36	2.72	5.79	4.50

Different letters within the same row indicated significant differences ($P < 0.05$) among variants

The same, DPPH radical scavenging activity decreased as the fertilization dose increased from 188.44 μMTE/100 g, (Lignohumat 100 g/ha) to 113.13 μMTE/100 g (Lignohumat 200 g/ha). The unfertilized variant recorded the lowest value of 91.46μMTE/100 g.

Parađiković et al. (2011) have claimed that biostimulators improve antioxidant activity, vitamin C content and phenols, compared to

untreated fruits. Some authors said that cultivars selection, climate conditions, fertilizer types, culture system (conventional or organic) may influence the antioxidant activity of vegetables (Apahidean, 2017; Dinu et al., 2016; Salandanan et al., 2009; Salman et al., 2005). Other authors state that irrespective of the analysed index, variants organically fertilized are significantly superior to the control variant (Draghici et al., 2016). Also, the maturing stage is an important factor in the antioxidant properties of melon, and the cultivar must determine the appropriate harvesting time for fruits with high antioxidant potential (Wulandari et al., 2016).

Regarding the correlation coefficient between some quality characters, positive correlation coefficient values were obtained between ABTS and total polyphenols (0.935), between DPPH and total polyphenols (0.962) and between DPPH and ABTS (0.996) (Table 3). These results agreed with those reported by Selale (2012) which showed significant correlation between antioxidant capacity and phenolic content.

Table 3. Coefficients of correlation (r) between some quality characters of melon

Specification	Total phenolics (mg GAE/100 g)	Total carotene (mg/100 g f w)	ABTS µMTE/100 g	DPPH µMTE/100 g	Reducing sugars (%)	Vitamin C (mg/100 g)
Total phenolics	1					
Total carotene	-0.970	1				
ABTS	0.935*	-0.822	1			
DPPH	0.962*	-0.867	0.996*	1		
Reducing sugars	-0.900	0.767	-0.995	-0.984	1	
Vitamin C	0.891*	-0.974	0.675	0.735	-0.605	1

Correlation coefficient statistically significant *p≤0.05

CONCLUSIONS

The differences between the variants determined by the Lignohumat biofertilizer were due both to the quality, the balance of the nutrients contained in the composition and also to the treatment doses. Thus, the 200 g/ha Lignohumat dose significantly influenced the quality attributes (TSS, acidity and reducing carbohydrates) and the 100 g/ha Lignohumat dose significantly influenced the antioxidant capacity compared to the untreated variant. Although the study was limited only to the influence of biofertilization on a single variety, the differences between the studied variants regarding the antioxidant properties and quality attributes suggest that research can contribute to fertilization opportunities with this product in optimal doses to the melon culture.

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EFFICACY OF PLANT BASED BIOPRODUCTS AGAINST TOMATO SEEDLINGS DAMPING-OFF DISEASE-SHORT OVERVIEW

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Abstract

Most European countries are investing in research to reduce reliance on pesticides and the risks associated with their use. At this moment, farmers' access to a wide range of pesticides is predicted to become limited due to legal regulations regarding sustainable use of pesticides. Therefore, the modern farmers will have to incorporate innovative pest and disease management approaches to reduce their dependency on pesticide use. This study presents an overview about the recent results on the efficacy of natural products obtained from plant extracts to control tomato seedlings damping off disease. It was already demonstrated that several plants contain secondary metabolites that are toxic to plant pathogenic microorganisms infecting horticultural crops, especially on the early emergence of the disease. The rich phenolic compound solutions possess antimicrobial effects and serve as plant defense mechanisms against plant pathogens. For using these products with reproducible efficiency, it is important to compare their mode of action for the optimization of the manufacturing process, the stabilization of these preparations, dates and rates of application.

Key words*: plant bioproducts, tomato disease, plant extract.*

INTRODUCTION

Plant diseases cause significant economical loses in horticultural production every cultivation season. Despite the continuous research on resistant cultivars, the crop losses are still being observed. More often, the intensive use of chemical pesticides resulted in inappropriate application of doses and has not only created fungicide resistance and increased soil contamination, but may also trigger imbalance in the microbial community (On et al., 2015) and a degradative effect on the ozone layer (Goudjal et al., 2014). Moreover, the access of farmers to a wide range of pesticides is predicted to become limited due to legal regulations regarding the implementation of integrated pest management and sustainable use of pesticides. As a result, most countries assign important resources to search for solutions to reduce reliance on pesticides and the risks associated with their use. The occurrence of serious plant diseases can occur at the early stage of seed germination and seedling emergence. Damping off disease and rot of crown and root of seedlings is caused by several different phytopathogens. This disease causes emerging seedlings to collapse and once infected, the seedlings rarely survive to produce a vigorous plant. The period of growth between planting and maturity, is considered as critical, and special attention should be paid to protect sensitive seedlings. As a potentially useful protection strategy, the use of plant products such as essential oils and plant extracts against several plant pathogens has already been demonstrated. Tomato (*Lycopersicon esculentum* Mill.) belongs to nightshade family *Solanaceae*. It is the second extensively grown vegetable crop after potato due to its tangy fruit, taste and high nutritive value containing vitamins particularly vitamin A, C, β-carotene and essential minerals (Chohan et al., 2017; Goudjal et al., 2014).

This study presents an overview about damping off tomato seedlings pathogens and the reported efficacy of natural products obtained from plant extracts to control this disease.

CAUSAL PATHOGENS FOR DAMPING-OFF OF TOMATO SEEDLINGS

Almost all species of vegetables (tomatoes, cucumber, eggplants, radishes, beans) can be

affected by damping off. The young leaves, roots and stems of newly emerged seedlings are highly susceptible to fungal or fungus like infections.

When meeting proper conditions, damping-off pathogens may also cause the rot of root or crown rot in mature plants. Cool and cloudy weather, excessive irrigation resulting in wet or compacted soil, poor ventilation and high humidity due to overcrowding are just few of the prerequisites for damping-off.

Damping off may occur before or after seed germination. In pre-emergence, the seeds will basically fail to germinate after sowing. They appear soft, dried or mushy, with necrotic radicle. In post-emergence damping-off, the seedlings will decay shortly after they have emerged from the soil wards Table 1 (Kato et al., 2013; Pagoch et al., 2015).

The causal agents of damping off are soil borne pathogens that are also able to survive quite well in plant debris, on contaminated tools, pots and potting media. Their spores can be carried by insects like fungus gnats, or can be transported in in irrigation pipes and drainage water. Some pathogens can be introduced on dirty hands, contaminated tools or by hose ends that have been in contact with dirt and debris.

Rhizoctonia spp. and *Fusarium* spp., along with the water mold *Pythium* spp. and *Phytophthora* spp. are the most common pathogens responsible for damping off of many vegetables seedlings, including tomato (*Lycopersicon esculentum*) (Chohan et al., 2017).

Table 1. Key features of fungi or fungal like organisms responsible for attacks on tomato seeds and seedlings (adapted from Blancard, 2012)

Pathogen	Symptoms	Attack frequency	Differentiating structures
***Pythium* spp.** (damping-off)	Soft wet brown rot on the stem base and roots.	Frequently	Oospores, sporangia, and chlamydospores
***Phytophthora* spp** *P. erythroseptica, P. citricola, P. capsici, P. cryptogea, P. nicotianae* (damping-off)	Brown rot on the stem and roots.	Frequently	Oospores, sporangia, and chlamydospores
Sclerotium rolfsii (damping-off, stem base rot)	Brown rot surrounding the stem and extending to the roots level.	Mainly in traditional nurseries in tropical areas.	White mycelium covering seedlings and soil. Spherical sclerotia (1-3 mm), white to brown.
Rhizoctonia solani (damping-off, stem base rot)	Reddish brown wounds on hypocotyl and epicotyl before emergence. After emergence, reddish brown to black spots, on the seedlings	Quite frequently	Compartmentalized mycelium, with a constriction at branches.

The control and prevention of these pathogens is mainly done by application of agrochemicals. However, the present movement towards environmentally safe methods in sustainable agriculture calls for reducing their use.

Recent research directions are focusing on developing environmentally friendly products for the management of plant diseases.

Natural plant products are considered a potential solution as they are important sources of new agrochemicals for the control of plant diseases (Nashwa et al., 2012). Furthermore, plant-based products are easily biodegradable.

PLANT PRODUCTS WITH ANTIFUNGAL ACTIVITY

In a study reported by Singh et al. (2012), four plant products obtained from leaf extracts were tested for their potential antifungal activity against *Pythium aphanidermatum*, the causal pathogen of damping-off of tomato seedling. All products showed significant inhibitory effect on the mycelial growth of the test pathogen, as compared to chemical fungicide control (Bavistin 0.2%). The leaf extract from *Eucalyptus globulus* (92.78%) showed maximum mycelial growth inhibition followed

by *Azadirchta indica* (72.91%) extract (Singh et al., 2012).

The antifungal activities of plant products are attributed to different chemical compounds like phenols, flavonoids, isoflavonoids, coumarins, pyrones, alkaloids etc. present in these plants which effect the growth of pathogenic fungi. For example, the main components of *Eucalyptus* sp. essential oil, determined by GC-MS are β-Cymene (32.1%), Eucalyptol (36.59%), Cryptone (4.35%) and Spathulenol (3.82%) (Rusu et al., 2014).

In a recent study, *Ocimum* species essential oils were evaluated for their *in vitro* antifungal effect through poison food technique against *Rhizoctonia solani* and *Choanephora cucurbitarum*. *Ocimum tenuiflorum, O. gratissimum,* and *O. kilimandscharicum* exhibited complete growth inhibition of both pathogens (24 and 48 h after treatment). Nevertheless, *O. basilicum* showed variable levels of fungal growth inhibition (63.0%-100%). Moreover, the composition of the tested essential oils was analyzed and compared by using capillary gas chromatography (GC/FID) and GC-mass spectrometry (GC/MS). Phenyl propanoids (upto 87.0%) and monoterpenoids (upto 83.3%) were prevalent constituents distributed in the studied O*cimum* taxa (Padalia et al., 2014).

An essential oil of *O. basilicum* and *Cymbopogon citratus* was also tested against three *Phytophthora* species under *in vitro* and greenhouse conditions compared to fungicides. However, *C. citratus* had the lowest EC_{50} values for in vitro inhibition of the mycelial growth of *Phytophthora* spp (between 31.5 and 69.1). In greenhouse, *C. citratus* essential oil reduced disease severity from 47.4% to 60.5% compared to the untreated control (Amini et al., 2016). More recently, another *Ocimum* specie (namely *Ocimum gratissimum*) essential oil was tested for antifungal activity against several isolates of *Fusarium* spp., *Rhizoctonia solani*, and *Macrophomina phaseolina*. The broth microdilution method revealed that *F. oxysporum* f. sp. *lycopersici* and *R. solani* were the most sensitive strains (Mohr et al., 2017).

Rhizoctonia solani is very common soil fungus and is widespread in the world. It can be considered as a biomarker of so called 'sick' soil (Blancard, 2012). In a more extensive research, fifty five species of medicinal plants were employed for *in vitro* antifungal activity testing against *Rhizoctonia solani* AG 2-1 and *Trichoderma harzianum.* The aim was to improve the biocontrol efficacy of *T. harzianum*. *Cinnamomum loureirii* stem bark extract inhibited mycelial growth of *R. solani* AG 2-1 by 73.7%. The combination of *T. harzianum* and *Cinnamomum loureirii* stem bark powder reduced the severity of radish damping-off by 80.6%, suggesting that it can be employed for the control of *R. solani* development (Lee et al., 2011).

Islam et al. (2012) evaluated the seed treatment with neem leaf (*Azadirachta indica*), garlic clove (*Allium sativum*), allamanda leaf (*Allamanda cathartica*), ginger rhizome (*Zingiber officinale*), kalijira seed (*Nigella sativa*), bel leaf (*Aegle marmelos*), turmeric rhizome (*Curcuma longa*), katamehedi leaf (*Lawsonia alba*) and onion bulb (*Allium cepa*) against damping-off, seed germination and growth characters of tomato (*Lycopersicon esculentum* L.), eggplant (*Solanum melongena* L.) and chilli (*Capsicum annum*) seedlings. The most effective against seedling damping off was neem leaf extract followed by garlic clove and allamonda. The highest seed germination of tomato seeds (86.67 %) was observed under neem leaf extract effect (Islam et al., 2012).

Therefore, several papers were identified for presenting results in controlling a wide range of tomato soil borne pathogens by different plant products but still very few studies went further to report the efficacy of botanicals against soil borne pathogens in field level (Islam et al., 2012). After reviewing the literature on antimicrobial activity, it can be concluded that the vegetal material is representing an inexhaustible source of biologically active compounds. It was already demonstrated that secondary metabolites of plant origin have practical application in the food industry and (Arsene et al., 2015) and agriculture, for sustainable crop disease management as well.

CONCLUSIONS

Due to international trend for environmentally safe crop production, the modern farmers will have to incorporate innovative pest and disease management approaches to reduce their

dependency on pesticide use. Plant products have shown promising results for the control of soil-borne pathogens. The antifungal and antimicrobial effects are due to synergistic activity of constituents present in plant-based products.

Nevertheless, for using these products with reproducible efficiency, it is important to compare their mode of action for the optimization of the manufacturing process, the stabilization of these preparations, dates and rates of application.

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DETERMINATION OF STRENGTH PROPERTIES FOR MECHANICAL HARVEST OF PARSLEY (*PETROSELINUM CRISPUM*)

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Abstract

Parsley (Petroselinum crispum) is a vegetable from Umbelliferae family (or Apiaceae) that eats leaves as a salad. Although parsley vegetable to produce small areas our country, it has started to make production in large and larger areas in recent years. This study aimed to determine the strength of Parsley (Petroselinum crispum) specifications for mechanical harvesting. For this purpose, properties as the maximum force, stress in the maximum force point, work at maximum force point, shearing force, deformation at maximum force, bioyield force, and shearing stress of Parsley (Petroselinum crispum) stalk, flower have determined. Average values for maximum force, stress and energy in maximum force were determined as 4.535 N, 0.566 MPa and 0.015 J at stalk, respectively. The shearing force and shearing stress were found to be as 1.170 N and 0.14 MPa, respectively. Average values for bioyield force were determined to be 3.628 N. These features can be used in determining the design and operating conditions for the mechanical harvester cutting blade.

Key words*: Parsley (Petroselinum crispum), strenght properties, mechanical harvesting.*

INTRODUCTION

Petroselinum crispum (Parsley) is a bright green, biennial herb, which belongs to the family *Apiaceae* (Figure 1). Native to the central Mediterranean region (Southern Italy, Algeria and Tunisia) and naturalised elsewhere in Europe, Africa and Asia. There are also plenty of wild parsley in Spain, Greece, Morocco and Turkey. It is commonly used as a garnish in soups, salads, meats, vegetables and sauces (Lis-Balchin, 2006). Traditionally, the leaf, seed and root are being used in herbal medicine (Simon and Overley, 1986).

Figure 1. Parsley (*Petroselinum crispum*) plant

It has 23 000 (1000 ha) of farmland in Turkey. 3.4 percent of this area (809 000 ha) used for vegetable production.

Vegetable production has been increasing in recent years.

According to 2016 data, the parsley production area is 49296 ha, the production volume is 58160 tons in Turkey.

Depending on the variety and the season, 60-70 days after seeding, the plants are came to the harvesting stage. Parsley harvest can be done in 6-7 forms per a year.

The vegetable mechanization is mostly conducted by hand in Turkey. Mechanization is needed due to the increase in production area.

The studies generally focused on chemical, medicinal and culinary of parsley (*Petroselinum crispum*) (Charles, 2004). However, studies on strength properties of parsley (*Petroselinum crispum*) are limited. This study covers determination of maximum force, bioyield force, shearing force, stress and energy in maximum force, shearing deformation and shearing stress of parsley (*Petroselinum crispum*) stalk, leaf.

MATERIALS AND METHODS

For this study, parsley (*Petroselinum crispum*) plants were harvested by hand from the parsley plant harvested from a greenhouse in the Isparta province, Turkey.

Diameter and cross-sectional area of the experimental samples were measured before the shearing tests. Moisture content of the plants was determined at harvest time. Specimens were weighed and dried in an oven at 102°C for 24 h and then reweighed (ASABE, 2006). It was provided concise but complete information about the materials and the analytical and statistical procedures used.

A universal testing machine (LF Plus, UK) with a 500 N load cell and a computer-aided cutting and picking apparatus (Figures 2 and 3) was used to measure the strength properties of the parsley (*Petroselinum crispum*) plant. Knife material was hardened iron. All the tests were carried out at a speed 0.8 mm s^{-1}, and data were recorded at 10 Hz. All data were analyzed by nexygen software program.

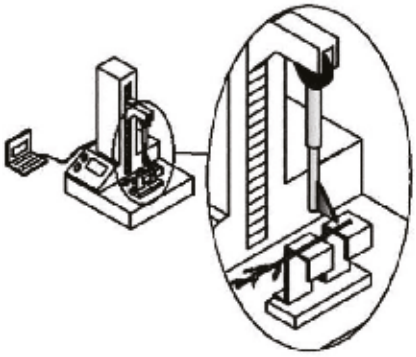


Figure 2. Cutting system

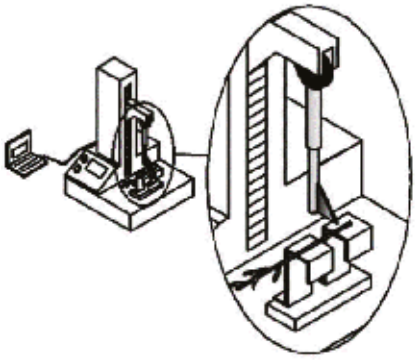


Figure 3. Picking system

The shearing forces on the load cell with respect to knife penetration were recorded by computer.

The shearing stress in N/mm^2 was calculated using the equation of Shahbazi et al. (2012):

$$\tau = \frac{F_{s\max}}{A} \quad (1)$$

Where F_{smax} is the maximum shearing force of the curve in N, and A is the area of the stalk at the deformation cross-section in mm^2.

The parsley plants were attached to the apparatus from its stalks (Figure 4). The shearing tests were conducted with 0.8 mm s^{-1} knife speed progress (Simonton, 1992).

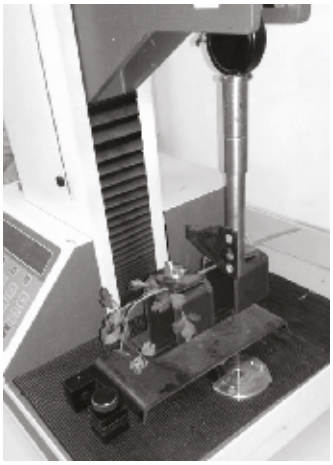


Figure 4. Measuring the cutting of parsley (*Petroselinum crispum*) plant

Picking force can be defined as the force required to separate leaf stalks from ovary point (picking force of leafs). The load cell of the machine was then pulled upward to determine the picking force of the parsley (*Petroselinum crispum*) leaf (Figure 5).

Maximum force, bioyield force, shearing force, stress and energy in maximum force, shearing stress and shearing deformation were calculated from the force-deformation curves at the inflection point as defined by ASAE Standard (1985). S368.1 (ASAE Standards, 1985) was obtained from all curves (Figure 6).

The energy of shearing was determined as the area under these curves (Chen et al., 2004; Srivastava, 2006).

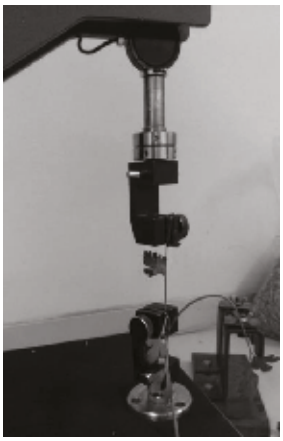
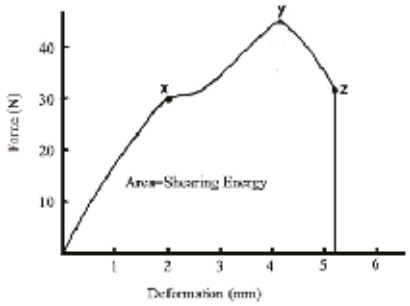


Figure 5. Measuring the picking force of parsley (*Petroselinum crispum*) leaf stalk



Note. Labels on the graph indicate the following points:
x - bioyield force, y - maximum force, z - shearing force (Liu, 2012)

Figure 6. Typical force-deformation curve of parsley (*Petroselinum crispum*) plant stalk during shearing loading

RESULTS AND DISCUSSIONS

Moisture content of the parsley plants was determined as 85.6 % at harvest time and all tests were conducted at harvest moisture. The strength measurements of rocket (parsley) stalks are given in Table 1.

The maximum force was observed as 4.535 N at parsley stalk. The bioyield force of 3.628 N was observed at stalk.

Shearing force is one of the most important plant characteristics affecting plant harvesting. If the weight of the plant is known, the shearing force and the shearing height can be used to determine the speed of the blade to be used in harvesting (Igathinathane et al., 2010; Taghijarah et al., 2011).

The maximum shearing force was observed as 1.170 N at stalk. The stress value in maximum force (0.566 MPa) was observed at stalk.

The energy at maximum force was found to be as 0.015 J.

Deformation has an important place among the strength characteristics of the plant. The maximum shearing deformation (24.323 mm) was observed at stalk. The average cross-sectional area of parsley (*Petroselinum crispum*) was determined as 7.884 mm^2 at harvest moisture (85.6%). The strength measurements of parsley (*Petroselinum crispum*) leaf are given in Table 2.

Table 1. Average strength properties of parsley (*Petroselinum crispum*) stalk

	Maximum force (N)	Bioyield force (N)	Shearing force (N)	Stress in maximum force (MPa)	Energy in maximum force (J)	Shearing stress (MPa)	Shearing deformation (mm)	Area (mm 2)
Stalk	4.535	3.628	1.170	0.566	0.015	0.14	24.323	7.884
Standard Deviation	2.994	2.393	1.406	0.176	0.018	0.09	3.465	3.575

Table 2. Average strength properties of parsley (*Petroselinum crispum*) leaf

	Maximum force (N)	Bioyield force (N)	Shearing force (N)	Stress in maximum force (MPa)	Energy in maximum force (J)	Shearing stress (MPa)	Shearing deformation (mm)
(Leaf) Flower	1.773	1.418	0.904	0.125	0.006	0.03	6.308
Standard Deviation	0.823	0.658	0.002	0.009	0.001	0.01	1.063

The maximum force required to separate leaf from stalk was determined as 1.773 N. As a function of the maximum force the bioyield force was found to be 1.418 N. Lower shearing forces required for mechanical harvesting leads to savings in power and energy usage. Leaf shearing force of parsley observed 0.904 N is lower than stalk shearing force. The maximum stress in maximum force value (0.125 MPa) was observed at leaf. The energy at maximum force was found to be as 0.006 J. The stress shearing value was observed as 0.03 MPa. The average shearing deformation value of parsley leaf was found as 6.308 mm.

CONCLUSIONS

This study was carried out to determine the strength properties of parsley (*Petroselinum crispum*) at leaf and stalk sections in the harvest moisture. Properties as the maximum force, bioyield force, shearing force, stress in maximum force, energy in maximum force, shearing stress, shearing deformation of parsley (*Petroselinum crispum*) leaf and stalk have determined at moisture content of 85.6%.

The strength parameters measured at root section higher than that of the stalk and leaf sections. The lowest values were found at parsley (*Petroselinum crispum*) stalk. The strength parameters of stalk section should be considered for mechanical harvesting of rocket plant to provide data for the design machines for mechanized applications.

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WHICH IS CRUCIAL FOR HETEROSIS? TRAITS, GENETIC OR CHARACTERISTIC DIVERSITY: PUNGENCY PARADIGM

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Abstract

Commercially plant breeding and crop improvement is possible to know heterotic behavior of plant species and heredity of the traits. It is commonly accepted that heterosis has been positively correlated with distance between traits of the parents. Two pepper variety belongs different Capsicum species, non-pungent Santos Flame (Capsicum annuum L.) and Biquinho (Capsicum chinense Jacq.) which had very low capsaicinoid content were crossed to estimate mid parent heterosis, heterobeltiosis and standard heterosis. In this study extraordinary heterosis rates were calculated F_1 progeny of two different Capsicum species close to pungency trait but definitely divergent genetically. The capsaicin content of Santos Flame, Biquinho and F_1 progeny were 0 mg kg^{-1}, 2.1 mg kg^{-1} and 217.4 mg kg^{-1}, respectively. The highest mid parent heterosis was estimated from capsaicin content with 20605%. Similarly uncommonly heterobeltiosis and standard heterosis rate was estimated for capsaicin content with 10252% and 237%, respectively. Correlatively extreme heterosis rates were calculated for SHU pungency level, dihydrocapsaicin and total capsaicinoids content.

Key words*: pungency, heterosis, heterobeltiosis, pepper.*

INTRODUCTION

The importance of heterosis in agriculture is to improve high yielded varieties. Heterosis is not a trait expressed by specific genes or alleles therefore distinction is important for heterosis (Lippman and Zamir, 2007). Genetic diversity could be as great value as combining ability or greater than it (Hayes and Immer, 1942). Allard (1999) indicated that single-cross hybrids diverged maximally in allelic frequencies produce most heterozygoty in most loci in F_1 hybrids.

The most important quality characteristics in pepper are color, pungency, dry/fresh yield ratio and flesh thickness (Abak, 1995). Capsaicinoids are colorless, odorless and flavorless substances only found in pepper. They have burning feature and no nutrition value. Cause of heat feeling substances are capsaicinoids consist of the most important seven different substances structurally similar pungent compounds including capsaicin, dihydrocapsaicin, nordihydrocapsaicin, norcapsaicin, homocapsacin, nornorcapsaicin and homodihydrocapsaicin (Greenleaf, 1986; Collins and Bosland, 1994).

The capsaicinoid content of the pepper cultivars varies greatly and related with maturity. A subjective assessment Scoville Heat Unit (SHU) based on panel evaluation also is used to determine pungency of peppers. ASTA (American Spice Trade Association) prefers Scoville Heat Unit determining of pungency (Scoville, 1912; ASTA, 1985).

With this study two different *Capsicum* species distinct from genetically but close to pungency trait were crossed and inheritance of pungency component calculated.

MATERIALS AND METHODS

Two pepper cultivars 'Santos Flame', PanAmerican Seed Illinois USA (*Capsicum annuum* L.), as female, and 'Biquinho' (*C. chinense* Jacq.), as male, were crossed and capsaicinoid contents of parents, F_1 48 F_2, progenies and check variety Jalapeño were measured by high pressure liquid chromatography.

Extraction of capsaicin and dihydrocapsaicin

The whole fruits dried at 50°C for 2 days and ground to fine powder. One gram of dried and ground fruit stirred in 10 ml sodium acetate saturated ethanol during 3 hours in 60°C. The mixture was filtrated and analyzed.

Determination of capsaicin and dihydrocapsaicin contents

HPLC: was (Model LC-20 Shimadzu, Kyoto, Japan) consisting of a high-pressure pumps, column (C18 100-5 250 × 4.6 mm), oven (35°C) and UV-VIS detector (at 280 nm). Isocratic mode (methanol 48.4%, water 30.2% dioxan 13.3%, acetonitrile 7.9% and perchloric acid 0.2% [% 2]) was performed at 1.5 ml min^{-1} of flow rate with 20 µL sample volume (ASTA, 1985).

Standards of capsaicin and dihydrocapsaicin were purchased from Sigma-Aldrich Co. Pungency values were converted to SHU (Scoville Heat Unit) multiplying total capsaicinoids by 15 (Mathur et al., 2002).

The plants of parents and progenies were grown in field conditions during the 2015 pepper growing season in EMTZARI (East Mediterranean Transitional Zone Agricultural Research of Institute Kahramanmaraş, Turkey).

Calculating heterosis

Mid parent, better parent also known as heterobeltiosis and standard also called as economic heterosis (Sharma et al., 2013) calculated by using formula.

$$\text{Mid Parent} = \frac{F_1 - MP}{MP} \times 100$$

$$\text{Better Parent} = \frac{F_1 - BP}{BP} \times 100$$

$$\text{Standard} = \frac{F_1 - \text{Check (Jalapeño)}}{\text{Check (Jalapeño)}} \times 100$$

Where: F_1 was mean of F_1; MP was mean of two parents; BP was mean of better parents and Jalapeño was used for control variety.

RESULTS AND DISCUSSIONS

The chromatogram peaks for capsaicin and dihydrocapsaicin of parents, F_1, Acc. 97, Acc. 103 and 270) were exhibited in Figure 1. The mean of retention time was 5.56 min. for capsaicin and 7.47 min. for dihydrocapsaicin.

Cultivar Biquinho which had a little amount (2.10 mg kg^{-1}) capsaicin content crossed non-pungent Santos Flame and hybrid exhibited 217 mg kg^{-1} capsaicin. An extraordinary heterosis over mid parent, better parent and standard check (Jalapeño 64.60 mg kg^{-1}) was observed in F_1 generation for capsaicinoid content and pungency level from interspecific crosses of *Capsicum*. The magnitudes of heterosis, better parent and standard check for capsaicin were 20605, 10252 and 237%, respectively (Table 1.)

Mid parent heterosis and heterobeltiosis was 3877 and 1889%, respectively while the economic heterosis was 228% over standard check Jalapeño cultivar on account of dihydrocapsaicin.

The percentage of mid parent heterosis related sum of the capsaicin and dihydrocapsaicin contents was 7855%. Heterobeltiosis and standard heterosis was 3877 and 233%, respectively. Correlatively pungency level had similarities with sum of the capsaicin and dihydrocapsaicin concerning mid parent, better parent and economic heterosis were found same.

The capsaicin contents ranged between 0.00 - 1279 mg kg^{-1} and the dihydrocapsaicin contents ranged from 0.00 - 912 mg kg^{-1}. The Accession 97 had the highest capsaicin content with 1279 mg kg^{-1} while the highest dihydrocapsaicin was obtained from Accession 103 with 912 mg kg^{-1}. Ten accessions from F_2 progeny of Santos Flame × Biquinho had no capsaicin content. Dihydrocapsaicin was not detected from 11 accessions of F_2 progeny of Santos Flame × Biquinho. Despite low level capsaicin content than Acc. 97, Acc. 103 was the most pungent genotype with 30793 SHU because of its high amount of dihydrocapsaicin. Segregation of all of the pungency components transgressively in F_2 progeny demonstrated that pungency was expressed dominantly (Figures 2 and 3).

Biquinho exhibited 140 SHU pungency level while it was 0 in Santos Flame. The interspecific hybrid of these two cultivars produced 349.61 mg kg^{-1} sum of capsaicin and dihydrocapsaicin content and exposed 5594 SHU pungency level. Pungency mean of parents was 70 SHU and the check variety Jalapeño had 1678 SHU level.

Table 1. Capsaicinoid content of hybrid, parental and check varieties and heterosis percentage of pungency components

	Capsaicin (mg kg^{-1})	Dihydrocapsaicin (mg kg^{-1})	Capsaicin + Dihydrocapsaicin (mg kg^{-1})	Pungency (SHU)
Biquinho	2.10	6.65	8.79	140
Santos Flame	0.00	0.00	0.00	0
Mean	1.05	3.33	4.40	70
F_1	217.40	132.25	349.61	5594
Jalapeño	64.60	40.26	104.85	1678
		Heterosis (%)		
Mid Parent	20605	3877	7855	7855
Better Parent	10252	1889	3877	3877
Standard	237	228	233	233

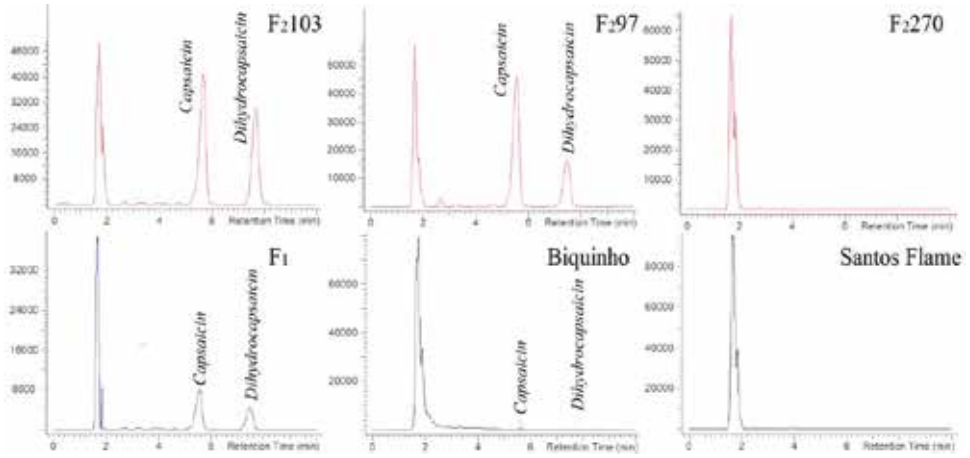

Figure 1. Capsaicin and dihydrocapsaicin HPLC chromatogram of Biquinho, Santos Flame and their F_1 and 103, 97,270 lines from F_2 progenies

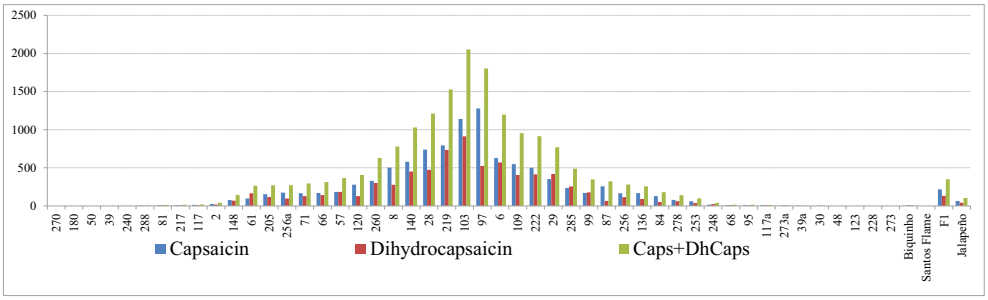

Figure 2. Capsaicin, dihydrocapsaicin (mg kg^{-1}) content of Biquinho, Santos Flame and their F_1 and F_2 progenies

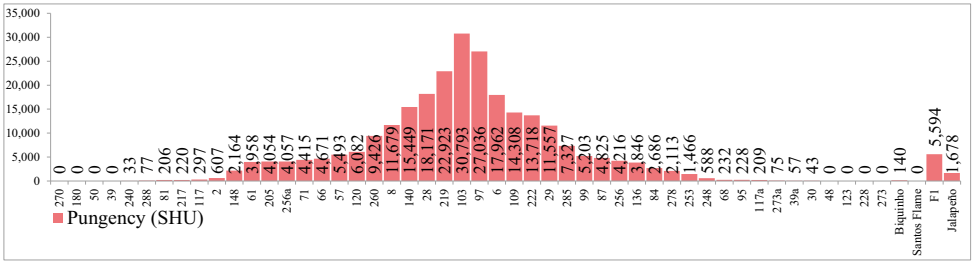

Figure 3. Pungency level (SHU) of Biquinho, Santos Flame and their F_1 and F_2 progenies

The pungency components in pepper were first extracted by Thresh (1846) and named as capsaicin. Substances of pungency in peppers are highly influenced by environmental conditions such as high temperatures and water stress (Lindsey and Bosland, 1996). Ahmed et al. (1982) emphasized that the pungency is a dominant character and additive gene effect was very important in their inheritance. According to our results transgressive segregation of pungency indicated capsaicin, dihydrocapsaicin content and pungency was inherited dominantly.

Biquinho (*Capsicum chinense* Jacq.) is known as non-pungent sweet pepper cultivar (Alves et al., 2014; Sganzerla et al., 2014). Nevertheless de Aguiar et al. extracted (2014) low concentration of capsaicinoids in Biquinho pepper. We found a low concentration of capsaicin and dihydrocapsaicin in Biquinho. These two compounds were not detected from Santos Flame. The main objective of the research was to manifest that genotypes close to concerned trait could be exhibited heterotic behavior if they were distinct from genetically. Prasad and Singh (1986) indicated that extremely divergent parents create high magnitude of heterosis harmonizing by alleles. Genetic diversity is important related with heterosis and inbred lines from different origin show greater heterosis (Hayes and Immers, 1942). Pearson (1983) reported that Peter and Singh (1976) declared 494% heterosis related with yield in eggplant and Shifriss and Rylski (1973) notified 195% for exported quality in bell pepper. Tu et al. (2007) find 672.7% better parent heterosis for kernel yield in rice. However there is no extraordinary heterosis and heterobeltiosis percentage as we found in our experiment for the literally we know.

CONCLUSIONS

The extreme heterosis and heterobeltiosis percentages were observed from hybrid of two species *Capsicum chinense* Jacq. and *Capsicum annuum* L. distinct from genetically but closed to pungency trait and its component. Therefore genetic diversity was found important related with heterosis. However it is believed that quality traits such as pungency could be more heterotic compared yield and yield component.

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THE EFFICACY OF DIFFERENT TREATMENTS FOR PATHOGENS CONTROL ON THE EGGPLANT CROPS IN THE FIELD

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In the field, eggplants are frequently attacked by Alternaria porri f. sp. solani, Botrytis cinerea and Phytophthora parasitica pathogens, which diminishes yield in quantitative terms and depreciates qualitatively. For the control of these pathogens three variants of fungicide treatments with different active ingredients were tested: chlorotalonil 500 g/l, pyraclostrobin 5% + metiram 55%, metiram 80%, iprovalicarb 8.4% + Cu oxychloride 40.6%, azoxystrobin 200 g/l + diphenoconazole 125 g/l. The best efficacy was obtained at variant 2 with the following schedule of treatments: treatment 1 - pyraclostrobin 5% + metiram 55% - 0.2%; treatment 2 - iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%; treatment 3 - pyraclostrobin 5% + metiram 55% - 0.2%; treatment 4 - iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%; treatment 5 - azoxystrobin 200 g/l + diphenoconazole 125 g/l - 0.1%; treatment 6 - pyraclostrobin 5% + metiram 55% - 0.2%. In this variant, the average efficacy was 85.5% and the yield 39.7 t/ha (123.17%) compared to 31.8 t/ha in the untreated control variant.

Key words: *Alternaria porri f. sp. solani, Botrytis cinerea, pathogens control, Phytophthora parasitica, Solanum melongena.*

INTRODUCTION

Although eggplants (*Solanum melongena* L.) are characterized by their adaptation to varied pedoclimatic conditions, crop yield is restricted by the large number of pests whose attack affects the productive biological potential, causing the decrease of quantity and quality of fruits (Tomescu et al., 1992; Costache and Roman, 2007). In the field eggplants are frequently attacked by the pathogens *Alternaria porri* f. sp. *solani*, *Botrytis cinerea* (Buzatu et. al., 2017), *Phytophthora parasitica* and soil borne fungal pathogen including *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *melongenae* (Buzatu et. al., 2017). *Alternaria porri* f. sp. *solani* occur frequently in eggplant cultures, especially in years with high average temperatures and heavy rainfall, producing dark brown spots on leaves, stems and flowers that coalesce and form large necrotic areas (Leite, 1997; Oliveira et al., 2004; Cristea, 2005). *Alternaria* spp. has also been identified on seeds of various plant species (Cristea et al., 2008; Radu et al., 2011; Pochon et al., 2012; Mardare et al., 2014; Pană et al., 2014; Cristea et al., 2015; Manole and Cristea, 2015; Gruia et al., 2016; Dudoiu et al., 2016). The distribution of *Alternaria* spp. on various plant species (Berca et al., 2015) was also studied in the literature.

Under laboratory conditions comparative measurements were made on enzymantic content of the leaves of *Momordica charantia* (both healthy and infected with pathogenic fungi *Aspergillus niger* and *Alternaria* sp.).

Amylases, proteases and lipases content of the biological material were determined.(Cozea and Cristea, 2011). In our country, this disease was first reported in 1958 by Tr. Săvulescu (Docea, 2012). *Botrytis cinerea* occurs frequently in crops of eggplants in protected areas, but also in the fields in years of abundant precipitation or if the culture is irrigated by sprinkler irigation.

Under favorable environmental conditions to the onset and evolution of the attack, the losses can reach up to 15-20%.

Symptoms present in flowers range from browing to fall off. On the fruit, the symptoms begin at the base of the calyx through a small round lesion that develops into a soft rot.
The fruit is covered by a mass of gray spores, characteristic of the disease (Compendium of Strawberry Diseases, 1998).
Phytophthora parasitica appears more frequently on well - developed fruits at the base of plants that come in contact or are near the surface of the soil. The tissues of the attacked fruit is rotting.
Under high temperature and humidity conditions, the rot is expanding and progressing affecting the whole pulp (Docea, 2012).
Based on the results obtained in the world and presented in the literature (Dillard et al., 1996; Anthony et al., 1998), the work aims to highlight the possibilities to monitor the complex of pathogens in the eggplant crops by using different treatment schedule.

MATERIALS AND METHODS

The experiments were carried out in 2017, under field conditions, using the Luiza eggplant variety. The culture was planting on May 12, 2017, with a density of 28,000 plants/ha. Four variants of treatments (Table 1) were used for the control of pathogens *Alternaria porri* f. sp. *solani*, *Botrytis cinerea* and *Phytophthora parasitica*. Technological control variants have been establisheed according to the sequence of pathogens in culture. During the growing season were applied 6 foliar treatments, at intervals of 12-16 days, in correlation with the climatic factors. Observations were made on the frequency (AF%) 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% × I%)/100 and the efficacy with the formula (untreated DA% – treated DA%) × 100/untreated DA.

Table 1. Variants to pathogens control on the eggplant crops in the field (Vidra, 2017)

Variant	22.06.2017	06.07.2017	18.07.2017	04.08.2017	22.08.2017	06.09.2017
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
I.	chlorotalonil 500 g/l - 0.2%	iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%	chlorotalonil 500 g/l - 0.2%	iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%	azoxystrobin 200 g/l + diphenoconazole 125 g/l - 0.1%	chlorotalonil 500 g/l - 0.2%
II.	pyraclostrobin 5% + metiram 55% - 0.2%	iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%	pyraclostrobin 5% + metiram 55% - 0.2%	iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%	azoxystrobin 200 g/l + diphenoconazole 125 g/l - 0.1%	pyraclostrobin 5% + metiram 55% - 0.2%
III.	metiram 80 % - 0.2%	iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%	metiram 80 % - 0.2%	iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%	azoxystrobin 200 g/l + diphenoconazole 125 g/l - 0.1%	metiram 80% - 0.2%
IV.	Untreated control	-	-	-	-	-

RESULTS AND DISCUSSIONS

The first pathogen in culture was *Botrytis cinerea*, followed by *Alternaria porri* f. sp. *solani* and *Phytophthora parasitica*. The attack of the pathogens *Botrytis cinerea* and *Alternaria porri* f. sp. *solani* began in the third decade of July (21.07. and 24.07., respectively) and the *Phytophthora parasitica* in the first decade of August (7.08.). Abiotic factors (temperature, atmospheric humidity and light) are important factors in the evolution of pathogen attack.
The influence of these abiotic factors on the growth and development of *Alternaria* spp. has been studied in the literature (Mardare et al., 2015).
The attack of the 3 pathogens had a slow evolution due to the low relative atmospheric humidity in the period July - August (52.4 - 74.7%, mean = 61.2%; Table 2).
Under these conditions, the average efficacy of treatment variants was between 79.7% (V **III**) and 85.5% (V **II**, Table 3).
The yields of field eggplants were 38.7 t/ha (V **III**), 39.7 t/ha (V **II**) and the untreated control variant was 31.8 t/ha (Table 4).
The highest yields were obtained in variants II (39.7 t/ha - 124.8%) and I (39.2 t / ha - 123.1%.

Table 2. The occurrence and evolution of the pathogens attack on the eggplants from the field in correlation with climatic factors (Vidra, 2017)

The pathogens/ climatic factors	Date of the attack	Degree of attack/frequency of attack (%)/month, decade												
		May	June			July			August			September		
		III	I	II	III	I	II	III	I	II	III	I	II	III
Alternaria solani	24.07	0	0	0	0	0	0	1,1	2.3	4.2	5.8	6.7	8.8	10.9
Botrytis cinerea	21.07	0	0	0	0	0	0	1.4	1.9	2.5	3.4	3.9	5.8	8.1
Phytophthora parasitica	7.08	0	0	0	0	0	0	0	0.8	1.5	2.3	3.2	4.4	5.9
Minimum temperature (ºC)	-	12.4	15.0	14.0	16.3	15.9	15.9	17.5	21.1	18.3	15.1	13.7	15.3	10.5
Maximum temperature (ºC)	-	21.4	27.0	26.0	31.9	28.4	28.9	31.0	36.6	31.8	28.7	28.8	29.4	19.2
Average temperature (ºC)	-	16.5	20.1	19.8	23.8	22.0	22.0	24.0	30.8	25.6	21.3	20.8	21.5	14.2
Minimum relative humidity (%)	-	57.0	47.5	43.8	36.4	46.0	37.8	35.7	26.9	27.4	30.2	29.3	30.8	41.7
Maximum relative humidity (%)	-	79.6	77.8	74.4	82.5	74.7	63.6	63.3	52.4	57.0	56.4	72.3	61.7	64.6
Average relative humidity (%)	-	66.9	59.5	56.8	56.4	57.9	47.6	46.8	36.7	38.7	41.7	46.9	44.8	50.7
Precipitation (mm)	-	7.5	20.0	22.5	1.0	84.0	8.5	6.5	0	0	45.0	30.0	1.0	2.0

Table 3. Efficacy of some schedules of trearments to control pathogens on the eggplant culture (Vidra, 2017)

Variant	Degree of attack / frequency of attack (%) and efficacy (%)						
	Alternaria solani (DA%)	E (%)	*Botrytis cinerea* (AF%)	E (%)	*Phytophthora parasitica* (AF%)	E (%)	Average of efficacy (%)
I	2.2	79.8	1.3	83.9	1.0	83.0	82.2
II	1.9	82.6	1.0	87.6	0.8	86.4	85.5
III	2.4	78.0	1.5	81.5	1.2	79.7	79.7
IV (Untreated control)	10.9	-	8.1	-	5.9	-	-

Table 4. The yield of eggplant in correlation with the experimental treatments (Vidra, 2017)

Variant	Yield			
	kg/sqm	% as compared to the untreated control	Difference (kg/sqm)	Significance
I	3.920	123.1	+ 0.74	***
II	3.972	124.8	+ 0.79	***
III	3.872	121.6	+ 0.69	**
IV (untreated control)	3.182	100.0	0	

LSD 5% - 0.359 kg/ sqm, LSD 1% - 0.504 kg/sqm, LSD 0.1% - 0.712 kg/sqm

Analyzing the obtained results, compared to the untreated control variant, it was found that the best results were obtained in the variants I and II, with very significant yield differences compared to the untreated control variant.

CONCLUSIONS

Alternaria porri f.sp. *solani*, *Botrytis cinerea* and *Phytophthora parasitica* are more frequent occured pathogens that attack the foliage and fruits of the eggplants.

In the climatic conditions of 2017, in Vidra, Ilfov region, these pathogens decreased the yield with 22-25%.

The best efficacy was obtained at variant II with the following schedule of trearments: treatment 1 - pyraclostrobin 5% + metiram 55% - 0.2%; treatment 2 - iprovalicarb 8.4% + Cu oxychloride 40.6 % - 0.2%; treatment 3 - pyraclostrobin 5% + metiram 55% - 0.2%; treatment 4 - iprovalicarb 8.4% + Cu oxychloride 40.6% - 0.2%; treatment 5 - azoxystrobin 200 g/l + diphenoconazole 125 g/l - 0.1% and treatment 6 - pyraclostrobin 5% +

metiram 55% - 0.2%. In this variant the mean efficacy was 85.5%. The highest yields were obtained in variants II (39.7 t/ha - 124.8%) and I (39.2 t/ha - 123.1%).

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PATHOGENS WITH ECONOMIC IMPORTANCE FOR TOMATO CROPS GROWING IN THE FIELD AND THEIR CONTROL

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Abstract

During 2017, at the RDIFG Vidra, was organized a bifactorial experience, placed on the subdivision parcels method, with 12 variants and 4 repetitions, using Pontica 102 tomato variety. During the growing period on tomatoes have been identified following pathogens on foliage: Pseudomonas syringae pv. tomato, Xanthomonas campestris pv. vesicatoria, Alternaria porri f. sp. solani, Fulvia fulva and Phytophthora infestans. Their attack has influenced production in terms of quantity and quality. In order to reduce yield losses, different treatment variants were tested using the following fungicide-bactericids: copper hydroxide 50% (Copper Max 50 WP 0.25%), chlorothalonil 500 g/l (Bravo 500 SC 0.2%), azoxystrobin 200 g/l - difenoconazole 125 g/l (Ortiva Top 0.1%), iprovalicarb 8.4% + Cu of oxychloride 40% (Melody Compact 49 WG 0.2%), difenoconazole 250 g/l (Score 250 SC 0.05%), copper hydroxide with 50% metallic Cu (Champ 77 WG 0.25%), metiram 80% (Polygram DF 0.2%), dimethomorph 9% + mancozeb 60% (Acrobat MZ 69 WG 0.2%), mefenoxam 4% + macozeb 64% (Ridomil Gold MZ 68 WG 0.25%), mancozeb 80% (Dithane M 45 WP 0.2%). Foliar fertilizers have also been used Crop Max 0.3%, Agroleaf Power Total (20.20.20) 0.5% and Agroleaf Power HK (15.10.31) 0.5%.

Key words*: pathogens, Pseudomonas syringae* pv. *tomato, Xanthomonas campestris* pv. *Vesicatoria.*

INTRODUCTION

From the vegetable species, tomatoes (*Lycopersicon esculentum* Mill.) represent the largest area of culture.

Thus, in 2014, tomatoes occupied an area of 5 million hectares worldwide, with an average production of 33,988 t/ha (FAO, 2014).

Alternaria spp. colonizes different plant species with unfavorable effects on production, both quantitatively and qualitatively (Cristea, 2005).

An important incidence of *Alternaria* spp. was also reported on the seeds of certain species of crop plants (Cristea et al., 2008; Cristea (Manole) et al., 2015; Dudoiu et al., 2016; Gruia et al., 2016; Manole (Cristea) et al., 2015; Mardare et al., 2014; Pana et al., 2014). Research on the influence of abiotic factors on the biological parameters of fungi belonging to the genus *Alternaria* spp. (Mardare et al., 2015; Radu et al., 2011), was also carried out, the distribution of these fungi, depending on the level of the attack (Berca et al., 2015) and their influence on some seed indicators (Cristea et al., 2013).

A particular aspect of the attack of *Alternaria* spp. is the pathogenic-enzymatic interrelation between plant species (Cozea et al., 2011).

Pathogens responsible for the occurrence of tomatoes diseases and their description are presented by Docea et al. (2012) and Gheorghies et al. (2001).

For tomato attack (*Phytophthora infestans*) in field conditions, prognosis and warning measures are recommended (Gheorghies et al., 2001). Pathogens attacking tomato crops cause considerable economic damage, which can be direct (quantitative reduction of the harvest and damage to its quality) or indirect (social or economic effects - import from other countries; Severin et al., 2001).

In tomato field crops, the following pathogens are frequently attacked: *Xanthomonas campestris* pv. *vesicatoria* (staining the leaves and blistering the fruits), *Pseudomonas syringae* pv. *tomato* (pustular fruit stain), *Alternaria*

porri f. sp. *solani* (brown spotting of the leaves or alternarioza), *Fulvia fulva* (brown hair staining) and *Phytophthora infestans* (hand) (Mandru et al., 2017).

Alternaria porri f. sp. *solani* can cause signify-cant economic damage, the main symptoms being collar rot in the basal part of the seedlings, leaf and stem stains and rotting fruit (Walker, 1952).

The reported production losses can reach 79% and have been reported in Canada, India, USA, Nigeria (Basu., 1974; Datar et al., 1981; Sherf et al., 1986; Gwary et al., 1998).

"Collar" rot occurs at a frequency of 20-40% in seedlings after planting in the field (Sherf et al., 1986).

Fulvia fulva only attacks the plant foliage, but in favorable conditions it may cause premature defoliation (Babadoost, 2011).

Attack of bacteria *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* is favored by high atmospheric humi-dity, and is manifested on leaves and fruits, on petiole, on stems, and can also cause plant defoliation and fruit degradation (Dafna Tamir-Ariel et al., 2007).

The most dangerous attack is caused by *Phytophthora infesters* which, under favorable conditions (moderate temperatures, maximum atmospheric humidity, the presence of drops of water on the foliage and fruits) may lead to crop failure if adequate control measures are not taken (Costache et al., 2007).

For the control of these pathogens, products with different active substances are frequently used: copper hydroxide 50% (Copper Max 50 WP 0.25%), chlorothalonil 500 g/l (Bravo 500 SC 0.2%), azoxystrobin 200g/l-difenoconazole 125g/l (Ortiva Top 0.1%), iprovalicarb 8.4% + Cu of oxychloride 40% (Melody Compact 49 WG 0.2%), difenoconazole 250 g/l (Score 250 SC 0.05%), copper hydroxide with 50% metallic Cu (Champ 77 WG 0.25%), metiram 80% **(**Polygram DF 0.2%), dimethomorph 9% + mancozeb 60% (Acrobat MZ 69 WG 0.2%), mefenoxam 4% + mancozeb 64% (Ridomil Gold MZ 68 WG 0.25%), mancozeb 80% (Dithane M 45 WP 0.2%).

The research undertaken at RDIVFG Vidra, in 2017, aimed to establish treatments for the simultaneous control of pathogens present in tomato field crops.

MATERIALS AND METHODS

During 2017, at the RDIFG Vidra, it was organized a bifactorial experience, placed on the subdivision parcels method, with 12 variants and 4 repetitions, using Pontica 102 tomato variety.

Treatment variants including foliar fertilizers are presented in Table 1.

Table 1. Experimental variants

V	Foliar fertilizers	Phytosanitary tratments	June	July	August
			Treatments 1, 2	Treatments 3, 4	Treatments 5, 6
1.	A1 Crop Max 0.3%	B1	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%
2.		B2	1. Champ 77 WG 0.25% 2. Polyram DF 0.2%	3. Ortiva Top 0.1% 4. Acrobat MZ 69 WG 0.2%+ Score 250 SC 0.05%	5. Ortiva Top 0.1% 6. Acrobat MZ 69 WG 0.2%
3.		B3	1. Copper Max 50 WP 0.25% 2. Dithane M 45 WP 0.2%	3. Ortiva Top 0.1% 4. Ridomil Gold MZ 68 WG 0.25%+ Score 250 SC 0.05%	5. Ortiva Top 0.1% 6. Ridomil Gold MZ 68 WG 0.25%
4.		B4	Ut.	Ut.	Ut.
5.	A2 Agroleaf Power Total (20.20.20) 0.5% + Agroleaf Power HK (15.10.31) 0.5%	B1	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%
6.		B2	1. Champ 77 WG 0.25% 2. Polyram DF 0.2%	3. Ortiva Top 0.1% 4. Acrobat MZ 69 WG 0.2%+ Score 250 SC 0.05%	5. Ortiva Top 0.1% 6. Acrobat MZ 69 WG 0.2%
7.		B3	1. Copper Max 50 WP 0.25% 2. Dithane M 45 WP 0.2%	3. Ortiva Top 0.1% 4. Ridomil Gold MZ 68 WG 0.25%+ Score 250 SC 0.05%	5. Ortiva Top 0.1% 6. Ridomil Gold MZ 68 WG 0.25%
8.		B4	Ut.	Ut.	Ut.
9.	A3 (Untreated control)	B1	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%
10.		B2	1. Champ 77 WG 0.25% 2. Polyram DF 0.2%	3. Ortiva Top 0.1% 4. Acrobat MZ 69 WG 0.2%+ Score 250 SC 0.05%	5. Ortiva Top 0.1% 6. Acrobat MZ 69 WG 0.2%
11.		B3	1. Copper Max 50 WP 0.25% 2. Dithane M 45 WP 0.2%	3. Ortiva Top 0.1% 4. Ridomil Gold MZ 68 WG 0.25%+ Score 250 SC 0.05%	5. Ortiva Top 0.1% 6. Ridomil Gold MZ 68 WG 0.25%
12.		B4	Ut.	Ut.	Ut.

To reduce the losses caused by the attack of pathogens, the following fungicides–bactericides alone or in combination: copper hydroxide 50% (Copper Max 50 WP 0.25%), chlorothalonil 500 g/l (Bravo 500 SC 0.2%), azoxystrobin 200 g/l + difenoconazole 125 g/l (Ortiva Top 0.1%), iprovalicarb 8.4 % + Cu of oxychloride 40 % (Melody Compact 49 WG 0.2%), difenoconazole 250 g/l (Score 250 SC 0.05%), copper hydroxide with 50% metallic Cu (Champ 77 WG 0.25%), metiram 80% (Polyram DF 0.2%), dimethomorph 9% + mancozeb 60% (Acrobat MZ 69 WG 0.2%), mefenoxam 4% + mancozeb 64% (Ridomil Gold MZ 68 WG 0.25%), mancozeb 80% (Dithane M 45 WP 0.2%).

These have been established according to the sequence of pathogens, and the interval between them in correlation with climatic factors.

There were applied 6 foliar treatments at intervals of 8-17 days.

Foliar fertilizers were used: Crop Max 0.3% (4 foliar treatments at 10 days intervals), Agroleaf Power Total (20.20.20) 0.5% (3 foliar treatments at 10 days intervals) and Agroleaf Power HK (15.10.31) 0.5% (one treatment after Agroleaf Power Total).

Dynamic observations have been made on the occurrence and evolution of pathogen attack (frequency and severity of the attack) in relation to climatic factors.

The best treatment variants have been established according to the average efficacy and the obtained production.

Qualitative determinations for total dry substance, soluble dry matter, carbohydrate content and vitamin C in tomato fruits from variants A1B4 (fertilized with Crop Max foliar 0.3%), A2B4 (fertilized with Agroleaf Power Foliar Total (20.20.20) 0.5% + Agroleaf Power HK (15.10.31) 0.5% and A3B4 (untreated control).

RESULTS AND DISCUSSIONS

During 2017, in the Vidra area, the fall - summer tomato crops, the Pontica variety 102, the following pathogen attack could be seen: *Pseudomonas syringae* pv. *tomato, Xanthomonas campestris* pv. *vesicatoria, Alternaria porri* f. sp. *solani, Fulvia fulva* and *Phytophthora infestans.*

The earliest attack of *Pseudomonas syringae* pv. *tomato* (29.05.) was followed by *Xanthomonas campestris* pv. *vesicatoria* (8.06.), *Alternaria porri* f. sp. *solani* (13.06.), *Fulvia fulva* (16.06.), and *Phytophthora infestans* (20.06.).

The rise and evolution of the attack was favored by rainfall in May (71.0 mm), June (43.5 mm) and July (99.0 mm) and maximum atmospheric humidity of over 70%, so at the end the third decade of August recorded values between 8.4% (*Fulvia fulva*) and 13.7% (*Phytophthora infestans* - Table 2).

Mandru et al. (2017) also carried out research on tomato culture, which identified the following pathogens on the foliage: *Pseudomonas syringae* pv. *tomato, Alternaria porri* f. sp. *solani, Fulvia fulva* and *Phytophthora infestans.*

Symptoms produced by the pathogens on the tomato plant foliage are shown in Figures 1, 2, 3, 4 and 5.

Figure 1. Attack by *Pseudomonas syringae pv. tomato* on the foliage

Figure 2. Attack by *Xanthomonas campestris* pv. *vesicatoria* on the foliage

Figure 3. Attack by *Alternaria porri* f. sp.*solani* on the foliage

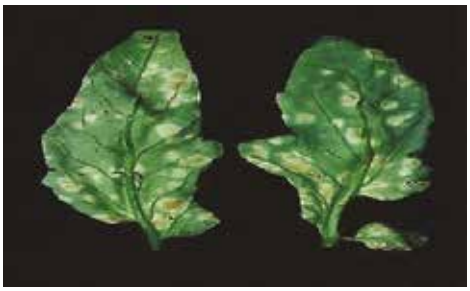

Figure 4. Attack by *Fulvia fulva* on the foliage

a) on foliage

b) on leaves

Figure 5. Attack by *Phytophthora infestans*

Table 2. Influence of climatic factors on the occurrence and evolution of pathogen attack to the tomato field crop (Vidra, 2017)

Pathogenic agents and climatic factors	Date of the attack	The degree of attack/month/decade											
		May			June			July			August		
		I	II	III	I	II	III	I	II	III	I	II	III
Pseudomonas syringae pv. *tomato*	29.05	0	0	0.2	0.5	3.3	5.2	5.8	6.4	7.9	8.5	10.1	12.2
Xanthomonas campestris pv. *vesicatoria*	8.06	0	0	0	0.3	2.7	4.6	5.2	5.8	6.7	7.0	7.9	9.5
Alternaria porri .f. sp. *solani*	13.06	0	0	0	0	0.7	4.9	5.5	7.3	8.1	10.0	11.3	12.7
Fulvia fulva	16.06	0	0	0	0	0.2	2.8	3.5	4.8	6.0	6.8	7.7	8.4
Phytophthora infestans	20.06	0	0	0	0	0.7	5.8	7.5	8.5	9.6	10.3	12.2	13.7
Temperature minimum (^{0}C)	-	10.9	10.5	12.4	15.0	14.0	16.3	15.9	15.9	17.5	21.1	18.3	15.1
Temperature average (^{0}C)	-	14.7	15.3	16.5	20.1	19.8	23.8	22.0	22.0	24.0	30.8	25.6	21.3
Temperature maximum (^{0}C)	-	19.4	20.9	21.4	27.0	26.0	31.9	28.4	28.9	31.0	36.6	31.8	28.7
Minimum relative humidity (%)	-	60.2	53.7	57.0	47.5	43.8	36.4	46.0	37.8	35.7	26.9	27.4	30.2
Average relative humidity (%)	-	70,3	63.1	66.9	59.5	56.8	56.4	57.9	47.6	46.8	36.7	38.7	41.7
Maximum relative humidity (%)	-	85.2	76.3	76.6	77.8	77.4	82.5	74.7	63.6	63.6	52.4	57.0	56.4
Precipitation (mm)	-	43.6	19.9	7.5	20.0	22.5	1.0	84.0	8.5	6.5	0	0	45.0

All variants of experiments have shown good results in controlling the pathogens *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria*, *Alternaria porri* f. sp. *solani, Fulvia fulva* and *Phytophthora infestans* (Table 3). Among them were A3B1 (E = 88.3%), A3B3 (E = 86.3%) followed by A1B1 (E = 87.4%), A1B3 (E = 85.3%), A2B1 (E = 86.3%) and A2B3 (E = 84.9%).

Table 3. Influence of phytosanitary treatments and foliar fertilizers on the attack of pathogens on field tomato culture (Vidra, 2017)

V	The degree of attack on the foil (%)						
	Pseudomonas syringae pv. *tomato*	*Xanthomonas campestris* pv. *vesicatoria*	*Alternaria porri* f. sp. *solani*	*Fulvia fulva*	*Phytophthora infestans*	Total	Effectiveness (%)
1.	1.3	1.2	1.1	1.8	1.7	7.1	87.4
2.	1.7	1.5	1.3	1.9	2.2	8.6	84.8
3.	1.5	1.4	1.4	1.5	2.5	8.3	85.3
4.	12.2	9.5	12.7	8.4	13.7	56.5	-
5.	1.5	1.4	1.4	2.0	2.4	8.7	86.3
6.	1.9	1.7	1.5	2.1	2.9	10.1	84.1
7.	1.8	1.6	1.7	1.7	2.8	9.6	84.9
8.	13.7	10.7	14.1	9.9	15.2	63.6	-
9.	1.0	0.9	0.8	1.5	1.9	6.1	88.3
10.	1.5	1.3	1.1	1.7	2.4	8.0	84.6
11.	1.2	1.1	1.2	1.3	2.3	7.1	86.3(2)
12.	11.6	8.7	11.5	8.0	12.2	52.0	-

Good results were observed at variant B1 variants with 6.13 kg/m^2 (134.1%) and B3 with 6.04 kg/m^2 (132.2%, Table 4) compared with B4 (the control untreated) at which production was 4.57 kg/m^2. Good results were also obtained in variant B^2 with 5.96 kg/m^2.

Regarding the differences in production obtained in addition to the untreated control variant, these are very significant in all three

Research on the control of pathogens *Alternaria porri* f. sp. *solani*, *Botrytis cinerea, Fulvia fulva* and *Phytophthora infestans* in tomato crops were also carried out by Costache et al. (2017), which established the efficacy and influence on the production of combinations of fungicides in simultaneous control thereof.

Table 4. Influence of phytosanitary treatments on production (Vidra, 2017)

Factor B	Production			
	kg/m^2	(%)	The difference from untreated varian	Signification
B1	6.13	134.1	+1.56	***
B2	5.96	130.4	+1.39	***
B3	6.04	132.2	+1.47	***
B4	4.57	100.0	-	-

DL 5%=0.021; DL 1%=0.029; DL 0.1%=0.039

The analysis of the data presented in Table 5 shows that treatments with foliar fertilizers (A1 fertilized with Crop Max 0.3%, A2 fertilized with Agroleaf Power Total (20.20.20) 0.5% and Agroleaf Power HK (15.10.31) 0.5% did not significantly influence the quantity of the obtained production, the values being very close: at A1 5.70 kg/m^2 was obtained, at A2 5.67 k/m^2 and at A3 (Mt) 5.65 kg/m^2.

Table 5. Influence of foliar fertilizer treatments on production (Vidra, 2017)

Factor A	Production			
	kg/m^2	(%)	The difference from untreated varian	Signification
A1	5.70	100.88	+ 0.05	-
A2	5.67	100.35	+ 0.02	-
A3 (Ut.)	5.65	100.00	-	-

DL 5%=0.070; DL 1%=0.100; DL 0.1%=0.172

Analyzing the data presented in Table 6, it was found that in all cases, in the variants treated (1, 2, 3, 5, 6, 7, 9, 10, 11), the differences in production obtained in addition by the untreated witness (4, 8, 12) are very significant.

Among the parameters analyzed for determining the influence of foliar treatments on Crop Max 0.3%, Agroleaf Power Total (20.20.20) 0.5% and Agroleaf Power HK (15.10.31) 0.5%, it was found that compared to the untreated control without foliar treatments,

fruit content in total dry substance (TDS), soluble dry matter (SDM), carbohydrate content and vitamin C content were clearly influenced (Table 7).

Thus, the total dry fruit content of the fruit was higher by 13.4-15.1%, the dry substance content soluble by 33.3-44.4%, the carbohydrate content by 37.8-44.4%, and the vitamin C content by 8.3-29.2%.

Table 6. Influence of phytosanitary treatments and foliar fertilizers on production (Vidra, 2017)

Variants	Foliar fertilizers	Phytosanitary tratments	Production			
			kg/m^2	%	The difference from untreated variant	Signification
1.	A1	B1	6.15	134.6	+1.58	***
2.		B2	6.02	131.7	+1.45	***
3.		B3	6.07	132.8	+1.50	***
4.		B4 (Ut.)	4.57	100.0	-	-
5.	A2	B1	6.12	132.5	+1.50	***
6.		B2	5.90	127.7	+1.28	***
7.		B3	6.05	130.9	+1.43	***
8.		B4 (Ut.)	4.62	100.0	-	-
9.	A3 (Ut.)	B1	6.13	135.6	+1.61	***
10.		B2	5.96	131.9	+1.44	***
11.		B3	6.01	132.9	+1.49	***
12.		B4 (Ut.)	4.52	100.0	-	-

DL 5%=0.065; DL 1%=0.087; DL 0,1%=0.110

Table 7. Influence of foliar fertilizers on the quality of tomato fruits

V.	Variation of fertilization	Water	(%)	TDS	(%)	SDM	(%)	Acidity (g citric acid at 100 g s.p).	(%)	Glucids	(%)	Vitamin C (mg/ 100g s.p)	(%)
1.	Crop Max 0.3%	93.56	99.2	6.44	113.4	3.9	144.5	0.77	100.0	3.25	144.4	27.28	129.2
2.	Agroleaf Power Total (20.20.20) 0.5% + Agroleaf Power HK (15.10.31) 0.5%	93.46	99.1	6.54	115.1	3.6	133.3	0.70	90.9	3.10	137.8	22.88	108.3
3.	Untreated control	94.32	100.0	5.68	100.0	2.7	100.0	0.77	100.0	2.25	100.0	21.12	100.0

CONCLUSIONS

In the field tomato crops, the pathogens *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesication*,, *Alternaria porri* f. sp. solani, *Fulvia fulva* and *Phytophthora infestans* diminish production in terms of quantity and qualitatively impair it. Among the variants of experienced treatments were B1 (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%) with production of 6.13 kg/m^2 (134.1%; E medium = 87.3%) and B3 (T1: Copper Max 50 WP 0.25%; T2: Dithane M 45 WP 0.2%; T3: Ortiva Top 0.1%; T4: Ridomil Gold MZ 68 WG 0.25%+ Score 250 SC 0.05%; T5: Ortiva Top 0.1%; T6: Ridomil Gold MZ 68 WG 0.25%) with production of 6.04 kg/m^2 (132.2%; E medium = 85.5%).

Treatments with foliar fertilizers Crop Max 0.3%, Agroleaf Power Total (20.20.20) 0.5% and Agroleaf Power HK (15.10.31) 0.5% did not significantly influence production in terms of quantity but only qualitatively: the total dry substance (TSS) increase by 13.4-15.1%, the dry substance soluble (DSS) by 33.3-44.4%, the carbohydrate content by 37.8-44.4% and the vitamin C content with 8.3-29.2%.

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EXOGENOUS CYTOKININ APPLICATION INCREASED THE CAPSAICIN AND ASCORBIC ACID CONTENT IN PEPPER FRUIT

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Abstract

Chili peppers synthesize capsaicin and accumulate it in the fruits. Many biochemical studies have been done in the field of capsaicin biosynthesis in chili peppers. The levels of capsaicin vary among chili pepper cultivars, and it is also affected by environmental conditions. The present study assayed the effect of cytokinin on the capsaicin and ascorbic acid content of Capsicum annum var 'Ilica' (pungent pepper) in the greenhouse. Cytokinin levels include 0, 50 and 100 µM of Benzyl amino purine and the treatments were applied at every 10 days until the harvest beginning 5 days after planting. The results of this study monitored that cytokinin (benzyl amino purine) treatment increased the capsaicin and ascorbic acid content in pepper fruits as compared to the control but decreased the fruit number per plant.

Key words*: ascorbic acid, capsaicin, fruit, pungent pepper.*

INTRODUCTION

Hot pepper burning sensation induced by the presence of acid amides, collectively known as capsaicinoids, that are formed from phenylalanine and valine or leucine. Capsaicin and dihydro indicate more than 90% of the total capsaicinoid content in most pungent peppers (Ben-Chaim et al., 2006). Capsaicinoid biosynthesis is restricted to the genus *Capsicum* (Stewart et al., 2007). Capsaicin content raises slowly during fruit development reaching peak levels at 40 to 50 days after planting (Contreras-Padilla and Yahia, 1998), after which it gravitates to deteriorate into secondary compounds due to peroxidase action (Bernal and Barceló, 1996). For increasing or improving pungent compound production, a research has revealed that hydric stress ameliorates capsaicinoid levels, because water deficit affects the phenylpropanoid pathway (Estrada et al., 1999).

Hydric stress can raise capsaicin levels by increasing activity of the enzymes phenylalanine ammonia-lyase (PAL), cinnamic acid-4-hydroxylase (C4H), and capsaicinoid synthetases (CS), all engaged in capsaicin biosynthesis (Sung et al., 2005). Monforte-González et al. (2010) found that application of nitrate can increase the capsaicin biosynthesis, but potassium application had not a significant effect on it. Ascorbic acid is very abundant in pepper fruits and is the main component of vitamin C. Its content is highly varying among cultivar and ripening stage (Bae et al., 2014).

Cytokinin is a hormone of plants that adjusts growth and developmental cycles. It has an important role on the chlorophyll status in plants (Lim et al., 2007). Exogenous treatment of N6-benzyl amino purine (BAP) on the eggplant increased its tolerance under salt stress by decreasing the O_2^- and malondialdehyde production rate (Wu et al., 2014). Application of BAP in the *in vitro* culture increased the comptothecin content in shoots and roots of *Ophiorrhiza rugosa* var. *decumbens* (Vineesh et al., 2007). It was reported that the application of BAP increased the shikonin formation in *Onosma paniculatum* cultured cell (Ding et al., 2004). So BAP has an exclusive effect on the plant growth and secondary metabolites production in the plants.

The results of the literature review suggest that abiotic and biotic parameters could affect the secondary metabolites production in plants. Up to now a research has not been done on the

effect of cytokinin on the capsaicin production, so in this study, we investigated the effect of BAP on the capsaicin and ascorbic acid content in pepper.

MATERIALS AND METHODS

The experiment was performed in the Ataturk University under controlled greenhouse conditions. Seeds of *Capsicum annum* var. Ilica (pungent pepper) were germinated on the 86-celled styrofoam trays filled with peat, then the homogenous and healthy seedlings were transplanted to the pots in size 30 x 20 x 20 cm after thirty days. The pots were filled with a ratio of 2: 1: 1 (v: v: v) of soil: sand: manure having around 1.30 g cm^3 bulk density.

The greenhouse mean temperature and relative humidity were 24°C and 74%, respectively during the experiment. The cytokinin treatment included 0, 50 and 100 µM of N6-benzyl amino purine (BAP). The BAP solutions were prepared with distilled water containing 0.02% Tween 20 as a surfactant, and the control (without BAP) treatment just was contained distilled water and surfactant. Five days after seedling transplanting, the BAP solutions were sprayed to leaves in the afternoon and the foliar spraying continued every 10 days until the harvest. Half-strength Hoagland nutrient solution was used with irrigation (Rubio et al., 2011).

Marketable fruits were selectively harvested weekly from 55 days after transplanting until the end of the experiment. In addition, at the end of the experiment, one representative marketable pepper fruit (18 fruits) was selected from different plants and used for determining chemical analysis. Yield was determined by counting and weighing all fruits on each plant. Marketable fruit yield was determined according to the color, the health state, the shape, and the weight.

Capsaicin analysis

The samples were separated in a SHIM-PACK VP-ODS column (150 mm × 4.6 mm; Shimazu). For capsaicin, the eluent was a mixture of acetonitrile/1% acetic acid (2:1 v/v), and the flow rate was 1 ml/min. All eluates were monitored at 280 nm using a UV detector. External standards were prepared by dissolving commercial capsaicin (Sigma-Aldrich) in methanol and acetonitrile (Ogawa et al., 2015).

Ascorbic acid analysis

Five grams of well-homogenised sample were disrupted in a crucible mortar with quartz sand. To the macerate 50 mL of meta phosphoric acid (analytical grade) was gradually added and the mixture was then transferred to a 100 mL Erlenmeyer flask, closed with stopper and then filtered. The filtrate was purified in addition by passing through a 0.45 mm PTFE syringe filter before injection on HPLC column.

The analytical determination of ascorbic acid was performed on C18 Nautilus, 100-5, 150 × 4.6 mm column with gradient elution of 0.01 M KH_2PO_4 (A) and acetonitrile (B). The gradient elution started with 1% B in A and changed to 30% B in A in 15 min.; then, it turned to 1% A in B in 5 min. The flow rate was 0.7 mL/min. The highest absorption maximum of ascorbic acid under these conditions was detected at 265 nm. For quantitative determination of ascorbic acid, standard materials (Sigma-Aldrich, Budapest, Hungary) were used.

Stock solutions and then working solutions were prepared for each compound to make the calibration between concentration and peak area (Nagy et al., 2015).

Data analysis

A completely randomized experimental design was used in this study. Each treatment had 4 replications with 5 plants for each replication. Statistical analysis was carried out by SPSS version19 at $P \leq 0.001$, the mean separation was done following Duncan's Multiple Range Test.

RESULTS AND DISCUSSIONS

As shown in the Figure 1, BAP treatments significantly increased the capsaicin content in fruits of pepper. Treatment of BAP (100 µM) had the highest effect on the capsaicin content, as compared to the untreated ones. Exogenous BAP treatment had a significant effect on the ascorbic acid content in the pepper fruits, by increasing the BAP concentration its content enhanced. Higher concentration of BAP (100 µM) increased the ascorbic acid content 22% in comparison to the untreated one (Figure 2). The cytokinin (BAP) application had not a positive effect on the fruit number in pepper plant. The BAP treatment (100 µM) decreased the fruit number but its lower concentration (50 µM)

had not a significant effect as compared to the control plants (Figure 3).

Previous studies have shown that capsaicin biosynthesis exclusively is occurred in the fruit (Stewart et al., 2005; Stewart et al., 2007). Cytokinin has an important role in the adjustment of different biological processes, involving growth and development, furthermore, acclimation/adaptation to environmental conditions in plants. Li et al. (2016) reported that cytokinin application increased secondary metabolite production in *Morinda citrifolia*.

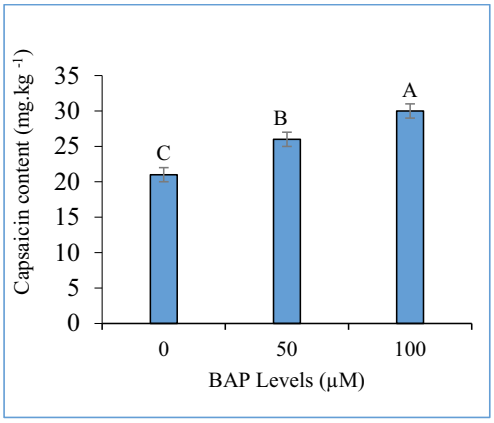

Figure 1. Effect of N6-benzyl amino purine (BAP) levels on capsaicin content in fruits of pepper. Data followed by a different letter were significantly different (P≤ 0.001) according to the Duncan Multiple Range Test

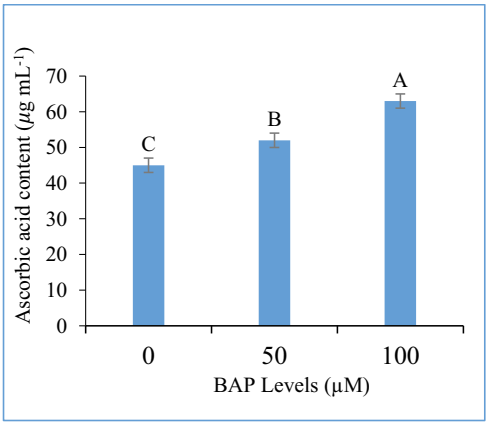

Figure 2. Effect of N6-benzyl amino purine (BAP) levels on ascorbic acid content in fruits of pepper. Data followed by a different letter were significantly different (P≤ 0.001) according to the Duncan Multiple Range Test

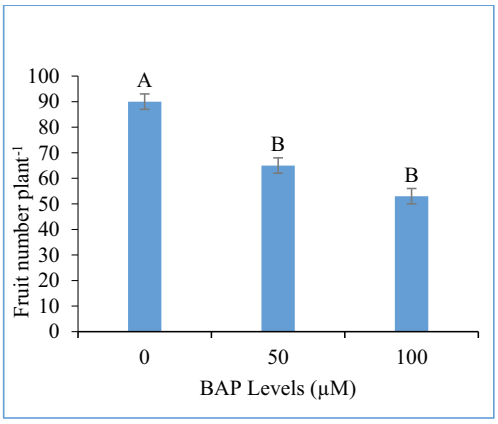

Figure 3. Effect of N6-benzyl amino purine (BAP) levels on number of fruit in pepper. Data followed by a different letter were significantly different (P≤ 0.001) according to the Duncan Multiple Range Test

Similarly, Govindaraju and Indra Arulselvi (2016) reported that BAP treatment enhanced the PAL and subsequently secondary metabolites production on *in vitro* media in *Coleus aromaticus*, without changes in plant genetic. In this study, exogenously BAP application increased the capsaicin content in fruits. It could be due to role of cytokinin on growth and development including meristem maintenance, vascular development, modulation of sink-source relationships and nutrient acquisition in roots.

BAP also play an important role in gene expression in capsaicin production. Moreover, Govindaraju and Indra Arulselvi (2016) reported that BAP application on *in vitro* propagation of *Coleus aromaticus* increased PAL gene expression, PAL directs phenylalanine (Phe) to secondary metabolism, deamination this amino acid to generate cinnamic acid, that could be converted to various phenolic compounds, involving capsaicin (Castro-Concha et al., 2016).

The ascorbic acid content with a gradual increase in fruit ripening was detected in hot pepper (Iqbal et al., 2013). Navarro et al. (2006) revealed that salinity decreased ascorbic acid content in pepper and methyl jasmonate application increased antioxidant capacity of strawberry fruits (Ayala-Zavala et al., 2005). In this study, BAP treatment increased ascorbic acid in Ilica pepper. It means that cytokinins

have a role in ascorbic acid biosynthesis in pepper plants.

The previous study have been shown that cytokinin application had not a significant effect on the yield and dry matter accumulation in the ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (Ghani et al., 2014). However, we found that BAP had a significant effect on the fruit number. In this regard, the cytokinin higher levels (50 and 100 µM of BAP) decreased the fruit number of pepper. The decline of fruit/plant could be due to its role in regeneration of shoot which it leads to the shadowing of plants on each other and it ultimately causes to decrease in the plants yield.

CONCLUSIONS

The literature review shows that capsaicin is responsible for pungent sensation in pepper cultivators and the fruit placenta is a main place for capsaicin production. BAP treatment had a significantly effect on capsaicin (CAP) production and increased CAP and ascorbic acid content in pepper.

ACKNOWLEDGMENTS

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ESSENTIAL OILS AND HOMEMADE FUNGICIDES AGAINST *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* TOMATO PATHOGEN

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Abstract

The destructive disease of tomato worldwide, fusarium wilt, is caused by Fusarium oxysporum f. sp. lycopersici (Sacc) W. C. Synder & H. N. Hans., a vascular wilt pathogen. The fungus affects greenhouse and field grown tomatoes in warm vegetable production areas. Yellowed leaves and wilted plants with low or absent crop yield are the main symptoms of the disease. In this paper we have analyzed possible substitutes to synthetic fungicides in controlling the pathogen. Since the potential of essential oils as antimicrobial agents is well established and farmers around the world already use traditional recipes, we have decided to test a fair amount of essential oils and four homemade fungicides against the growth of Fusarium oxysporum f. sp. lycopersici mycelium. For the experiment, 1 ml of essential oil serial dilutions of 0.1%, 1% and 10% were used along with the most used worldwide homemade fungicides. Fungal growth measurements were taken every 24h for 13 days, until no fungal growth was registered. Results show that 0.1% essential oils concentration had no inhibitory effect, while at 1% oil concentration only oregano essential oil was 61% effective. At 10% cinnamon, cloves, thyme, oregano and lemon essential oils showed cidal effects.

Key words*: essential oils, fusarium wilt, in vitro, tomato disease.*

INTRODUCTION

The unifying principle behind most supporters of organic farming is the belief that soil health is extremely important for life and is the only sustainable way of cultivating land and creating a safe future for humanity.

The use of biopesticides has been possible since the 1960s, but has only increased recently due to society's awareness of the issues of intensive use of chemicals such as human health, soil pollution and water resources, such as and the resistance acquired by pests and pathogens. Biocontrol products are used in both conventional and organic farming.

Tomatoes (*Lycopersicon esculentum* Mill.) are highly susceptible to pathogens, such as fungi, viruses, bacteria and nematodes, which cause severe yield losses (Barone and Frusciante, 2007).

The destructive disease of tomato worldwide, fusarium wilt is caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc) W. C. Synder & H. N. Hans., a vascular wilt pathogen. The fungus affects greenhouse and field grown tomatoes in warm vegetable production areas. Yellowed leaves and wilted plants with low or absent crop yield are the main symptoms of the disease (Sudhamoy et al., 2009). The disease may lead to a 30 to 40% yield loss and it can even go up to 80% under favorable climate conditions (Kirankumar et al., 2008).

The pathogen enters the root and further into the vascular tissue. The xylem vessels are colonized by mycelium and conidia. As a result, the vessels are clogged and plants suffer of severe water stress, leading to the characteristic wilt symptoms (Beckman, 1987). There are three physiological pathogen races (1, 2 and 3) known to affect tomato cultivars (Kawabe et al., 2005).

MATERIALS AND METHODS

The antifungal activity of 22 commercially purchased essential oils, i.e. anise (*Pimpinella anisum* L.), basil (*Ocimum basilicum* L.), Indian frankincense (*Boswellia serrata* T.), cinnamon (*Cinnamomum aromaticum* L.), camphor tree (*Cinnamomum camphora* L.), lemongrass (*Cymbopogon winterianus* L.),

cloves (*Syzygium aromaticum* L.), coriander (*Coriandrum sativum* L.), May Chang (*Litsea cubeba*), fennel (*Foeniculum vulgare* M.), oil grass (*Cymbopogon citratus* DC.), lavender (*Lavandula angustifolia* Mill.), tea tree (*Melaleuca viridiflora* Sol.), orange (*Citrus* x *sinensis* L.), palmarosa (*Cymbopogon martinii* Roxb.), turmeric (*Curcuma longa*), rosemary (*Rosmarinus officinalis* L.), clary sage (*Salvia sclarea* L.), spearmint (*Mentha spicata* L.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.) and lemon (*Citrus limon* L.), along with homemade fungicides based on baking soda, garlic and hydrogen perodixe were investigated against *F. oxysporum* f. sp. *lycopersici.*

For the homemade fungicides, we have selected the most used recipes by international farmers: mix 5 tablespoons of baking soda with 1 teaspoon of liquid soap in 1 gallon of water (B1); mix 1 tablespoon of baking soda with 1 teaspoon of castor oil in 1 gallon of water (B2); chop 100 g of garlic cloves in 1 L of water Us (Ga); hydrogen peroxide 3% AO (HP).

For the experiment, 1 mL of EO dilution or homemade fungicide was pipetted in each Petri dish (85mm), after which 17 mL of potato dextrose agar (PDA, Scharlau, Spain) were added at temperature of 50ºC, to avoid volatilizing or denaturing the aromatic compounds in the oils, then the dishes were stirred for 20s. EO concentrations of 0.1%, 1% and 10% were expressed using Percent Composition by Mass (%), in which the mass of the solute is divided by the mass of the solution (mass of solute plus mass of solvent), then multiplied by 100. Media was allowed to cool and solidify. After 2 h, a 7 mm mycelial plug of *F. oxysporum* f. sp. *lycopersici* was centered onto each Petri dish. The *F. oxysporum* f. sp. *lycopersici* cores were taken from the edge of individual 14 days old colonies. All Petri dishes were left inside the laminar-flow hood for 24 h then stored inverted so that water would not condense on the agar surface. Dishes were incubated in the dark inside a 28ºC germination chamber. A total of 66 dishes were used per replication, with 3 replications. Control plates were included in each replication: 3 PDA media plates inoculated with the pathogen, to determine the viability and growth.

Fungal growth measurements were taken every 24 h for 13 days, until no fungal growth was registered. One Way Within Subjects ANOVA was used to determine the effect of treatments on each concentration on growth measurements. Statistical analysis was performed with the IBM SPSS Amos v20.

RESULTS AND DISCUSSIONS

The maximum growth of *F. oxysporum* f. sp. *lycopersici* at the end of the 13 days experiment is outlined in Figure 1.

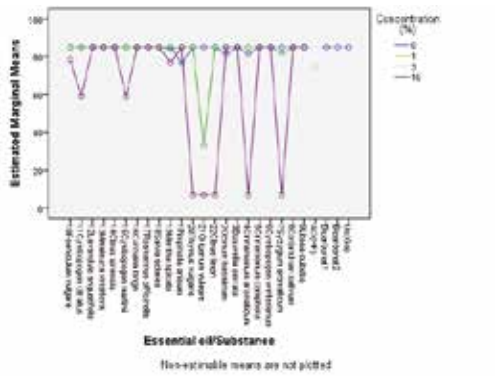

Figure 1. Effect of EOs and homemade fungicides on growth of *Fusarium oxysporum* f. sp. *lycopersici*, at different concentrations (original)

The oregano (*Origanum vulgare*) EO is the only oil that has the most aggressive effect on the growth of the fungus: both at 1% and 10% concentration, the mycelium was stopped from developing (Figure 2); on the other hand, the 0.1% oil concentration did not prevent growth in any way. The main components of the oil are carvacrol, thymol, p-cymene, cis-omen, caryophyllene and linalool.

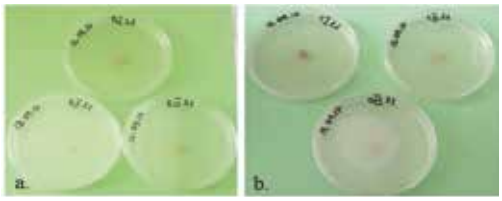

Figure 2. Cidal effect of oregano EO on growth of *Fusarium oxysporum* f. sp. *lycopersici*, at 1% (a) and 10% (b) oil concentrations (original)

There are a number of essential oils that did not allow the fungus any growth at 10% oil

concentration, i.e. cinnamon (*Cinnamomum aromaticum*), cloves (*Syzygium aromaticum*), thyme (*Thymus vulgaris*) (Figures 3 and 4), and lemon (*Citratus limon*) EOs.

Figure 3. Cidal effect of cinnamon (*Cinnamomum aromaticum*) (a), cloves (*Syzygium aromaticum*) (b) and thyme (*Thymus vulgaris*) (c) EOs on *Fusarium oxysporum* f. sp. *lycopersici*, 10% oil concentration (original)

Cinnamon and cloves EOs have eugenol and eugenol acetate. As distinct elements, cinnamon oil also contains cinnamic aldehyde and benzyl benzoate, and cloves oil also has iso-eugenol and caryophyllene. The α-pinene, camphen, β-pinene, α-terpinene and linalool are found to be both in thyme and lemon EOs.

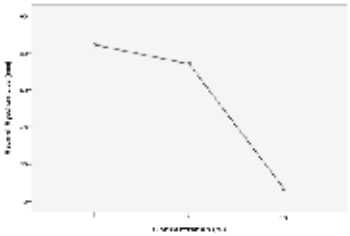

Figure 4. Average size of *Fusarium oxysporum* f. sp. *lycopersici* mycelium under treatment with cinnamon (*Cinnamomum aromaticum*), cloves (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) EOs at 0.1%, 1% and 10% concentrations (original)

Effects of EOs treatments on growth of *F. oxysporum* f. sp. *lycopersici* are significant, Wilks'Lambda = 0.71, F (2, 20) = 3.95, p = 0.036 (Table 1).

Table 1. Multivariate Tests[a] - *Fusarium oxysporum* f. sp. *lycopersici*

Effect		Value	F	Hypothesis df	Error df	Sig.
Factor 1	Pillai's Trace	.283	3.954[b]	2.000	20.000	.036
	Wilks' Lambda	.717	3.954[b]	2.000	20.000	.036
	Hotelling's Trace	.395	3.954[b]	2.000	20.000	.036
	Roy's Largest Root	.395	3.954[b]	2.000	20.000	.036

a. Design: Intercept
Within Subjects Design: factor1
b. Exact statistic

Since the Sig. value for Wilks' Lambda variation is lower than 0.05, there is a significant difference between the effect of each oil concentration.
The Paired T-Test (Table 2) was further used to compare the *post hoc* effect of each oil concentration on the fungus development.

Table 2. Paired Samples Test - *Fusarium oxysporum* f. sp. lycopersici

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
concentration=0.1% - concentration=1%	-2.227	13.349	2.846	-8.146	3.691	-.783	21	.443
concentration=0.1% - concentration=10%	-25.455	41.977	8.950	-44.066	-6.843	-2.844	21	.010
concentration=1% - concentration=10%	-23.227	38.295	8.164	-40.206	-6.248	-2.845	21	.010

The T-test indicates there are significant differences between 0.1% - 10%, as well as between 1% - 10% EOs concentrations, since the Sig. value, also known as the p value, is lower than 0.017.

CONCLUSIONS

All the EOs showed low influences at 0.1% concentrations, up to 14%.

The oregano (*Origanum vulgare*) EO treatment demonstrated a 61% efficacy on the *F. oxysporum* f. sp. *lycopersici* mycelium at 1% oil concentration.

The fungus was influenced by a reduced number of EOs at 10% concentration, but they all demonstrated a cidal effect, i.e. cinnamon (*Cinnamomum aromaticum*), cloves (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) and lemon (*Citrus limon*).

Usually, the effects are seen in a time- and dose-dependent manner; higher concentrations cause severe effects more rapidly.

As the present paper showed, the oil concentrations necessary to kill *F. oxysporum* f. sp. *lycopersici* are most of the times much higher than those required to inhibit its growth.

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POSITIVE ASPECTS OF AN ORNAMENTAL VEGETABLE GARDEN AND ITS EFFECTS TOWARDS FAMILY AND COMMUNITY SUSTAINABILITY

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Abstract

Sustainability is based on three important pylons, the environment, the social component and the economic component. Design based on landscape principles and rules and by applying the correct crop technology, the vegetable garden presents many functions, which increase its impact on the community and on the environment. To reach the aim and objectives of this research based on the obtained results during 2015-2017 regarding crop productivity, plant development, economic aspects and ornamental criteria a survey regarding sustainability of the vegetable garden was done. From an economic point of view, the studied garden has a positive impact on the family budget. The garden also has a big impact on the owners bringing benefits to their health and also on the education of the young generation regarding horticultural practices. The results of the study show that the ornamental vegetable garden, design in an intercropping system has a positive impact on the owners, contributing to the sustainability of the community and its surroundings.

Key words*: function, family garden, aesthetic.*

INTRODUCTION

The first vegetable gardens were situated on the valleys of Tigre, Eufrat and Indus rivers (Iliescu, 2008), initially their purpose was to offer fresh vegetables and, in some celebration, they had an ornamental use (Sima, 2009).

Landscape design is a phenomenon that was used in vegetable growing for a long time, due to the desire to create an area where you can relax and admire beautiful views. This landscape offers food for the body and soul (Galea et al., 2017).

Father Ignatius said „a garden should look like a beautiful arranged table". In a vegetable garden, the soul of the owner is represented by an element in the design, usually a flower (Sima, 2017).

In public spaces, in Romania, ornamental vegetable garden are rare, but because of the importance that vegetable crops have and the desire for healthy vegetables in private gardens, this type of design has slowly entered Romanian households (Galea, 2017).

Vegetable plants have many ornamental elements that in an intercropping system are underlined and contribute to the general aesthetic of the garden. An intercropping system besides its ornamental values has a positive impact in attracting useful insects.

Using this system in a family vegetable garden can have many advantages that can have a good impact on the community and on the environment.

In this context, the paper presents an analysis of the effects of an ornamental vegetable garden on the sustainability of the family and community in which it exists, in the period 2014-2017.

MATERIALS AND METHODS

In order to reach the aim and the objectives of this research six experimental gardens were conceived and based on the analyses obtained in different studies (Galea et al., 2017) one garden was chose as the best from an ornamental and sustainable point of view.

The experimental garden was done/ established in the experimental field of the vegetable growing department from USAMV Iasi, between 2014-2017.

The studied family ornamental vegetable garden was designed in a geometrical style with

a planted area of 100 m^2. The family garden was evaluated by a panel of experts in a survey regarding its ornamental value and in another, regarding its sustainability value. The two surveys had scale from 1 to 5, in wich 1 represented highley disagree, 2 represented disagree, 3 neutral, 4 agree and 5 represented higly agree.

In the composition of the surveys were taken into consideration the following: the combining method, the proposed species, the ornamental layout, garden functions (educational, cultural etc.), the influence of the used intercropping system on plant growth, development and production, pest and disease attack.

The achieved results were evaluated using SWOT analysis to help determine the dree of ornamental and agronomical value.

From an economic point of view, plant production and production quality was assessed.

Due to the fact that the vegetable garden has in its composition 34 different vegetable plants (13 intercropping systems), 6 samples (tomatoes, hot pepper, eggplant and squash) were analysed regarding production quality using the device DR 301-95 (digital refractometer-for sucrose) and a vacuum drying oven (for dry substance and humidity).

In order to realize the purpose of this study, besides increasing the ornamental value, one the main objectives was to increase the impact of the vegetable garden on its owners and on the environment.

RESULTS AND DISCUSSIONS

The climatic conditions from the experimental field were suitable for vegetable growing. From a development point of view the habitus of the plants was harmonious.

The chosen intercropping systems had a positive impact on plant production (Table 1). The average values obtained for the studied plants were close to the values found in the existing literature (Stan et al., 2003; Stan and Munteanu, 2001; Fălticeanu and Munteanu, 2003; Savițchi et al., 2013; Multeanu, 2003; Ciofu et al., 2003; Carvalho et al., 2012; Tringovska et al., 2015; Tincă, 2011; Hamburdă, 2015; Bavec et al., 2010). The results optained regarding chemical composition, of the six analyzed samples had values in line with those in known literature, fact that proves that the applied intercropping system has an evident effect in obtaining a qualitative production (Table 2).

The percentage of sucrose for cherry tomatoes varied from 6.09% for Aristan purple bumble bee cv. to 8.15% for Ema de Buzau cv.

Regarding the hot pepper the obtained result were higher than the ones in the known literature with an average value of 8.35%.

The obtained result for egg plants was 3.6-3.8%, values that are in between the known data (2.5-4.0%) (Bodea and Enăchescu, 1984).

Table 1. Comparative results regarding the obtained production

Nr. Crt.	Plant name	Plant production (g)	Standard production (g)
1.	Lollo Rossa lettuce	180 - 200	200-300
2.	Aristan Puple Bumble Bee tomatoe	1380	1500
3.	Beam's yellow pear tomatoe	1200	1500
4.	Ema de Buzău tomatoe	1400	1500
5.	Ovari feher squash	1700	430-450
7.	Bordi pea	3	3
8.	Runner bean	80	70
9.	Thyme	200	125-175
10.	Pascal celery	300-342	120
11.	Black beauty eggplant	3000	900-1800
12.	Kayene pepper	280	63-105
13.	Sweet Thing F1 sweet corn	775	750
17.	Scarlet kale	522	792
18.	Kadet kale	540	792
19.	Chard	3500	41,66
20.	Dalmatziano beans	40	20
21.	Clemson Spineless okra	210	105
22.	Cap de Negru 2 cabbage	2315	1400-1800
23.	Calabrese Natalino broccoli	450	500
24.	De Ciorani hysopus	200	193
25.	Violet basil	190	105-126
26.	Onions	26	26
27.	Piccolo verde di Parigi cucumber	800	1050
28.	Sage	550	360
30.	Di Sicilia Violetto cauliflower	450	480-800
31.	Bulgăre de zăpadă cauliflower	600	480-800
32.	Radish	425	200-300
33.	Pumpkin butternut	10500	10000-12000
34.	Rosa di trevise 4 cicory	220	177-222

Regarding dry matter the values obtained for cherry tomatoes were between 8.09-13.20%. The highest values was for Ema de Buzau cv.

The average values obtained for eggplant were 6.8% for dry matter for Black beauty cultivar and 7.2% for Albe de Buzău cv.

The obtained results for squash had an average of 5.4% for dry matter.

Based on the two surveys the strong points and the weaknesses of the garden were underlined using SWOT analysis providing an overview of the ornamental degree of the garden and over its effects regarding the sustainability of the community and its surroundings.
The obtained results regarding the ornamental value of the studies garden revealed that the ornamental vegetable garden has many functions, with beautiful perspectives. The usage of different intercropping systems (lettuce + runner bean; eggplant + pepper; okra + beans etc.) contributed in a positive way to the ornamental degree (Figure 1).

Figure 1. Garden detail

One of the week point of the garden was represented by the alternative mean of plant protection, that need to be improved (Table 2).

Table 2. SWOT Analysis - Model 6

Strengths	Weaknesses
• many functions; • beautiful perspectives; • the style of the garden enhances the ambiance of the household; • the intercropping system brings aesthetic value;	• alternative mean of plant protection;
Opportunities	**Threats**
• landscape design principles correctly used ; • color and volume game - increased ornamental impact	• reduced communication.

The surveys regarding the sustainability of the ornamental family vegetable garden revealed that according to the panel of experts the garden is considered economically affordable.
The studied intercropping systems had a positive impact on attracting useful insects that had a good influence in plant production (Figure 2).

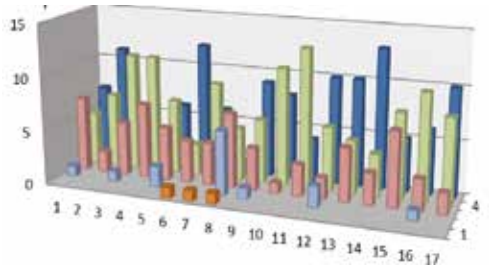

Figure 2. Evaluation forms analysis for garden sustainability degree

Based on the SWOT analysis the garden has many strengths, such as the education of the young generation, high yield, high ornamental value, the increase of useful insects and fresh vegetable for a long period.
The analysis also revealed that the garden needs many carrying practices. By using an intercropping system, the number of disease attack was reduced.
From an economic point of view the obtained results reveal that the highest cost in the ornamental garden were the ones with materials, fallowed by seeds and seedlings cost.
In the first year of study the profit rate was 11 % due to the initial investments (Table 3). The profit rate was obtained din the second year of study.

Table 3 Correlation study between costs and revenue

Nr.crt.	Specification	U.M.	1st year	2sd year	3rd year
1.	Total production costs	lei	1759	1028	1229
2.	Yeald value	lei	1856	1663	1600
3.	Profit	lei	204	635	370
4.	Profit rate	%	11.65	61.77	30.08

CONCLUSIONS

The proposed intercropping systems had a positive effect on plant growth and development. The ornamental perspectives created in the garden were beautiful and contributed to the enhancement of the environment. The many function the garden has (ornamental, aesthetic, cultural and educational) increase community communication and help the owners maintain a healthy life style.

From an economic point of view the ornamental vegetable garden contributes to the economy of the family budget.

By using an intercropping system, the garden offers fresh vegetable for a long time.

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FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE


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PRELIMINARY RESEARCH ON THE INFLUENCE OF THE PLANTING TIME ON THE GROWING AND BLOOMING OF VARIETY OF HERBACEOUS PEONY IN THE FIELD

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Abstract

The present paper refers to the results of the research on the influence of the different planting times of some varieties of herbaceous peony in the field on the blooming and growing. The research was performed in the experimental field of USAMV Bucharest and in the town of Singureni, Giurgiu County. The studies made are based on the data obtained both by visual observation and by biometric measurements on the varieties of herbaceous peony. The biologic material war represented by a variety of herbaceous peony with white flowers, with pink flowers and with red flowers. The data was processed in the following indicators: growth stages (emergence, springing, percentage of shoots starting vegetation, percentage of springs, vegetation rest) and blooming stages (percentage of flower springs, appearance of buds, stages of blooming, percentage of blooming) as well as the dynamics of growth and blooming. There were results related both to the growth and blooming stages and to the dynamics of the growth and blooming.

Key words*: blooming, growth, herbaceous peony, planning times*.

INTRODUCTION

The peony is native of Europe, Asia and America. The peony grows spontaneously in numerous countries with temperate climate in the Northern hemisphere. Peonies are perennial ornamental plants of the genus *Paeonia*, pertaining to the family of *Paeoniaceae* and it consists of 33 species (Rogers, 1996).

The herbaceous peonies are grown mainly for the use in horticultural industry like a home garden plant and also, the peonies are commercially grown as cut flowers. Peonies are, also cultivated for their medicinal properties (Dong-Yi et al., 2011) and even for their use as ornamental plants (Nehrlingand, 1978).

The research made by Cucu et al.(2009) on the study of the behaviour in crop of some species and the growing of the peony in the southern part of Romania, under the influence of local climate factors.

The study started with the plants coming at rest in the autumn of 2005. It was observed that the first signs of senescence are given away by the species *Paeonia officinalis* L. and its growing (late August), followed closely by the species *Paeonia peregrina* Miller and *Paeonia mollis* Hort., as well as the herbaceous hybrids. The species *Paeonia lactiflora* Pallas and its growing are the last to come at rest on the ecologic conditions of the area, as well as the *Itoh* hybrids.

At the same time, the crops of the species *Paeonia lactiflora* Pallas became remarkable by the decorative colour of the foliar apparatus, especially 'Rosy Down' and 'Auguste Dessert'.

In a research study published in 2002 by Barzilay et al., with the topic *The annual life cycle and flower development of 'Sarah Bernhardt' under the conditions of Israel*, they present the life cycle and the morphogenesis of the floral sprouts of *Paeonia lactiflora* Pallas. The cultivar 'Sarah Bernhardt' was studied on the conditions of Israeli climate.

The formation of the buds starts at the beginning of spring. The stems grow rapidly and they reach heights of 50-70 cm in 60-70 days. The flowering starts in April and it continues until the end of May. After the flowering, the stem remains green until September-October, when the leaves age and the plant comes at vegetation rest for 3-4 months.

Toma (2009) mentions that the herbaceous species of peony reproduce by the division of the bush, operation made by the mature plants, at the age of 4-5 years. After part of the soil is removed from the roots, the mother plants are cut by hand or with a very sharp knife in 3-6 parts, depending on the size of the mother plant.

After the division, the very large leaves are shortened to 2-3 foils in order to assure a good reproduction of the plants. The plant divisions will be put in planting holes, after mudding the roots, so that the area of the collar is covered with 2-3 cm of soil (Cantor, 2016). Deeper planting results in the delay and the diminishing of the flower or even its compromise for the next 2-3 years from the planting (Toma, 2009).

Peonies need a long period of cold of at least 900 hours around or under the freezing point (Jacob et al., 2006), in order to pass through the rest period and start the vegetation in the next season. In case the temperature does not drop enough, the peonies will fail to make flowers.

MATERIALS AND METHODS

The planting material used in the research was constituted of herbaceous peony with white flowers, with red flowers and with pink flowers. The varieties of peonies were planted both at USAMV Bucharest and in Singureni in 3 planting times, this resulting 9 variants.

The planting was made with plants coming from 3-4 years old bushes and 7 years old bushes as divided and undivided bushes (Figures 1-4). The planting occurred in the autumn of 2016 at USAMV Bucharest and at Singureni. The data was processed using the biometric measurements and the visual observations made during 2017.

The indicators used in the study of the research were: start of vegetation, the appearance of shoots, the senescence, the appearance of the sprouts, the percentage of shoots, the percentage of flowering shoots, the phenophases of growth and flowering, the dynamics of growth and flowering, the earliness of the flowers and the flowering percentage.

The experimental variants, the cultivar the plating dates and the features of the planting materials are given in Table 1.

Table 1. Experimental variants

Variant	Cultivar	Date of planting	Type of material	Number of sprouts min/ max	Diameter of the ballot of the roots min/max (cm)
V1	Cultivar with pink flowers in the collectionUS AMVB	05.11.2016	Divided bushes	1 - 7	2 - 16
V2	Cultivar with pink flowers in the collectionUS AMVB	14.11.2016	Divided bushes	2 - 16	5 - 21
V3	Cultivar with pink flowers in SINGURENI	05.11.2016	Divided bushes	4 - 9	8 - 15
V4	Cultivar with pink flowers SINGURENI	14.11.2016	Undivided bushes	8 - 24	20 - 27
V5	Cultivar with pink flowers in SINGURENI	25.11.2016	Divided bushes	2 - 14	6 - 17
V6	Cultivar with pink flowers in SINGURENI	25.11.2016	Undivided bushes	9 - 16	22 - 27
V7	Cultivar with white flowers in SINGURENI	05.11.2016	Divided bushes	4 - 9	9 - 15
V8	Cultivar with white flowers SINGURENI	14.11.2016	Divided bushes	3 - 12	5 - 14
V9	Cultivar with red flowers in the collection USAMVB	05.11.2016	Divided bushes	5 - 13	5 - 16

Figure 1. Undivided bushes, pink cultivar in Singureni

Figure 2. Divided bushes, white cultivar in Singureni

Figure 3. Divided bushes, pink cultivar in Singureni

Figure 4. Divided bushes, red and pink cultivar in USAMV Bucharest

RESULTS AND DISCUSSIONS

The obtained results as far as the phenophases of growth of the varieties of herbaceous peony studied were made by the measurement of the following indicators: date of appearance of the sprouts, end or appearance of the sprouts', appearance of the shoots and the end of the growth of the shoots.

When starting the vegetation, the following results were obtained: early start in the variants V6, V7 and V8 and late starts in the variants V2, V4, V5 and V9 according to figure 5. In the early appearance of the shoots, the variants V6, V7 and V8 stood out, and in the late appearance, the variants V2, V4, V5 and V9.

In her research Barzilay et al. (2002) at the *'Sarah Bernhardt' paeony cultivar,* the shows that on the conditions of Israeli climate the formation of the buds starts at the beginning of spring.

In Figures 6 and 7 it is observed the appearance of the sprouts in the two locations of the research on 2017, and in Figures 8 and 9 it is presented the growth of the shoots with the late start of sprouts.

The growth of shoots in the two research locations of the varieties of herbaceous peony studies is presented in Figures 10-13.

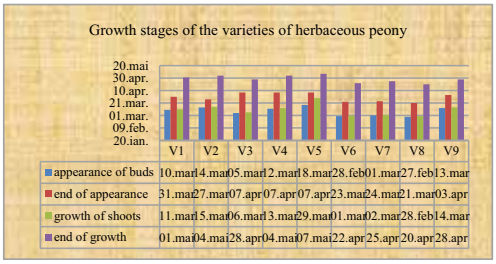


Figure 5. Stages of growth of the varieties of herbaceous peony planted in the field in 2017

Figure 6. Appearance of sprouts in the herbaceous peony, 2017

Figure 7. Start of vegetation in the varieties of herbaceous peony at USAMV Bucharest, 2017

Figure 8. Start of vegetation and the growth of shoots, pink cultivar of Singureni, 2017

Figure 9. Appearance of the sprouts and of the shoots in white cultivar of Singureni, 2017

Figure 10. Growth of shoots in the varieties of herbaceous peony planted in USAMV Bucharest, 2017

Figure 11. Growth of shoots, white and pink cultivar of Singureni, 2017

Figure 12. Aspect of e bush and shoots, pink cultivar of Singureni, undivided bushes, 2017

Figure 13. Aspect of the shoots towards the end of growth, pink cultivar of Singureni, 2017

Researching the stages of growth and flowering, the following indicators were analysed: percentage of vegetation start, percentage of shoots, percentage of flower shoots, percentage of plants with floral shoots, percentage of plants with blooming floral shoots and the blooming percentage.

The percentage of vegetation start in all the variants is 50%, the same with the percentage of shoots. The percentage of flower shoots was in two variants under 50%, and the rest above 50%. As far as the percentage of plants with floral shoots, the obtained results being under 100% in two variants and 100% in the others.

The blooming percentage was under 50% in variants V1, V2, V4, V5, V6, V7, and above 50% in variants V3, V8 and V9. The results are given in Figure 14.

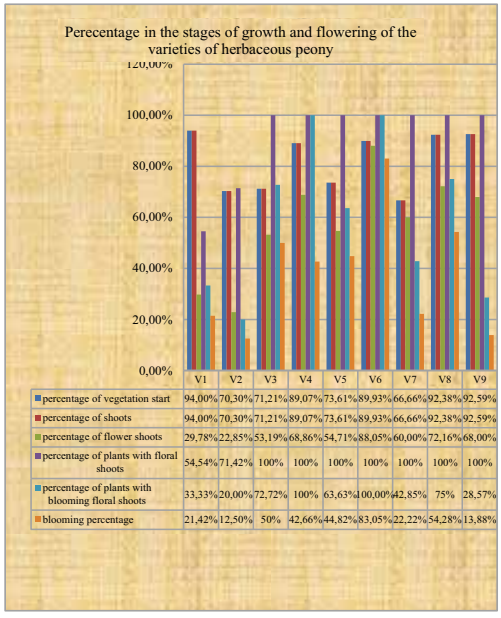


Figure 14. Percentage of growth and flowering in the varieties of herbaceous peony planted in the field, 2017

The aspect of the mass flowering of the varieties of herbaceous peony is presented in Figures 15 and 16. In Figures 17 and 18 are given the plants of herbaceous peony with floral shoots. Figure 19 presents the aspect of the flower in the cultivar of peony with pink flowers in USAMV Bucharest.

Figure 15. Aspect of flowering in pink cultivar at USAMV Bucharest, May 24, 2017

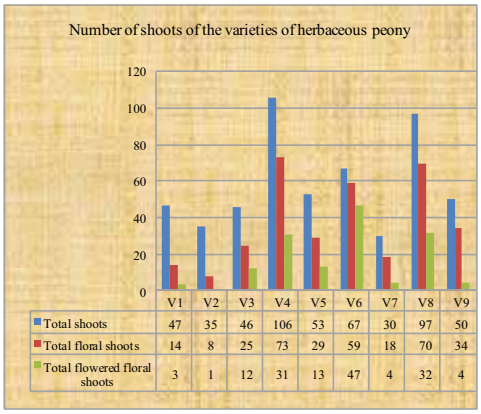
Figure 16. Aspect of flowering in white cultivar Singureni, May 24, 2017

Figure 17. Floral shoots in pink cultivar in Singureni, May 28, 2017

Figure 18. Floral shoots in pink cultivar at USAMV Bucharest, May 15, 2017

Figure 19. Aspect of flowering in pink cultivar USAMV Bucharest, May 24, 2017

We went on to analyse also the number of shoots in the varieties of herbaceous peony studied in the indicators: total shoots, total floral shoots and total flowering floral shoots.
The number of shoots in the variants of divided bushes was a minimum of 35 in V2 and a maximum 97 in V8, and in the variants with undivided bushes they were 67 in V6 and 106 in V4.
The minimum number of floral shoots was in V2 and the maximum in V8 and V4. The minimum number of flowering floral shoots was in V2, and the maximum in V6. The obtained results are given in figure 20.
Figures 21 and 22 present shoots of peony before the flowering with semi-open buds in the two research locations.



	V1	V2	V3	V4	V5	V6	V7	V8	V9
Total shoots	47	35	46	106	53	67	30	97	50
Total floral shoots	14	8	25	73	29	59	18	70	34
Total flowered floral shoots	3	1	12	31	13	47	4	32	4

Figure 20. Number and types of shoots of the varieties of herbaceous peony planted in the field, 2017

Figure 21. Start of flowering in the white cultivar in Singureni, 16.05.2017

Figure 22. Floral shoots in the red cultivar USAMV Bucharest, May 10, 2017

The early emergence of the bud appeared in variants V6, V7 and V8 and the late emergence in variants V2, V3, V5, and V9.
The closed bud phase May 4 in V6, May 10 in V8, May 12 in V3, May 13 in V5,V7 and V9, May 15 in V1,V2,V4.
The opening of the flower emerged in May 10 in V6, May 15 in V8, May 17 in V3, V5; May

18 in V7 and V9; May 19 in V1 and May 20 in V2.

The end of blooming occurred on May 25 in V8; May 27 in V6; May 30 in V5; June 2 in V3 and V7; June 4 in V2; June 5 in V4 and V9; June 6 in V1.

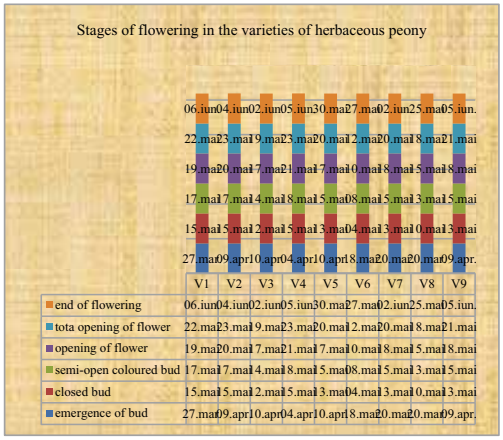


Figure 23. Stages of flowering in the varieties of herbaceous peony planted in the field, 2017

Figures 24-28 present the aspects of flowering and flowers of varieties of herbaceous peony in 2017.

Figure 24. Aspect of flowering in the red cultivar USAMV Bucharest, May 24, 2017

Figure 25. Red cultivar flowered in USAMV Bucharest, May 2017

Figure 26. Pink cultivar flowered in USAMV Bucharest, May 2017

Figure 27. Aspect of flowering in the red cultivar USAMV Bucharest, May 24, 2017

Figure 28. Aspect of flowering in the pink cultivar USAMV Bucharest, May 24, 2017

Analysing the duration of the stages of growth and flowering of the varieties of herbaceous peony, it was observed that the shortest duration of budding was in variants V2, V5 and V9, and the longest in variant V3. The duration of flowering was of 13 days in V5 and V8, 14 days in V4 and V7, 15days in V2, V3, V6, and 17 days in V1 and V9 (Figure 29).

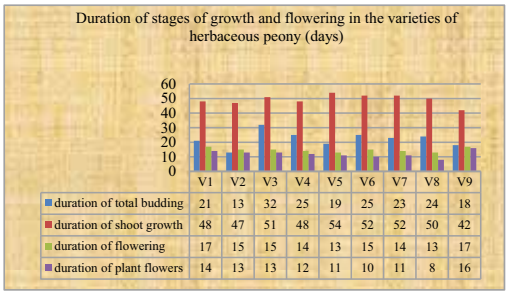


Figure 29. The duration of stages of growth and flowering in the varieties of herbaceous peony planted in the field, 2017

Figures 30-38 present the aspect of flowering and the aspect of the flower in the cultivars of herbaceous peony with pink flowers in

USAMV Bucharest, with pink flowers in Singureni and with white flowers in Singureni.

Figure 30. Aspect of flower, pink cultivar USAMV Bucharest, May 24, 2017

Figure 31. Aspect of flower, pink cultivar USAMV Bucharest, May 24, 2017

Figure 32. Aspect of flower, pink cultivar Singureni, May 24, 2017

Figure 33. Aspect of flower, pink cultivar Singureni, May 18, 2017

Figure 34. Aspect of flower, white cultivar Singureni, May 20, 2017

Figure 35. Aspect of flower, white cultivar Singureni, May 20, 2017

Figure 36. Aspect of flower, white cultivar Singureni, May 22, 2017

Figure 37. Aspect of flower, pink cultivar Singureni, May 20, 2017

As far as the dynamics of the growth and of the inflorescence of the types of studies herbaceous peony, the indicators in Table 2 were analysed. The short vegetation was observed for the variants V1, V6, V7 and V9, and a longer vegetation period was observed in variants V2, V3, V4, V5 and V8.

Table 2. The dynamics of growth and flowering in the varieties of herbaceous peony planted in the field, 2017

Variant	Sprouting	Shooting	Budding	Blooming	End of blooming	End of vegetation
V1	10.03	13.03	27.03	19.05	06.06	III October
V2	01.03	03.03	20.03	18.05	02.06	I November
V3	05.03	07.03	10.04	17.05	02.06	I November
V4	12.03	16.03	04.04	21.05	05.06	I November
V5	18.03	21.03	10.04	17.05	30.05	I November
V6	14.03	17.03	09.04	20.05	04.06	III October
V7	13.03	16.03	09.04	18.05	05.06	III October
V8	27.02	01.03	20.03	15.05	25.05	I November
V9	28.02	01.03	18.03	10.05	27.05	III October

Figure 38. Total opening of the flowers and the aspect of the flowering in the pink cultivar in Singureni, May 24, 2017

CONCLUSIONS

The research made shows that some varieties of herbaceous peony may be planted in a period different from August or September, increasing thus the planting period in autumn of the herbaceous peony. This research came to the following conclusions:

The percentage of the start of vegetation in all the variants was over 50%, respectively 66.66% in the cultivar with white flowers in Singureni by 94% in the cultivar with pink flowers in the collection USAMV Bucharest.

The percentage of shoots is the same with the percentage of start of vegetation.

The percentage of floral shoots in two variants was under 50% respectively in the cultivar with pink flowers in the collection of USAMV Bucharest cu 29.78% and 22.85%, and the rest of the variants is over 50%, starting with the variety with pink flowers in Singureni with 53.19% by 88.05% in the cultivar with red flowers in the collection of UASVM Bucharest.

The percentage of plants with floral shoots is over 50% in all the variants, of which two variants with 54.54% and 71.42% in the cultivar with pink flowers in the collection of USAMV Bucharest and the rest of 7 variants of 100% respectively in the cultivars with white flowers in Singureni, with red flowers in the collection of USAMV Bucharest and with pink flowers in Singureni.

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PRELIMINARY RESEARCH ON THE GROWTH AND FLOWERING OF DIFFERENT VARIETIES OF HERBACEOUS PEONY UNDER CONDITIONS OF CONTAINERIZED AND FORCED CULTIVATION

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Abstract

This research has been focused on the studies on the growth and flowering of some varieties of herbaceous peony under conditions of containerized and forced cultivation. The research was conducted at USAMV Bucharest using as biologic material split bushes with white, pink and red flowers originated from USAMV Bucharest and Singureni, Giurgiu County. The split bushes being planted in pots of 30cm in diameter in autumn in October. After planting, the pots were kept in cold conditions a different number of weeks, after which they have been brought in the greenhouse to be forced. For each of the forced stages, observations have been made, regarding the following phenophases of growing and developing: starting in vegetation, number of sprouts, growth of the sprouts, appearance of the buds, opening of the flowers, fading of the flowers, the flowering time, the flowering percentage. The results obtained show a good behaviour of the herbaceous peony in containerized and forced cultivation, the percentage and the quality of blooming being variable, depending on the variety and the moment of the introduction of the pots in the greenhouse.

Key words*: containerized and forced culture, early flowering, herbaceous peony.*

INTRODUCTION

Peonies are among the most popular garden plants in temperate regions. Herbaceous peonies are also widely sold as cut flowers, although they are generally available only at the end of spring and early summer. The annual growth is predetermined: if the growing tip of a sprout is removed, no new buds will develop in that season (Kamenetsky et al., 2003, 2012).

In temperate regions of the Northern hemisphere, the peonies are blooming in May - July. Modern reproduction researches from the last years lead to obtain of cultivars with flowers with very different shapes and colours (Cantor, 2016).

Some of these new cultivars were studied also in the climatic areas of the Southern Romania (Cucu et al., 2009).

In the Northern hemisphere there is a growing demand to extend the flowering period of the peony (Toma, 2009). For example, Alaska peonies are nowadays foremost only for export (Fitzgerald, 2004). Another strategy is to use cold artificial environments as well as hormonal treatments to induce flowering at different times of the year (Halevy et al., 2002).

Herbaceous peonies are grown successfully in moderate climates with cold winters, where they bloom in May-July. Anyway, there are some researches that prove the capacity of the herbaceous peony to bloom under warmer climatic conditions, e.g. California and Israel (Byrne et al., 1986; Halevy et al., 1995).

Other researches prove that peonies can be forced to bloom in greenhouse conditions (Wilkins et al., 1985; Evans et al., 1990) for the early production of cut flowers and potted plants.

Gregory et al. (2015) elaborated a database for herbaceous peony cultivated in warm climate zones, in the "Effects of temperature on stagnation and plant growth" article.

These authors introduce the first database for development parameters for the herbaceous peony cultivated in a warm areas. The data on the temperature effect during the rest and growth period are presented for 2,232 plants of two commercial varieties exposed to 14 different temperature regimes.

Recently, the possibility of cultivating peony in warm climates has been reported also by the authors Fulton et al. (2001) and Catley et al. (2001).

Evans et al. (1990) and Wilkins et al. (1985) showed that in milder areas the peonies may be forced to produce early cut flowers in greenhouse.

In order to increase early forcing and to improve the profitability of growing peonies in warmer conditions, in-depth knowledge of temperature requirements is required at different stages of the annual growth cycle Gregory et al. (2015).
In a previous study, Gregory et al. (2015) established the key relationships between the thermal regime in the rest period, the stem growing and blooming.
The cooling regime for the release from resting, was studied on peony plants grown in the field by exposing them to a cold winter climate in northern Israel (8-10°C by night, 16-26°C by day, average about 17°C from November to February).
The greenhouses were covered with plastic films on different dates, looking for the accumulation of cooling units (Fishman et al., 1987; Erez et al., 1988). The flowering occurred two months after the resting of the plants has been over (Halevy et al., 2002).
The peonies cultivated in the pots have been artificially cooled by refrigeration to 2°C for 60 days ('Sarah Bernhard', 14) or 1-7°C for 3-12 weeks ('Coral Sunset', 'Monsieur Jules Elie', 'Sarah Bernhardt') (Barzilay et al., 2002).
The time until the start of vegetation after the completion of the cooling treatment has decreased proportionally with the increase in the duration of the cooling treatment (Hall et al., 2001).
In Chinese varieties, stem height, flower size and flowering rate were also positively affected by the cooling temperature during the resting period (Cheng et al., 2009).

MATHERIALS AND METHODS

The biological material used in these researches is made up of divided herbaceous peony varieties with white, pink and red flowers. These were obtained from mother plants aged 6-7 years, from USAMV Bucharest and Singureni, Giurgiu County.
The plant divisions were planted in October, in pots with a diameter of 30 cm. The subsoil of planting was consisting of 40% peat, 30% soil, 20% soil naturally fertilised, 5% sand and 5% perlite with a granulation of 4 mm.
After planting, the pots were left outside in a shaded area in conditions corresponding to the ones in open field (9°C average daily temperature and 2°C the average nightly temperature). Here, they have been kept for 10 weeks, until the frost.
From this moment, the pots were moved in a basement with an average temperature of 5°C, where they have been kept a variable number of weeks, until the movement in the greenhouse for forcing period.
The movement of the plants pots in the greenhouse for forcing was made at four different dates: 14th of February, 21st of February, 28th of February and 7th of March 2017. By combining these dates with the varieties of colours and provenance, resulted 11 experimental varieties (Table 1).

Table 1. Experimental Variants

Variant	Cultivar	No. of cold weeks	Date of forcing	Number of buds min - max	Diameter of roots bale min – max (cm)
V1	White flower peony from SINGURENI	16	14.02.2017	2 - 9	3 - 10
V2	White flower peony from SINGURENI	17	21.02.2017	5 - 7	3 - 10
V3	White flower peony from SINGURENI	18	28.02.2017	5	8 - 9
V4	Pink flower peony from SINGURENI	16	14.02.2017	3 - 10	4 - 13
V5	Pink flower peony from SINGURENI	17	21.02.2017	3 - 11	4 - 12
V6	Pink flower peony from the USAMVB collection	16	14.02.2017	4	6 - 9
V7	Pink flower peony from the USAMVB collection	17	21.02.2017	1 - 7	2 - 4
V8	Red flower peony from the USAMVB collection	16	14.02.2017	3 - 8	4 - 15
V9	Red flower peony from the USAMVB collection	17	21.02.2017	2 - 10	4 - 11
V10	Red flower peony from the USAMVB collection	18	28.02.2017	3 - 12	5 - 9
V11	Red flower peony from the USAMVB collection	19	07.03.2017	2 - 6	2 - 4

The observations on plants had in view the following elements: starting of vegetation, number of sprouts, the growing of sprouts, the apparition of flower buds, the number of shoots with flowers, the opening of flowers, the fading of flowers. Based on these observations, the duration of blooming and the flowering percentage were calculated. Also, the possible

correlations between the plant growing elements of the plants and between them and the duration of the cold period administrated before the introduction of the plants in the greenhouse.

RESULTS AND DISCUSSIONS

The starting on vegetation was faster on the variants V6 and V8 on 09.02., besides V1 on 10.02., V4 on 11.02., V2,V5, V9 on 15.02., V3 and V7 on 17.02., V10 on 19.02. and V11 on 28.02. (Figure 1).
The appearance of the sprouts was observed starting with 10.02. at V6 and V8 until 20.02. at V10 and 01.03. at V11 (Figure 1).

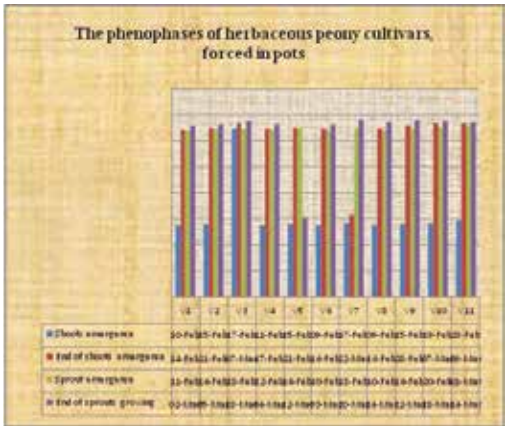

Figure 1. The phenophases of herbaceous peony growing, forced in pots, 2017

We are concluding that the varieties with pink and red flowers from USAMV Bucharest are starting earlier in vegetation compared to the white and pink flowers varieties from Singureni. Also, a bigger number of weeks of cold between the introduction of the pots in the greenhouse lead to a quicker vegetation starting on all varieties.
Details regarding the growth phenophases are shown in Figures 2-6.
Duration of buds starting was between 6 days at V7 and 21 days at V9, and the sprouts growth lasted between 16 days at V10 and 38 days at V9 (Figure 7).
The growing of sprouts lasted from 16 days at V11 up to 38 days at V9 (Figure 7).

Figure 2. Growth of sprouts, red cultivar, UASVM Bucharest, V10, 2017

Figure 3. Growth of sprouts, red cultivar, UASVM Bucharest, V8, 2017

Figure 4. Starting of vegetation of the pink cultivar from UASVM Bucharest, V6, 2017

Figure 5. Growing aspect of sprouts of pink cultivar from Singureni, 2017

Figure 6. Growing and trellising on peony sprouts, 2017

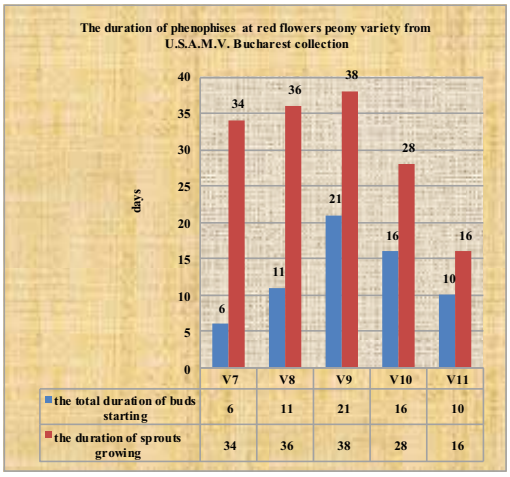

Figure 7. Dynamics of flowering, red flowers peony cultivar, UASVM Bucharest collection, 2017

It is observed a shorter period from total starting of the buds at the pink flowers variety from USAMV Bucharest compared to the red flowers variety from USAMV Bucharest.

Also, the growing period of the sprouts was shorter at the red flowers variety compared to the pink flowers variety from USAMV Bucharest.

The growth dynamics of the herbaceous peony varieties in containerized and forced cultivation is presented in Table 2.

An early vegetation start at V1, V6 and V8 and a later occurrence at V3, V9 and V11 were observed.

The percentage of the vegetation start was 33.33% and 36.36% at V1 and V2 up to 95% at V7 and 100% at V6.

The growth of sprouts started at V1 on 11.02, at V6 on 10.02 and V8 on 10.02 and continued until 20.02 at V10, and 01.03 at V11.

So, we are observing an early start of the vegetation on the pink and red flowers varieties from USAMV Bucharest compared to the white and pink flowers varieties from Singureni. Also, the maximum vegetation starting percentage was observed at the pink flowers variety from USAMV Bucharest compared to the white and pink flowers varieties from Singureni and with red flowers from USAMV Bucharest.

The beginning of buds vegetation lasted between 5 days at V1 and 7 days at V2 and V4 (Figure 8).

Table 2: Growing dynamics of the herbaceous peony varieties planted in pots and forced, 2017

Variant	Cultivar	Buds appearance	% vegetation start	Start of sprouts growth
V1	White flowers peony from SINGURENI	February 10	33.33%	February 11
V2	White flowers peony from SINGURENI	February 15	36.36%	February 16
V3	White flowers peony from SINGURENI	February 17	90%	February 18
V4	Pink flowers peony from SINGURENI	February 11	64%	February 12
V5	Pink flowers peony from SINGURENI	February 15	86%	February 16
V6	Pink flowers peony from USAMVB collection	February 9	100%	February 10
V7	Pink flowers peony from USAMVB collection	February 7	95%	February 18
V8	Red flowers peony from USAMVB collection	February 9	63.61%	February 10
V9	Red flowers peony from USAMVB collection	February 15	63.12%	February 16
V10	Red flowers peony from USAMVB collection	February 19	91.90%	February 20
V11	Red flowers peony from USAMVB collection	February 28	66.66%	March 1

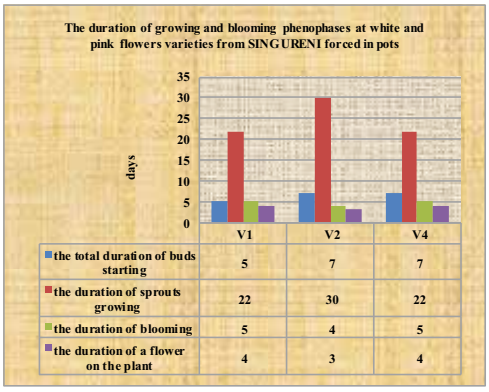

Figure 8. The dynamics of growing and flowering at the cultivars with white and pink flowers from Singureni, forced in pots in greenhouse, 2017

Regarding the growing of sprouts, the growth lasted 22 days at V1 and V4, and 30 days at V2 (Figure 8).

The flowering period as well as the duration of the flowers on the stem were short, between 3 and 5 days (Figure 8).

We are concluding that the white flower variety from Singureni had a shorter period for starting the buds compared to the pink flowers variety from Singureni. Also, the sprouts development

period, the blooming period and the duration of the flower on the plant is the same with the pink flower variety from Singureni.
The percentage of the vegetation starting was 33.33% at V1, and 36.36% at V2, up to 95% at V7 and 100% at V6 (Figure 9).

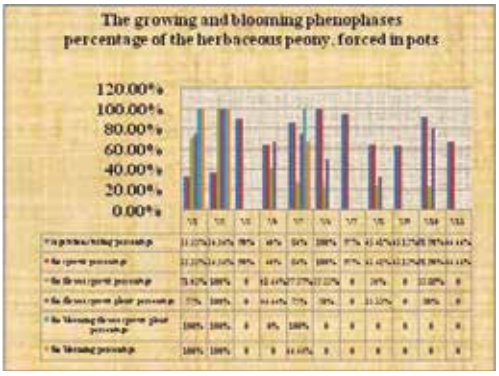

Figure 9. The growing and blooming phenophases percentage of the herbaceous peony, forced in greenhouse, 2017

The percentage of floral sprouts has been shown in 7 variants out of 11, with values between 22.22% at V6 up to 100% at V2.
The percentage of plants having floral sprouts has been shown in 7 variants out of 11, with values between 33.33% at V8 and 100% at V2 (Figure 9).
The percentage of flowering has been reached in 3 variants out of 11, at V5 with 66.66%, and 100% at V1 and V2, respectively (Figure 9).
It is determined that the variety with pink flowers from USAMV Bucharest had a maximum percentage related to the white and pink from Singureni and red from USAMV Bucharest. Also, a maximum percentage of blooming sprouts and plants with blooming sprouts was shown at the white flowers variety from Singureni related to pink and red varieties. A maximum blooming percentage is observed at the white flowers variety from Singureni related to pink and red varieties.
The influence of the cold season on the percentage of vegetation starting at the Singureni white flower variety is shown in Figure 10. There is a positive influence of the cold period on the start of vegetation of the cultivar with white flowers by Singureni. There is a correlation between the cold period and the start of vegetation: the higher the herbaceous peony plants at a higher cold season and percentage of vegetation starting.

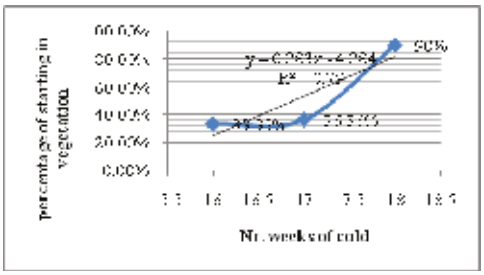

Figure 10. Influence of the cold period on the vegetation starting percentage at the variety with white flowers by Singureni, 2017

The phenophases of flowering were only conducted in 3 variants and 2 cultivars, V1 and V2 cultivars with white flowers of Singureni, and V4 cultivar with pink flowers of Singureni (Figure 11).

Figure 11. Flowering phenophases of Singureni cultivars with white and pink flowers, 2017

The appearance of the bud occurred at V1 and V4 on 18.02 and V2 on 24.02 (Figure 11).
The opening of the flower was started at V1 on 14.03, at V2 on March 21, and on V4 on 17.03, and the blooming ended at V1 on 20.03, V2 on 25.03 and V4 on 22.03. (Figure 11).
So, we are observing that only the Singureni flowers varieties were blooming and passed through the blooming phenophases. An early blooming is observed at the white flowers variety compared to the pink flowers variety from Singureni.
Details regarding the growing phenophases are found in the Figures 12-18.

Figure 12. Floral sprouts, white flowers cultivar, Singureni, V1, 2017

Figure 13. Floral sprouts, white flowers cultivar, Singureni, V2, 2017

Figure 14. Bud opening, pink flowers cultivar, Singureni, V5, 2017

Figure 15. The aspect of flower and blooming, pink flowers cultivar, Singureni, V5, 2017

Figure 16. Flower opening, white cultivar, V1, 2017

Figure 17. Flowering appearance of Singureni cultivar with pink flowers, V5, 2017

Figure 18. Flowering of white flowers cultivar, Singureni, V1, 2017

The influence of the cold period on flowering was observed in Figure 19, resulting in a correlation between plant exposure to cold and the beginning of inflorescence in herbaceous peony, which shows how much the cold period increases and the date of flowering increases and vice versa decreases the period of exposure to cold peony blooms earlier. So, the cold period has a positive influence on peony bloom.

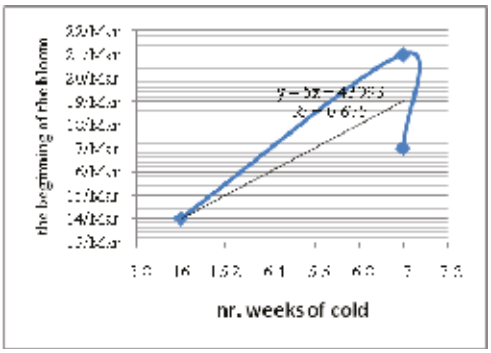

Figure 19. Influence of the cold period on blooming in the varieties with white and pink flowers by Singureni, 2017

After flowering, the plants continued to grow, forming rich bushes.
The largest begetting increases were recorded at cultivars which red flowers from USAMV Bucharest and the cultivars which white flowers from Singureni (Figures 20-21).

Figure 20. Vegetative growth of plants after flowering at cultivar of red flower from USAMV Bucharest, 2017

Figure 21. Vegetative growth of plants after flowering at cultivar of white flower from Singureni, 2017

CONCLUSIONS

The research carried out led to an early flowering compared to the culture in the field by almost two months.
In the containerized and forced culture of the herbaceous peony varieties, the researches and observations made, lead to the following conclusions:

The earliness of the passing through the growing phenophases was highlighted on all varieties of herbaceous peony studied.
The varieties with pink and red flowers from USAMV Bucharest had the earliest vegetation starting, compared to the white and pink flowers varieties from Singureni.
All varieties studied had a percentage of vegetation starting over 50%.
The increase in the cold period positively influences the percentage of starting the peony vegetation.
The shortening of the cold period positively influences the early eruption of the herbaceous peony, and the increase of the cold period influences the early blooming.
The early blooming and the earliness of the passing through the growing phenophases was highlighted at the white and pink flowers variety from Singureni.
The white flowers variety from Singureni was highlighted by a 100% percentage of blooming, compared to the pink flowers variety from Singureni (66.66%).
The earliness of the blooming was highlighted at the white flowers variety from Singureni (20.03), compared to the pink flowers variety from Singureni (22.03).

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TREE SPECIES SELECTION GUIDELINES FROM THE PERSPECTIVE OF BIOCLIMATIC LANDSCAPE DESIGN

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Abstract

In landscape architecture, numerous researches carried out in different climatic regions around the world show that arborescent vegetation has the most significant role in the improvement of microclimate. The study is focused on the adaptation and integration of human bioclimatology and bioclimatic design principles in planting and landscape design in order to develop tree species selection methods. Thus, in order to identify the most valuable tree species, a number of criteria have been identified considering the impact of trees on the main factors influencing microclimate, such as heat, wind speed, air humidity, light, air ionization and air quality. The results consist of a comparative analysis of tree species from the temperate continental plain area. A ranking of the species was generated considering their potential favourable impact on the microclimate as well as the eco-climatic resilience of the trees. The bioclimatic values of the analysed species were attributed according to biological and ecological characteristics such as tree habitus, canopy density, longevity, carbon storage capacity, air ionization level, resistance to wind, drought and air pollution, etc. The tree species were assessed by researching specialized dendrological studies and through field observations. The conclusions reveal the most valuable bioclimatic trees species, which are classified according to the way they can be used in different types of green areas.

Key words*: tree species selection, bioclimatic landscape design, urban microclimate improvement.*

INTRODUCTION

A comparative analysis of arborescent vegetation was initiated in order to identify which tree species presents the highest potential for urban microclimate improvement in the case of four different types of green areas: A - shelterbelts, B - mobility areas, C - green areas adjacent to buildings and D - green cores (green areas over 1 ha). Classification of green areas was carried out in previous studies (Boc, 2017) depending on the differential impact of the vegetation on urban microclimatic variables.

In the present study, the analysed species are ranked according to the microclimatic impact and to the ecological and climatic resilience in the context of local conditions in Bucharest. The hierarchy of the species has been established taking into account specific criteria that vary in each type of green area such as habitus, sunlight requirements, compatibility with built environment or paved areas, emissions of volatile organic substances; but also by general criteria, valid for all types of urban green areas, such as leaf area index (LAI), carbon storage capacity, crown (canopy) density, pollutants bioaccumulation capacity, air ionization, wind resistance, resistance to urban air pollution and tolerance to drought conditions.

MATERIALS AND METHODS

The assessed vegetation consist of frequently encountered tree species in urban areas in the Bucharest-Ilfov metropolitan area were selected and a number of rarely encountered species, which are well adapted to the local climatic conditions. The evaluated tree species were divided into three major categories:

- Conifers trees (persistent foliage);
- Deciduous trees I-II height level;
- Deciduous trees III height level.

For each green area typology, the evaluation criteria were selected according to the indicators mentioned in the introduction. For each criterion, a score was attributed to the degree of bioclimatic value of the species. Thus, in the case of a low value, 1 point was

assigned, for an average value - 2 points, and for a high value - 3 points. Also, for each criterion, the limits of each score have been set. The values have been attributed by integrating research results of specialized studies, data provided by large tree nurseries in Europe and visual observations in the north of Bucharest (such as Chitila, Bucureştii Noi, Domenii, Băneasa, Herăstrău). The assessment methods and the source of the documentation are presented in the list of analysis criteria. Also, for each criterion, the classification and ranking of the assigned values are presented.

The general criteria for analysis are divided into criteria for the bioclimatic impact of tree species (Boc, 2017) and criteria for climatic and ecological resilience in the case of the temperate-continental urban microclimate, which is specific to Bucharest.

The criteria for bioclimatic impact assessment include:

1. The relative Leaf Area Index (LAI) - the species were ranked according to Asner et al. (2003) and to visual observations[1]:

- Low LAI (1 point) = LAI between 1 and 2;
- Average LAI (2 points) = LAI from 3 to 4;
- High LAI (3 points) = LAI between 5 and 6;

2. Canopy density (species ranked following visual observations and according to Iliescu, 2005, Van den Berk, 2017):

- Rare canopy (1 point) = Density below 40%;
- Average canopy (2 points) = Canopy density between 40 and 75%;
- Dense canopy (3 points) = Canopy density over 75%;

3. Longevity of the species (species ranked according to Iliescu, 2005; Iliescu, 2006; Chira and Bolea, 2008; Van den Berk, 2017):

- Low longevity (1 point) = under 50 years;
- Average longevity (2 points) = between 50 and 100 years;
- High longevity (3 points) = over 100 years;

4. Security against allergens (species ranked following visual observations and according to Iliescu, 2005; Carinanons and Casares-Porcel, 2011; Van den Berk, 2017):

- High risk (1 point) = strong allergic species;
- Moderate risk (2 points) = strong allergenic dioecious species (only masculine specimens are allergenic) or moderate allergenic species;
- Low risk (3 points) = species without significant allergenic risk or moderately allergenic dioecious species;

5. Carbon storage capacity - directly proportional to the growth rate and LAI, except for VOC (Volatile organic compound) emissions in air polluted areas (species ranked according to Nowak, 2002; Nowak et al., 2007; Iliescu, 2005; Costăschescu et al., 2010; Van den Berk, 2017):

- Low capacity (1 point) = slow growth rate and low LAI;
- Average capacity (2 points) = average growth rate and average LAI or fast growth rate and high LAI or slow growth rate and high LAI;
- High capacity (3 points) = fast growth rate and high LAI;

6. Air ionization (species ranked according to Iliescu, 2005; Costăchescu et al., 2010; Teodoreanu, 2011)

- Reduced ionization (1 point) = deciduous species emitting positive ions;
- Average ionization (2 points) = other deciduous species;
- High ionization (3 points) = coniferous trees and species with lanceolate leaves;

7. Bioaccumulation of chemical pollutants (according to Nowak, 2002; Iliescu, 2005; Chira, Bolea, 2008; Costăchescu et al., 2010):

- Low capacity (1 point) = reduced bioaccumulation compared to analysed species;
- Medium capacity (2 points) = moderate bioaccumulation, close to the average of the studied species;
- High capacity (3 points) = high bioaccumulation compared to other species;

8. Dust and particle retention capacity (species ranked according to Iliescu, 2005; Chira, Bolea, 2008; Costăchescu et al., 2010; Van den Berk, 2017 and following field observations):

- Reduced capacity (1 point) = species with rare canopy and small leaves;
- Medium capacity (2 points) = species with rare canopy and large leaves or dense canopy and small leaves;
- High capacity (3 points) = species with large canopy and large leaves.

The general criteria for the assessment of climatic and ecological resilience include (Ranking according to visual observations, Iliescu, 2005; Iliescu, 2006; Chira, Bolea, 2008; Costăchescu et al., 2010; Van den Berk, 2017):

[1]It was estimated for trees aged 20-40 years

9. Resistance to wind;
10. Resistance to drought;
11. Resistance to air pollution.
The following hierarchy applies to all criteria concerning environmental and climatic resilience: low resistance (1 point), medium resistance (2 points), high resistance (3 points).
The specific criteria are listed below, separately for each green zone typology. Following the evaluation of the species, the arithmetic mean of the values for each of the two categories of criteria was established. Thus, the final score resulted by achieving the arithmetic mean of the microclimatic impact general score and the climatic and ecological resilience score. Species were ranked according to the final score (global average) according to which they were grouped into three major categories within each green zone typology:
Species recommended as dominants - high bioclimatic impacts and high climatic and ecological resilience (impact and resilience scores ≥ 2.25, species which obtained over all the criteria above 1 point) - total score represented in green in Tables 1, 2, 3, 4 and 5;
Complementary species - average bioclimatic impact values and climate and ecological resilience (impact and resilience scores ≥ 1.75, species which have received no more than two marks of 1 point for the general criteria or a maximum of 1 point for the specific criteria) - represented in yellow in the tables;
Not recommended species (average impact and resilience scores ≤ 1.75, species which obtained more than two marks of 1 point on the analysed criteria) - represented in orange.

RESULTS AND DISCUSSIONS

Bioclimatic value of tree species according to general criteria

The species identified with the highest bioclimatic potential following assessment based only on the general criteria are:
- Coniferous tree species recommended as dominants (Table 1): *Pseudotsuga menziesii* ssp. *glauca, Juniperus virginiana, Pseudotsuga menziesii* ssp. *menziesii, Picea pungens, Abies concolor;*
- Deciduous tree species (height level I-II) recommended as dominants (Table 2): *Carpinus orientalis, Fraxinus angustifolia, Fraxinus excelsior, Fraxinus americana, Celtis australis, Acer platanoides, Quercus rubra, Carpinus betulus, Quercus robur, Ulmus carpinifolia, Ulmus glabra;*
- Deciduous tree species (height level III) recommended as dominants (Table 3): *Elaeagnus angustifolia, Corylus avellana, Quercus pubescens, Morus alba, Pyrus communis, Pyrus nivalis, Fraxinus ornus, Prunus cerasifera Pissardii, Salix babylonica, Salix matsudana, Acer tataricum, Cercis canadensis, Sorbus aria, Ginkgo biloba, Quercus cerris.*

Zone A - Protection green areas. Shelterbelts
In the case of relatively narrow urban shelterbelts, the use of dense canopy species and a predominantly vertical habitus is recommended, allowing the formation of compact planting compositions. Considering the high density of plantations, the adaptation of the species for partly shaded areas is also an important condition for climate and ecological resilience. Following the analysis, in the group of coniferous trees, three species obtained scores from 2 up to all criteria and, implicitly, the highest score. Thus, after the evaluation, the species were divided into the following three categories:
Coniferous trees (Table 5):
-Recommended species: *Pseudotsuga menziesii* ssp. *glauca, Juniperus virginiana, Pseudotsuga menziesii* ssp. *menziesii;*
- Not recommended species: *Pinus silvestris, Pinus strobus.*
Deciduous tree species (height level I-II) (Table4):
- Recommended species: *Carpinus orientalis, Fraxinus angustifolia, Ulmus glabra, Celtis australis, Fraxinus excelsior, Fraxinus americana, Carpinus betulus, Ulmus carpinifolia, Maclura pomifera, Acer platanoides, Ginkgo biloba*[2]*, Quercus rubra;*
- Not recommended species: *Populus tremula, Populus x canadensis, Platanus x hybrida, Populus alba, Betula pendula, Robinia pseudacacia, Paulownia tomentosa, Ailanthus altissima.*
Deciduous tree species (height level III) (Table 5):

[2]Ginkgo biloba was placed in the deciduous trees category considering it is not an evergreen coniferous.

- Recommended species: *Corylus avellana, Elaeagnus angustifolia, Crataegus monogyna, Fraxinus ornus, Prunus cerasifera 'Pissardii', Sorbus aria;*
- Not recommended species: *Salix babylonica, Acer negundo, Prunus avium, Prunus cerasus, Malus baccata, Malus domestica, Prunus 'Accolade', Koelreuteria paniculata, Malus silvestris, Prunus domestica, Albizzia julibrissin, Prunus serrulata 'Kanzan';*

Zone B - Mobility green areas. Greenways, trees alignments and planted platforms

In the case of the green areas designed for mobility, the density of planted areas is relatively low and the main bioclimatic role of vegetation is to provide shade and retain the heat reflected by the paved surfaces. In this regard, species with a dense and wide canopy will be chosen to cover larger areas. Regarding the ecological resilience of species, it is essential to choose species compatible with road and pedestrian infrastructure from the point of view of the root system and fruit that can affect the paved areas.

In zone B, the coniferous tree species were not taken into account as the ability to provide shade and resistance to air pollution are generally low. Considering the results, the following species for mobility areas were identified:

Deciduous tree species (height level I-II) (Table 4):

- Recommended species: *Carpinus orientalis, Fraxinus angustifolia, Fraxinus excelsior, Acer platanoides, Carpinus betulus, Fraxinus americana, Celtis australis, Quercus rubra, Ginkgo biloba, Quercus robur, Quercus cerris, Ulmus carpinifolia;*
- Not recommended species: *Ulmus glabra, Salix alba, Populus tremula, Paulownia tomentosa, Populus nigra, Aesculus hippocastanum, Sophora japonica, Populus alba, Populus x canadensis, Betula pendula, Ailanthus altissima, Robinia pseudacacia;*

Deciduous tree species (height level III) (Table 5):

- Recommended species: *Quercus pubescens, Elaeagnus angustifolia, Corylus colurna, Prunus cerasifera 'Pissardii', Fraxinus ornus, Sorbus aria, Acer tataricum;*
- Not recommended species: *Salix babylonica, Ulmus pumila, Prunus 'Accolade', Acer negundo, Malus baccata 'Street Parade', Albizzia julibrissin, Prunus serrulata 'Kanzan', Malus silvestris.*

Zone C - Buffer green areas.

Plantations adjacent to the buildings

In the green areas planted adjacent to the buildings, the density of the vegetation should be high and the planted areas consist of relatively narrow strips (Boc, 2017). In this case, we will choose species with vertical habitus compatible with the built environment from the point of view of tree crowns, root systems and fruits that may affect paved surfaces. Subzone C1 is a type of buffer green infrastructures located near the southern facades. Subzone C2, situated near the northern facades and subzone C3, located between buildings, it was taken into account the complementary criterion of resistance to semi-shade conditions. The following species have been hierarchized for subzone C1:

Deciduous tree species (height level I-II) (Table 4):

- Recommended species: *Fraxinus angustifolia, Carpinus orientalis, Carpinus betulus, Fraxinus excelsior, Fraxinus americana, Acer platanoides, Celtis australis, Quercus rubra, Ulmus carpinifolia, Ginkgo biloba, Quercus cerris, Quercus robur;*
- Not recommended species: *Ulmus glabra, Acer pseudoplatanus, Salix alba, Populus tremula, Populus nigra, Platanus x hybrida, Aesculus hippocastanum, Acer campestre, Populus x canadensis, Paulownia tomentosa, Populus alba, Betula pendula, Acer negundo, Robinia pseudacacia, Ailanthus altissima;*

Deciduous tree species (height level III) (Table 5):

- Recommended species: *Elaeagnus angustifolia, Corylus avellana, Pyrus communis, Pyrus nivalis, Fraxinus ornus, Prunus cerasifera 'Pissardii', Sorbus aria;*
- Not recommended species: *Salix babylonica, Acer tataricum, Acer negundo, Malus baccata, Malus domestica, Prunus 'Accolade', Prunus domestica, Ulmus pumila, Malus silvestris, Albizzia julibrissin, Prunus serrulata 'Kanzan'.*

The following semi-shade species, identified for subzones C2 and C3, have been identified:

Coniferous trees (Table 5)

Since coniferous trees are compatible with the built environment and the rest of the analysis criteria are similar to those in the case of the shelterbelts, then the results from zone A are valid also for subzones C2 and C3.

Deciduous tree species (height level I-II) (Table 4):

- Recommended species: *Carpinus orientalis, Fraxinus angustifolia, Carpinus betulus, Fraxinus excelsior, Fraxinus americana, Celtis australis, Acer platanoides, Ginkgo biloba, Ulmus carpinifolia, Quercus rubra;*
- Not recommended species: *Tilia tomentosa, Acer pseudoplatanus, Ulmus glabra, Salix alba, Quercus cerris, Quercus robur, Gleditsia triacanthos, Aesculus hippocastanum, Sophora japonica, Populus tremula, Populus nigra, Platanus x hybrida, Populus x canadensis, Paulownia tomentosa, Liriodendron tulipifera, Populus alba, Acer negundo, Robinia pseudacacia, Ailanthus altissima;*

Deciduous tree species (height level III) (Table 5):

- Recommended species: *Elaeagnus angustifolia, Corylus avellana, Fraxinus ornus, Populus simonii, Prunus cerasifera Pisardii, Sorbus aria, Prunus padus;*
- Not recommended species: *Pyrus communis, Pyrus nivalis, Salix matsudana, Salix babylonica, Prunus avium, Ulmus pumila, Prunus Accolade, Acer negundo, Koelreuteria paniculata, Prunus domestica, Malus silvestris, Prunus serrulata Kanzan, Albizzia julibrissin.*

Zone D - Green cores

Green cores include consistent planted areas and do not impose specific restrictions regarding road infrastructure and buildings. Thus, in this case it is recommended to choose the dominant species from among the trees with the most bioclimatic impact resulting from the assessment based on general criteria. In this case, other species except those mentioned in the selection analysis may be inserted separately, provided that the recommended dominant species are used in the planting composition.

CONCLUSIONS

Following the ranking of the species, it was found that trees with the highest bioclimatic potential belong to the deciduous tree group (height level I-II) (Table 1). The genera characterized by the highest values are *Fraxinus, Carpinus, Celtis* and *Acer*, which are compatible with all four types of green areas (Table 4).

Among the deciduous tree of height level III, there is a high degree of compatibility with all types of green areas in the case of the species *Eleagnus angustifolia, Fraxinus ornus, Prunus cerasifera* and *Sorbus aria* (Table 5).

From the coniferous category, the species with the highest score are *Juniperus virginiana* and *Pseudotsuga* sp. (Table 5). It should be noted that although they have a high degree of adaptability to environmental conditions, the two coniferous species are rarely used in Bucharest.

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Table 1. Assessment and ranking of coniferous tree species according to the relative bioclimatic value

	FACTORS INFLUENCING THE BIOCLIMATIC IMPACT OF DENDROLOGIC VEGETATION													
GENERAL CRITERIA	**IMPACT ON EOLIEN FACTORS**			**IMPACT ON AEROCHEMICAL AND AEROELECTRIC FACTORS**					**CLIMATE AND ECOLOGICAL RESILIENCE**					
Coniferous tree species	Estimated LAI	Canopy density	Longevity	Allergens security	Carbon storage	Air ionization	Accumulat-ion of chemical pollutants	Dust particles retention	Wind resistance	Drought resistance	Resistance to air pollution	Total 1 - Impact	Total 2 - Resilience	TOTAL
Pseudotsuga menziesii ssp. glauca	3	2	3	3	2	3	2	3	3	3	3	2,63	3,00	2,81
Juniperus virginiana	2	3	3	3	2	3	2	2	3	3	2	2,50	2,67	2,58
Pseudotsuga menziesii ssp. menziesii	3	2	3	3	2	3	2	3	3	2	2	2,63	2,33	2,48
Picea pungens	2	3	2	2	2	3	3	2	3	2	2	2,38	2,33	2,35
Abies concolor	2	3	3	2	2	3	2	2	3	2	1	2,38	2,00	2,19
Abies nordmanniana	3	2	3	2	2	3	3	3	3	1	1	2,63	1,67	2,15
Abies alba	3	2	3	3	2	3	2	3	3	1	1	2,63	1,67	2,15
Cupressocyparis leylandii	2	3	2	2	2	3	3	3	1	2	2	2,50	1,67	2,08
Thuja plicata	3	3	2	2	3	3	2	3	1	1	2	2,63	1,33	1,98
Picea abies	3	2	3	3	2	3	3	2	2	1	1	2,63	1,33	1,98
Chamaecyparis lawsonianna	2	3	2	2	2	3	3	3	1	1	2	2,50	1,33	1,92
Pinus nigra	2	2	3	2	2	3	1	2	2	2	1	2,13	1,67	1,90
Pinus strobus	2	2	3	2	2	3	2	2	2	1	1	2,25	1,33	1,79
Pinus silvestris	2	1	3	2	2	3	1	1	2	2	1	1,88	1,67	1,77

Relative bioclimatic value

1 - low | 2 - medium | 3 - high | no data

Table 2. Assessment and ranking of deciduous tree species (height level I-II) according to the relative bioclimatic value

Deciduous tree species (Height level I-II)	Estimated LAI	Canopy density	Longevity	Allergens security	Carbon storage	Air ionization	Accumulat-ion of chemical pollutants	Dust particles retention	Wind resistance	Drought resistance	Resistance to air pollution	Total 1 - Impact	Total 2 - Resilience	TOTAL
Tilia tomentosa	3	3	3	1	2	2	3	3	3	3	3	2,50	3,00	2,75
Carpinus orientalis	3	3	3	2	2	2	3	3	3	3	2	2,63	2,67	2,65
Fraxinus angustifolia	3	2	3	2	3	2	3	3	3	2	3	2,63	2,67	2,65
Fraxinus excelsior	3	2	3	2	3	2	3	3	3	2	3	2,63	2,67	2,65
Fraxinus americana	2	2	3	2	3	2	3	3	3	2	3	2,50	2,67	2,58
Celtis australis	2	2	3	2	2	2	2	2	3	3	3	2,13	3,00	2,56
Acer platanoides	3	3	2	2	3	2	3	3	2	3	2	2,63	2,33	2,48
Carpinus betulus	3	3	3	2	2	2	3	3	3	2	2	2,63	2,33	2,48
Quercus rubra	2	2	3	2	2	3	2	2	3	2	3	2,25	2,67	2,46
Acer pseudoplatanus	3	3	2	2	3	2	3	2	3	1	3	2,50	2,33	2,42
Salix alba	2	2	3	2	3	3	3	2	3	1	3	2,50	2,33	2,42
Ulmus carpinifolia	2	2	3	2	2		2	2	3	2	3	2,14	2,67	2,40
Ulmus glabra	2	2	3	2	2		2	2	3	2	3	2,14	2,67	2,40
Maclura pomifera	2	2	2	2	3	2		2	2	3	3	2,14	2,67	2,40
*Ginkgo biloba**	2	2	3	3	2		3	2	2	2	3	2,43	2,33	2,38
Quercus robur	2	2	3	2	2	3	3	2	3	2	2	2,38	2,33	2,35
Populus nigra	3	2	2	1	3	3	3	2	3	2	2	2,38	2,33	2,35
Populus tremula	2	2	2	1	3	3	3	3	3	1	3	2,38	2,33	2,35
Quercus cerris	2	2	3	2	2		3	2	3	2	2	2,29	2,33	2,31
Platanus hybrida	3	2	3	1	3		2	2	2	2	3	2,29	2,33	2,31
Sophora japonica	2	2	1	3	3		3	2	2	2	3	2,29	2,33	2,31
Populus canadensis	2	1	2	1	3	3	3	2	3	1	3	2,13	2,33	2,23
Populus alba	2	1	2	1	3	3	3	2	2	2	3	2,13	2,33	2,23
Aesculus hippocastanum	3	3	3	1	2		2	3	2	2	2	2,43	2,00	2,21
Gleditsia triacanthos	1	1	3	3	2	1	2	1	2	3	3	1,75	2,67	2,21
Quercus palustris	2	2	3	2	2	1		2	3	1	3	2,00	2,33	2,17
Juglans regia	2	2	3	2	2	3	2	2	3	1	2	2,20	2,00	2,10
Robinia pseudacacia	1	1	2	1	1		3	1	2	3	3	1,43	2,67	2,05
Paulownia tomentosa	2	1	2	3	3		3	3	1	1	3	2,43	1,67	2,05
Liriodendron tulipifera	2	2	3	3	3	2	2	2	2	1	2	2,38	1,67	2,02
Acer campestre	2	2	2	2	2	2	2	2	2	2	2	2,00	2,00	2,00
Tilia platyphyllos	2	2	3	1	2	1	3	2	2	2	2	2,00	2,00	2,00
Betula pendula	1	1	2	1	1	3	3	1	2	2	3	1,63	2,33	1,98
Ailanthus altissima	2	1	1	2	2	1	2	2	2	2	3	1,63	2,33	1,98

Relative bioclimatic value

1 - low 2 - medium 3 - high no data

Table 3. Assessment and ranking of deciduous tree species (height level III) according to the relative bioclimatic value

GENERAL CRITERIA	IMPACT ON EOLIEN FACTORS			IMPACT ON AEROCHEMICAL AND AEROELECTRIC FACTORS					CLIMATE AND ECOLOGICAL RESILIENCE					
Deciduous tree species (Height level III)	Estimated LAI	Canopy density	Longevity	Allergens security	Carbon storage	Air ionization	Accumulat-ion of chemical pollutants	Dust particles retention	Wind resistance	Drought resistance	Resistance to air pollution	Total 1 - Impact	Total 2 - Resilience	TOTAL
Elaeagnus angustifolia	2	2	3	2	2	3	3	2	3	3	3	2,38	3,00	2,69
Corylus avellana	2	3	3	2	2		3	3	3	2	3	2,57	2,67	2,62
Quercus pubescens	2	2	3	2	2			2	3	3	3	2,17	3,00	2,58
Crataegus monogyna	2	2	3	2	1		2	2	3	3	3	2,00	3,00	2,50
Populus simonii	3	3	2	2	3	3	3	2	3	1	3	2,63	2,33	2,48
Morus alba	2	2	3	2	2		3	2	2	3	3	2,29	2,67	2,48
Pyrus communis	2	2	3	3	2		2	2	3	3	2	2,29	2,67	2,48
Pyrus nivalis	2	2	3	3	2		2	2	3	3	2	2,29	2,67	2,48
Fraxinus ornus	2	2	3	2	3	2	3	3	2	3	2	2,50	2,33	2,42
Prunus cerasifera Pisardii	2	3	2	3	2		2	3	2	2	3	2,43	2,33	2,38
Salix babylonica	2	2	2	2	3	3	3	2	3	2	2	2,38	2,33	2,35
Salix matsudana	2	2	1	2	3	3	3	2	3	2	2	2,25	2,33	2,29
Acer tataricum	2	2	2	3	2	2	2	2	2	2	3	2,13	2,33	2,23
Cercis canadensis	2	2	1	3	2		2	3	2	3	2	2,14	2,33	2,24
Sorbus aria	2	2	3	3	2		2	2	2	2	2	2,29	2,00	2,14
Prunus padus	2	3	2	2	2			3	1	2	2	2,33	1,67	2,00
Ulmus pumila	2	1	2	2	2			1	2	2	3	1,67	2,33	2,00
Acer negundo	2	2	1	2	2	2	3	2	1	2	3	2,00	2,00	2,00
Prunus Accolade	1	1	2	2	2		2	1	2	2	3	1,57	2,33	1,95
Catalpa bignonioides	2	2	2	3	2		2	2	2	1	2	2,14	1,67	1,90
Prunus avium	1	2	2	2	2		1	2	2	2	2	1,71	2,00	1,86
Albizzia julibrissin	1	1	1	3	1		2	2	2	2	2	1,57	2,00	1,79
Koelreuteria paniculata	2	2	1	2	2	2	2	2	1	2	2	1,88	1,67	1,77
Prunus cerasus	1	2	2	2	2		2	2	2	1	2	1,86	1,67	1,76
Malus baccata	1	2	2	2	1		2	2	2	1	2	1,71	1,67	1,69
Malus domestica	1	2	2	2	1		2	2	2	1	2	1,71	1,67	1,69
Prunus domestica	1	2	2	3	2		2	2	1	2	1	2,00	1,33	1,67
Prunus serrulata Kanzan	1	2	2	1	2			2	1	2	1	1,67	1,50	1,58
Malus silvestris	1	2	2	2	1		1	1	2	1	2	1,43	1,67	1,55

Relative bioclimatic value

1 - low **2 - medium** **3 - high** **no data**

Table 4. Compatibility level between deciduous tree species (height level I-II) and green areas typologies

Deciduous trees height I-II	Zone A	Zone B	Subzone C1	Subzone C2, C3	Zone D
Acer campestre					
Acer negundo					
Acer platanoides					
Acer pseudoplatanus					
Aesculus hippocastanum					
Ailanthus altissima					
Betula pendula					
Carpinus betulus					
Carpinus orientalis					
Celtis australis					
Fraxinus americana					
Fraxinus angustifolia					
Fraxinus excelsior					
*Ginkgo biloba**					
Gleditsia triacanthos					
Juglans regia					
Liriodendron tuilipifera					
Maclura pomifera					
Paulownia tomentosa					
Platanus hybrida					
Populus alba					
Populus canadensis					
Populus nigra					
Populus tremula					
Quercus cerris					
Quercus palustris					
Quercus robur					
Quercus rubra					
Robinia pseudacacia					
Salix alba					
Sophora japonica					
Tilia platyphyllos					
Tilia tomentosa					
Ulmus carpinifolia					
Ulmus glabra					
Compatibility level	**Low**	**Medium**	**High**		

Table 5. Compatibility level between coniferous tree species and green areas typologies (up and down)

Coniferous trees	Zone A	Zone B	Subzone C1	Subzone C2, C3	Zone D
Abies alba					
Abies concolor					
Abies nordmanniana					
Chamaecyparis lawsonianna					
Cupressocyparis leylandii					
Juniperus virginiana					
Picea abies					
Picea pungens					
Pinus nigra					
Pinus silvestris					
Pinus strobus					
Pseudotsuga menziesii ssp. glauca					
Pseudotsuga menziesii ssp. menziesii					
Thuja plicata					

Deciduous trees height III	Zone A	Zone B	Subzone C1	Subzone C2, C3	Zone D
Acer negundo					
Acer tataricum					
Albizzia julibrissin					
Catalpa bignonioides					
Cercis canadensis					
Corylus avellana					
Corylus colurna					
Crataegus monogyna					
Elaeagnus angustifolia					
Fraxinus ornus					
Koelreuteria paniculata					
Malus baccata					
Malus domestica					
Malus silvestris					
Morus alba					
Populus simonii					
Prunus Accolade					
Prunus avium					
Prunus cerasifera Pisardii					
Prunus cerasus					
Prunus domestica					
Prunus padus					
Prunus serrulata Kanzan					
Pyrus communis					
Pyrus nivalis					
Quercus pubescens					
Salix babylonica					
Salix matsudana					
Sorbus aria					
Ulmus pumila					

Compatibility level	Low	Medium	High

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ANALYSIS OF CHANGES IN GROWING DEGREE-DAY VALUES BY ALTITUDE: OIL ROSE (*ROSA DAMASCENA* MILL.) CASE

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Abstract

Times such as optimum sowing, planting, germination time and duration, fertilization, agricultural pest control, irrigation time, harvest, plant growing stages can be determined by using the Growing Degree-Day (GDD) values. The Isparta region has an ideal climatic area for oil rose growing. For this purpose, meteorological stations were chosen as the study area for Isparta provinces and districts having the most suitable growing ecology in oil rose cultivation. Oil rose is a perennial plant with an active growing period of about 120 days depending on the phenological periods. Different GDD values for different phenological periods were acquired when the GDD values for the active growing period of the oil rose plant were correlated with the elevations of meteorological stations at different locations. It was determined that the study area was divided into 4 groups by the statistical Duncan test, which was made according to the growing degree-day values and optimum temperatures. It was determined that the Sütçüler district is the most suited growing area for the oil rose plant followed by Atabey, Eğirdir, Isparta, Keçiborlu, Senirkent and Uluborlu.

Key words*: Isparta, phenological periods, rose plant, temperature.*

INTRODUCTION

Volatile oils are obtained from the flowers, leaves, fruits, seeds and roots of plants. Rose is the most popular and most important among all volatile oil plants grown in Turkey (*Rosa damascena* Mill.). Turkey is the biggest global producer of oil rose (Ikiz and Demircan, 2013). Roses have an important place among medicinal and aromatic plants. Some genotypes of *Rosa damascena* have been grown for industrial purposes in Turkey (Region of Lakes) since 1888. Oil production started in 1892 and it has been processed as an industrial plant since 1935 (Özçelik et al., 2013). Turkey and Bulgaria are the countries with the highest amount of oil rose growth in the world. These two countries meet 80% of the total oil rose production in the world. According to 2016 data, a total production of 12,267 tons has been completed on 2,975.3 hectares in Turkey (Tuik, 2017).

Temperature and humidity are among the most important factors with impacts on the flowering intensity of oil rose. Oil rose plants are quite resistant to cold weather during the winter when it sheds its leaves. Resting of mature plants during the winter season helps bud development and differentiation. However, it is very sensitive to low temperatures and frost during the budding period (starting in March). It is desired that the temperature varies between 5-20^{o}C during the flowering period of oil rose. Volatile oil content decreases when nighttime temperatures drop below 5^{o}C or when daytime temperatures exceed 20^{o}C (Baydar and Baydar, 2005; Sangwan et al., 2001).

Climate data should be analyzed accurately since plant growth depends solely on climate conditions. Changes in temperature and rainfall play a determining role on yield especially during phenological periods of plant growth. Plant growing stages such as optimal plantation, germination time, fertilization, pest control, irrigation time and harvesting period, may be determined using the degree day method. The purpose of the study was to determine and interpret the relations between

the altitude values of locations included in the study area for the oil rose plant and the calculated GDD values.

MATERIALS AND METHODS

In the study, long term maximum and minimum daily temperature values with different record lengths, measured at different meteorological stations (16 in total) in the Isparta city limits have been used (Table 1). Since meteorological stations in the city of Isparta are built in districts or close-by regions, they are known by the names of these districts. Properties for the 16 meteorological stations in the city of Isparta and its districts used in the study have been given in Table 1. Average altitude of the province was about 1,065 m (Anonymous, 2018).

Temperature values given in Table 2 (Baydar and Kazaz, 2013) have been used for calculating the GDD values of oil rose in different phenological periods. Changes between the calculated GDD values and the altitudes of meteorological stations (H, m) were tested by way of regression analysis. Statistical Duncan test was used for grouping the GDD values of oil rose plant calculated in different phenological periods and the altitudes of meteorological stations.

Table1. Characteristics of Meteorological Stations used in the study

Meteorological stations	Years	Period duration (years)	Latitude	Longitude	Altitude (m)
Aksu	1983 – 2003	21	37°47'	31°04'	1,240
Atabey	1968 – 2015	48	37°57'	30°38'	1,000
Bağkonak	1987 – 1996	10	38°14'	31°17'	1,397
Barla	1987 – 1992	6	38°01'	30°78'	1,085
Eğirdir	1968 – 2015	48	37°50'	30°52'	917
Gelendost	1983 – 1988	6	38°07'	31°01'	952
Isparta	1929 – 2015	87	37°47'	30°34'	997
Kasımlar	1987 – 1993	7	37°53'	31°19'	1,070
Keçiborlu	1971 – 1990	20	37°57'	30°18'	996
Kumdankı	1984 – 1995	12	38°32'	30°97'	1,029
Senirkent	1970 – 2015	46	38°06'	30°33'	959
Sütcüler	1968 – 2015	48	37°30'	30°59'	975
Sarkıkaraağaç	1976 – 2015	40	38°05'	31°22'	1,180
Uluborlu	1968 – 2015	48	38°05'	30°27'	1,025
Yalvaç	1972 – 2015	44	38°16'	31°10'	1,096
Yenisar-Bademli	1983 – 1994	12	37°42'	31°23'	1,183

Table 2. Optimum Temperature Requirements for Different Phenological Periods of Oil Rose

Phenological Periods	Temperature (°C)	Date	Period duration (days)
Bud break	5 – 10	1 March– 15 March	15
Shoot bud	10 – 15	16 March – 31 March	16
Leaf and Flowering bud	15 – 18	1 April – 30 April	30
Flowering and harvesting	15 – 25	1 May – 30 June	61
Total			122

Growing Degree-Day (GDD) Method

Agriculturally cultivated plants benefit differently from the temperature in each growth period. Temperature is one of the most important meteorological factors with impacts on plant growing. Various methods for estimating plant growing using temperature values have been used in agricultural studies. Growing Degree Day (GDD) method is the most frequently used method. In this method, calculations are made based on the daily maximum (T_{Max}) and daily minimum (T_{Min}) values measured in meteorological stations. GDD values are calculated using the below

equation (McMaster and Wilhelm, 1997; Kadıoğlu and Şaylan, 2001; Snyder et al., 2001; Matzarakis et al., 2007; Rulm et al., 2010; de Souza et al., 2011).

$$BDG = \sum_{i=1}^{n} \left(\frac{T_{Max} - T_{Min}}{2} - T_O \right) \quad (1)$$

where: T_{Max} stands for the daily maximum temperature value (°C), T_{Min} stands for the daily minimum temperature value (°C), whereas T_O denotes the temperature value suggested for different phenological periods for the rose plant (°C) and n represents the yearly number of days. In case $(T_{Max} - T_{Min})/2 > T_O$ growing degree-day (GDD) value is calculated. It means that growing of the plant is determined. On the contrary, when $(T_{Max} - T_{Min})/2 < T_O$ growing degree-day (GDD) not calculated. It means that there is no growing in the plant (McMaster and Wilhelm, 1997; Kadıoğlu and Şaylan, 2001; Snyder et al., 2001; Matzarakis, et al., 2007; Rulm et al., 2010; de Souza et al., 2011).

Statistical methods

Regression analysis. The objective with the dependent variable to be determined is to put forth an estimation method for determining the relationship between one or more independent variables. The method developed for this purpose can be used to make estimations. The general equation is expressed with the following equality.

$$Y = a + b.x \quad (2)$$

where: Y is the dependent variable, x is the independent variable, whereas a and b are equation regression coefficients. Regression analysis statistical fit is evaluated by; correlation coefficient (r), F test and probability (p) (Haan, 1977; Helsen ve Hirsch, 1993; Shammugasundram, 2012).

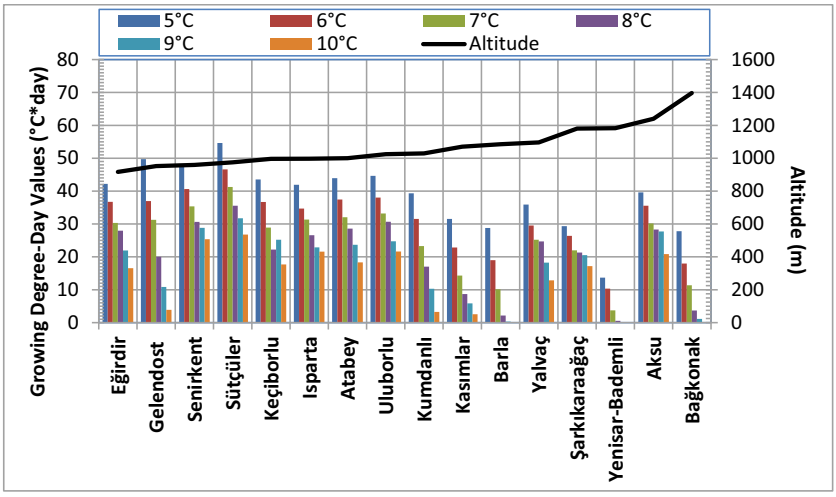
Duncan Multiple Comparison Test: It is one of the most frequently used methods in agricultural studies. It is used for determining whether there are statistically significant differences between the variables obtained as a result of statistical analyses such as Regression, Variance analyses. It takes into account the positions of the averages according to their magnitudes when comparing group averages for this purpose. The minimum difference between two group averages in this method is calculated by way of Duncan table. Group averages are evaluated according to the distances between them when ordered based on magnitude and it is one of the most frequently used methods (Duncan, 1955; Harter, 1960; Efe et al., 2000).

RESULTS AND DISCUSSIONS

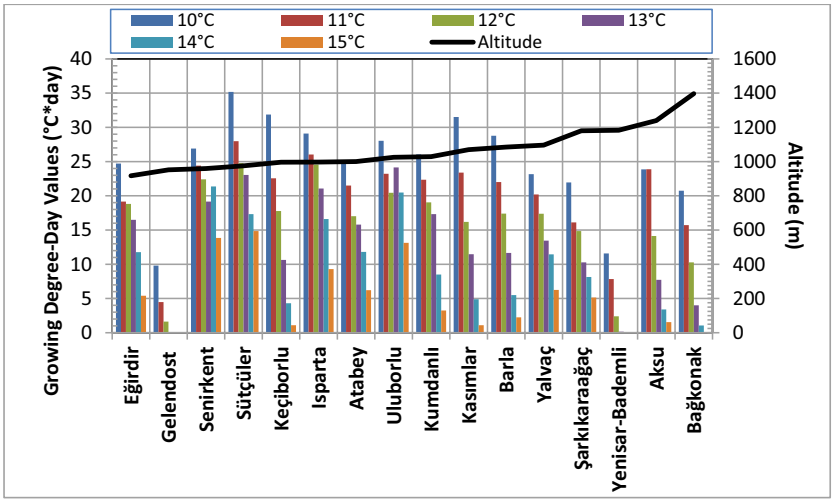
Altitude and temperature values acquired from measurement stations at 16 different locations in the city of Isparta and its districts have been used in the study. Figure 1 was prepared depicting the graphical relations between the GDD values for different phenological periods of the oil rose plant and the elevations of the districts. Oil rose is a perennial plant with an active growing period of about 120 days depending on the phenological periods (Baydar and Kazaz, 2013). Different GDD values for different phenological periods were acquired when the GDD values for the active growing period of the oil rose plant were correlated with the elevations of meteorological stations at different locations. The average elevation of the city of Isparta is 1,065 m and Sütçüler region with an elevation of 975 m was determined as the district with the highest GDD value during the bud break period. Whereas the lowest GDD values during the bud break period were determined in Yenişar Bademli. It was determined upon an examination of the shoot bud period that the Sütçüler region had the highest GDD values during this period as well. The lowest GDD values were determined in the Gelendost district. No linear relationship was determined between the phenological temperature demands of the oil rose plant and the elevation values of the districts during the shoot bud period as well. It was determined that the GDD values during the leaf and flowering bud periods are above 70 for the Sütçüler region. The lowest GDD value was observed at Yenişar Bademli. Sütçüler region was observed to have the highest GDD value during the final phenological period of oil rose which is flowering and harvesting period. It can again be

observed in this stage that GDD values do not have a linear relationship with elevation. Yenişar Bademli had the lowest GDD values during the flowering and harvest period.

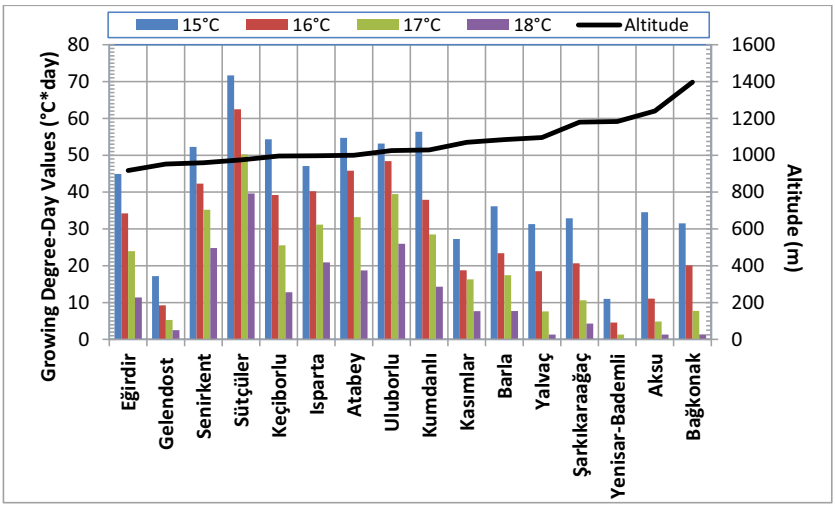
As put forth by Serter (2004), Nield and Smith (1997), determined during their study on the maturing of corn plant at different locations, maturity times varied according to locations. Thus, the GDD values acquired for 16 different locations in the study area were different for oil rose plant. It can be indicated that Sütçüler district is the best in the study area with regard to the temperature demands of the oil rose plant during its phenological periods. Local producers in the region along with Baydar and Kazaz (2013) indicate that Keçiborlu, Isparta central villages, Atabey, Eğirdir, Uluborlu, Senirkent and their environs, are the best areas for oil rose. Therefore, it was determined that all areas, excluding Sütçüler, were determined to be compatible with regard to GDD values and elevation.



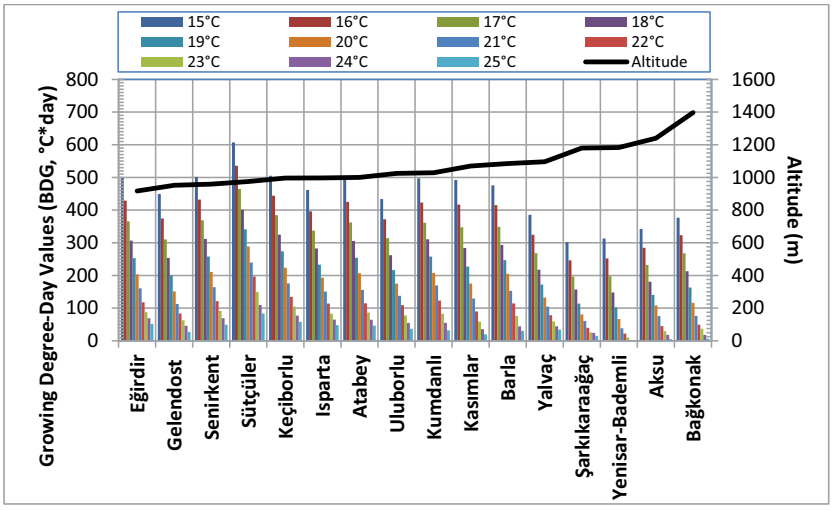
a. Bud break



b. Shoot bud



c. Leaf and flowering bud



d. Flowering and harvesting

Figure 1. Relationship between GDD values and altitude in different phenological periods of oil rose plant

The relationships between the elevation (H, m) values for the different meteorological stations at different locations in the study area and the GDD values calculated for the different phenological periods of oil rose plant, were examined by way of regression analysis and the statistical values for which two-parameter parabolic equation $(GDD = a + b.H + c.H^2)$ has been developed are given in Table 3.

Table 3. Relations between GDD values, altitude and statistical results

Suggested Temperature (T_o, °C)	Equation Coefficients			r	F_{Result}	F_{Table}	Probability (p)
	a	b	c				
Bud break							
5	0.000181	- 0.4676	328.70	0.754	8.58	3.810	0.004
6	0.000126	- 0.3378	246.80	0.709	6.55	3.810	0.011
7	0.000130	- 0.3474	246.50	0.666	5.18	3.810	0.022
8	0.000068	- 0.2039	159.60	0.563	3.02	3.810	0.084
9	0.000034	- 0.1171	102.80	0.465	1.79	3.810	0.205
10	0.000025	- 0.0893	79.30	0.415	1.35	3.810	0.293
Shoot bud							
10	- 0.000033	0.0590	- 0.40	0.307	0.68	3.810	0.526
11	- 0.000041	0.0813	- 19.80	0.261	0.47	3.810	0.633
12	- 0.000010	0.0021	26.00	0.415	1.35	3.810	0.292
13	- 0.000014	0.0006	28.50	0.536	2.62	3.810	0.111
14	0.000010	- 0.0527	53.80	0.531	2.56	3.810	0.116
15	0.000012	- 0.0473	41.45	0.474	1.89	3.810	0.190
Leaf and Flowering bud							
15	0.000145	- 0.3904	290.60	0.501	2.18	3.810	0.152
16	0.000178	- 0.4782	334.60	0.589	3.46	3.810	0.063
17	0.000112	- 0.3262	240.10	0.633	4.34	3.810	0.036
18	0.000083	- 0.2427	175.10	0.602	3.68	3.810	0.054
Flowering and harvesting							
15	0.001226	- 3.2680	2520.00	0.774	9.71	3.810	0.003
16	0.001198	- 3.1630	2375.00	0.756	8.66	3.810	0.004
17	0.001143	- 3.0080	2213.00	0.758	8.74	3.810	0.004
18	0.001012	- 2.6680	1968.00	0.759	8.84	3.810	0.004
19	0.000888	- 2.3820	1734.00	0.752	8.44	3.810	0.004
20	0.000708	- 1.9460	1431.00	0.744	8.06	3.810	0.005
21	0.000550	- 1.5510	1152.00	0.754	8.54	3.810	0.004
22	0.000433	- 1.2380	919.80	0.749	8.29	3.810	0.005
23	0.000448	- 1.2190	854.20	0.762	9.03	3.810	0.003
24	0.000358	- 0.9810	683.50	0.806	12.00	3.810	0.001
25	0.000262	- 0.7303	511.70	0.794	11.05	3.810	0.002

Equations which reflect the relationships between elevation and the GDD values of oil rose plant in different phenological periods have been obtained, which were then examined at a statistical significance value of 5% by way of correlation analysis (r), F test and probability (p) values. It was determined that temperatures of around 8, 9, 10°C suggested for the bud break period, all temperatures for the shoot bud period and temperatures of around 15, 16, 18°C during the leaf and flower bud period, had a relationship with temperature which was not statistically significant. In short, it should be taken into consideration when developing equations for the growing periods of the oil rose plant that factors such as temperature and elevation may be effective factors but that other factors (frost, humidity, rain, fertilization, irrigation, diseases etc.) may also be effective. Therefore, a more exact determination can be made by considering other factors as well in addition to the factors of temperature and elevation.

In addition, the changes in GDD values of oil rose plant at different phenological periods with the elevations of meteorological stations, have been examined via Duncan test (Table 4). Duncan test was used for determining and classifying the changes between the location elevations in the study area and the GDD values for these locations.

Table 4. The results of Duncan test grouping according to the relationship between GDD values and altitude for oil rose

Meteorological stations	Phenological Periods			
	Bud break	Shoot bud	Leaf and Flowering bud	Flowering and harvesting
Aksu	30.40 b	12.40 c	12.90 d	133.00 d
Atabey	30.70 b	16.20 b	38.10 b	228.50 b
Bağkonak	10.30 d	8.60 c	15.20 d	149.70 c
Barla	10.10 d	14.60 b	21.20 c	218.40 b
Eğirdir	29.30 b	16.10 b	28.60 c	231.20 b
Gelendost	25.50 b	2.70 d	8.60 d	188.20 c
Isparta	29.80 b	21.10 a	34.80 b	214.90 b
Kasımlar	14.30 c	14.80 b	17.50 c	206.90 c
Keçiborlu	29.00 b	14.70 b	33.00 b	246.10 b
Kumdanlı	20.80 c	16.10 b	34.30 b	229.00 b
Senirkent	34.70 a	21.40 b	38.60 b	234.40 b
Sütçüler	39.40 a	23.90 a	56.00 a	310.70 a
Şarkıkaraağaç	22.80 b	12.70 c	17.10 d	114.60 d
Uluborlu	32.20 a	21.60 b	41.70 b	199.00 c
Yalvaç	24.40 b	15.30 b	14.70 d	165.50 c
Yenisar-Bademli	4.70 d	3.70 d	4.20 d	104.60 d

According to the Duncan test applied for elevation and the GDD values calculated according to the optimum temperature demands for different phenological periods of oil rose plant (Table 4), it was determined that the study area can be classified into 4 different homogeneous growing groups. The groups were determined as such: 1. (a) group growing area: Sütçüler district; 2. (b) group growing area: Atabey, Eğirdir, Isparta, Keçiborlu, Senirkent, Uluborlu districts; 3. (c) group growing area: Barla, Bağkonak, Gelendost, Kasımlar, Kumdanlı, Yalvaç districts; 4. (d) group growing area: Aksu, Şarkıkaraağaç, Yenisar-Bademli districts. It was determined that the Sütçüler district is the most suited growing area for the oil rose plant followed by Atabey, Eğirdir, Isparta, Keçiborlu, Senirkent and Uluborlu.

CONCLUSIONS

It was concluded upon an examination of the relationships between elevations and the GDD values calculated at 16 different locations for the oil rose plant according to different phenological periods that the best growing locations would be Sütçüler, Atabey, Eğirdir, Isparta, Keçiborlu, Senirkent and Uluborlu. It was also determined that there is no linear relationship between the GDD values calculated for the oil rose plant and elevations. It has led to an opinion that temperature and elevation in equations developed for the different growing periods of the oil rose plant, may be effective factors for determining the growing areas. However, it was also concluded that other factors with impacts on growing (frost, humidity, rain, fertilization, irrigation, diseases etc.) should also be taken into consideration.

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IMPROVING BIOLOGICAL AND PHYSIOLOGICAL PARAMETERS OF *NERIUM OLEANDER* L. CUTTINGS BY USING BIOSTIMULATING SUBSTANCES

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Abstract

Nerium oleander is a perennial shrub native to the eastern Mediterranean basin and Southeast Asia. As an ornamental plant grown in pots, it reaches heights of 1-2 m, with the appearance of a bushy shrub, with fragrant flowers, simple or double, colourful, blooming from spring until the end of autumn. With a very large adaptive potential, the species is one of the most popular in floral plants, as an indoor plant and where the climate allows, it can be grown on terraces and balconies, in hedges, alignments etc. The purpose of this study was to evaluate the impact of treatments with various rhizogenic biostimulators on the rooting potential of different types of Nerium oleander cuttings in greenhouse conditions. The experiment was performed in the HORTINVEST greenhouses of the USAMV Bucharest and there were determined the rooting percentage, the development of the root system and the aerial part, as well as some physiological indicators: photosynthesis, transpiration and stomatal conductance.

Key words: *cuttings, rhizogenic biostimulators, physiological indicators.*

INTRODUCTION

Nerium oleander L. is an evergreen shrub or small tree (with 2-5 m in height) from the *Apocynaceae* family, cultivated worldwide as an ornamental flowering plant, in different geographical and ecological places (Sinha and Biswas, 2016) thanks to an abundant and long-lasting flowering period from summer until late autumn (Argiropoulus and Rhizopoulou, 2013) and due to its moderate hardiness, tolerance to different soil characteristics and low nutrients consumption (Kiran and Prasad, 2014).

The plant value is due not only by its evergreen leaves, but also by the terminal flowers organized as clusters, with different beautiful colors, such as white, red, pink, salmon or light yellow.

Besides the above mentioned utilities, some of the secondary metabolites produced by this plant have also a pharmacological interests (Zibbu and Batra, 2010; Chaudhary et al., 2015) due to their antibacterial, antimicrobial, anti-inflammatory, antinociceptive, and antitumor activity (see review Sinha and Biswas, 2016). In addition, oleander has a promising potential for use in phytoremediation programs (Elloumi et al., 2017). Moreover, results obtained by Doganlar et al. (2012) and Vázquez et al. (2016) also highlights the importance of the species as a bio monitoring tool for airborne metal pollution in urban areas, thanks to its resistance to metals and its exclusion capacity.

Even if oleander is native to northern Africa and to the eastern Mediterranean region (Bailey, 1976), it is naturalized very easily (Zibbu and Batra, 2010) and grown as pot plant, on and around terraces and balconies, in hedges and screen plantings (Simion and Anton, 2009).

As Comeaux (1991) noticed, oleander may look like a small tree if the suckers are removed and a few stems are kept. Moreover, by growing it in container can be a good choice to plant grown even in cooler climates into greenhouses and conservatories, or as indoor plant, that can be grown outside during the summer, as it was practiced for many years.

Due to the great importance of this species and increased demands for seedlings, during the time, breeders have had many preoccupation to obtain new cultivars using different plant propagation techniques such as: generative propagation, vegetative propagation by cuttings

(softwood or semi-hardwood cuttings in the spring or summer) or *in vitro* culture (Ochoa et al., 2003; Simion and Anton, 2009; Vila et al., 2010; Aryan and Rani, 2016), but are missing studies on the interaction between the multiplication methods and physiological performances of the obtained plants.

As regard as oleander leaves behavior, the researchers were concerned to study the influence of stress factors such as water stress (Björkman et al., 1981; Lenzi et al., 2009), freezing injury (Syros et al., 2005; Miralles-Crespo et al., 2011), ozone pollution (Lorenzini et al., 1999) or temperature acclimation (Badger et al., 1982) on some physiological and biochemical indicators.

To our knowledge, in our country has not until now done research regarding the oleander gas exchange indicators as related to the vegetative multiplication techniques. The objectives of the present study were to evaluate the cuttings type influence on rooting performance under different stimulation treatments and to determine some physiological indicators.

MATERIALS AND METHODS

Plant material

Oleander plants (*Nerium oleander* L.) were grown in a computer-controlled greenhouse of the HORTINVEST Centre at USAMV Bucharest, Romania (44º 26' N and 26º 06' E latitude and longitude, respectively).

The plants were obtained by vegetative propagation, using two different type of stem cuttings, the segment ones (4-6 cm long) and the peak ones (length of 8-10 cm).

After sampling, the cuttings were cut out by removing the leaves from the lower node for rooting. A number of 12 cuttings of each category were used for each variant.

As rhizogenic biostimulators were used three different commercial substances and three replicates were prepared:

- Clonex (a gel, a powerful formula of hormones, vitamins and minerals);
- Radistim (a powder containing non-hazardous bioactive preparations);
- BioRoot (contains vitamins, enzymes, organic acids, humic acids to stimulate root mass growth) (Table1).

The substrate for rooting cuttings was perlite, in alveolar plaques.

Table 1. Experimental distribution

Experimental variants	Description
Vm	Untreated
V_1	Clonex
V_2	Radistim
V_3	BioRoot

Rooting and Growth Parameters

The following parameters were determined: percentage of rooting, roots length, shoots length.

Leaf gas exchange physiological indicators such as photosynthesis rate (A_n), transpiration rate (E), and stomatal conductance (g_s), respectively have been quantified using the LCIpro + photosynthesis system equipped with a square analysis camera of 6.25 cm^{-2}, between 7:00 and 10:00 h a.m. All measurements were made in four replicates, three times, by analyzing the same leaves. The greenhouse temperature was below 32°C and the light intensity was maintained between 500-700 μmol m^{-2} s^{-1} to avoid the photo inhibition process. The results were expressed as: A_n (μmol CO_2 m^{-2} s^{-1}), E (mmols H_2O m^{-2} s^{-1}) and g_s (mol H_2O m^{-2} s^{-1}) and represent the mean values for the two cuttings types.

RESULTS AND DISCUSSIONS

Rooting and Growth

The experimental results show that oleander cutting rooting was improved by the stimulating substances.

The effect of the cuttings treatments was noticed for both cuttings types, and the medium and hormone applied to the cutting modified all the studied parameters.

The rooting percentage (Figure 1), for the peak shoot cuttings recorded the highest value on V_3 (100%), followed in decreasing order by V_2 (83.33 %), V_1 (75%) and Vm (66.67 %). The order for the other type of cuttings was the same, with the mention that on V_1 the value of the parameter was equal on both types of cuttings (75%). The rooting percentage for the peak shoot cuttings was higher, with the mentioned exception of V_1.

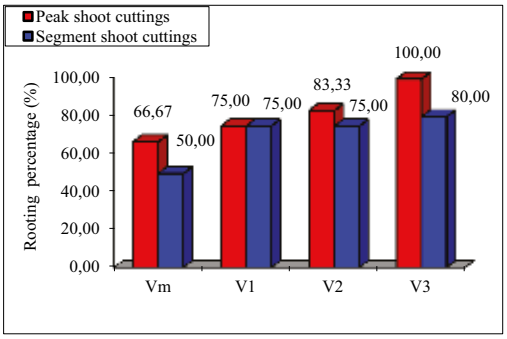

Figure 1. The rooting percentage of the cuttings

For the root length, as it is showed in the Figure 2, the value was highest on the same type of cuttings (the peak shoot ones), this time for all the experimental variants. The order was also the same as for the rooting percentage: V_3, followed by V_2, V_1 and Vm.

The difference between variants was from 21.49% (V_3 vs. Vm) to 12.40% (V_3 vs. V_2) on the new plants obtained from peak shoot cuttings and from 28.45% (V_3 vs. Vm) to 18.10% (V_3 vs. V_2) in the case of the segment shoot cuttings new plants.

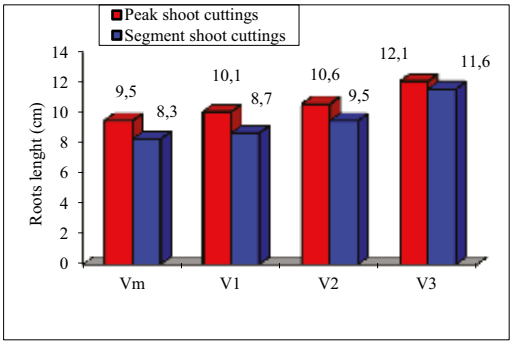

Figure 2. The roots length on the new plant

Regarding the result for the third parameter, the shoot length on new plants obtained from cuttings (Figure 3) it can be seen that the tendency is preserved: the highest value are on V_3 for the both type of cuttings (15.1/10.5 cm peak shoot/segment shoot cuttings), followed by V_2 (13.75/10.25 cm), V_1 (12.3/9.7 cm) and finally Vm (11.1/7.6 cm).

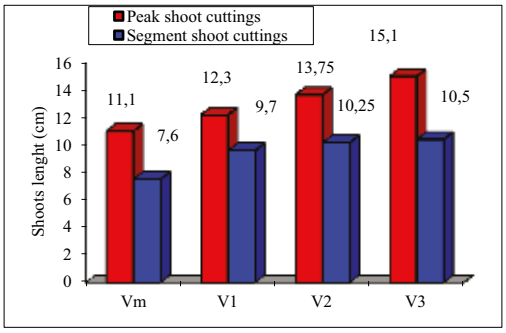

Figure 3. The shoots length on the new plant obtained by cuttings

Leaf gas exchange

Photosynthesis rate (Table 2) varied between 1.90 µmol CO_2 m^{-2} s^{-1} and 3.90 µmol CO_2 m^{-2} $s^{-1}$and presented higher values in the case of V_3, as compared with the others treated variants (V_1 and V_2), only in the case of the first analysis data. Similarly, transpiration rate (E) had higher values in the case of V_3.

For stomatal conductance, the values were generally low and near to those reported by Delaney (2012) (0.03 mol H_2O m^{-2} s^{-1} - 0.06 mol H_2O m^{-2} s^{-1}).

As regard as oleander multiplication, previous studies found that the seeds germination percentage varied between 82% and 100% (corresponding to two determination periods) and the germination time was five days in the both study cases.

A better rooting was obtained in the case of tip cuttings on parapet and rooting system for cuttings with temperature control, in peat and perlite (2:1) in 22°C warming culture medium (Simion and Anton, 2009).

As authors emphasized, the best period for micro propagation was July, on the Murashige & Skoog medium.

According to Ochoa et al. (2003) the larger root growth was obtained by using basal cuttings, while in the case of the apical cuttings, a longer roots number and a higher homogeneity in their distribution was noticed.

Micro propagation by using axillary shoot breaking of wild plants and commercial cultivars allowed higher multiplication rates than the propagation by cuttings, and rooting and acclimatization did not limited the efficient production of plants (Vila et al., 2003).

Table 2. Leaf gas exchange parameters (mean values ± standard errors)

Parameter	Data	Control	V_1	V_2	V_3
A_n (μmol CO_2 m^{-2} s^{-1})	25th May	3.30±0.30	3.00±0.36	3.00±0.15	3.90±0.11
	11th July	3.62±0.29	3.05±0.25	3.21±0.24	3.89±0.41
	12th Sept	2.65±0.66	1.90±0.38	2.51±1.33	2.18±0.10
E (mmol H_2O m^{-2} s^{-1})	25th May	0.54±0.07	0.50±0.07	0.50±0.05	0.73±0.04
	11th July	0.93±0.09	0.90±0.29	1.21±0.09	1.49±0.04
	12th Sept	0.55±0.08	0.78±0.25	0.52±0.15	0.62±0.09
g_s (mol H_2O m^{-2} s^{-1})	25th May	0.04±0.008	0.02±0.004	0.03±0.003	0.03±0.002
	11th July	0.043±0.004	0.039±0.01	0.046±0.004	0.05±0.004
	12th Sept	0.028±0.005	0.027±0.01	0.02±0.007	0.03±0.003

Legend: An= Net photosynthesis rate; E= Transpiration rate; g_s = Stomatal conductance.

The four studied variants showed some minor differences in leaf gas exchange parameter. Temperature increase determined a higher oleander leaves photosynthesis rate thanks to improved stability of enzymes involved in photosynthesis (Badger et al., 1982).

Transpiration is performed mainly by stomata complexes and their position, density and opening degree have generally a major impact on this process rate, interacting with the stomata conductance. Given that the oleander leaf stomata are located in crypts filled with trichomes, it was expected that water loss to be reduced, but studied carried out by Losch et al. (1982) emphasize that as compared with other species, stomatal crypts were not necessarily linked with greater leaf resistance.

According to the results of Roth-Nebelsick et al. (2009), in the case of water stress situations at the soil level, when the stomata will close to preserve the absorbed water, crypts influence is one minor. However, if the growing conditions are some that allow a higher stomata conductance, crypts presence and their effect on reducing water loss are important factors. In such a context, in a previous paper, Gollan et al. (1985) noticed that there was not a critical relationship between leaf water potential and leaf conductance, thus, gas exchange decreased was a consequence of soil water content, while making reference to abscisic acid hypothesis, about the influence on stomatal movement and indirectly on photosynthesis. According to Miralles-Crespo et al. (2011), a rapid method to characterise the impact of freezing injury in oleander is measuring chlorophyll fluorescence. Also, Syros et al. (2004) tested the ability of six-month-old oleander grown in pot, and obtained from propagation by cuttings, to cold hardening. The values obtained for the photosynthesis rate in the case of the control plants were positive, around 2.21 (photosynthesis higher than respiration) and negative values in the case of temperature levels under 0°C. Also, in the case of transpiration, the values were positive (0.32), while at negative temperatures the values were negative, up to -0.72 at -8°C. Higher photosynthesis rate was reported by Lenzi et al. (2009) (above 8 μmol CO_2 m^{-2} s^{-1}) in the case of fully irrigated plants and in the case of Angiolo Pucci cv. it was registered the highest carbon dioxide assimilation (An- 10.97 μmol CO_2 m^{-2} s^{-1}), as well as the highest transpiration rate of 3.24 mmol m^{-2} s^{-1} was registered. In the same time, for this cv. stomatal conductance was higher too, 114.72 mmol m^{-2} s^{-1}.

It was reported a significant decrease of photosynthesis in *Nerium oleander* in the case of ozone treatments and this behavior was associated to a partial stomatal closure (Lorenzini et al., 1999). In terms of light intensity impact, the fotoinhibition effect on oleander chloroplast activity caused by a decrease in leaf water potential was lower in the case of shade conditions, compared to the plants growth of in full sun (Bjorkman and Powles, 1981). On the other hand, experiments performed by Badger et al. (1982) led to the conclusion that in the case of oleander, temperature acclimation it was not required the activation state of Rubisco enzyme. The influence of different temperatures was explained as follows: at low temperatures it has accumulated a higher amount of proteins involved in photosynthesis, to ensure higher rates of photosynthesis and on the other hand, leaves grown at higher temperatures have

managed to achieve higher photosynthetic rate, due to increased heat tolerance of some enzymes, which are active in the carbon dioxide reduction cycle (Badger et al*.,* 1982).

CONCLUSIONS

The experimental data show that oleander cutting rooting was improved by the stimulating substances and the best results were particularly obtained by BioRoot.
The effect of the cuttings treatments was noticed for both cuttings types, and the culture medium and hormone applied to the cutting modified all the biological studied parameters.
The physiological indicators varied especially in relation to the leaf age, but generally higher values were registered in the case of BioRoot stimulation, in a close interrelation with the particularities of plant rooting and growth.

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EFFECT OF RHIZOGENIC BIOSTIMULATORS ON *ROSMARINUS OFFICINALIS* ROOTED CUTTINGS BIOCHEMICAL COMPOSITION

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Abstract

Originally from the Mediterranean area, Rosmarinus officinalis (Fam. Lamiaceae) is a species widespread in most temperate regions of Europe and America with a significant economic impact due to the trivalent effect (ornamental, aromatic and medicinal). The purpose of these researches was to evaluate the impact of rhizogenic biostimulator Clonex-gel treatments on the rooting potential and some biochemical parameters of different types of cuttings in greenhouse conditions. The experiments carried out in the HORTINVEST greenhouses of USAMV Bucharest aimed to evaluate the rooting percentage, the development of the root system and the aerial part after rooting. Also, some biochemical parameters (assimilatory pigments and total soluble sugars content) were analyzed to assess the potential effect of the substances used to stimulate rooting on the biochemical composition of leaves formed on cuttings. The researches performed indicated that maximum percentage of rooting cuttings was determined in treated variants. The stimulation of the roots growth determined also an increased accumulation of assimilatory pigments and soluble glucids in the leaves.

Key words*: carotenoids, chlorophyll, cuttings, rooting potential, rosemary.*

INTRODUCTION

Rosemary (*Rosmarinus officinalis*) belongs to the family *Lamiaceae* (*Labiatae*) and is native to southern Europe and the Mediterranean area. Nowadays, rosemary is grown in almost all countries around the Mediterranean but also in England, USA and Mexico. In the countries of the Mediterranean, it is a popular spice especially in Italy and France, and less in Greece and Spain.

If for centuries it has been used as a herbal aroma, natural medicine and a basic ingredient in the perfume industry today rosemary can be defined as a trivalent species, in addition to its medicinal uses. So, it is cultivated as an aromatic plant in culinary art and also as a decorative plant for fragrances, protection from pests and gardening.

Rosemary has been longtime considered an important plant for its essential oil used not only in perfumes and as food spice (Moss et al., 2003), but also in medicine (Miguel et al., 2007).

The whole plant was reported to possess several medicinal properties like cardiac, antiseptic, antispasmodic, carminative, cholagogue, nervine spasmodic, astringent, stomachic, and tonic (Grieve, 1984; Polunin and Huxley, 1987; Chakravarti et al., 2005) and also it was used as a natural antimicrobial and antifungal against *E. coli, Pseudomonas, Aspergillus, Staphylococcus* or an insecticide (Duke, 2001). Besides this, it was reported that the leaves have high antioxidant activity attributed to its phenolic compounds (Singh and Guleria, 2013) therefore rosemary extracts have been widely used as a preservative in the food industry (Bozin et al., 2007).

One hundred grams of fresh rosemary leaves contain approx. 67.77 g of water, 3.31 g of protein, 20.70 g of carbohydrates, and 14.1 g of fiber. Among the minerals are Ca (317 mg), Mg (91 mg), P (66 mg), K (668 mg), Na (26 mg), Fe (6.65 mg), Zn (0.93 mg). Vitamins are mainly B6 (0.336 mg), Thiamin (0.036 mg), C (21.8 mg), Riboflavin (0.152 mg) and others (USDA, 2016).

Oils and vinegar are flavored with rosemary branches. It can be found in tea blends (Tisane) or spices (herbs of Provence). In combination with most Mediterranean herbs (oregano, basil etc.) it tastes good but can also be associated with garlic and thyme. The rosemary leaves from the culinary point of view can be used fresh, but also dry (the aroma is not lost, even if the plant is treated for a long time).

Pleasure-smelling foliage shrubs are appealing in the garden, especially for visually impaired people. Sometimes the scent feels only in the vicinity of the bushes, sometimes it has to touch or crush the leaves between the fingers to release the perfume (Noordhuis Klaas T., 2008).

The propagation by cuttings obtained from the stem is a frequently used way of vegetative multiplication of many ornamental plant species. Cutting is well-known as common and relatively cheap method because it overcomes the difficulties of multiplication by plant seeds (Elhaak et al., 2015).

It is well known that the plant-produced phytohormones like auxins play a key role in stimulating the adventitious root development of stem cuttings and the roots' branching. Treating cuttings with auxins aims to increase the percentage of rooting, root initiation, number and uniformity of rooting (Elhaak et al., 2015). Besides these, cytokinin and gibberellins also contribute to the intensification of metabolic processes, cell division or growth in length. Associating such compounds in rooting substrates allows the increase of the rooted percentage of the cuttings and in the rooting of cuttings (Georgescu et al., 2012).

Previous study related that rosemary has a good rooting ability and the effect of the root-forming solution (standard macrosalt formulation) induces better results, both in terms of the rooting percentage and the qualitative features of the new root system (Talia et al., 2004).

The purpose of these researches was to evaluate the impact of the treatment with rhizogenic stimulator on the rooting potential and on some biochemical parameters in different types of cuttings in greenhouse conditions.

The rooting percentage, the development of the root system and the aerial part after rooting together with the content of assimilatory pigments and sugars were analyzed to assess the effect of this treatment.

MATERIALS AND METHODS

The experiment was conducted in greenhouses at the Hortinvest Center of the USAMV Bucharest (44º 26' N and 26º 06' E latitude and longitude, respectively).

Long stems from selected mother plants were harvested. Two types of stem cuttings were made: of the segment (4-5 cm long) and peak (length 5-6 cm).

After sampling the cuttings were cut out by removing the leaves from the lower node for rooting. A number of 15 cuttings of each category were used for each variant.

The substrate for rooting cuttings was river sand in alveolar plaques.

Experimental variants were established: 50% of the cuttings were subjected to a treatment by immersing the base in rooting stimulators (Clonex-gel, a powerful formula with a 0,3% concentration of 4-indol-3-yl-butyric acid, containing also vitamins and minerals). As control variants there were V_2 and V_4 (Table 1).

Table 1. Experimental variants

Variants	Description	Number of plants
V_1	Shoot peak cuttings treated with Clonex	15
V_2	Shoot peak cuttings without Clonex	15
V_3	Shoot segment cuttings treated with Clonex	15
V_4	Shoot segment cuttings without Clonex	15

Throughout the period of rooting there were applied specific works complex care for directing microclimate factors.

After rooting were made observations and measurements on rooted cuttings, on the percentage of rooting, root length, shoot length.

For the rooting percent comparison between variants, it was used the Fisher exact test and for comparison between variants for shoot length and root length it was used the Student test.

The biochemical parameters were analyzed using proper methods:

• *Determinations of the assimilatory pigments content* in the active leaves**:** *chlorophyll* and *carotenoid pigments* were extracted in 80% acetone and the absorbance of the extract was measured at three wavelengths (663 nm, 647 nm and 480 nm) with a UV/Visible ThermoSpectronic Helios spectrophotometer. The results were calculated using the extinction coefficients and equations described by Schopfer (1989) and were expressed in mg/g fresh weight.

• *Determination of total soluble sugars* was performed according to the Nelson-Somogyi method (Iordachescu, 1988; Somogyi, 1952). For determination of the total soluble glucid non-reducing glucids were first transformed in reducing glucids by hydrolysis with hydrochloric acid. The reducing glucids when heated with alkaline copper tartrate reduce the copper from the cupric to cuprous state and thus cuprous oxide is formed. When the cuprous oxide is treated with arsenomolybdic acid, the reduction of molybdic acid to molybdenum blue takes place. The measurements of absorbance were achieved at 540 nm with a UV/Visible ThermoSpectronic Helios spectrophotometer. The results were expressed in mg/g fresh weight.

RESULTS AND DISCUSSIONS

The rhizogenesis process at *Rosmarinus officinalis* cuttings has been carried out quickly, so that after approximately 30 days since the placement of the cuttings on the rooting medium, they have formed roots.

The number of new plants (rooted cuttings) obtained differs depending on the variant. Also, as the rooting process takes place, there are new vegetative growths of the cuttings' terminal bud.

Study of biometric parameters in the rosemary cuttings in all experimental variants

The data on the number of cuttings obtained after rooting were summarized in Table 2.

Table 2. The parameters of new plants obtained by cuttings

Variants	Rooting percent (%)	Shoot length (cm)	Root length (cm)
V_1	93.33	10.84	13.22
V_2	80.00	8.60	12.10
V_3	86.66	9.43	13.14
V_4	66.66	6.90	9.90

Thus, it can be seen that the values for the main parameters are superior in the variants treated with Clonex (V_1 and V_3). Regarding the values for each type of seedlings, it is noticeable that the peak ones (V_1) gave better results.

According to the Fisher exact test results, there is no significant difference for the rooting percent between variants. Comparing the shoot length mean with the Student test, there was nonsignificant differences between V_1 and V_3 ($df = 28$, $p = 0.10 > 0.05$).

Similar results have been obtained when comparing means between variants for root length.

Roots system is significantly involved in plant growth due to their role in water and nutrient uptake, therefore maintaining an active roots growth is essential for plant development and productivity.

It is expected that the bioregulators treatment to maintain viability and functionality of the roots system during the growth period.

Study of biochemical parameters in the rosemary cuttings in all experimental variants

Assimilatory pigments

Chlorophylls a *and* b represent the major photosynthetic pigments in plants, playing an important role in the photochemical reactions of photosynthesis (Taiz and Zeiger, 2009), while *carotenoids* are considered as accessory components providing photoprotection (Torres-Netto et al., 2005; Simkin et al. 2008).

Previous studies have been reported increased amounts of assimilatory pigments under applications of some growth biostimulators in tomato leaves (Khan et al., 2009), *Physocarpus opulifolius* stem cuttings (Pacholczak et al., 2016), dogwood cuttings (Pacholczak et al. 2012), wheat (Vician and Kováčik, 2013) or tomato (Mikiciuk and Dobromilska, 2014). On the contrary, Tsai and Arteca (1985) noticed that applications of gibberellic acid decreased the chlorophyll content in barley, squash, pepper, sorghum, pigweed and kochia, while in oat, wheat, mung bean, corn, millet and gomphrena it remained unchanged. Also, Vasca-Zamfir et al. (2012) found that treatment of the plants with root stimulators like Clonex, Coralite and Radistim did not significantly influence the content in assimilatory pigments in the leaves of *Pelargonium peltatum*, while Elhaak et al. (2015) made similar observation regarding the treatment with IBA (indole-3-butyric acid) of rosemary.

The researches made on rosemary cuttings showed that generally the biostimulator treatment influenced the contents of organic compounds. The contents of total chlorophyll in the cuttings treated with Clonex (V_1 and V_3) increased relative to the control plants (V_2 and V_4) in both experimental variants (Table 3).

However, the cuttings provided from peak registered higher values of the chlorophyll total content by comparison with shoot segment cuttings.

Table 3. The impact of rhizogenic biostimulator treatments on total chlorophyll and glucids content in rosemary cuttings

Variants	Total chlorophyll (mg/g f.w.)	Total soluble glucids (mg/g f.w.)
V_1	2.03±0.04	1.32±0.03
V_2	1.41±0.03	0.92±0.02
V_3	1.48±0.05	0.82±0.05
V_4	1.36±0.03	0.56±0.03

It can be noted higher amounts of both chlorophylls *a* and *b* in the cuttings which received stimulatory treatments, while the ratio chlorophyll *a*/*b* was not significantly influenced by the Clonex application (about 2.8 in the cuttings from the peak, respectively 2.9 in the cuttings from segment whether treated or not) (Table 4).

Table 4. Ratio of assimilatory pigments in the variants

Variants	Chlorophyll a/b	Total chlorophyll / carotenoids
V_1	2.82	23.36
V_2	2.88	20.67
V_3	2.97	21.45
V_4	2.95	20.90

Regarding the carotenoids content, the amounts determined showed a slight variation in the analyzed rosemary leaves in all experimental variants (Figure 1) indicating that Clonex treatment not affected this parameter.

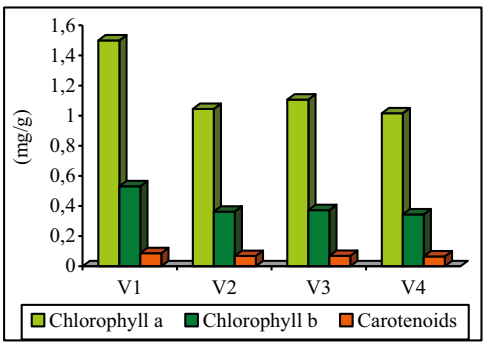

Figure 1. Accumulation of assimilatory pigments in the experimental variants

However, the chlorophyll (*a+b)*/carotenoids ratio increased in the variants treated with the biostimulator due to an increase of chlorophylls *a* and *b* rather than of carotenoids. Increased chlorophyll (*a+b*)/carotenoids ratio found also Elhaak et al. (2015) in rosemary cuttings after soaking for one and three hours in IBA but the prolonged time of soaking (six hours) decreased the ratio probably because of an inhibition in the metabolism of chlorophylls *a* and *b*. Sosnowski et al. (2016) reported after treating *Medicago* plants with different growth regulators that the highest concentration of carotenoids was found in the *Medicago* leaves sprayed twice with cytokinin while auxin decreased their content and a mixture of auxin and cytokinin raised the ratio of total chlorophyll content to carotenoids.

Total soluble glucids

Sugars are considered important metabolites, is not only the first organic complex compounds formed in the leaves as a result of photosynthesis, but also a major respiratory substrate. Also sugars are involved in plant protection against wound and infections, as well as in cell detoxification (Kaur et al., 2000). The results of the present researches indicated that the stimulation of the roots growth determined an increased accumulation of glucids in the leaves of rosemary cuttings (Table 3). Both experimental variants showed higher amounts of total soluble glucids in the stem cuttings treated with Clonex comparing to the control cuttings: about 1.43 times higher in the shoot peak cuttings, respectively 1.46 times higher in the segment cuttings.

Scientific literature noted that higher photosynthetic pigment concentrations in cuttings treated with growth regulators like auxin significantly contribute to increased photosynthetic activity and enhanced production of photoassimilates. Consequently, higher glucids levels during rooting are positively correlated with increased root formation and more nutrients uptake of cuttings (Krajnc et al., 2013).

Also, Pacholczak et al. (2016) found that the treatments with Goteo biostimulator increased total soluble and reducing sugar contents in *Physocarpus opulifolius* stem cuttings. Similar results obtained Rathore et al. (2009) in *Glycine max* treated with a preparation based

on alga *Kappaphycus alvarezii* and Sivasankari et al. (2006) in *Vigna sinensis* after application of seaweed extracts. Also, IBA (indole-3-butyric acid) applications led to increased chlorophyll accumulation in plant leaves (Ludwig-Muller, 2000).

CONCLUSIONS

In conclusion, the analysis of the accumulated data allows the conclusion that treatment with Clonex led to obtaining of rosemary new plants of high quality and with strong roots, with real chances to solve the problem of setting up a commercial culture. It is available for the plants obtained on both types of cuttings.

The biochemical analysis made on rosemary cuttings showed that generally the biostimulator treatment significantly influenced the contents of organic compounds: total chlorophyll and total soluble glucids increased in the rosemary cuttings treated with Clonex both in the cuttings from the peak and from the segment. The carotenoids content in the leaves of rosemary cuttings was not influenced by the Clonex treatment but the chlorophyll (*a+b)*/carotenoids ratio increased in the variants treated with the biostimulator due to an increase of chlorophylls *a* and *b*.

In summary, it can be noted that all types of rosemary stem cuttings showed good growth and development regardless of which stem section they were provided, so the whole rosemary stem can be used for the obtaining of cuttings.

The results presented in this work show that biostimulators may enhance rooting and may be helpful for other researchers which aim to evaluate the effects of growth regulators on growth parameters and biochemical processes occurred during plants growth and development.

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COPPER EFFECT ON SEED GERMINATION AND PLANT SPROUTING OF *ALYSSUM MURALE* SPECIES

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Abstract

The studies aimed at establishing the level of copper concentration in the soil, which Alyssum murale species can tolerate without significantly affecting the percentage of germination and plant sprouting. The experience was conducted in 4 variants with 3 replicates, each replicate having 100 seeds. The humidity of the substrate for sowing was performed with water for the control sample (C) and with $CuSO_4$ solutions for the other variants (it was calculated the required amount of $CuSO_4$ to obtain a contamination with Cu of 20 ppm, 100 ppm, 200 ppm and, respectively, 500 ppm). The influence of copper on seed germination was assessed by the percentage of plant sprouting, germination rate, seedling rate and velocity. The significant decrease in the percentage of normal sprouts and the increase of percentage of dead seeds in variants V3 and V4 suggest the level of supportability of these species. The increase of copper concentration in the germination substrate caused the decrease of the seedling rate and of the velocity of seedling.

Key words*: Alyssum murale, copper, germination, sprouting percentage.*

INTRODUCTION

Pollution has become a major concern in all industrialized countries, at a global level, since every year, millions of tons of toxic pollutants originating from natural sources, but more particularly from anthropogenic sources, are released into the environment. As a result, the research carried out over the years has highlighted the favourable effect that some plants have on the depollution of the environment by absorbing the pollutants and accumulating them in different parts of the plant (Wenzel and Jockwer, 1999; Clemens, 2001; Wierzbicka, 2002; Baranowska-Morek, 2003). Phytoremediation is the use of spontaneous and/or cultivated plant species in order to extract, stabilize and/or neutralize pollutants in soils. Over time, research in phytoremediation showed favorable effect of certain plants species, including ornamental plants, indicating pollution reduction by absorbing pollutants by means of plant roots and accumulating them inside the plant. Lately, phytoremediation has become a well-studied scientific subject, which has led to an interdisciplinary cooperation between biochemists, biologists, soil chemists, agronomists and environmental engineers (Salt et al., 1995).

Copper (Cu) represents one of the first metals that were extracted and used by humans in different activities, thus providing a major contribution to the evolution of human society since ancient times. Research done on the toxicity of this particular metal has highlighted a series of anthropogenic sources of emission of copper in the environment, such as: copper extraction and processing, the agriculture, the electric power industry, the plastics industry, wastewater sludge (animal farms) and steelworks.

In low quantity, copper constitutes the essential element for a proper development in plants, playing an important role in photosynthesis, respiration, lignification and growth, as well as being a constituent of proteins and enzymes.

In excess, copper can become extremely toxic, affecting seed germination and causing a weak root and stem growth, as well as leaf chlorosis and necrosis (Yruela, 2005; Xu et al., 2005; Muhammad A. et al., 2015).

Various studies have reported the germination test as being a basic process of determining the toxicity effects of Cu in numerous plant species. The reduction of the germination percentage as a result of the presence or increased concentrations of copper in the germination environment was highlighted in different species: Sunflower (Pena et al., 2011;

Boroş et al., 2015); *Vicia sativa* L. (Muccifora and Bellani, 2013); *Minuartia hirsuta*, *Silene compacta*, *Alyssum montanum* (Ouzounidou et al., 1994); *Phaseolus vulgaris* L. (Ashagre et al., 2013); *Oryza sativa* L. (Ahsan et. al., 2007; Mahmood et al., 2007)

The results of the studies suggest that Cu toxicity in relation to the seed germination process in different plants can present a remarkable variability of tolerance, both within the genus as well as between different species.

MATERIALS AND METHODS

For the experiment, the biological material used was represented by *Alyssum murale* seeds taken from the collection of the Faculty's Floriculture department, a species that is conserved ex-situ. In the experiment were used containers of 2 kg capacity, containing the substrate made of two parts peat and one part gardening soil, in which 100 seeds were sown per container, three replicates being done for each experimental variant.

Watering was realised with tap water for control sample, and for the other three samples, with solutions of different $CuSO_4$ concentrations.

The necessary quantity of $CuSO_4$ was calculated, in order to obtain certain copper concentrations in the substrate, that represented the maximum admitted limit of Cu - 20 ppm (V_1); the warning threshold - 100 ppm, (V_2); the intervention threshold - 200 ppm, (V_3); exceeding the intervention threshold - 500 ppm (V_4).

During the experiment, the temperature of the vegetation room was constantly maintained at 22 ± 1°C for 8 hours, and at 24 ± 1°C for 16 hours. The seed germination was done under conditions of relative humidity of approximately 80% during the first 3 days and of 87% until the end of the germination period, at a level of light intensity over 8000 lux.

During the entire germination period, daily determinations and observations were made regarding the germination percentage, the number of normal sprouts, the number of abnormal sprouts, the number of dead seeds, the velocity of seedling and the coefficient of velocity (Kotowski, 1962).

RESULTS AND DISCUSSIONS

The observations regarding the influence of copper on the *Alyssum murale* seed germination highlight a decrease of the germination percentage occurring at the same time as an increase in the copper concentration.

By comparing the results obtained in all experimental variants, the influence of copper pollution on *Alyssum murale* seed germination is highlighted by the more accentuated decrease of the germination percentage. The highest germination percentage was obtained in the control variant (93%), and the lowest germination percentage was obtained by the V_4 variant (64%) (Table 1).

Compared with the control variant, for all the other experimental variants, there was a decrease of the germination percentage observed, the highest values being recorded for the V_3 variants, of 20%, and V_4 variants, of 29%.

The determinations regarding the seed germination period have revealed that the *Alyssum murale* seed germination was influenced by the concentration of copper.

Table 1. Influence of copper on the seed germination of the *Alyssum murale* species

Variant	Sowing date	Germination onset date	End of germination date	Total germination*
C	19.03.2014	22.03.2014	26.03. 2014	93
V_1	19.03.2014	22.03.2014	27.03. 2014	88
V_2	19.03.2014	25.03.2014	02.04. 2014	80
V_3	19.03.2014	24.03.2014	03.04. 2014	73
V_4	19.03.2014	29.03.2014	09.04. 2014	64

*The values represent % of normal sprouts

The seed germination began after 3 days in the case of the control variant and V_1, the increase of the copper concentration determining a bigger delay of the onset of germination, the biggest delays being recorded in the case of the V_3 variant (4 days, compared to the control variant) and V_4 variant (4 days, compared to the control variant) (Table 1).

Regarding the influence of copper on the germination period (from the onset until the end), there is a bigger delay observed in the case of the variants that were exposed to higher doses of copper (Table 1).

Figure 1 illustrates that, under conditions of copper contamination, the number of days from

the onset of germination of the *Alyssum murale* seeds, up until its end, ranged between 4 days in the case of the control variant and 12 days in V_4.

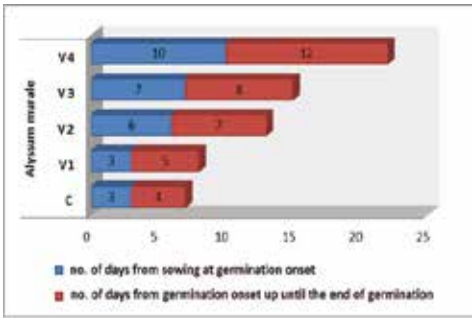

Figure 1. Seed germination duration under conditions of copper contamination (number of days)

The period of time from sowing until the onset of germination was of 3 days, in the case of the seeds originating from the control variant and V_1 variant, of 6 days for the V_2 variant and of 8 days for the V_3 variant.

Regarding the V_4 variant, the results regarding the seed germination were obtained during a period of 12 days.

Compared to the control variant, the biggest delay regarding the number of days from the onset to the end of germination, was observed in the case of the V_4 variant (8 days), followed by the V_3 variant (4 days).

In the case of the variants contaminated with copper, there may be observed an increase in the number of abnormal sprouts, as well as the ratio of dead seeds (Figure 2).

In the case of the *Alyssum murale* species, under conditions of copper contamination, there may be observed an increase in the number of abnormal sprouts, for the variants that were exposed to the highest doses of Cu. The observations made also highlight a change in the number of abnormal sprouts, the differences compared to the control variant being highly significant.

In the case of the control variant, out of the total number of seeds used in determining the germination, 93% have generated normal sprouts, 4% have generated abnormal sprouts and 3% were dead seeds. The increase in the copper concentration has determined an accentuated increase in the percentage of abnormal sprouts and dead seeds (Figure 2).

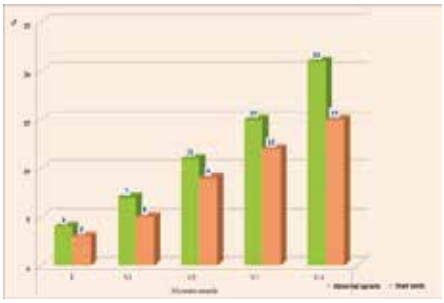

Figure 2. Ratio of abnormal sprouts and dead seeds (%)

Compared to the control variant, the number of abnormal sprouts was much higher in the case of the contaminated variants, the most evident increases in numbers being obtained for the V_4 variant, by 17%, followed by the V_3 variant, with an increase of 11%.

By comparing the number of dead seeds obtained in the case of the contaminated variants with the one obtained by the control variant, the highest increases in numbers can be observed in the case of the variants that had exhibited the highest number of abnormal sprouts as well (12% for V_4 and 9% for V_3).

The results regarding the seedling growth rate (Table 2) indicate the tendency of a decrease in germination percentages, in the case of the variants that show a metal content that represents the intervention threshold and a concentration over the intervention threshold (V_3 and V_4).

Table 2. General characterization of the seedling of the *Alyssum murale* species under exposure to different doses of copper

Variant	Seedling (%)	Seedling duration	Velocity of seedling (%)	Velocity coefficient (%)
C	82.5	3	27.5	4.71
V_1	77.5	3	25.8	3.88
V_2	67.5	6	11.3	2.08
V_3	20.0	7	2.9	1.33
V_4	16.0	10	1.6	0.73

The long time span necessary from sowing until the onset of seedling, correlated with the small number of seedlings obtained in the first days, in the case of the variants exposed to high doses of copper, determine reduced values of velocity and coefficient of velocity.

The velocity (Table 2) registered the lowest

value in the case of the V_4 variant (0.73%) and the highest value in the case of the control variant (4.71%). A similar situation may be observed in the case of the coefficient of the velocity of seedling, where the lowest value is recorded in the case of the V_4 variant (1.6%) and the highest value in the case of the control variant (27.5%).

CONCLUSIONS

The influence of copper pollution on the *Alyssum murale* seed germination has been highlighted by the more accentuated decrease of the germination percentage in the case of the variants with contaminated substrate, the highest values being recorded in the V_3 variant, of 20% and the V_4 variant, of 29%.

Higher concentrations of copper have determined a bigger delay regarding the number of days from the onset until the end of germination, which was more evident in the case of the V_4 variant (8 days) and V_3 variant (4 days).

The development of the sprouts was affected by the increase in the concentration of copper, resulting in a higher number of abnormal sprouts and dead seeds in the case of V_4 and V_3 variants.

The long time span necessary from sowing until the onset of seedling, correlated with the small number of seedlings obtained in the first days, in the case of the variants exposed to high doses of copper, determine reduced values of velocity and coefficient of velocity. The research conducted targeted the practical check of some methodologies for the copper soil decontamination by means of the phytoremediation method, monitoring the influence of copper metal on seed germination of the *Alyssum murale*.

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DECODING THE SIGNIFICANCES OF BUILT LANDSCAPE PROFILES

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Abstract

Perceived from a distance, any built landscape emits a specific energy personified by the silhouettes of its profiled buildings on the background of the environment. Overall images of human settlements have appeared in medieval artwork, being further perfected. These representations had a symbolic value, contributing to strengthening the respect for tradition and the local history. Towards the middle of the 20th century, architect G.M. Cantacuzino created several sketches of built landscape profiles, both historic and modern. The theme of this paper is based on these sketches. The research aims to argue that by elaborating certain built landscape profiles and then by decoding, analysing and by comparative assessment of the meanings transmitted by silhouettes, valuable information can be obtained about the essence, identity, personality and specificity of those human settlements. Research has been made on the basis of selected sketches of Cantacuzino and on the basis of the creation of original profile sketches of historical settlements. The arguments in this paper on decoding the profiles of built landscape contribute to highlighting a valuable tool useful for landscape researches and urbanism studies.

Key words*: decoding, features, landscape, profiles, settlements.*

INTRODUCTION

It is important to understand that human settlements can be considered as "the most conclusive possession" of a natural space and its transformation according to the aspirations and needs of the respective human society (Gheorghiu, 2002).

Analysing the history of an epoch and its influence on the evolution of urban tissue and the built framework, we observe that some important political-social-economic-religious-cultural processes in the local history are imprinted in the volumetric silhouette of human settlements.

Perceived from a distance, any built landscape emits a special message, personified by the building's overall silhouettes.

Based on judicious documentation, studying stamps, manuscripts, frescoes, paintings, photographs and old maps, we can sketch or draw the outline of built landscape profile, extracting and deciphering certain characteristic features, essential for the respective human settlement, such as identity, personality, specificity and value.

We can also discover synthetic features of the environmental elements involved in historical evolution: the natural environment, the built environment, and the socio-economic-cultural environment (Dascălu and Cojocariu, 2016).

The idea of this work has its roots in the historical and modern built profiles, drawn by the architect George Matei Cantacuzino in his youth. Sketching the silhouettes, he tried to characterize the historical personality of human settlements he visited and loved. His creations were impregnated with great passion for the built landscape, nature playing a very important role in his life (Dascălu, 2017).

In one of the volumes of Art and Criticism Dictionaries entitled Symmetry, written between 1939 and 1946, the architect manages to define the profile in general, either in art or in architecture, urbanism or landscaping: „The profile is the line that includes the appearance of an object (or a settlement) ... It is a line that expresses its self schematically ... The profile suggests forms without expressing them ... and tends to ideogram" (Vasilescu, 1993).

So at first glance, a built landscape profile transmits a synthetic idea about that human settlement and its inhabitants.

In this context the paper aims to demonstrate that certain features specific to historic cities, such as defensive or aggressive aspect,

dominant or peaceful ambience etc., can be clearly decoded by drawing the general profile of a built landscape, outlined on the sky and on the background of the natural setting.
In order to extract more detailed information from the silhouette of a profile, it is necessary to study the history of the places but also to analyse the environmental elements involved in the evolution of those human settlements. Besides all this, on the basis of written documents and old images, it is necessary to create several profiles on successive periods. The information extracted through sketching and through correlated and comparative research can finally reveal unexpected features that could be necessary in the contemporary studies of urban and landscape development of localities and territories.
The ideas and the research in this work are original. No other similar research is known.
Different studies about city abstraction as icons or lines and its skylines have been aimed at either clarifying certain aspects of aesthetic or psychological perception of the urban or natural landscape, or pursuing certain socio-spatial aspects.
Of these studies we mention Gassner's (Gassner, 2009) specifying the difference between his study and ours. Gassner-type studies and sketches aim to improve the city's current silhouettes, impacting cognitively and socially.
We underline that our research aims to provide a tool useful for deciphering the study of historical evolution of human settlements, correlating the local history, the natural chosen location and the historical silhouettes offered by different authors.

MATERIALS AND METHODS

Two profiles outlined by G.M. Cantacuzino for the cities of San Gimignano and New York-Manhattan were selected for analysis.
The analysis investigate the correlation between the historical truth based on the documentation and the profiles drawn by Cantacuzino.
Based on the documentary material, the reality and the veracity of the synthetic features declared by the architect and decipherable in his drawings, were analyzed and substantiated for these profiles.
The demonstration continued with the researches of two case studies of historical cities from France, chosen as a result of documentary research: the city of Carcassone, which has some resemblance to San Gimignano and the small settlement of Castelnau-le-Lez.
For these settlements were studied historical frescoes, paintings and engravings representing eloquently the built frame and their natural landscape. The old images have been checked and compared with current photos and images.
In order to complete the decoding of these settlements personality it was necessary a documentary on the history of places and on environmental elements.
The elaboration of original profiles, based on selected paintings and photographs, was the most important part of the research, contributing to the deciphering of the personality of the studied settlements.

RESULTS AND DISCUSSIONS

The first analyses were made on the basis of selected silhouettes from G.M. Cantacuzino's drawings and on the basis of the elaboration of historical settlement profiles, after the research on the history of the places and on the environmental elements.
Regarding deciphering the meanings of the lines and forms, there is a wide range of aesthetic studies correlated with the psycho-logical ones. According to these studies, the sharp, agitated, angular lines can be the result of either conflicting states or dynamic tendencies (from natural, artificial or human environment), while the sinuous, with quiet curves unfolded suggests situations of stability and peaceful development (Reid, 2007).
There were chosen two silhouettes for which the architect Cantacuzino finds similarities in terms of styling of the profile line and the size scale of the constructions: San Gimignano from Italy (Figure 1) and Manhattan the heart of New York USA (Figure 2).
In the dictionary Symmetry - Books and Criticism Cantacuzino draws attention that „San Gimignano preceded by several centuries the American ambitions... By comparing the two profiles, we can be convince that it is both ambition and pride” (Vasilescu, 1993).

Figure 1. The profile of San Gimignano city, sketched by G.M. Cantacuzino
(source: processed from Vasilescu, 1993)

Figure 2. The profile of New York City, sketched by G.M. Cantacuzino
(source: processed from Vasilescu, 1993)

San Gimignano is a medieval Italian town whose historic nucleus is protected by UNESCO. The purpose of the documentation was to check why San Gimignano might have a proud and high-aimed profile.

A first observation, extracted from the history of architecture and urbanism, is the fact that, in general, ascending architectural volumes suggests ambition and pride (Gheorghiu, 2009). Despite this fact, history demonstrates often that ascending features are chosen to be expressed because impressive and massive volumes can demoralize any attacks of enemies (Gheorghiu, 2009).

Related to these two possibilities, studying the conditions for choosing the location of the early urban core, we noted the special defense possibilities were offered by the local relief, proving their importance. The city is located on one of the hills of Tuscany, therefore the site have a good natural defense. As a result of the research, we discovered a painting of George Howard at the end of the 19th century, clearly distinguishing the territorial strategic location, the city's silhouette being legible on the impressive hillside (George Howard's watercolour „La citta della belle torre-San Gimignano", 1877, exhibited at London, Grosvenor Gallery).

Analyzing the representations of San Gimignano over time, we find that characteristic features such as massive and ascending volumetric trends have been perceived and presented similarly from Renaissance time to the 20th century, indicating that the city has always developed in the same architectural and constructive manner. The following images were selected and compared: the frescoes of the saints Gimignano and Fina, who both hold and protect the town of San Gimignano in their arms (exhibited at Pinacoteca of San Gimignano Palazzo Comunale); a Benozzo Gozzoli's Renaissance fresco (exhibited at the apse of the Sant Agostino-San Gimignano chapel); a picture in the Cubism manner of 1912 by Pyotr Konchalovsky (exhibited at State Russian Museum of Saint Petersburg).

As mentioned above, the fortified volumetric expression of the city could suggest a defensive attitude. But, investigating the history of the city we discover that during the medieval period there was a rivalry between two noble families that dominated economically the city. This conflict has generated the competitive construction of buildings with higher towers, more and more grandiose, in the egotic attempt to leave a trace in the history of the city.

In connection with Cantacuzino's comparison of San Gimignano and New York, we examined many artistic representations of the two cities and selected two artists whose works, in Expressionist manner, crystallize the essence of the profiles: San Gimignano by M.C. Escher painted in 1922 and Manhattan by Howard Cook painted in 1930. The comparison of their drawings is eloquent to distinguish the same ascending traits in which the message of ambition and pride is transmitted.

Research has continued with the study of New York history, which has highlighted that over time the city has tended to play an increasingly important economic role in the USA. Consequently, towards the end of the nineteenth century, the first skyscrapers in New York appeared to be the tallest, in competition with Chicago buildings for example.

The tendency to build upright has increased over time, making Manhattan the heart of New York and the symbol of USA economic power. Unfortunately, this trend is tragically linked to

the 2011 attacks that aimed at destroying the volumetric symbols of New York at the time - the twin towers of the World Trade Center.

In conclusion, these researchs on the San Gimignano and New York-Manhattan silhouettes demonstrates the veracity of G.M. Cantacuzino's assertion. The message of both profiles is aggressive, revealing the desire for domination in a competition of economic power.

In order to deepen the argumentation about the sketching and deciphering of built landscape profiles, the analyses were completed by two original case studies, with the extraction and verification of the characteristic features of some historic French cities.

The settlements selected on the basis of the documentation were Carcassone and Castelnau-le-Lez. In order that analyzes can be compared as clearly and without confusion, the most important criterion of the selection was that the two cities must be different as human settlements personality, in terms of environmental, volumetric and urban tissue elements.

An examination of characteristic images for these cities was made and as a result, a few vintage paintings, photographs and aerial images were selected. The old images were checked and compared to the actual photos and on their basis the profiles of the two cities were sketched. It was also necessary a documenttation regarding the elements of the environment and on the evolution of the historical stages.

The first case study is the historic city of Carcassone, which has some resemblance to San Gimignano because it is fortified and has a massive volume dominated by many towers.

It is a city located in southern France on the hills of Languedoc. In contrast to the San Gimignano evolution, Carcassone has a rather agitated history with tragic episodes.

The medieval citadel was built on the basis of a preconceived urban plan being at present protected by UNESCO.

It was erected in the thirteenth century in order to be an intangible fortress to defend the ever-changing regional medieval frontiers, attacked by infighting regional.

The analysis of the historical pictures of the citadel highlights the fact that the ascending characteristic features were similarly represented over time, from the 13th century to the 19th century, with small variations due to the construction of new buildings, but raised in the same style to keep the city's personality. The most representative images are the following: „Expulsion of the inhabitants from Carcassone in 1209" image from Grandes Chroniques de France 1415; „La Délivrance des emmurés de Carcassonne" painting of Jean-Paul Laurens 1879; old maps of Carcassone. The elaboration of the profile was based on several selected, compared and overlaid photos (Figures 3 a and b).

Figure 3a. Drawing the Carcassone profile by overlapping the picture. Graphics Cojocariu M. (source: https://www.blog.thelittleprince.com/a-little-prince-shop-in-the-medieval-city-of-carcassone-franc)

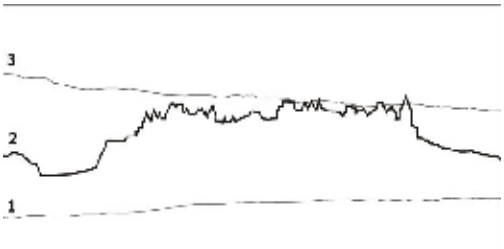

Figure 3b. Final profile of Carcassone (source: Graphics Cojocariu M.)

The energetic line of the silhouette highlights features characteristic of a defensive urban organization. This strategic feature it is certified by historical documents and is confirmed by the qualities of landscape elements: the city is located on top of the hill, with a significant difference in level compared to the rest of the unfortified settlement, located to the left and right of the fortress (Figure 3b). The three important lines of the profile are: 1-the line from which the perception was made; 2-the citadel profile; 3-the background of the hills. The image of the profile on the background of the surrounding hills makes a valuable asset of the medieval settlement,

namely its adaptation to the natural environment. The line no. 2 stands out through the multitude of defense towers, visible in the density of the zigzags lines that evokes anxiety of the defensive status.

The second chosen case study is the small settlement of Castelnau-le-Lez, located also in southern France, on the river Lez, in the Occitan region.

Research on the evolution of the site shows that, dating from antiquity, the settlement was a village with a quiet history and a continuous economic development.

In terms of the natural environment, the landscape is hilly with rich vegetation.

The overall image of the old area is picturesque both as buildings and as a natural landscape. From the historical images were selected, as the most representative, two paintings done by the impressionist artist Frédéric Bazille in 1864 and 1868 (Figure 4a). Both paintings have on their background a gentle image of the quiet settlement. Checking and comparing paintings with current photos revealed that the old 19th century core still retained its appearance.

Figure 4a. The final selected painting of Frederic Bazille - View of the Village of Castelnau-le-Lez, 1868 (source:https://www.wikiart.org/en/frederic-bazille/view-of-the-village-of-castelnau-le-lez-1868)

The profile sketch was created by overlapping the 1868 painting (Figures 4b and 4c).

The three major lines for the basic features are: 1-the river line; 2-the built profile; 3-the natural landscape with hilly relief, the river and the vegetation (Figures 4b, 4c).

The final profile reveals the harmonious integration of all construction into the landscape, suggesting both the beauty of the peaceful life and the attachment to nature. These are the most important features that have contributed to the development of the locality, keeping a tradition of respect for history and the natural environment.

Figure 4b. Drawing the Castelnau-le-Lez profile by overlapping the picture.
(source: Graphics Cojocariu M.)

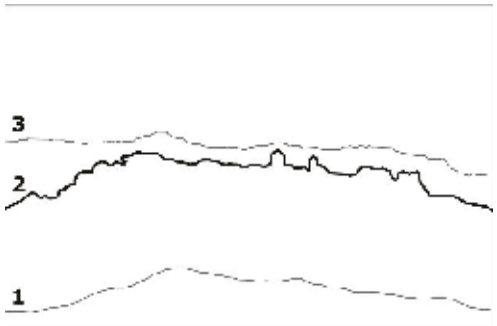

Figure 4c. Removal of background paint for profile decoding.
(source: Graphics Cojocariu M.)

These studies and researches revealed that by analyzing only the old images and the present photos as a whole we cannot decipher all subtle features.

Consequently, it was necessary to draw some original profiles based on scientific documentation.

These analyze, in their entirety, highlighted the features of the personality of the studied settlements, impregnated by the history of the places and their natural landscape.

CONCLUSIONS

The sketching and deciphering of built landscape profiles must be based on comparative studies and analyses of the natural environment, of the built and of socio-economic-cultural environment.

Elaborating silhouettes is especially useful if it is created by following the succession of various historical periods.

The decoding, analysis and comparative evaluation of the meanings transmitted by them, generates valuable information about the essence, identity, personality and specificity of human settlements.

The research in this paper provides an original and particularly useful tool for studies of urban and landscape evolution of human settlements.

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STUDIES REGARDING THE BEHAVIOUR OF ORNAMENTAL SPECIES *LAGURUS OVATUS* IN CROPPING CONDITIONS FROM N-E AREA OF ROMANIA

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Abstract

The current paper aimed to analyse some aspects about behaviour and multiplication of Lagurus ovatus species in cropping conditions from Iaşi County, Romania. Research material was represented by Lagurus ovatus. Were established five experimental variants represented by different substrate types utilised for sowing: V1 garden soil, V2 garden soil + leaves soil (1:1), V3 garden soil + leaves soil + peat (1:1:1), V4 leaves soil + peat (1:1) and V5 jiffy pots. Observations and determinations were carried out in the didactic field of Floriculture Discipline from UASVM Iaşi, Romania, during 2015-2016 and had in view aspects regarding seedlings obtaining and also plants behaviour. At the end of the current research we observed that for seedlings production the best results were obtained at variant V3, followed by V2 and V4; and in cropping conditions at variants V3, V2 and V4 the results were quite close as values. In conclusion we can affirm that Lagurus ovatus is an ornamental grass which could be easily multiplied through seeds and for establishing crops the utilisation of seedlings is recommended.

Key words*: capitalization, Lagurus ovatus, ornamental (grass).*

INTRODUCTION

At world level plant species, generally named ornamental grasses, are utilised in floral art and also in vegetal compositions in landscape designs.
In Romania, ornamental grasses are less known and utilised in landscape designs and in floral art. In the last years, interest for those species increased, but due to lack of information regarding cropping technologies and utilisation modalities in pedo-climatic conditions of Romania, their utilisation is not always adequate (Chelariu, 2013).
Lagurus ovatus L. belongs to *Poaceae* botanical family and is cultivated in rustic areas 8-10 (11) (Mills-Hicks, 2007). It is a species with origins in South Europe, Mediterranean area. It's an annual species with a bush aspect, height of 30-50 cm and leaves of a light green colour (Brickell and Cathey, 2004; Şelaru, 2007). Flowers are grouped in small compact spikes, with a green white-yellow colour. Fruits appearance and maturation took place in stages from summer till autumn (Chelariu, 2013).
This species prefers fertile soils, permeable, with a pH between 6.6 and 7.5. Have a well development on sunny exposure fields (Brickell and Cathey, 2004; Chelariu, 2013; Colborn, 2006; Ondoño et al., 2016). Multiplication of this species is realised on a generative way. Seeds germination could be influenced by different parameters of some ecological factors (light, temperature, salinity etc.) (Carreño et al., 2004).
In the current paper are presented the aspects regarding seedlings obtaining and also the behaviour in cropping conditions of *Lagurus ovatus* species in pedo-climatic conditions from Iaşi, Romania.

MATERIALS AND METHODS

Research took place during 2015-2016 in the didactic collection (greenhouse and field) of Floriculture Discipline from UASVM Iaşi,

Romania. Biological material was represented by *Lagurus ovatus* species and the seeds used for establishing the experiments were achieved from Holland. Experiments were organised in five variants, each variant being represented by a substrate type utilised for sowing (Table 1).

Table 1. Experimental design

Studied species	Biological material	Seeds number	Variant	Substrate for sowing
Lagurus ovatus 	Seeds 	100	V_1	garden soil
		100	V_2	garden soil + leaves soil (1:1)
		100	V_3	garden soil + leaves soil + peat (1:1:1)
		100	V_4	leaves soil + peat (1:1)
		100	V_5	jiffy-pots

The experimental variants were established having in view the ecological demands of the studied species and data from literature (Buta and Cantor, 2009; Cantor, 2009; Draghia and Chelariu, 2011; Chelariu, 2013).

In each year sowing was realised on 10th of February and the establishment of field crops was done on 10th of May.

Research aimed the obtaining of seedlings (seed germination percent, necessary period from sowing till ending of plants' emergence), as well as the behaviour in cropping in pedo-climatic conditions from Iaşi, for species.

In field, were studied separately plants obtained in different types of substrate, aiming aspects regarding the growing rhythm, phenology and morphology of plants.

The obtained results were statistically processed and were presented as a mean of two years of study.

RESULTS AND DISCUSSIONS

Seeds' germination at species *Lagurus ovatus* could be influenced by light, temperature, soil salinity (Carreño et al., 2004).

The research highlighted that the percent of germinated seeds at *Lagurus ovatus* varied between 75% and 100% (Figure 1). The best results were recorded at variant V_3 (1 part garden soil + 1 part leaves soil + 1 part peat), followed by variants V_2 (1 part garden soil + 1 part leaves soil) and V_4 (1 part leaves soil + 1 part peat), and the lowest values were obtained at variant V_5 (jiffy-pots) (Figure 1).

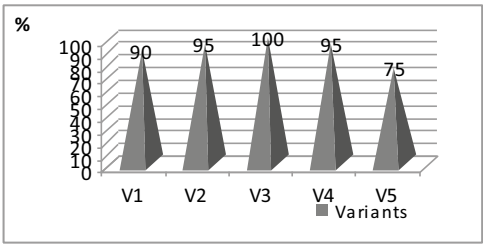

Figure 1. *Lagurus ovatus* seeds germination (%)

From statistic point of view at variants V_2, V_3 and V_4 were observed very significant positive differences comparing with the control variant (V_1). At variant V_5 the negative difference value comparing with the control was very significant (Table 2).

Table 2. Results regarding seed germination

Variant	Number of seeds (pieces)	% face to control	Difference	Signification
V_1	90.0	100.00	0.0	control
V_2	95.0	105.56	5.0	***
V_3	100.0	111.11	10.0	***
V_4	95.0	105.56	5.0	***
V_5	75.0	83.33	-15.0	000
LSD 5% = 1.4 pieces				
LSD 1% = 2.0 pieces				
LSD 0.1%= 3.1 pieces				

Regarding the necessary time period till plants' emergence, were observed differences between experimental variants. So, at variants V_1, V_2, V_3 and V_4 the emergence started after 3 days from sowing and at variant V_5 after 5 days

(Figure 2). Necessary time for a complete emergence of plants, calculated from sowing, was of 10 days at variant V_3, 11 days at variant V_2, 12 days at variants V_1 and V_4, for variant V_5 were necessary 17 days from sowing to end of emergence (Figure 2).

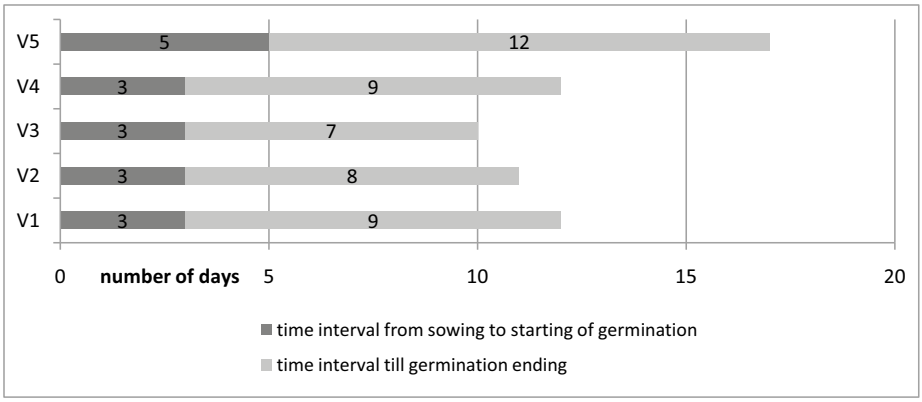

Figure 2. Duration of germination (number of days from sowing)

The utilised seedlings for establishing the field crops had different features function by experimental variant (Table 3).

Table 3. Morphological features of seedlings planted in experimental field

Variant	Mean number of roots per plant	Mean length of seedlings (cm)	Mean height of plant (cm)	Mean number leaves/plant
V_1	6.3	14.2	11.2	14.4
V_2	6.8	14.4	11.6	14.6
V_3	7.2	15.1	12.5	14.8
V_4	6.7	14.3	12.0	14.7
V_5	5.4	9.6	8.1	13.0

Seedlings obtained in greenhouse, on different types of substrates were planted in field on 10th of May. In according with literature (Brickell and Cathey, 2004; Colborn, 2006; Ondoño et al., 2016) plants of *Lagurus ovatus* reach a height between 30 and 50 cm; flowering in summer. During research period, in pedo-climatic conditions from Iaşi, Romania, plants had a normal rhythm of growing. Flowering took place in the second decade of June (V_1, V_2, V_3, V_4) or in the third decade of June (V_5), and fructification in the second decade (V_1, V_2, V_3, V_4) or in the third decade of August (V_5). Vegetation period ended in the first decade of October (Table 4).

In Table 5 is presented the morphological characterization of plants, realised based on field observations made on 1st of October.

Table 4. Phenology of *Lagurus ovatus* specie in cropping conditions from Iaşi County

Variant	Growing rhythm	Flowering (decade/ month)	Fructify (decade/ month)	Ending of vegetation period (decade/month)
V_1	normal	II/06	II/08	I/10
V_2	normal	II/06	II/08	I/10
V_3	normal	II/06	II/08	I/10
V_4	normal	II/06	II/08	I/10
V_5	normal	I/07	III/08	I/10

Table 5. Morphological characterization of *Lagurus ovatus* plants

Variant	Mean height of plant -cm-	Diameter of bush -cm-	Mean length of leaves -cm-	Mean width of leaves -cm-	Mean length of inflorescence -cm-	Mean number inflorescence per plant -pieces-
V_1	38.8	23.3	14.1	1.3	5.3	24.3
V_2	40.1	23.5	14.2	1.3	5.4	24.6
V_3	40.9	24.2	14.5	1.4	5.5	25.1
V_4	40.3	23.9	14.4	1.4	5.5	25.0
V_5	34.9	21.9	14.0	1.3	5.3	23.9

Regarding mean height of plants were observed very significant positive differences comparing with the control (V_1) at variant V_3 and distinct significant positive differences at variants V_2 and V_4. At variant V_5 the negative difference comparing with the control variant is very significant (Table 6).

Regarding flowering capacity at variants V_3 and V_2 differences comparing with the control

(V_1) were positive distinct significant while variant V_5 recorded a significant negative difference (Table 7).

Table 6. Results regarding plants' growing

Variant	Mean height of plant (cm)	% face to control	Difference	Signification
V_1	38.8	100.00	0.0	control
V_2	40.1	103.35	1.3	**
V_3	40.9	105.41	2.1	***
V_4	40.3	103.87	1.5	**
V_5	34.9	89.95	-3.9	000
LSD 5% = 0.8 cm LSD 1% = 1.1 cm LSD 0.1% = 1.7 cm				

Table 7. Mean number of inflorescences/plant

Variant	Number of inflorescences (pieces)	% face to control	Difference	Signification
V_1	24.3	100.00	0.0	control
V_2	24.6	101.23	0.3	-
V_3	25.1	103.29	0.8	**
V_4	25.0	102.88	0.7	**
V_5	23.9	98.35	-0.4	0
LSD 5% = 0.4 pieces LSD 1% = 0.5 pieces LSD 0.1% = 0.8 pieces				

In landscape design, *Lagurus ovatus* assures decoration due to its bush habitus, from spring till autumn, and by inflorescences in period June-September (Chelariu, 2013). Due to fine texture and plants' elegance these species could be utilised in vegetal compositions from different types of landscape designs such as: decorative pots, rounds, flats, stone designs etc. (Brickell and Cathey, 2004; Chelariu, 2013; Colborn, 2006; Şelaru, 2007; Tomaškin et al., 2015), green roofs (Ondoño et al., 2016). Also, floral rods could be utilised in floral art as cut flowers both in fresh state as well as in dry state (Chelariu, 2013; Tomaškin et al., 2015).

CONCLUSIONS

In conclusion we can affirm that *Lagurus ovatus* is an ornamental grass which could be easy multiplied through seeds and for establishing of crops is recommended the utilisation of seedlings.

To obtain a 100% percent of germination and a quality seedling at species *Lagurus ovatus* is recommended as substrate for sowing the mixture between garden soil + leaves soil + peat (1:1:1).

In crop conditions, at plants obtained at variants V_3, V_2, V_1, and V_4, morphological characteristics were quite similar, differences being observed at plants from variant V_5.

Lagurus ovatus is an ornamental grass species which in cropping conditions from North-East area of Romania acts as an annual plant, having a normal growing rhythm and decorates through habitus and inflorescences.

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THE RESPONSE OF BULGARIAN SPRAY-CARNATION (*D. CARYOPHYLLUS* F. *SPRAY,* HORT.) CV. 'RUSALKA' TO DROUGHT - *IN VITRO* INDUCED BY DIFFERENT PEG CONCENTRATIONS

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Abstract

In our study, to simulate water deficit induced by osmotic stress, different concentrations of polyethylene glycol (PEG-6000) were used: 10%, 20%, 30% and 40% at different durations of treatment (1, 3 and 6 days) in vitro conditions. The model plant was Bulgarian spray-carnation (D. caryophyllus f. spray, Hort.) flowers, cv. 'Rusalka'. The response to drought stress was studied based on the following end-points: plant growth reactions, relative water content (RWC %), and electrolyte leakage (conductivity). The water deficit varied from 16% (control) to 75% (40% PEG-6 days). The growth of the explants proportionally decreased with the increase of polyethylene glycol concentration from 10% to 40% and the fresh weight was below 50% vs. the control at 30% and 40% PEG. The relative water content of the plant tissues decreased depending on PEG quantity, the lowest values - 25.16 ± 2.06% being reported at 40% PEG concentration on the 6th day. The highest values of electrolyte leakage up to 1712 µS/g fresh weight were reported on the 6th day at 40% PEG concentration.

Key words*: spray-carnation, drought, polyethylene glycol (PEG), water deficit, growth.*

INTRODUCTION

The ongoing worldwide climate changes enforced multiple studies of how the plants react towards them. One of the main components of the drought is the water deficit, an abiotic factor that causes multiple morphological and physiological changes in the plants that reduce their quality and economic value.

The research on the physiological mechanisms of plant resistance in laboratory conditions gives an opportunity to monitor the specific response of the plants to one of the impact factors (Yordanov et al., 2000; Alexieva et al., 2003).

The drought simulation was done by means of an osmotic agent with high molecular weight (>3000) such as polyethylene glycol (PEG) (Murillo Amador et al., 2002). The use of PEG in liquid media allowed achieving precisely and recreating the necessary osmotic potential of the environment (Song et al., 2013).

The response of the *in vitro* cultures to induced stress enabled the selection of water deficit tolerant plants at an early stage. This was made possible by the existing correlation in the response of the plants to stress on cellular level-*in vitro* and *in vivo* (Song et al., 2013)

The purpose of the investigation was to establish the physiological and adaptive response of the Bulgarian spray-carnation cultivar to drought.

MATERIALS AND METHODS

For the purpose of the experiment, the plant material of the *D. caryophyllus* f. *spray,* Hort.*)* cv. 'Rusalka' was reproduced *in vitro* on MS nutritive medium with added sacharose - 30 g/l and agar 6 g/l at pH = 5.7-5.8 prior to autoclaving (Murashige and Skoog, 1962). Then it was grown in a phytostatic room at a temperature of 22°C, photoperiod of 16: 8 (day: night) hours and light intensity 30 µmol m^{-2} s^{-1}.

For the induction of the experiment, an MS medium was used containing salts and vitamins, saccharose - 30 g/l and polyethylene glycol (PEG 6000) in the following concentrations - 10%, 20%, 30% and 40% with pH = 5.7-5.8 prior to autoclaving.

The explants were placed in test-tubes on control (0) and stress inducing liquid medium (PEG - 10%, 20%, 30% and 40%) on filter

paper bridges, 10 for each concentration and the control in 3 replications. The duration of stress impact was short (one day), medium (three days) and long-term (six days).

The explants used were 2-3 cm long and had a weight of 100-300 mg, measured in sterile conditions prior to the initiation of the stress inducing medium.

In order to establish the effect of water deficit on plant tissues, we measured the explants' growth and rooting, the rate of cell membranes damage and the relative water content.

In *in vitro* research, growth was expressed as the percentage of micro explants' weight increase after being cultivated for a certain period of time (1, 3 and 6 days) on 10%, 20%, 30% and 40% PEG, compared to the initial weight.

The rate of the membrane damage was defined by the electrolyte leakage from the leaves with accounting for conductivity only after stress and was expressed as μS/g fresh weight.

The relative water content (RWC) was measured simultaneously with electrolyte leakage and calculated by the following formula:

RWC % = (fresh weight – dry weight)/(turgor weight – dry weight) × 100 - according to Turner's method (Turner, 1981).

The water deficit (WD) was expressed by the following formula:

WD % = 1 – RWC.

Following the stress period, the explants were transferred to an MS medium without a stress agent (PEG) in order to establish their capacity for recovery and report the rooting percentage.

The data on the figures below were expressed as an average value ± SE of two independent experiments, carried out in 10 replications per variant. They were analyzed for significance by means of the t-test of the GraphPad Prizm software. The results were statistically significantly different at $P<0.05$ (*), $P<0.01$ (**), $P<0.0001$ (***), respectively, as compared to the control.

RESULTS AND DISCUSSIONS

Following the exertion of a short-term osmotic stress (1 day), the explants, Grown on the control medium, showed a slight growth and reached values up to 102.8 ± 5.8 (Figure 1). The growth decreased proportionally to the increase of PEG concentration in the nutritive medium, it was less than 50% vs. the control in the high PEG concentrations (30% and 40%), 58.94 ± 4.4 and 59.96 ± 2.5, respectively, with significance rate $P<0.0001$. Following short-term (one-day) osmotic stress, the explants showed little growth demonstrated by its gain in fresh weight (Figure 1).

The results were statistically significantly different at $P<0.05$ (*), $P<0.01$ (**), $P<0.0001$ (***), respectively, as compared to the control.

The reported growth values were 33.78 ± 6% at 30% PEG and 30.32 ± 2.7% at 40% PEG in the extended treatment periods (3 and 6 days) (Figure 1). The control plants were fresh, green and in normal turgor condition and showed initial rooting on the 6th day. The plants placed on 10% and 20% PEG concentration showed slight wilting, compared to the higher wilting rate on 30% and 40 % and no explants necrosis. At higher PEG concentrations and longer exposure times (3-6 days), a progressive decrease in the growth was observed: down to 50% below the control at 30% and 40% PEG. The control plants were fresh, green and with normal turgor on day 6 while the plants exposed to 10% and 20% PEG showed withering signs which were even more notable in the 30% and 40% PEG plants.

The suppression of growth was related to the reduced capacity of the plant to uptake water (Shabani et al., 2013). The effect of the osmotic stress on cellular level is expressed by slowing down cell division, they lose their turgor and this leads to weight loss (Levitt, 1980; Heyser and Nabor, 1981). Due to the low turgor pressure cell expansion and cell growth suppresses under water stress (Jaleel et al., 2009). In *chrysanthemum*, the values of the fresh weight decreased up to 50% vs. the control. The plants were wilting in low PEG concentrations (10% and 20%), while 20% of the trial explants became necrotic in addition to wilting in the high concentrations (30% and 40%) (Zapryanova and Nencheva, 2013).

In Bulgarian spray carnation (*D. caryophyllus* f. *spray,* Hort.) flowers, cv IRA a progressive decrease in the growth was observed: down to 50% below the control at 30% and 40% PEG (Zapryanova et al., 2015).

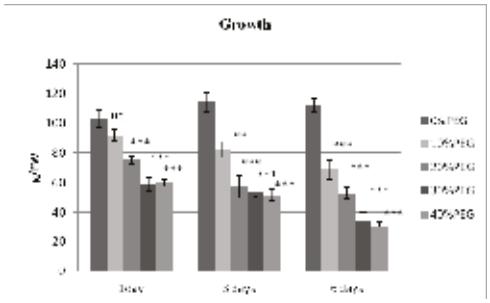

Fig. 1. Growth (gain in fresh weight) of *spray-carnation* explants cv. 'Rusalka' *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The index of the Relative water content (RWC) is often used to define the water status. It decreases with the increase of the PEG concentrations in plant tissues. The control plants maintained a high percent of water content in their tissues - about 80% (Figure 2). The results of day 1 showed that the exerted PEG stress lead to the gradual decrease of plant water content, the lowest values of 44.62 ± 2.3, being reported at 40% PEG concentration. The difference between the separate variants and the control were statistically significant at P<0.0001. That tendency was maintained on both the 3rd (29.25 ± 2.3) and 6th days (25.16 ± 2.06) lowest results for PEG. The results for all PEG concentrations vs. the control were statistically significant at P<0.05 and P<0.0001 (Figure 2).

A similar response was observed in callus culture of *Carthamus tinctorius* L. - the lowest growth values and RWC percentage were reported for 40% PEG concentration (Kakaei et al., 2013).

The long-term drought stress in chrysanthemum strongly reduced the water potential of the cell, lead to the decrease of tissue turgor and final wilting of the plants, especially in the higher PEG concentration. Whereas the values of the control plants were constant at about 70%, they reached 30% on the 6th day at 40% PEG concentration (Zapryanova and Nencheva, 2013).

In experiments with Bulgarian spray carnation (*D. caryophyllus* f. *spray,* Hort.) flowers, cv. 'IRA', the control explants (without PEG) maintained high RWC in their tissues: about 80%, regardless of the cultivation time (1, 3 or 6 days). The results clearly indicate that PEG-induced stress led to a gradual decrease in the explants' RWC. The lowest RWC values (28.52 ± 5.2) were obtained in the 40% PEG variant on day 6. Prolonged drought resulted in low RWC of cells and in low tissue turgor and irreversible withering, especially at higher concentrations (Zapryanova et al., 2015).

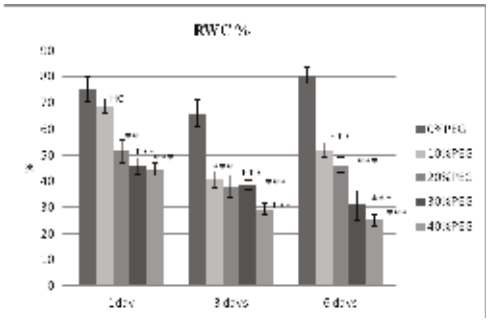

Fig. 2. Relative water content (RWC) of *spray-carnation* explants cv. 'Rusalka' *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The damage of cell membrane organization and content is one of the first responses of the plant organism to the impact of the stress agent. Electrolyte leakage in control plants was observed in low rates on the 1st day after trial initiation and was maintained throughout the trial duration (μS/g fresh weight): 396.78 ± 54.8 - 1st day, 226.53 ± 27.9 - 3rd day and 292.478 ± 37.58 - 6th day μS/g fresh weight that corresponded to the normally expected response of the studied material.

The simulated experimental drought showed a sharp increase of electrolyte leakage during short-term stress (1 day) in all the used PEG concentrations (Figure 3). The highest values – 1712 ± 363 μS/g fresh weights were reported at 40% PEG concentration - 6th day (Figure 3). The values of the electrolyte leakage at different PEG concentrations during the 3 and 6 days stress were constantly higher than the control plants but lower in comparison to the results of the short-term stress. The results had a good statistical significance between the separate variants and the control at P<0.05 and P<0.0001 (Figure 3).

Electrolyte leakage in the Bulgarian spray carnation (*D. caryophyllus* f. *spray,* Hort.) flowers, cv IRA - control plants was very low at day 1 and remained under 200 μS g^{-1} during the whole experiment, which is in good

correspondence with the expected normal values for the chosen plant material. The highest values 2633 ± 521 µS/g fresh weight were reported at 40% PEG concentration (Zapryanova et al., 2015).

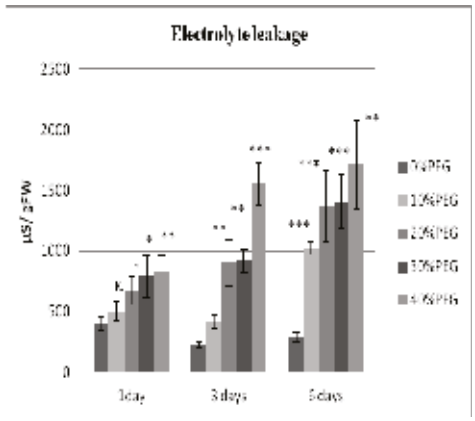

Fig. 3. Electrolyte leakage from *spray-carnation* explants cv. 'Rusalka' *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The highest values of water deficit (WD %) were reported during the long-term stress (6 days period), namely 69% and 76% at 30% and 40% PEG (Figure 4). These results indicate that the drought stress induced in our experiments was of a moderate degree but developed rapidly. According to the concept of Cornic G., Fresneau C. (2002), dehydration that causes up to 30% water deficit in plants, is assumed as mild or moderate stress.

A similar response was observed in experiments with Bulgarian *spray carnation* (*D. caryophyllus* f. *spray,* Hort.) flowers, cv. 'IRA'. Dehydration of plant tissues to 69% for the PEG 30% variant and to 71% for the PEG 40% variant on day 6th was observed, whereas the values on day 1 were 46.2% and 63.62%, respectively (Zapryanova et al., 2015).

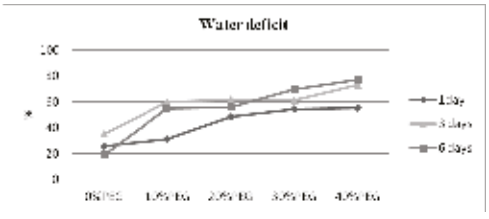

Figure 4. Water deficit (%) in the plant tissues of *spray-carnation* explants cv. 'Rusalka' *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The *spray-carnation* explants were placed on MS medium after the different stress periods for recovery**.** The results showed that the recovery is 100% after the short-term stress (1 day). Rooting was reported for all the concentrations, regardless of PEG quantity: 100% for 10% and 20% PEG; 75% for 30% PEG and 60% rooting - for 40% PEG.

The plants recovered 100% after the medium - term stress (3 days) at PEG concentrations - 10%, 20% and 30%, while only 90% recovered at 40% PEG concentration. The best manifestation of the rooting - 90% - was at 10% PEG, where it was expressed to a smaller degree at the higher PEG concentrations of 30% - 50% rooting and 40% - 30% rooting.

The long-term 6 days stress of PEG showed 100% recovery of the plants at 10%, 20% and 30% PEG concentrations but the percentage of rooted plants went down to 50. The recovery at higher PEG concentrations of 40% was 30% and rooting only 10%.

The explants of *spray-carnation* cv. 'IRA' showed a good adaptive response that was confirmed by the high recovery percentage - 60% and 40%, reported for the high PEG concentrations - 30% and 40%, respectively (Zapryanova et al., 2015).

In conditions of moderate PEG-induced drought stress- *in vitro*, the explants of Bulgarian *chrysanthemum* cv. 'Zhoro' showed an adaptive response, rather than stress-induced damage, as inferred from the relatively high recovery rate (80% and 60%) following 30% and 40% PEG treatment (Zapryanova and Nencheva, 2013).

CONCLUSIONS

The growth of the explants proportionally decreased with the increase of polyethylene glycol concentration from 10% to 40% and the fresh weight was below 50% vs. the control at 30% and 40% PEG.

The drought, simulated by means of different polyethylene glycol concentrations, caused changes in the cell membranes of *spray-carnation* cv. 'Rusalka'. The highest values of electrolyte leakage up to 1712 ± 363 µS/g fresh weight were reported on the 6th day at 40% PEG concentration.

The relative water content of the plant tissues decreased depending on PEG quantity, the lowest values – 25.16 ± 2.06% being reported at 40% PEG concentration on the 6th day.

The water deficit varied within 18% - 76% depending on PEG concentration and durations of treatment.

The explants of *spray-carnation* cv. Rusalka showed a good adaptive response that was confirmed by the high recovery percentage - 100% and 30 %, reported for the high PEG concentrations - 30% and 40%, respectively on the 6th day.

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ASPECTS REGARDING THE ORNAMENTAL VALUE OF SOME ROSE NURSEY SPONTANEOUS IN IAŞI DISTRICT CONDITIONS

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Abstract

Wild species of the genus Rosa L. have importance for: creating green spaces, getting roses nobles and their use as rootstock for cultivated varieties. The purpose of this paper is to highlight the possibilities of identifying potential ornamental species in the genus Rosa sp. Observations were made at 6 species of wild rose "Rosa californica", "Rosa caudata", "Rosa centifolia", "Rosa damascena", "Rosa multiflora" and "Rosa rugosa". To achieve that goal were made observations and measurements on the main morphological characters, growth of stems, number of buds and abundance of flowers.

Key words*: roses, stems, buds, flower.*

INTRODUCTION

One of the basic components of green spaces which assure a esthetical aspect of localities and contributes to the welfare and good mood of people, which also assure a favourable working and living climate is represented by floral and roses landscapes designs (Wagner Șt., 2002).
Rose was considered from ancient times "Queen of flowers", due to its multiple qualities and particularly great wealth and beauty flowers, scented and with various colours and shapes (Zaharia D. et al., 2003; Wagner Șt., 2002).
In the multitude of dendrological species which contains ornamental shrubs, spontaneous species of *Rosa* L. have a real importance for design of green spaces, obtaining of noble roses, and many of them are utilised as parent stocks for cultivated sorts. (Mohan et al., 2010)
The conditions from Romania are generally favourable for this specie.
Necessity for diversifying the assortments is a priority, having in view the diversity of biological material and the results obtained in the world. Literature shows, which in this genus are known over 400 species with more than 20,000 sorts (Bernardis R., 2011; Mohan et al., 2010; Zaharia D. et al., 2003; Wagner Șt., 2002).
Being known the diversity importance of *Rosa* L. spontaneous species, in the research method was proposed to be realised phenological observations on: stems, leaf, flowers and fruits.

MATERIALS AND METHODS

Observations on roses were carried out at "Tudor Neculai" - Iaşi nursery. Nursery is placed outside Iaşi City, into a field belonging to Miroslava village, being destined for production of planting dendrological and floral material for decoration and beautification of Iaşi City green spaces. Nursery have also a rich assortment of spontaneous rose species from which we mention: *Rosa californica*, *Rosa canina, Rosa carolina, Rosa caudata, Rosa centifolia, Rosa corymbifera, Rosa damascena, Rosa davidii, Rosa davurica, Rosa foetida, Rosa gallica, Rosa multiflora, Rosa rugosa*, *Rosa virginiana* (Bernardis R., 2011; Zaharia D. et al., 2003; Wagner Șt., 2002).
Observations were made on 6 spontaneous rose species:

- *Rosa californica* (Figure 1);
- *Rosa caudata* (Figure 2);
- *Rosa centifolia* (Figure 3);
- *Rosa damascena* (Figure 4);
- *Rosa multiflora* (Figure 5);
- *Rosa rugosa* (Figure 6).

The aim of the current paper is to highlight the ornamental potential and identification possibi-

lities of *Rosa spontaneous* species in the genus (Bernardis R., 2011; Milică C. et al., 2010).
Were realised observations and determinations regarding: growing rhythm of stems; number of buds; abundance of flowers.
Observations and determinations regarding phenological particularities were realised for six *Rosa* L. *spontaneous* species, in three repetitions and in five different days, as follows: (28.04, 7.05, 9.06, 11.07 and 10.08). Observations were carried out during 2015-2016 and were studied the growing rhythm of stems, number of buds and abundance of flowers.

Figure 1. *Rosa californica*

Figure 2. *Rosa caudata*

Figure 3. *Rosa centifolia*

Figure 4. *Rosa damascena*

Figure 5. *Rosa multiflora*

Figure 6. *Rosa rugosa*

RESULTS AND DISCUSSIONS

From measurements made at the end of observation period (10.08) at analysed species regarding height of stems were recorded low growing rates of 11.33 cm at *Rosa californica*, 14 cm at *Rosa rugosa*, and the greatest growing rates were recorded at *Rosa damascena* with a stem growing of 19.66 cm and 16.33 at *Rosa caudata* (Table 1).

Table 1. Average of stem height (cm) during 28.04.2015 - 10.08.2015

Nr.	Species	28.04	7.05	9.06	11.07	10.08
1	*Rosa californica*	5.33	32	11.66	9.03	11.33
2	*Rosa caudata*	6	7.33	13.66	10.66	16.33
3	*Rosa centifolia*	6	7.66	21.66	1.8	15.33
4	*Rosa damascena*	5	12.66	13.66	14	19.66
5	*Rosa multiflora*	12	12	15	9.66	15.66
6	*Rosa rugosa*	11.66	5.33	14.33	13.66	14

Analysing the number of buds, the lowest values were recorded at specie *Rosa californica* (4.66), and the greatest values were recorded at species *Rosa multiflora* (8.66) and at *Rosa damascena* (10.66) (Table 2).

Table 2. Average of buds number during 28.04.2015 - 10.08.2015

No	Species	28.04	7.05	9.06	11.07	10.08
1	*Rosa californica*	1	1	4.33	6.66	4.66
2	*Rosa caudata*	-	3.33	1	-	-
3	*Rosa centifolia*	1	2	3.33	-	-
4	*Rosa damascena*	-	1.33	3.33	6	10.66
5	*Rosa multiflora*	-	3.33	11.33	6.66	8.66
6	*Rosa rugosa*	1	1	3	5.33	7

Study realised regarding recorded abundance of flowers at the end of observation period appreciate that the lowest values were recorded by *Rosa californica* (1.33) and the highest values were recorded at *Rosa damascena* (8.66) and *Rosa multiflora* (4) (Table 3).
Also was realised a total mean regarding stems height, buds number and flowers abundance in period 28.04.2015 and 10.08.2015 (Table 4) and average for period 09.06.2015 - 09.06.2016 (Table 5), and based on them were designed 6 graphs for year 2015 (Figures 7-12) and 6 graphs for year 2016 (Figures 13-18).

Table 3. Average of flowers abundance during 28.04.2015 - 10.08.2015

No	Species	28.04	7.05	9.06	11.07	10.08
1	*Rosa californica*	-	-	1.66	3.33	1.33
2	*Rosa caudata*	-	-	2.33	-	-
3	*Rosa centifolia*	-	-	3.66	-	-
4	*Rosa damascena*	-	-	2	2.33	8.66
5	*Rosa multiflora*	-	-	8	3	4
6	*Rosa rugosa*	-	-	1.33	2.33	3

Table 4. Total average stems heigt, buds number, flower abundance during 28.04.2015 - 10.08.2015

Nr.	Species	Height of stems (cm)	Number of buds	Abundance of flowers
1	*Rosa californica*	13.93	3.53	1.26
2	*Rosa caudata*	10.79	0.86	0.46
3	*Rosa centifolia*	13.66	1.26	0.73
4	*Rosa damascena*	13	4.26	2.60
5	*Rosa multiflora*	21.44	6	3
6	*Rosa rugosa*	11.79	3.46	1.33

Table 5. Average stems height, buds number, flower abundance during 9.06.2015 - 9.06.2016

No.	Species	Average at 9.06.2015			Average at 9.06.2016		
		Stems height (cm)	Buds number	Flowers abundance	Stems height (cm)	Buds number	Flowers abundance
1	*Rosa californica*	11.66	4.33	1.66	13.33	6.33	2.33
2	*Rosa caudata*	13.66	1	2.33	15	2	4
3	*Rosa centifolia*	21.66	3.33	3.66	24	5.33	5.33
4	*Rosa damascena*	13.66	3.33	2	15.66	5	3.33
5	*Rosa multiflora*	15	11.33	8	16.66	13	10.33
6	*Rosa rugosa*	14.33	3	1.33	15.66	4.33	3

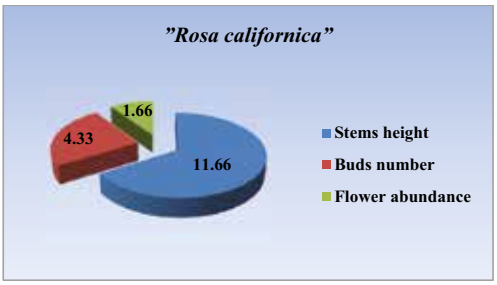

Figure 7. Average stems height, buds number, flowers abundance at 09.06.2015 for *Rosa californica* variety

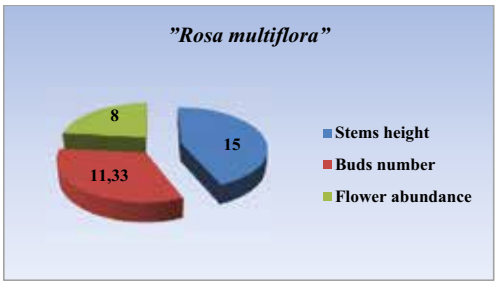

Figure 11. Average stems height, buds number, flowers abundance at 09.06.2015 for *Rosa multiflora* variety

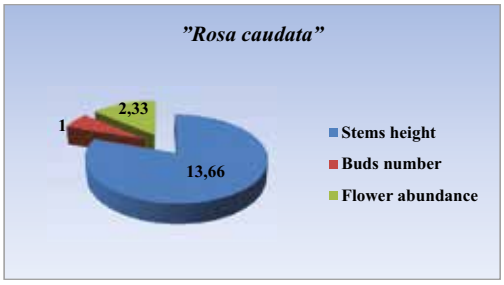

Figure 8. Average stems height, buds number, flowers abundance at 09.06.2015 for *Rosa caudata* variety

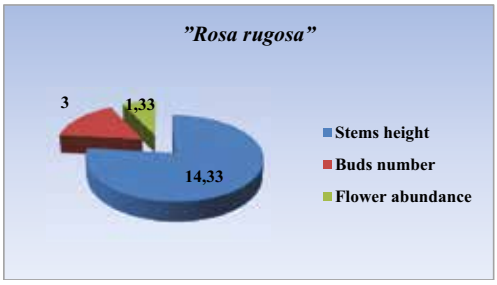

Figure 12. Average stems height, buds number, flowers abundance at 09.06.2015 for *Rosa rugosa* variety

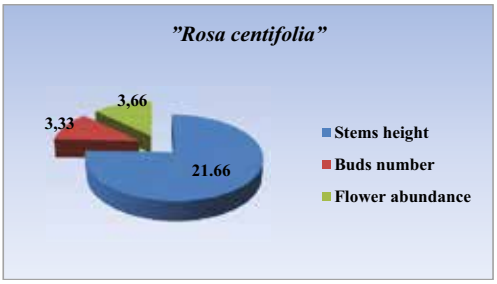

Figure 9. Average stems height, buds number, flowers abundance at 09.06.2015 for *Rosa centifolia* variety

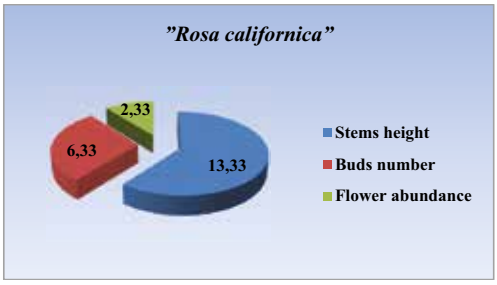

Figure 13. Average stems height, buds number, flowers abundance at 09.06.2016 for *Rosa californica* variety

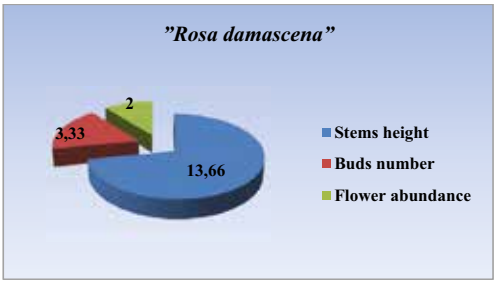

Figure 10. Average stems height, buds number, flowers abundance at 09.06.2015 for *Rosa damascena* variety

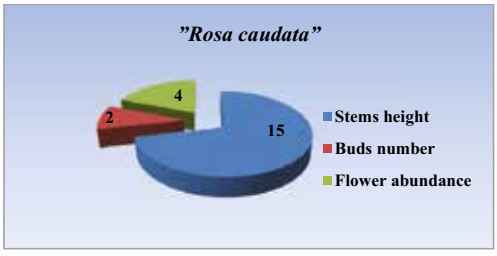

Figure 14. Average stems height, buds number, flowers abundance at 09.06.2016 for *Rosa caudata* variety

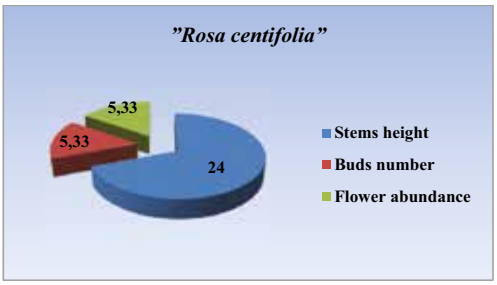

Figure 15. Average stems height, buds number, flowers abundance at 09.06.2016 for *Rosa centifolia* variety

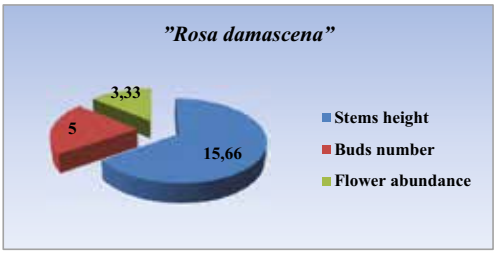

Figure 16. Graphs regarding average stems height, buds number, flowers abundance at 09.06.2016 for *Rosa damascena* variety

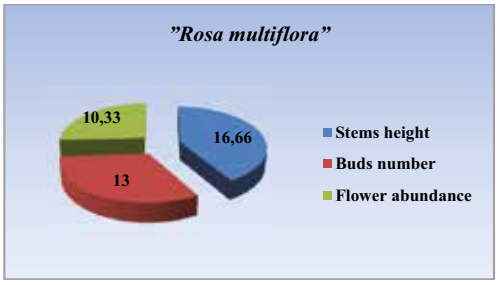

Figure 17. Average stems height, buds number, flowers abundance at 09.06.2016 for *Rosa multiflora* variety

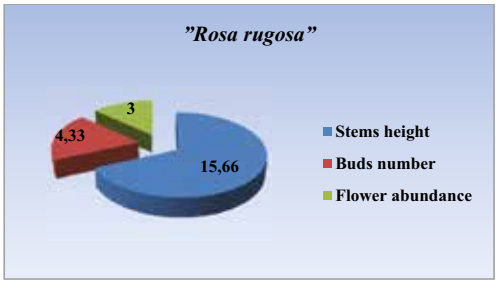

Figure 18. Average stems height, buds number, flowers abundance at 09.06.2016 for *Rosa rugosa* variety

CONCLUSIONS

Based on the effectuated determinations, observations and analyse of the obtained results we could draw the following conclusions:

1. Regarding the stems growing rhythm:

- spontaneous species of *Rosa* have a different growing rhythm;
- the highest growing rate: *Rosa multiflora* with an annual mean of 21.4 cm, and the lowest one at *Rosa caudata* 10.79 cm.

2. Regarding number of buds:

- buds number is different function of their appearance period on stems and function of specie;
- at *Rosa californica*, *Rosa centifolia* and *Rosa rugosa,* buds appear in the second decade of April and have a mean of 1 bud. At *Rosa multiflora* and *Rosa caudata* buds appear on stems in the first decade of May with a mean of 3.33 buds.

3. Regarding abundance of flowers:

- flowers differs through abundance and decoration period;
- *Rosa centifolia*, decoration period is in June with a flowers abundance mean of 3.66, and *Rosa damascena* have a decoration period between June-July-August with an average of 2.60.

4. Regarding development of *Rosa* spontaneous species between 9.06.2015 and 9.06.2016 we could appreciate that:

- growing rhythm of stems is between 1.33 cm (*Rosa rugosa*) to 2.34 (*Rosa centifolia*);
- buds number in these period increase in average from 1 (*Rosa caudata*) to 2 (*Rosa centifolia* and *Rosa californica*);
- flowers abundance at majority of studied *Rosa* species increases with a mean value around 1.67.

Having in view the tradition of rose cultivation, their biological potential and high decorative value, it is recommended utilisation of *Rosa* genus spontaneous species for:

- parent stock for noble sorts;
- decorative purposes in the majority of landscape designs.

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STUDIES ON GROWTH AND DEVELOPMENT OF *HEDERA HELIX* L. ON DIFFERENT WOODY SPECIES

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Abstract

The most known spontaneous species of woody climber, common ivy, it was found to prefer some species more then others as host for its growth and development. Different trees and shrubs from the biggest urban park in Bucharest were investigated to find the presence of spontaneous growth of Hedera helix on their trunks and branches. Seven species of trees and three shrubs were labeled as preferred hosts (100% presence) for Hedera helix, no matter their age or health condition, while four species of trees and nine shrubs were found as totally inconvenient (0% presence) for ivy growth. It was concluded that some species of woody plants create better conditions for Hedera helix to grow as an invasive plant, with considerable repercussions on the plantations management programs.

Key words*: Hedera helix, host species, invasive growth, plantations management.*

INTRODUCTION

Ivy (*Hedera helix* L.) is a natural presence in the European temperate forests, especially broadleaves ones (Rizzetto et al., 2016; Moser et al., 2017). Unlike other lianas species of these habitats, such as *Clematis vitalba* or *Lonicera caprifolium*, ivy can grow at the ground level, covering the soil, or at the canopy layer, climbing the trees. Thus, *Hedera* species were considered having a positive impact on the forest, in general, but mainly on the host tree, because of nutrients inputs in spring with the foliage fall, tree stability and attracting and hosting desirable organisms (Trémoliéres et al., 1988; Bell et al., 2012; Smets et al., 2016; Ruggeri et al., 2016).

In urban environment, green areas are made by a mixture of native and exotic species, for ornamental reasons but also as a consequence of preventing the cities problems such as pollution or poor soils (Barrico et al., 2018; Müller et al., 2018; Vieira et al., 2018). Furthermore, the remaining urban forests were sometimes completed with exotic species of trees and shrubs. However, some of the native species, such as *Hedera helix* shows local invasions in these plantations, especially when the human interference is absent. Originally, ivy appears here from the seeds brought by various birds, which consume the fruits (Mitchell, 1975; Reichard, 2000). Then, the ivy grows covering the ground and climbing the trees, in some cases became invasive (Beekman, 1984; Trémoliéres et al., 1998; Badre et al., 1998; Schnitzler and Heuzé, 2006). On some species of trees *Hedera* may add weight and increase the storm damage (Reichard, 2000; Schnitzler and Heuzé, 2006). Invasive growth necessitates costly eradication programs in some countries.

The objectives of this work were to examine ivy population dynamics in forest-like plantations of one of the biggest urban park in Europe and provide information about host species that ivy prefers to attach and develop.

MATERIALS AND METHODS

The study was conducted in Herăstrău Park, situated in the northern side of Bucharest, Romania. Opened in 1936, the park covers an area of 187 ha and is bordering by high traffic streets. For this reason, the peripheral limits of the park were designed as a protective zone, made from massive trees and shrubs plantations.

In these plantations, various species, mainly broadleaves, both native and exotic, grow together without any human intervention, as a

natural forest. The structure of these plantations is characterized by medium-low dense woody vegetation, developed in three levels: shrubs, small trees and medium-big trees.

We analysed in this study the ivy plants, from natural spreading, which appear frequently covering the ground or the plants (both trees and shrubs) in these massive plantations.

Area of study was limited at the south of the park (approximate 20 ha), where ivy is much more present, growing frequently on trunks and branches of trees and shrubs.

For evaluation of ivy preference for certain hosts, the proportion of invaded hosts and condition of the hosts (Table 1), this area was divided in plots. In each plots, all shrubs and trees of >5 cm diameter at breast height (dbh) were inventoried, identified and analysed for age, health condition and ivy presence.

Table 1. Category of woody plants condition

Class	Condition	Description
A	Excellent	Healthy, vigorous plants
B	Medium	Healthy, vigorous plants in general, but with maximum 25% wounds or dead branches. No disease or parasitic attack.
C	Poor	Plants vigour affected. Unhealed wounds or chronic parasitic attack to maximum 50%.
D	Irreversible decline	Plants with large dead branches, cavities or signs of internal decay to maximum 75%. Irremediable damaged.
E	Dead	-

Observations and measurements were carried out during the autumn-winter in 2016 and 2017. The relationship between quantitative data was examined using statistical analysis (Pearson's *R*).

RESULTS AND DISCUSSIONS

Ivy was found frequently growing on trees located at the edge of the tree massive, near main alleys or lawns. In some zones of the massive plantations, ivy was present only at the ground level. However, we establish a mean density of ivy hosted by shrubs and trees of 36.4 ivies per hectare of green area.

The host trees dbh values indicated that ivy was more present on mature trees (Figure 1). The number of host trees with small dbh values was expected to be lower, because of climbing of ivy on particular barks. Anyway, we observed that on young host trees the climbing behaviour of ivy was stronger in exotic species (68% at broadleaves species and 100% conifers). From these non-native species, we remarked ivy climbing more often: *Acer negundo*, *Celtis occidentalis*, *Robinia pseudacacia*, *Thuja orientalis* and *Pinus nigra*.

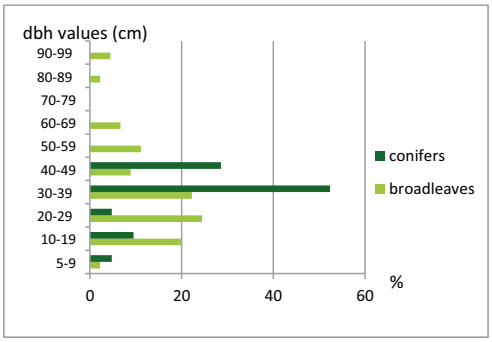

Figure 1. Frequency of ivy on different dbh of host trees

Independent of species, the diameters of large host tress were in positive correlation with the height of ivy on trunks (Pearson's $R = 0.54$, $P < 0.001$). The occurrence of ivy per layers of vegetation was considerably higher at shrub layer (Table 2).

Table 2. Distribution of ivy per layers of vegetation

	Shrub layer (1.5-5m)	Subcanopy (5-15 m)	Canopy (>15m)
Mean density of host plants (per ha) in different layers	5.9	18.9	11.6
Occurrence of ivy per layers of vegetation (%)	62	34	4

Although subcanopy is much more represented in the massive plantations of this park, ivy grows and develops better below these, particularly because of the light conditions offered at this level. Most of the trees growing in the subcanopy have small or medium leaves (57%, respectively 23%) and low density crowns.

Ivy proved to choose certain species as hosts (Figure 2). According with the ivy preference, four groups of host species were identified: the most attractive (100% of the trees support ivies), highly attractive (>60% of the trees support ivies), attractive (20-50% of the trees support ivies) and less attractive (<20% of the trees support ivies).

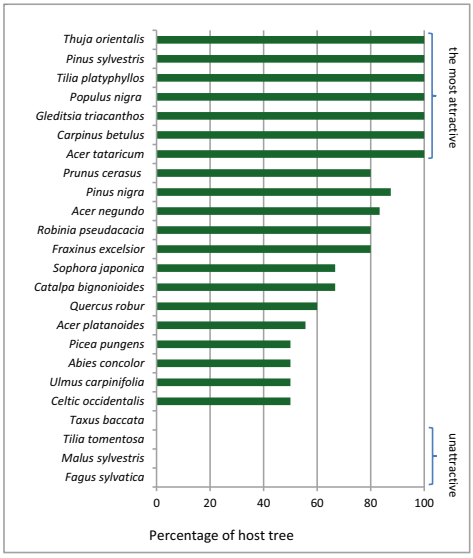

Figure 2. Preference of ivy for host tree species

Seven species of trees are the most preferred by ivy: *Acer tataricum, Carpinus betulus, Gleditsia triacanthos, Populus nigra, Tilia platyphyllos, Pinus sylvestris* and *Thuja orientalis*. Other eight tree species were found highly attractive for ivy, most of them exotic and much appreciated for their biological and ecological characteristics for this type of plantations. Four species (all native) proved to be unattractive for ivy: *Fagus sylvatica, Malus sylvestris, Tilia tomentosa* and *Taxus baccata*.

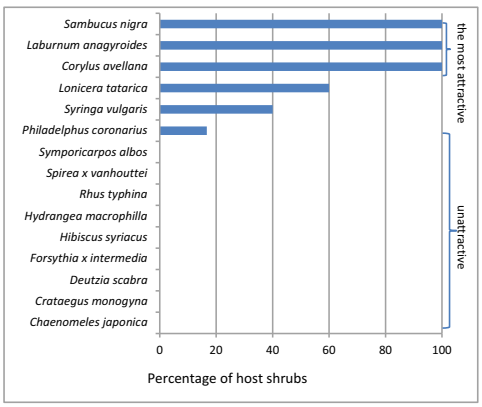

Figure 3. Preference of ivy for host shrubs species

For the shrubs placed near or in the massive tree plantations, most of them exotic species (65%), ivy proved also a different attractiveness (Figure 3). The majority of shrubs species were unattractive for ivy. In this case, even the ivy was present at the ground level or in trees nearby shrubs (in different light conditions) it was not covering at all their base or branches. However, three of them - *Corylus avellana*, *Laburnum anagyroides* and *Sambucus nigra*, all native species with vigorously growing, were highly preferred by ivy.

The evaluation of hosts' condition showed a tendency of ivy to climb trees in poor and irreversible decline more than the others (Table 3).

Table 3. State of vegetation of the host trees (%)

	Class A	Class B	Class C	Class D
Broadleaves species	14.5	20.8	20.8	43.7
Conifers species	11.1	33.3	55.5	-

Correlation between trees' state of vegetation and the incidence of ivy on their trunk showed that ivies are significantly more present in trees with poor condition (Pearson's $R = 0.63$, $P < 0.001$).

Still, in some species, such as: *Acer negundo, Acer tataricum, Carpinus betulus, Catalpa bignonioides, Fraxinus excelsior, Gleditsia triacanthos* and *Robinia pseudacacia*, even with a very good condition of trees (class A), ivy was found growing on their trunk. All of these species develop a rough bark since early stages of growth. These results confirm some other studies (Hegarty and Caballe, 1991; Schnitzler and Heuzé, 2006; Leicht-Young et al., 2010; Steinbrecher et al., 2010), which demonstrated that ivy, like other lianas, prefer the rough barks for support.

The tendency of invasive growth of ivy was observed at some species (Figure 4). A proportion of coverage over 60% of the total surface of host plant was remarked at 15 different species of trees and shrubs. Over 60% from these are exotic species and commonly present in urban green spaces.

Values of host coverage were extremely high (85%) at some species: *Catalpa bignonioides, Fraxinus excelsior, Gleditsia triacanthos, Lonicera tatarica, Quercus rubra, Robinia pseudacacia, Sambucus nigra, Syringa vulgaris* and *Thuja orientalis*.

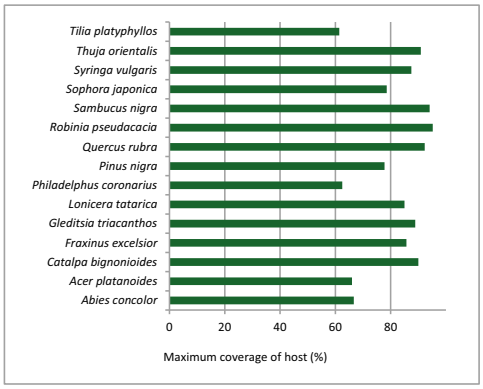

Figure 4. Percentage of ivy coverage at some species

The invasive growth of ivy is rare in urban plantations, but it was reported by some authors for ivies growing in natural forest (Schnitzler and Heuzé, 2006; Rizzetto et al., 2016).

CONCLUSIONS

Urban massive trees and shrubs plantations are made of native and exotic species. Ivy coming from natural sites can appear and populate dense plantations of green areas in certain conditions. Our results showed that some of the exotic species, especially trees, are more susceptible as host for ivy. In this case, maintenance of massive plantations can require further attention and cost more. For this reason, selection of unattractive or less attractive species for ivy or even reducing the proportion of attractive host species may be a future way to solve the problem of managing ivy propagation in urban plantations.

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POLLEN ANALYSIS IN SOME TULIP CULTIVARS

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Abstract

Viability and germination capacity of pollen grains is very important in breeding programs which require a minimum 30% germination level for the success of artificial hybridization. This paper presents the pollen viability and germination capacity analyses performed in nine tulip cultivars that belong to six different groups and the correlation between these two determinations. The viability of the pollen grains registered higher values in eight, out of nine cultivars ('Yokohama' - 81.7%, 'Veronique Sanson' - 93.9%, 'Tender Whisper' - 90.7%, 'Paul Scherer' - 86.0%, 'Davenport' - 84.8%, 'Fancy Frills' - 92.9%, 'Blue Parrot' - 96.1%, 'Red Riding Hood' - 89.8%), while in the case of germination capacity only four cultivars ('Red Riding Hood' - 35.9%, 'Blue Parrot' - 81.1%, 'Davenport' - 78.3% and 'Salmon Impression' - 27.7%) registered the necessary rate for the breeding programs. The lower germination percentage of pollen grains, in some cultivars, may be due to the duration of storage period of the pollen.

Key words*: breeding, pollen grain germination, pollen grain viability, tulips.*

INTRODUCTION

Although there are thousands of tulip cultivars over the world, breeders do not cease studying and creating new ones in order to satisfy the need for beauty of all mankind. Tulips, along with daffodils, hyacinths, gladioli are the most widely cultivated bulbous spring plants used to decorate green areas and also as cut flower in bouquets and other floral arrangements.

The study of germination capacity of pollen grains is very important in the selection of genitors and in a successful artificial hybridization, where its level should be at least 30% (Cordea, 2014). Germination capacity of pollen grains may be determined indirectly by testing the pollen viability and directly, by pollen germination *in vitro* on solid nutrient medium. Viability of pollen grains may be revealed with simple colouring tests (with potassium iodide or carmine staining method). It is well known that there is a very strong direct, positive correlation between germination and viability of pollen grains (Cordea, 2014).

The objective of this study was to evaluate the pollen germination and viability in nine tulip cultivars belonging to six classification groups in order to be used in breeding programs.

MATERIALS AND METHODS

Nine tulip cultivars belonging to six classification groups were tested in order to determine the germination capacity and viability of pollen grains (Brickell and Zuk, 1997; Raamsdonk and de Vries, 1996) (Table 1), tests having been performed in the Plant breeding laboratory of the Advanced Horticultural Research Institute of Transylvania, UASVM Cluj-Napoca.

Determination of germination

Germination capacity of pollen grains presumes that the fertile grains with viable cytoplasm and spermatia germinate and release pollen tube on an artificial nutritional medium enhanced with different sucrose concentrations (10-30%), with 70-90% humidity at a temperature between 20-22°C. There are different opinions on the concentration of the two ingredients which form the nutritional medium. Soares et al. (2013) has obtained the best results on a

medium with 15% sucrose and 0.8% agar in *Passiflora* sp. Some authors (Chagas et al., 2008) observe that higher concentrations of agar increase the germinated pollen grain ratio in pear cultivars while others (Luza and Polito, 1985) came to the conclusion that in nut cultivars a relative high concentration of agar inhibits pollen germination and decreases the pollen tube length.

Our tests were performed on solid nutritional medium according to the protocol described by Cordea (2014). In this regard anthers were collected from unopened flower buds before the pollen has reached maturity. The anthers have been placed on Petri dishes and maintained for a period of 48-72 hours in room temperature (22^{o}C) until they broke and released the pollen grains. The dried pollen grains have been sown on solid medium made of 1.5% agar and 15% sucrose with 85% humidity, preserved at 22^{o}C temperature.

The microscope readings have been performed in 24 hours after sowing the pollen grains on the nutritional medium. In order to obtain a precise counting of the examined grains there were chosen certain uncrowded microscopic fields and observations have been made with the 4x and 10x lenses (Figure 1).

Table 1. The tulips cultivars at UASVM Cluj-Napoca

No.	Cultivars	Groups	Flower colour
1	'Yokohama'	I – Single early tulips	Yellow
2	'Veronique Sanson'	III – Triumph tulip	Orange with red
3	'Tender Whisper'	III – Triumph tulip	Fuchsia with white
4	'Paul Scherer'	III – Triumph tulip	Dark purple
5	'Salmon Impression'	IV – Darwin hybrid tulips	Light pink salmon
6	'Davenport'	VII – Fringed tulips	Red with yellow
7	'Fancy Frills'	VII – Fringed tulips	Pink
8	'Blue Parrot'	X – Parrot tulips	Violet
9	'Red Riding Hood'	XIV – Greigii tulips	Red

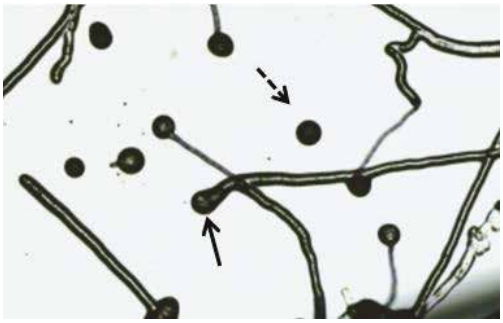

Figure 1. Pollen grain germination in tulips - magnification 10x (filled arrow - germinated grains; dashed arrow - non-germinated grains) (source:original)

Viability analysis

Viability of pollen grains was examined by means of the potassium iodide colouring test. In the presence of the colouring agent fertile grains with viable cytoplasm and spermatia will colour in black/dark brown while the sterile ones will remain colourless (Figure 2).

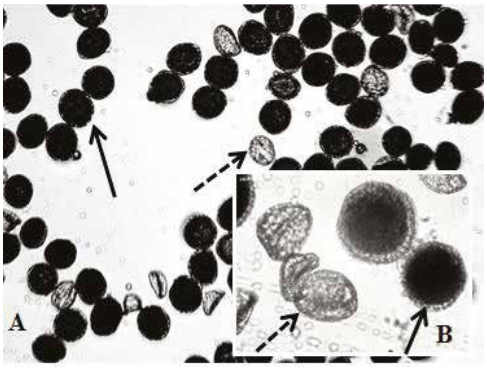

Figure 2. Pollen grain viability in tulips: A - magnification 10x; B - magnification 40x (filled arrow - viable grains; dashed arrow - non-viable grains) (source:original)

In this purpose, anthers were sunken immediately after collecting in the Carnoy fixative solution for a period of 1.5 hours then passed into alcohol until determination.

For high accuracy, in both determinations there were analysed about 300 pollen grains in several microscopic fields, then the rate (%) of germination and viability was calculated.

RESULTS AND DISCUSSIONS

In plant breeding the artificial cross pollination is a very important proceeding in creation of genetic variability, which will then be exploited by selection in order to obtain new cultivars with higher ornamental value than those on the market.

In tulip species there are multiple incompatibility barriers in interspecific hybridization as described by Kho and Baër (1971). To achieve a successful artificial hybridization, pollen grains should have a minimum 30% germination rate, very important to be taken in consideration when choosing the genitors in plant breeding programs.

The analysis of pollen grains of each cultivar of our tulip collection is presented in Figure 3.

Examining the results obtained there can be noticed that the highest germination capacity rate has been registered in 'Blue Parrot' cultivar (81.1%) while the lowest in 'Veronique Sanson' (7.8%).

It is also noticeable that only four cultivars ('Blue Parrot' - 81.1%, 'Davenport' - 78.3%, 'Red Riding Hood' - 35.9%, 'Salmon Impression' - 27.7%) have exceeded the 30% germination rate, the minimum level for a successful artificial hybridization.

Within the groups germination capacity varies as it follows: all the three cultivars belonging to group III - Triumph tulip, presented very low germination rate ('Veronique Sanson' - 7.8%; 'Tender Whisper' - 14.3% and 'Paul Scherer' - 11.0%) while in group VII - Fringed tulips - results are different. 'Devenport' records a rather high germination rate (78.3%) while in 'Fancy Frills' only 23.3% of the pollen grains germinated. This denotes that in cultivars of the same group pollen grains do not necessarily germinate in the same rate.

As far as viability is concerned data presented in Figure 3 reveal a higher rate than germination. Almost all cultivars, regardless the group they belong to, present a rather high viability rate between 81.7% ('Yokohama') and 96.1% ('Blue Parrot').

Cultivars of group III - Triumph, present a high viability level ('Veronique Sanson' - 93.9%; 'Tender Whisper' - 90.7% and 'Paul Scherer' - 86.0%) as compared to their germination rate which does not succeed to reach the necessary minimum 30%. The same situation is observed in 'Yokohama' and 'Fancy Frills' cultivars.

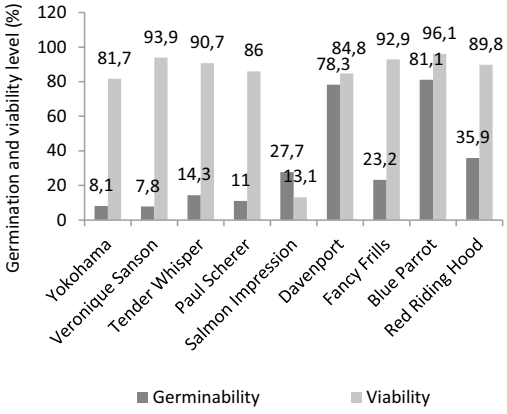

Figure 3. Pollen grain germination and viability in nine tulip cultivars, UASVM Cluj-Napoca, 2017

A special behaviour presents the 'Salmon Impression' cultivar (IV - Darwin hybrid tulip group) which encountered a rather low rate both in germination and viability.

According to the data presented in Figure 3 it can be noticed that the viability of pollen grains in all tested cultivars reached much higher levels than their germination rate, except the 'Salmon Impression'.

CONCLUSIONS

Based on these results there has been reached the conclusion that displaying a higher viability than germination rate might be due to the length of the drying and preservation period of pollen between collecting and analysis (4-5 days). This suggests that the fertile period of tulip pollen is rather short, only a few days.

As a consequence of the pollen viability and germination analysis in our tulip collection there can be concluded that only three cultivars ('Davenport', 'Blue Parrot' and 'Red Riding Hood') presented fairly high rates in both determinations, while the 'Red Riding Hood' cultivar slightly passed the required germination level. These results offer important information for selecting the best female/male genitor in tulip breeding programs for higher ornamental value.

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THE EFFECT OF DIFFERENT LIGHT CONDITIONS ON THE GROWTH AND DEVELOPMENT OF *CHLOROPHYTUM AMANIENSE* ENGL. 'FIRE FLASH'

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Abstract

Chlorophytum amaniense Engl. is a foliage plant, member of the family Liliaceae, originates from the rainforests of East Africa. 'Fire Flash', the only cultivar of this species, known by several common names including Fire Glory, Mandarin Plant and Tangerine, present unique decorative characteristics. C. amaniense, 'Fire Flash' do not support the direct action of the sun's rays and the placement in bright exposures. Given the claims of C. amaniense versus light and insufficient information in the literature, the objective of this research was to study the reaction of 'Fire Flash' under different lighting conditions. Plants attained the greatest leaf size and petioles when , 'Fire flash' were grown in low and medium light intensity while plants exposed to high light showed lowest values of growth parameters. In addition, high light intensity produced chlorosiss and leaf burn and plants have become unmarketable after a 3 months period. The best size and quality of 'Fire Flesh' plants, occurred when plants were grown at a medium light levels of 80 µmol m^{-2} s^{-1}.

Key words*: Chlorophytum amaniense, growth, light, photosynthetic parameters.*

INTRODUCTION

The genus *Chlorophytum* belongs to *Liliaccae* and encompasses about 200 species chiefly native to tropical Africa, Australia and Asia (Anton, 2009). Most species are evergreen perennials with rhizomatous roots either short and fibrous or thick and translucent.

Chlorophytum amaniense Engl., member of the family Liliaceae, is a foliage plant originates from the rainforests of East Africa. 'Fire Flash', the only cultivar of this species, known by several common names including Fire Glory, Mandarin Plant and Tangerine, present unique decorative characteristics (Anton Doina et al., 2006).

Bright coral petioles and midveins contrast with deep green, ovate-lanceolate leaves making an exotic appearing and exciting plant.

The flowers are white in a dense cylindrical panicle partially hidden by the foliage.

The inflorescence does not add any value to the aesthetic appearance. In fact, it is detrimental. Removal of the inflorescence at an early stage of flowering improves plant growth (Chen et al., 2002).

Unlike *C. comosum* (Thunb.) Jacques (spider plant), *C. amaniense* 'Fire Flash' does not produce stolons, and propagation could be realized through seed, division and *in vitro* regeneration (Cui et al., 2011).

In addition to ornamental value, the rhizomes of 'Fire Flash' form nearly oval tubers, which may contain antitumor steroidal saponins as do other species of *Chlorophytum* Ker. Gawl (Kaushik, 2005; Cui et al., 2011). Of the twelve species analyzed, *C. amaniense* Engl., showed highest phenol, flavonoid, saponin contents and *in vitro* antioxidant activity (Shinde et al., 2016; Patil, 2016).

'Fire Flash' is grown in shady greenhouses at temperatures between 18-32°C (optimum temperature between 24-29°C) and relative humidity between 50% and 100%. Water used for 'Fire Flash' production should be free of fluoride since it may cause leaf necrosis. Light intensity is extremely important for quality of ''Fire flash" plant production (Chen et al., 2002). *C. amaniense* 'Fire Flash'do not support the direct action of the sun's rays and the placement in bright exposures. Depending on the intensity of light, the leaves may have

different shades from intense green to light green. It also influences the color and length of petioles, these being the main decorative element. Higher light levels will cause leaf chlorosis and, eventually, scorching and necrotic lesions that can cause unsalable plants. Based on the evaluation study, Chen et al. (2005 a) recommended that 'Fire Flash' can be propagated through seed, division, or tissue culture and produced as a potted foliage plant under light levels from 114 to 228 μmol m^{-2} s^{-1} and temperatures from 18 to 32°C. After being placed in building interiors, plants should be located in interior light levels between 50 to 200 foot candles (10-40 μmol m^{-2} s^{-1}) (Chen et al., 2005 b). 'Fire Flash' plants are able to maintain their aesthetic appearances under a low light level of 8 μmol m^{-2} s^{-1} for 8 months or longer (Chen et al., 2005 a).

Given the claims of *C. amaniense* 'Fire Flash' versus light and insufficient information in the literature, the objective of this research was to investigate the response of *C. amaniense* plants under different light intensities. For this aimes a pot experiment was conducted to analyze growth parameters and some photosynthetic parameters.

MATERIALS AND METHODS

The experiment was established at the Floriculture Research Area, Faculty of Horticulture from Craiova (Romania), during February to August, 2016. The biological material consists of plants of *Chlorophytum amaniense* Engl. 'Fire Flash' from the didactic greenhouse of the Floriculture discipline.

Ambient temperatures ranged from 20°C-22°C and relative humidity from 60%-80%.

We studied the effect of different lighting intensity of the 'Fire Flash' plants to assess the effect on morphological, ornamental and some photosynthetic characteristics.

Young plants of *C. Amaniense* 'Fire Flash' (obtained from seeds), with 4-5 leaves, were selected and transplanted in the first week of February 2016 into black, 2.8 liter pots (17 cm diameter) filled with a substrate consisting of a 40:30:30 mix of peat, coconut fibre, and perlite. Controlled-release fertilizers (Osmocote 18-6-12) was added to the substrate. Plants were placed in a greenhouse with natural light for 8-10 hours per day and were maintained in moderate shade during the first month and then transferred to the different light treatments (in the first week of March 2016), by placing the plants in three areas of the greenhouse with different light level: low light intensity (LL): 7.98-9.12 μmol·m^{-2} s^{-1}; medium light intensity (ML): 77.9-82.46 μmol·m^{-2} s^{-1}; high light intensity (HL): 220.4-233.7 μmol·m^{-2} s^{-1}.

The light intensity inside the greenhouse was measured with a Lux meter at 10.30 am, 2.00 pm and 4.00 pm, in three zone of greenhouse with different light levels. These evaluations were performed on fivedifferent days in an interval of five months corresponding to the experimental time. The luminous flux per unit area was converted into photon flux density (PFD) using a conversion factor of 0.0185 μmol photons m^{-2} s^{-1} per lux valid for sunlight (Hershey D.R., 1991; Pedersen et al., 2016).

The experiment was conducted in a completely randomized design, using a monofactorial arrangement with three treatments represented by three levels of light availability (T1-LL; T2-ML; T3-HL), with three replicates, and five plant per experimental unit.

The observations on average height of the plants, number of leaves per plant, leaves size, lenght of petiole, width of petiole were recorded 90 days after the experiment was established. We also measured some photosynthetic parameters: the incident radiation in the leaf (Qleaf) expressed in μmol photons m^{-2} s^{-1}; stomatal conductance (gs), mol H_2O m^{-2} s^{-1}, transpiration (E), mmol H_2O m^{-2} s^{-1}, net photosynthesis (A), μmol CO_2 m^{-2} s^{-1}, on the third or fourth leaf counting from the apex to the base, by using Lcpro+® Portable Photosynthesis System. Water Use Efficiency (WUE; μmol CO_2 $mmol^{-1}$ H_2O) was calculated as A to E ratio (Ribeiro et al., 2009). The data were submitted to variance analysis and the averages compared by Tukey test at 5% error probability ($p < 0.05$) in MINITAB 16 software.

RESULTS AND DISCUSSIONS

Effects of Light Intensity on Plant Growth

In ornamental horticulture, the leaf forms and color, sizes, and shapes of pot plants is essential components of their visual quality that determines the commercial value of the

products (Boumaza et al., 2010). Light intensity influence plant characteristics and quality attributes (Runkle, 2013). Knowledge of the morphological and physiological characteristics of *C. amaniense* in response to various light conditions is still sparse. Clear external differences were observed among plants grown under five months under different light intensities.

Regarding the average height of the plants, the highest values were recorded in an exhibition characterized by low light intensity (LL-13,7 cm). Moreover, the plants grown in a high intensity area of light recorded the lowest average height (HL-9.7 cm). Similarly, shade induced more growth in height in *Passiflora edulis* f. *flavicarpa* plants (Valladares et al., 2000; Zanella et al., 2006). Apical dominance tends to increase when plants are submitted to high shade levels, due to a decrease in the production of photoassimilates and the highest level of auxin at the stem apex bud (Vanneste and Friml, 2009; Woodward and Bartel, 2005)

The average leaf number on the plant was maximum under medium illumination (ML - 9.7), and in variants grown under low or high light conditions, the values of this parameter were significantly reduced (LL - 8.3 leaves, respectively HL-7.3 leaves) (Table 1).

Placement of plants in areas with different luminous intensities caused significant differences in the average leaf length. Compared to plants grown in intense light, where the average value of this parameter was minimal (HL-21.5 cm), the plants placed in a low light intensity recorded the highest value (LL-32.5 cm), followed at a significant difference by the variant in which the plants received a medium light intensity (ML - 27.2 cm). In contrast, the increase of the leaf width was proportional to the decrease in the shade, with the highest average values occurring under high light intensity (HL-5 cm) (table 1). The lowest value corresponds to the plants grown in an area with a minimum intensity of light (LL - 4.5 cm).

Low light intensity may lead to increase in leaf number and leaf size and these changes may maximize the capture of available light to meet the demand for leaf photosynthesis (Steinger et al., 2003).

Regarding the average dimensions of the petioles, the main decorative element of this species, there were also significant differences according to the intensity of the light. The average length of petioles recorded the lowest value at HL (3 cm), and the highest value was recorded at LL (6.8 cm). Instead, average petiole widths were between 1.2 cm in low light intensity (LL) plants and 1.7 cm in intensive light (HL) plants.

In addition to the measurable parameters, the study of *C. amaniense* 'Fire Flash' plants under different illumination conditions had the following results: low intensity (7.98-9.12 µmol $m^{-2}s^{-1}$) caused a more intense color of the leaves and the petiole, the elongation and thinning of the main decorative element, the petiole; high intensity (220.4-233.7 µmol $m^{-2}s^{-1}$) produced chlorosiss and leaf burn and plants have become unmarketable after a 3 months period. Visual observations indicated that *C. amaniense* produced commercially acceptable plants at 7.98-9.12 µmol m^{-2} s^{-1}, however the optimal growth and development occurred from 77.9-82.46 µmol m^{-2} s^{-1}. Although Chen et al. (2005 a), recommends as optimal light interval 10-40 µmol m^{-2} s^{-1} for 'Fire Flash' production, our results suggest that a light level of about 80 µmol m^{-2} s^{-1} appeared to be optimal.

Effects of light intensity on plant photosynthetic parameters

Physiologically, light has both direct and indirect effects. It affects on metabolism directly through photosynthesis, whilst indirectly through growth and development (Zhang et al., 2011). Table 2 presents the effects of different light intensities on leaf photosynthetic parameters.

Net CO_2 assimilation (A) was highest in HL (7.73 µmol CO_2 m^{-2} s^{-1}) followed by ML (6.81 µmol CO_2 m^{-2} s^{-1}) treatment and, finally, LL treatment (5.44 µmol CO2 m^{-2} s^{-1}). Similar results were obtained on *Dieffenbachia longispatha* and *Camellia x williamsii* (Skillman et al., 2005; Fini et al, 2010). The authors have shown that that A was higher in full sun and mild shade plants if compared to heavy shaded plants.

The greatest leaf stomatal conductance (gs) was observed under medium light intensity (ML-0.12 mol H_2O m^{-2} s^{-1}), and the lowest under low light intensity (LL-0.03 mol H_2O m^{-2} s^{-1}).

Stomatal conductance (gs) was signifycantly higher in ML than that of all other treatments. As also reported in other works, plants grown at high light are characterized by greater stomatal conductances than plants grown at low light (Baroli et al., 2008; Niinemets et al., 2006). Previous studies also showed thatchanges in photosynthesis and transpiration were correlated with stomatal conductance (Greer, 2012; Miyashita et al., 2012).

Transpiration (E) was affected by different shading treatments and followed a similar pattern to A. Significant differences were observed between the HL irradiance treatment and all other treatments. There were significant differences on leaf transpiration rate (E) between the HL treatment (1.75 mmol H_2O m^{-2} s^{-1}) and all other treatments (0.71-1.68 mmol H_2O m^{-2} s^{-1}).

Water use efficiency (WUE) varied signify-cantly with light intensity in *C. amaniense* plants. Significant differences were observed between plants submitted to high shade levels (LL-7.61 µmol CO_2 $mmol^{-1}$ H_2O) compared to plants placed under medium and high intensity (ML-4.05 µmol CO_2 $mmol^{-1}$ H_2O, HL-4.46 µmol CO_2 $mmol^{-1}$ H_2O). No significant differences were observed between the ML and HL treatments (Table 2).

Intercellular CO_2 concentration (Ci) showed same evolution as WUE. Light reduction resulted in significantly higher values of Ci (LL-439.33 ppm) (Table 2). Previous studies showed that, in some species, the intercellular CO_2 concentration (Ci) declined with increases with the increase of light intensity (e.g. Hanba et al., 2002; Oguchi et al., 2005).

Our findings show the higher light levels have provoked severe leaf damage, characterized by leafchlorosis or scorching. Huang et al., 2015 show that under high light condition, excess absorbed light energy can induce photoinhi-bition explaining why shade-established species cannot survive under high light.

Table 1. Effect of different light conditions on mean vegetative growth parameters at the end of experiment

Treatments	Height of plants (cm)	Number of leaf (cm)	Length of leaf (cm)	Leaf area (cm^2)	Length of petiole (cm)	Width of petiole (cm)
LL	13.7a	8.3ab	32,5a	109,53a	6.8a	1,2b
ML	11.2b	9.7a	27,2b	95,1ab	4,5b	1,5ab
HL	9.7b	7.8b	21,5c	80,65b	3c	1,7a

Means comparison were done using Tukey's test ($p<0.05$). For each variable lowercase letters indicate comparison among treatments and uppercase ones comparison among species.

Table 2. Net photosynthetic rate (PN), stomatal conductance to water vapour (gs), leaf transpiration rate (E), intercellular CO_2 concentration (Ci) and water use efficiency (WUE) of *C. amaniense* leaves were subjected to different levels of irradiance

Treatments	A (µmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	Gs (mol H_2O m^{-2} s^{-1})	Ci (ppm)	WUE (µmol CO_2 $mmol^{-1}$ H_2O)
LL	5.44 b	0.71 c	0.03 c	439.33a	7.61a
ML	6.81 ab	1.68 b	0.12 a	354.33ab	4.05b
HL	7.73 a	1.75 a	0.08 b	320.33b	4.46b

Means comparison were done using Tukey's test ($p<0.05$). For each variable lowercase letters indicate comparison among treatments and uppercase ones comparison among species.

CONCLUSIONS

The cultivation of *C. amaniense* 'Fire Flash' in Romania is almost nonexistent, though is a exciting ornamental foliage plant as a result of its unique coral-colored midribs and petioles and tolerance to interior low light levels.

Light intensity had different effects on *C. amaniense* growth. The results showed that *C. amaniense* attained greatest hight of plant, number of leaf and leaf size when cultivated under low light intensity. Under high light intensity values of plants grown were the smallest. With the reduction in light intensity,

petioles have elongated by over 50% but their diameter has decreased significantly. Visual observations indicated that *C. amaniense* produced commercially acceptable plants at 7.98-9.12 $\mu mol \cdot m^{-2} \cdot s^{-1}$, however the optimal growth and development occurred from 77.9-82,46 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants exposed to full light conditions (220.4-233.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) become unmarketable within a 3 months period. Our findings suggests that the net photosynthesis (A) was higher in high (HL) and medium light (ML) and low light intensity (LL) can decrease photosynthesis. The results from this study show that a light level of about 80 $\mu mol\ m^{-2}\ s^{-1}$ appeared to be optimal for *C. amaniense* when both morphological and physiological performance were considered.

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TINERETULUI PARK – FUNCTIONAL AND COMPOSITIONAL COMPONENTS IN THE URBAN LANDSCAPE OF BUCHAREST

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Abstract

Tineretului Park is a component of the urban green spaces system of Bucharest, being one of the most important green entities of the city. The current study highlights the diversity of the park landscape functions, according to the urban needs regarding the population's loisir activities. Furthermore, the aspects of the landscape composition are analyzed, as well as the manner in which the socio-economic and urban development conditions have influenced the selection of the location for the future park; moreover, the particular conditions of the site have had a determinant role in the design of the actual landscape-architectural composition, as well in the options for choosing and distributing the loisir functions in the whole park area.

Key words*: urban park, landscape functions, landscape composition.*

INTRODUCTION

Tineretului Park is located in the southern part of Bucharest, the site being bordered by Şerban Vodă Hill, Piscului Hill, Olteniţei Highway and Tineretului Boulevard. During the 1960's the question was raised regarding the development of an urban park in this area, at that time the location being referred to as "Plângerii Valley" (Marcus, 1958). The park's surface is 83 ha, while the surface of the lake is 13 ha.

This enclosure of the site has been envisaged in the systematization and urban development plan of Bucharest since 1956; the park - as distinct green entity - was to be part of large green area ("feather") in the southern part of Bucharest. These aspects of urban development had been provided even since the Interbelic period (1935), when a sketch with general development guidelines of Bucharest was conceived (Marcus, 1958).

MATERIALS AND METHODS

The methods of research and evaluation of the green area "Tineretului" are based on establishing connections between a functional landscape approach (in which function is predominant) and the compositional - stylistic approach specific to the second half of the XX century. The connections target three approach levels for the design solution, which are reciprocally determinant: physical, social - functional and aesthetical. The physical level refers to the determination of the park's location, which was defined by the following considerations:

- the necessity to develop the urban and green landscape towards the southern part of the city;
- the nature of the soil in this area, which (except for Piscului Hill) did not allow the development in normal economical conditions of any type of urban constructions;
- the necessity to fill up the area of existing urban green spaces at that date (Carol Park, Bellu Cemetery) and the development - through this operation of the green area mentioned above;
- the construction of a valuable urban core as a point of perspective closure, which took place along side the important urban artery - Base Line North-South;
- highlighting the most favourable natural elements: the difference of topographic level between the cornice (high ground) and the Dâmboviţa Meadow, also the semi-circular shape - amphitheatre style - of Piscului Hill and Şerban Vodă Hill;

- eliminating an important harmful source in this area of the city, which was affected at that time by the presence of swamps and trash deposited on the site, aspects which led to the formation of a pest hole (Marcus, 1958).
The architectural-landscape composition was founded on the concept of optimal exploitation of the natural configuration of the land. Four study versions for composition design were created (Figure 1).

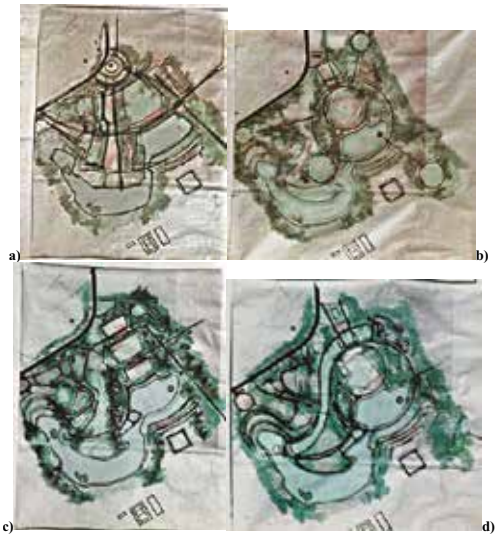

Figure 1. Study versions of Tineretului Park

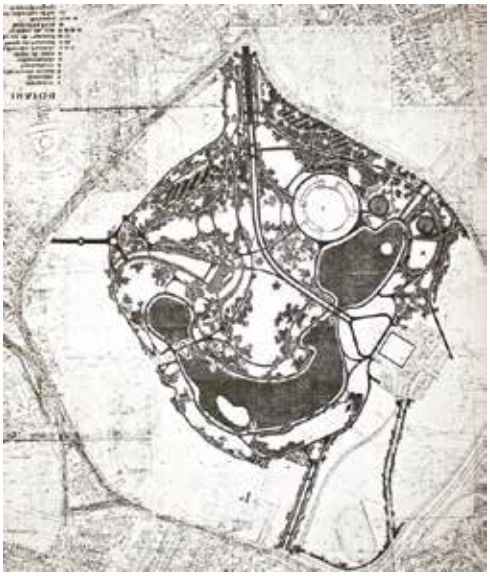

Figure 2. Final composition of Tineretului Park

The style of the final composition (approved by the beneficiary) is a mixt one, with a greater percentage of the free-landscape style (Figure 2). The complex parks of the 20th century find their best compositional solution within the mixt style, which includes and combines in resolution all the processing needs of different functions and areas of the park (Stănescu, 2008).
Regarding composition, the designers developed a complex of access paths, axes and perspective in tight correlation with the main circulation arteries in the area, by which the park is accessed. The access and the main axis of the park lead to the Sala Polivalentă building, where social cultural events are hosted, which involve a large audience. Thus, the most important alley is located alongside the main axis, which is approximately 28 meters high (Figure 2).
The landscape architect chose the preservation of the natural landscape, which is mostly the optimal solution (Stănescu, 2008). However, the terrain, being very degraded, required ample remediation works in order to assure the optimal levels of quality for the vegetation development. In this context, approximately 290000 m^3 of healthy soil were brought on site, quantity mixed in with the existing soil. By means of hydromechanical works, 236000 m^3 of excavation and compensational fillings were carried out.
In the place of the old swampland, a 13 ha lake was created (Figure 2), which is supplied by eight shafts of low depth (20 meters) upstream, while the excess waters are evacuated to the collector at the base of Piscului Hill.
The actual shape of the lake was created by correcting an existing profile, while pursuing the ample modification of the contour, which was supposed integrated in the general composition by shape, sensibility planning solutions conceived by the designer (Stănescu, 2008).

RESULTS AND DISCUSSIONS

The park's functionality is based on the terrain conditions and the particularities of the site. The landscape architect's options for choosing and distributing the loisir functions was determined by the specifications of the project theme and by the needs regarding the spending

of free time of the inhabitants. Regarding this, the functional aspects subscribed to the general trends of urban landscape design in the 20th century: the public gardens and parks have developed in various directions in the 20th century, while the dominant types were represented by recreational and amusement parks (Kluckert, 2005).

On the other hand, a garden should not be a substitute for nature, but an artistic expression for bringing the human being in close contact with nature - this aspect is met by a public park because nature is used as a frame for relaxation and fun (Kluckert, 2005).

By applying the connections method in the compositional approach, the present study demonstrates that Tineretului Park is the result of an integrator concept in the creation of the designing solution, concept which brings together at the same time physical, social – functional and aesthetic elements, all of which based on environmental factors, on the physical characteristics of the site, and also on the urban development and socio-economic factors.

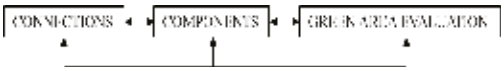

Figure 3. Diagram of the composition's integrated formation

The synthesis of the determinant compositional characteristics of Tineretului Park is presented in Table 1.

Table 1. Synthetic evaluation of the determinant compositional characteristics of Tineretului Park

CONNECTIVITY	COMPONENTS	GREEN AREA EVALUATION		
		QUANTITY	QUALITY	EFFICIENCY IN THE URBAN LANDSCAPE
PHYSICAL	- urban definition - extension of the city's green space system - area landscape - terrain composition	- distance to city centre - surface of the park: 83 ha	- personalization within the city's green space system - urban landmark - conformity with the site's natural character	- urban comfort - capitalization of natural potential - accessibility - diversification of urban texture - beneficial effects in the urban microclimate
FUNCTIONAL AND SOCIAL	- integrator character of functionality - population and area serviced - nature of use (individuals or groups) - average use time (days/week)	- landscape functions: loisir, rest, relaxation, sport, nautical recreation, parade - facilities, equipment and physical furnishing, distinct for each type of function	- coherent organization of recreational activities - differential application for categories of users - specificity of facilities in proportion to the park's profile and categories of users	- social utility - diversification of free time activities for the population - multiple possibilities for socialization
AESTHETICAL	- mixt composition type with predominant elements from the free style - composition principles: axis, perspective, symmetry and asymmetry, dominant, composition centre, contrast, accent, calibration	- optimum mineral/vegetal ratio - shapes of water bodies and alleys - contours of vegetation groups and clusters	- unity and variety - conformism and non-conformism - diversity of mineral and vegetal textures	- enrichment of the general urban ambiance - aesthetic diversity

The main function for Tineretului Park is that of rest - relaxation - promenade, materialized by the alleys system (including the lake's contour alley). Furthermore, the lake can be

used as an area for practicing nautical recreation activities (boat rides). The park also holds functions for practicing outside sports, playgrounds for children, and also the rose garden which corresponds to the functions of education and floral décor; the most diverse functions are found within most of the contemporary European parks, e.g. Els Pinetons Park from Ripollet del Valles - Spain. The project comprises the construction of a pedestrian alley which surrounds the park and unites a series of platforms with the dimensions 8 × 55 m, having various usages: barbeques, children's games, a bar for barbeque serving (Mostaedi, 2004).

CONCLUSIONS

The development of contemporary urban parks answers to a wide range of functional and aesthetical-compositional demands. All these comply with the whole composition of the park, spatial layout, the landscape functions included within the park, as well as with the vegetation components and design. This aspect affirms the landscape cogitation and also the evolution in good condition of all the functions, establishing at the same time a most valuable natural support for the facilities corresponding to each type of function.

ACKNOWLEDGEMENTS

The current paper was elaborated on the project for Tineretului Park, developed in the design institute "Proiect Bucureşti" by architect Valentin Donose, the author of the project and the coordinator of the entire construction of the park.

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NATURAL HERITAGE CONSERVATION IN BUILT-UP AREAS. STUDY ON THE CIRCUS PARK AS AN URBAN LANDSCAPE ASSET

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Abstract

Circus Park is one of the most distinguished green areas of Bucharest, being a veritable urban landscape resource. Measuring 17 ha of land, it was designed during 1960-1961 following the plans of the architect Valentin Donose, who formed and coordinated an interdisciplinary team within the Bucharest Project Institute, responsible with designing green areas much needed to the capital at the time. This document aims to analyse the essential components which form a park and bestow the attribute of an urban landscape resource: vegetation, water, alleys and ornamental furniture. Circus park is the quintessential example which points to the idea that, just as man is attracted to water, he is also instinctively attracted to trees and the spaces they compose. The distribution of foliage items contained in this study cover a wide spectrum of trees, shrubs, lianas and herbaceous plants which range from wild to natural or man-made architectural shapes. This inquiry recommends maintaining the concepts found in the initial plan of the park and taking the necessary measures to preserve this natural heritage of Bucharest.

Key words*: natural heritage, park, urban landscape resource, dendrological species.*

INTRODUCTION

This work aims to bring into attention the necessity of preserving the natural heritage in built-up areas

Green spaces contribute to the harmony of urban architecture and also add to the aesthetic of the urban landscape, thus being an essential element of the human habitat (Godeanu, 2013). In this regard we chose a park as the purpose of this study, which through surface, compositional elements and functions is an essential component of the natural heritage of any city.

Circus Park (17 ha) is one of outstanding green areas of Bucharest sector 2. It was created between 1960 and 1961 after the plans of architect Valentin Donose, within the Design Institute "Proiect București".

This park is a classic example (didactic even) of the idea that man is instinctively attracted by water and trees and the appealing spaces that these elements create and define. The studied elements cover a never-ending palette of trees, shrubs, bushes, lianas which vary from natural wild to clearly man crafted architectural shapes. All of these aspects bestow the value of an urban landscape resource upon this park.

MATERIALS AND METHODS

This study took place between March 2016-March 2017, the focus being on the compositional elements of Circus Park: vegetation (trees, shrubs, flowering plants), the water features, walkways - tracking their evolution in time through various photographs (photo archive architect Valentin Donose - Photos 1, 2). The vegetation study was based on both a quality and quantity inventory. The quality inventory is based on identifying all the plant species through field inspections during the four seasons of the year (2 field trips per season) to phenologically highlight the vegetal species and their viability (Felicia Iliescu, 1998; Doniţă N. et al., 2004; Ciocârlan, 2000). The quantity inventory was based on an established method: Braun-Blanquet (Van DerMaarel E., 1975) with which you can describe vegetal associations. Following this method led to identifying some valuable compositional areas of the park in which the plant material is predominantly made of trees and shrubs. The quantity was measured through surveys (4 surveys per field trip), each covering an area of 200 m^2 (the standard surface area

used in grouping trees and shrubs). This method concludes the structural and quantitative associations of plants through a combined scale of abundance - dominance after Braun - Blanquet: + - few elements with low coverage; 1 - many elements with low coverage or few elements with great coverage (up to 10% of the surface); 2 - many elements with coverage between 10-25%; 3 - various number of elements with cover between 25-50%; 4 - various number of elements with cover between 50-75%; 5 - various number of elements with cover between 75-100% (Doniță, Cocioabă, 2007).

General view of the park from Bd. Lacul Tei direction: photo 1- year 1960 (source: arh. V. Donose); photo 2- year 2000

RESULTS AND DISCUSSIONS

The initial project of Circus Park was outstanding through perfectly combining alley paths and combinations with planted areas which take the terrain architecture and the lake presence into account. The circulation areas respond to functional demands: granting access to visitors from carefully chosen key points (from Lacul Tei Blvd. and Stefan cel Mare Blvd.) in link with external circulation areas and with demands of neighbouring areas (ex: high population density of Lacul Tei and Stefan cel Mare quarters), linking these quarters and ensuring circulation flow by leading to areas which serve different functions (children playgrounds, rest areas etc.) The sparse vegetation which was in place before 1960 was kept as well. It was composed mainly from a few elements of pyramidal poplar trees which remain at the base of the hill and a white poplar at the edge of the park towards Lacul Tei Blvd. (this particular poplar is an outstanding element and is protected by law to this day).

In the present day, following this study we found 33 species of trees, 12 species of shrubs and 32 species of plants. Among the better represented species of trees are: *Tilia tomentosa*, *Platanus* × *acerifolia*, *Acer saccharinum*, *Betulla verucosa*, *Populus piramidalis*, *Quercus rubra*, *Salix alba*, *Ginkgo biloba, Taxodium distichum*, *Pinus nigra* etc. (Photos 3, 4, 5).

Photo 3 - Planted areas around the lake - year 2000 (source: archive photo arh. V. Donose

Photo 4 - Planted areas around the lake - year 2016 (original)

In the case of shrubs we identified 12 species, among which: *Buxus sempervirens*, *Spiraea vanhouttei, Forsithia* sp. *Hibiscus* sp., pictured below (Photo 6). The analysed shrubs are located near the main entrance into the park, which would currently require some reconditioning interventions.
A particular problem of the study was identifying valuable compositional areas through exact grouping of plant species (inland and foreign), areas in which not only the morphological characteristics of plants was taken into account, but also the rigors of landscape architecture (Table 1).

Photo 5 - *Taxodium distichum,* year 2017 (original)

Photo 6 - *Buxus sempervivens* - access area from Lacul Tei Blvd.(source: archive photo arh. V. Donose, 2000)

In table 1 we cover two examples of important compositional areas positioned along the alley which links the two quarters (Stefan cel Mare and Lacul Tei).

Table 1. Identifying valuable compositional areas - Circus Park (original, 2017)
Survey 1 (200m²): Trees and shrubs level (A/a) of access area from Lacul Tei Blvd:

Nr.crt.	Name	A/a	AD[1]
1.	*Ginkgo biloba*	A	60%
2.	*Quercus rubra*	A	35%
3.	*Crataegus monogyna*	a	5 %

Survey 2 (200 m²): Trees and shrubs level (A/a) of access area from Lacul Tei Blvd:

Nr. crt	Name	A/a	AD[1]
1.	*Quercus rubra*	A	60%
2.	*Tilia tomentosa*	A	25%
3.	*Crataegus monogyna*	a	10%
4.	*Forsithia* sp.	a	5%

[1]abundance - dominance

We consider that the viability of a park is kept in place by identifying, preserving and reconditioning, where necessary, of rich, aesthetically valuable vegetation areas.
This aspect must be present in any course of rehabilitating green areas which constitute fundamental elements of natural heritage in any city.

CONCLUSIONS

This study recommends that the original concept ideas of the initial plan of the park be maintained, as well as the variety of the existing dendrological species.
Circus Park is an important component of the Bucharest natural heritage. It's value, authenticity, and uniqueness deserve to be conserved.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the landscape architect Valentin Donose, the author of the Circus Park, and to the Design Institute "Proiect Bucureşti" in which he carried out his professional activity and which serves as a personal archive.

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LEAF STOMATAL PARAMETERS OF *IRIS GERMANICA* L. INFLUENCED BY CULTIVAR AND ARBUSCULAR MYCORRHIZAE INOCULATION IN FIELD CONDITIONS, ROMANIA

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Abstract

The aim of this study was to investigate if supplementary inoculation with arbuscular mycorrhizae of Iris germanica plants in field conditions has influence on key stomata parameters that are known to determine the maximum leaf diffusive conductance of CO_2 to the site of assimilation as well as water use efficiency. Six Iris germanica cultivars ('Black Dragon', 'Blue Rhythm', 'Sultan's Palace', 'Lime Fizz', 'Pinafore Pink', 'Pure As The') were inoculated at planting in autumn with the following arbuscular mycorrhizae fungi species: Funneliformis mosseae (Glomus mosseae), Funneliformis geosporus (Glomus geosporum), Claroideoglomus clarodeum (Glomus claroideum), Rhizophagus intraradices (Glomus intraradices), Glomus microaggregatum. Microscopic examination revealed that both inoculated and non-inoculated plants presented AM root colonization after entering in vegetation. Analysis of leaf imprints collected in spring showed that inoculation with arbuscular mycorrhizae determined a decrease in stomatal density but increase of guard cell length. Inoculated plants presented higher potential stomatal conductance index.

Key words: *physiological parameters, ornamental geophyte, AMF inoculation, root.*

INTRODUCTION

Stomata are involved in regulation of water and carbon dioxide balance of plants. Maximum stomatal conductance to water vapor and CO_2 is associated with stomata density and stomata size (Franks et al., 2017). During the evolution of plants, changes in stomata density and size were linked to fluctuations in atmospheric CO_2 levels (Franks et Beerling, 2009), this is why how future elevated CO_2 levels might affect plants is subject of high attention (Hepworth et al., 2015). In addition, recent studies showed stomata parameters to be reliably linked to drought-resistance in some cultivated monocot plants (Li et al., 2017; Hughes et al., 2017). Current understanding of molecular control of these leaf structures already allowed researchers to modify stomata density by manipulating epidermal pattering factor family of secreted signaling peptides which intervene in regulation of stomata development (Hepworth et al., 2015; Hughes et al., 2017). Previous studies indicate that AMF inoculation could have influence on primary physiological functions of leaves as well. For example, root colonization with AMF species was already shown to be accompanied by increase in stomatal conductance (Augé et al., 1992; Augé et al., 2008) or density (Chitarra et al., 2016) for several cultivated species. Regarding the effect of AMF on *Iris* plants, it was demonstrated the promoting effect of *Glomus mosseae* on photosynthesis rate (Chen et al., 2014). Also, inoculation with *Diversispora epigaea, Glomus aureum, Rhizophagus irregularis, Rhizophagus clarus* influenced physiological processes of *Iris pseudacorus* plants because it was observed an improved phytoremediation capacity (Wężowicz et al., 2015).

Previously, differences in stomatal density of *Iris* leaves were studied in terms of histological gradients within the leaves of the plant in different light conditions (Pazourek, 1970) or in order to highlight variations between *Iris* species, subspecies or clones (Miljkovi et al., 2013; Ghasemi et al., 2014; Kandemir et Çelik, 2017). Thus, although previous studies were able to put in evidence variations in stomatal parameters in *Iris* plants the influence of other factors was little explored so far.

Iris germanica leaves present epicuticular wax structures as film or platelets with regeneration type following concentric-layer formation and

platelets (Koch et al., 2009). In the genus *Iris* leaf stomata observed is of anomocytic and tetracytic type. In addition, under microscope *Iris* leaf can present papillae on the outer periclinal wall of epidermal cells as well as bulliform cells, while prism-shaped styloids of calcium oxalate occur in idioblasts in the mesophyll and are arranged parallel to long axis of the leaf often being visible through epidermis. Micro-morphology leaf features hold taxonomic importance in genus *Iris* (Wu et Cutler, 1985; Mitić et Pavletić, 1995; Çölgeçen and Tug, 2006; Wang et al., 2010; Kandemir et Çelik, 2017).

In all monocots stomatal development begins with an asymmetric mitosis in a meristemoid-mother cell, which gives rise to a larger stomatal-lineage ground cell and shorter meristemoid that directly forms a guard-mother cell. Each guard-mother cell undergoes symmetric mitosis to form a pair of guard cells that rapidly cease expansion compared with the greater enlargement of pavement cells as leaves grow. Because in monocots with linear leaves and parallel venation, stomata occur in epidermal cell files that develop basipetally from an intercalary meristem (Rudall et al., 2017), there is a histological gradient along the leaf blade.

The aim of the study was to investigate the influence of inoculation in field conditions with a consortium of five AMF species on key stomata characteristics such as stomata density and guard cell length for six *Iris germanica* cultivars.

Results of this study could help in identifying if AM inoculation might optimize basic physiological processes that could help plants cope with increasing atmospheric pollution and drought, both known to influence plant development within the context of current climate trend.

MATERIALS AND METHODS

The experimental field was located in the Agro-Botanical Garden of UASVM Cluj-Napoca Romania, elevation (AMSL) 380-430 m, with average annual temperature 8.1°C and average sum of annual precipitation 635 mm (Index Seminum USAMV Cluj).

The physical-chemical analysis of the soil collected when plants entered vegetation in spring 2017, conducted at the O.S.P.A. Cluj showed a clay loam soil type with 6.72 pH, low humus level (1.35%) and good NPK supply (N 0.461%, P 68 ppm, K 312 ppm). Sum of basic cations through Kappen method was 20.96 (me/100 g of soil), while hydrolytic acidity through extraction method was 1.92 (me/100 g soil). Basic cation saturation level determined by calculation was 92. Granulometric analysis using Kacinscki method was: coarse sand 14.42, fine sand 25.08, dust I 7.95, dust II 13.65 and clay 38.90. Low dose of NPK fertilizer was applied before bloom and wood ash was applied in autumn and spring.

The bifactorial experiment established in October 2016 was organized in randomized blocks with three replicates: factor A - *Iris germanica* cultivars, with six levels (a_1 = 'Black Dragon', a_2 = 'Blue Rhythm', a_3 = 'Sultan's Palace', a_4 = 'Lime Fizz', a_5 = 'Pinafore Pink', a_6 = 'Pure as The') and factor B – the treatment applied with two levels (b_1 = non-inoculated at planting and b_2 = inoculated at planting with 13 grams mycorrhizal products per rhizome). The two commercial mycorrhizal products special destined for use in ornamental plants were imported from U.K. and contained the following AMF species: *Funneliformis mosseae (Glomus mosseae), Funneliformis geosporus (Glomus geosporum), Claroideoglomus clarodeum (Glomus claroideum), Rhizophagus intraradices (Glomus intraradices), Glomus microaggregatum.* In addition to the AMF spores, the products contained excipients such as organic materials, humates and auxins along a few others. Between the three blocks of inoculated plants and the three blocks of non-inoculated plants was ensured 7 m, sufficient to prevent potential cross transport of AM propagules (Powell, 1979), also tools were washed after working with each set of plants.

Microscopic examinations were conducted with optical microscope (Figure 1c) ML-4M made in Romania. Images were taken with Bresser camera and microscope (Figures 2, 5).

The success of root colonization was confirmed in 2017 under microscope (Figure 2) using the staining technique of Stoian et Florian (2009) and colonization estimation method (https://www2.dijon.inra.fr/). Using clear polish and tape method (Palasciano et al., 2005), in

May 2017 were collected leaf imprints from middle segment of the leaf blade surface facing south (Figure 1c).
For each leaf imprint sample, the number of stomata was counted according to Vâtcă et al. (2007) and presented as density per square mm. Guard cell length was measured with eye piece reticle. Stomata were considered in the field of view only if the entire ostiole was visible.

Figure 1. *Iris germanica* 'Blue Rhythm': a) rhizome at planting; b) plants in spring; c) leaf imprint sample

In total for all six *Iris germanica* cultivars, stomata were counted on more than 1900 microscopic fields of view and over 5700 stomata were measured for guard cell length.
Leaf imprints were collected also from several other rhizomatous cultivated species *(Iris pallida, Iris pseudacorus, Iris sibirica* and *Iris chrysographes)* grown in Agro-Botanical Garden UASVM Cluj-Napoca, for comparison purposes and analyzed similarly.
Based on the microscopic examinations were calculated:

1. PCI = $L^2 \times SD \times 10^{-4}$ (Holland et Richardson, 2009), where PCI = potential conductance index, L = guard cell length (µm), SD = stomata density per 1 mm^2;
2. a% = $[(100 \times mA_3) + (50 \times mA_2) + (10 \times mA_1)]/100$ (Trouvelot et al., 1986), where a% = arbuscule abundance in mycorrhizal parts of the root fragments, based on percentages of *m* rated according to methodology.

Data was analyzed with Microsoft Excel, Mycocalc and Origin.

RESULTS AND DISCUSSIONS

In 2017 at the end of active vegetative growth of plants (end of spring) both inoculated and non-inoculated *Iris germanica* plants presented root colonization confirmed under microscope that looked rather discontinuous or patchy along root length.
The fresh, washed, and unstained thick roots of first order were white but thinner roots of higher order, were darker in color indicating root colonization causing a darkening of the cortical cells under the pigment released by collapsed arbuscules (Fester et al., 2002). All AMF structures were present: extraradical hyphae, hyphopodium and inner root coils at the entrance points, young and collapsed arbuscules, vesicles (oblong and round) and spores as well as a few loose sporocarps.
Under microscope was identified Arum type proliferation as well as intermediate Arum-Paris type proliferation. Spreading patterns varied slightly among cultivars. Thus, it was noticed in some segments a preponderant linear spreading within the roots of 'Pure As The', arbuscules preferentially along sieve elements in 'Lime Fizz' or in the outer root layers in 'Sultan's Palace', abundant hyphae coils within roots of some 'Black Dragon' plants.
Arbuscules abundance in mycorrhizal parts of root fragments of the six *Iris germanica* cultivars was slightly increased from average a% = 44.09 in non-inoculated plants to a% = 44.45 in inoculated plants, hinting to a higher transfer occurring between plants and arbuscular mycorrhizae fungi following inoculation treatment.

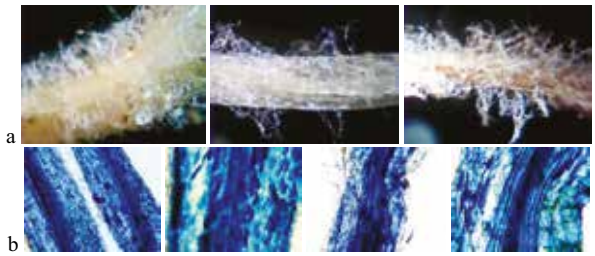

Figure 2. Microscopic view: a) colonized *Iris germanica* roots, some with pigmentation due to apocarotenoids released by collapsed arbuscules; b) roots after staining

In five out of six *Iris germanica* cultivars, stomata density decreased as a result of inoculation with arbuscular mycorrhizae (Figure 3). The only cultivar that presented a slight increase in stomata density due to inoculation was 'Black Dragon' from 61.41/mm^2 in non-inoculated plants to 61.95/mm^2 in inoculated plants. On average, stomata density decreased in inoculated plants with about 10 stomata/mm^2, from an average density of 63.83/mm^2 in non-inoculated plants to 53.12/mm^2 in inoculated plants. Highest decrease of stomata density due to inoculation was observed in 'Sultan's Palace' followed by 'Pinafore Pink' and 'Pure As The'. The smallest decrease in stomata density caused by inoculation was found in 'Lime Fizz' followed by 'Blue Rhythm'.

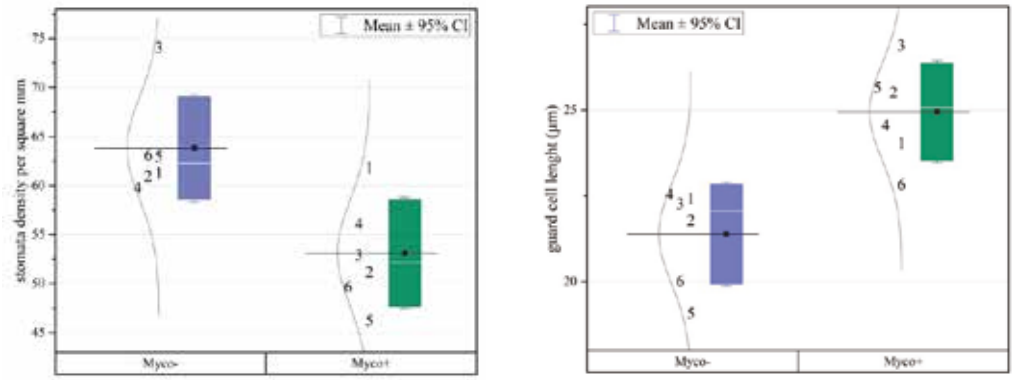

Figure 3. Stomata density (per mm^2) and guard cell length (µm) in six *Iris germanica* cultivars: 1) 'Black Dragon'; 2) 'Blue Rhythm'; 3) 'Sultan's Palace'; 4) 'Lime Fizz'; 5) 'Pinafore Pink'; 6) 'Pure as The' non-inoculated (Myco-) and inoculated with arbuscular mycorrhizae (Myco+)

In all inoculated *Iris germanica* cultivars guard cell length increased (Figure 3) on average with 3.56 µm. Non-inoculated plants on average had a stomata length of 21.39 µm while inoculated cultivars had an average stomata length of 24.95 µm. The largest increase in guard cell length linked to inoculation was observed in 'Pinafore Pink' that had the second highest decrease of stomata density as a result of inoculation followed by 'Sultan's Palace'. The slightest increase in guard cell length due to inoculation was observed in 'Black Dragon' of only 1.6 µm on average.

Potential stomatal conductance index increased due to inoculation in all studied *Iris germanica* cultivars (Figure 4), from the average of 2.93 in non-inoculated plants to 3.31 in the inoculated plants. The highest increase was observed in 'Pinafore Pink' (from 2.30 to 3.06). This cultivar also experienced one of the highest decrease in stomata density and highest increase in guard cell length due to inoculation. Also, it can be noted that non-inoculated plants of 'Pinafore Pink' presented the smallest guard cell length among all cultivars studied. Two cultivars presented very similar increase of potential stomata conductance due to

inoculation: 'Black Dragon' (from 3.10 to 3.59) and 'Blue Rhythm' (from 2.91 to 3.35). Both these cultivars had either a slight decrease in stomata density or a very small increase in stomata density due to inoculation. The least increase in potential stomata conductance index was observed in 'Pure As The', from 2.54 to 2.61, followed by 'Sultan's Palace' with an increase from 3.69 to 3.85. The cultivar 'Lime Fizz' with yellow flowers, situated between the dark-flowered cultivars and the light-flowered cultivars, with an increase of PCI due to inoculation from 3.06 observed in non-inoculated plants to 3.40 observed in inoculated plants (Figure 4).

Although stomata density did not increase, on contrary decreased in inoculated plants with one exception, the potential stomatal conductance increased in all studied *Iris germanica* cultivars due to inoculation, because of the increase in guard cell length.

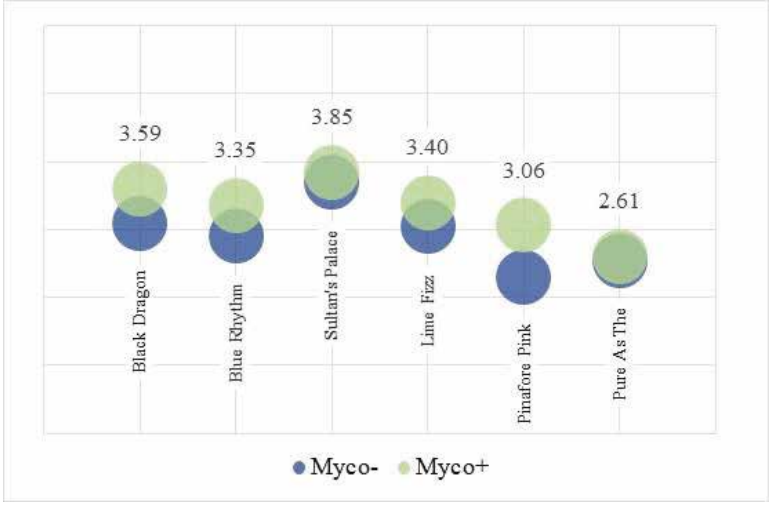

Figure 4. Potential stomatal conductance index (PCI) in six *Iris germanica* cultivars non-inoculated (Myco -) and inoculated (Myco +) with arbuscular mycorrhizae

During the vegetative months prior to flowering and before collecting the leaf imprint samples there was a warm spring with temperatures exceeding 20°C on no less than 17 days during the interval 15 March - 15 May 2017. The sum of precipitations was about 45 mm during same time interval, while the wind had intensities between 3-37 km/h (https://www.wunderground.com/history).

Plants were not irrigated and supported a natural water regime. Agro-Botanical Garden of UASVM is situated on the steep side of Someşul Mic river valley within Cluj-Napoca and experiences windy conditions in spring and fall, fact known to increase the transpiration and water loss in plants.

In the given conditions, it can be considered that plants had to find ways to cope with these environmental challenges by optimizing their physiological processes. Hepworth et al. (2015) citing previous researches mention that plants with low stomata density presented enhanced water use efficiency and reduced transpiration levels that allowed plants to grow larger especially under water restriction conditions. But reduced stomata could also mean lower transpiration. Yet, transpiration is driving the mass flow (Hepworth et al., 2015), fact that would suggest that if stomata density decreases under a certain level it would be expected to be made at the expense of nutrient accumulation. The increased guard cell length ensured the increase of potential stomata conductance index despite of a reduction in stomata density. Plants can rely on mycorrhizae mycelia network for increase uptake of both nutrients and water, but mycorrhizal fungi take in exchange part of the carbon fixed by the plant. Thus, the inoculated plant needs to be able to conduct photosynthesis at optimum levels, and this is perhaps

why the reduction in stomata density was accompanied by increase in stomata length. Since both sets of plants (inoculated and non-inoculated presented root colonization), the observed changes might be linked to inoculated species rather than those already present in the soil; also, some excipients could have played a beneficial role as well, either directly on plant rooting and nutrition or indirectly by stimulating the establishment of symbiose.

Micromorphology differences between studied *Iris germanica* cultivars and other rhizomatous species were observed under microscope on collected leaf imprint samples (Figure 5). Comparing the stomata density and guard cell length of non-inoculated *Iris germanica* plants (Figure 3) with those of *Iris pallida* (Table 1), it can be observed that values for *Iris germanica* are higher. It is known that current *Iris germanica* cultivars are tetraploids (Norris, 2012) and this cultivated species is actually a natural hybrid with diploid *Iris pallida* as one of its ancestors (Lim, 2016).

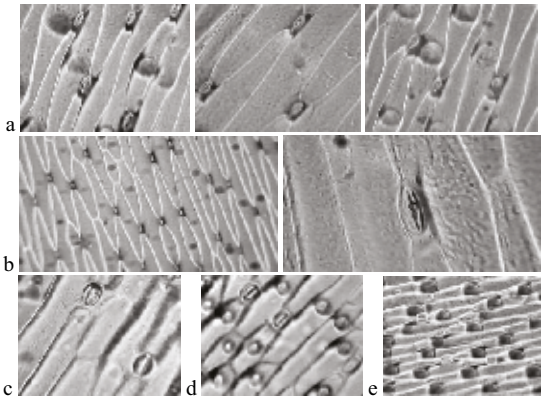

Figure 5. Leaf micromorphology on imprints from cultivated rhizomatous *Iris* species:
a) *Iris germanica*; b) *Iris pallida*; c) *Iris pseudacorus*; d) *Iris sibirica*; e) *Iris chrysographes*

Ploidy level influences stomatal characteristics, since guard cell size has been used to predict the haploid level of primitive angiosperms and the extent of polyploidy in the present-day angiosperms (Willmer et Fricker, 1996). On average both inoculated and non-inoculated plants of *Iris germanica* presented longer guard cell length, and polyploidy of *Iris germanica* could explain this characteristic compared to diploid *Iris pallida*. However, the values are too close for a clear indication of ploidy level in *Iris germanica* based on stomata parameters compared to *Iris pallida*, even more since inoculated *Iris germanica* plants had slightly smaller stomata density than *Iris pallida*. Ghasemi et al. (2014) trying to find if stomata parameters can be used in several *Iris* taxa from Iran to predict ploidy level, reached same conclusion that, environmental factors have a strong influence on stomata characteristics that would make this approach less exact in predicting ploidy level in *Iris*.

All three species from subgenus *Limniris* (*Iris pseudacorus*, *Iris sibirica* and *Iris chrysographes*) presented higher stomata density than the two species from subgenus *Iris* (*Iris germanica* and *Iris pallida*).

Iris germanica and *Iris pallida* are native to milder Mediterranean climate, with warm summer (Lim, 2016), and their stomata density and guard cell length have similar values. The lower stomata density compared to the other three species studied (Table 1) can be an indication of their known drought tolerance and their known preferences for dryer substrate (Beresford-Kroeger, 2004). On *Iris germanica* leaves were observed large papillae (no more than one per epidermal cell) while stomata over the leaf veins are present but disposed sparsely.

Iris sibirica and *Iris chrysographes* originate from colder regions of Eurasia and respectively Asia (White et al., 1997). Under microscope these two present smaller epidermal cells compared to other *Iris* plants from this study and abundant leaf papillae. Both present

smaller guard cell length and high stomata density. It is known that smaller stomata occur at higher frequency, fact that determines the total possible pore area for leaf to be similar (Willmer et Fricker, 1996). *Iris pseudacorus* presents both a large number of stomata per leaf unit area as well as longer guard cells. This species is used for water gardens and grows best in marshes (White et al., 1997), fact that can be linked to observed stomata parameters that indicates to higher water requirements known that this species has (Jacobs et al., 2011).

Table 1. Stomata parameters in four *Iris* species from Agro-Botanical Garden UASVM Cluj-Napoca

Species	SD (mm^2)	L (µm)	PCI
Iris pallida Lam.	56.33	20.83	2.44
Iris pseudacorus L.	82.11	23.77	4.64
Iris sibirica L.	97.59	17.28	2.91
Iris chrysographes Dykes	72.34	16.13	1.88

SD – stomata density, L – guard cell length, PCI – potential stomatal conductance index

Summarizing the observations and findings of this research, firstly can be suggested that patchy colonization pattern observed in both sets of *Iris germanica* plants (inoculated and non-inoculated) could be due to soil texture, particularly abundant clay component, that also might have caused poorer root ramification too. Previous studies showed that soil texture particularly clay rich soil along lime application are some of the most important factors influencing AMF root colonization in maize, sorghum and peanuts (Carrenho et al., 2007). Also, patchiness characterizes the distribution of mycorrhizal structures of members from genera *Ambispora*, *Archaeospora*, *Diversispora*, *Entrophospora*, *Intraspora* and *Paraglomus*, whereas members of the families *Gigasporaceae*, *Glomeraceae* and *Pacisporaceae* usually present a continuous mycorrhizal distribution along the root (Błaszkowski, 2012). This might as well explain the patchy colonization observed.

The different colonization patterns observed in some cultivars can be attributed both to some differences in root architecture between cultivars as well as to possible plant genotype-AMF colonizing specificity but could not be associated to inoculation treatment.

Previously in *Iris pseudacorus* plants grown in pots on sterile substratum and inoculated with *Diversispora epigaea, Glomus aureum, Rhizophagus irregularis* and *Rhizophagus clarus* obtained from trap cultures was reported typical Arum type spreading (Wężowicz et al., 2012), but in this study conducted in field conditions both Arum and intermediate Arum-Paris type proliferation was identified in *Iris germanica* cultivars either inoculated or non-inoculated. This comes to show that in field conditions colonizing patterns of plants can be different.

The yellowish tint of different intensities identified in colonized *Iris germanica* roots of higher order appears in response to AM fungi and various carotenoid degradation products (apocarotenoids) released during collapse of arbuscules. These accumulate as hydrophobic droplets in root cortical cells and were identified in many Liliopsida species analyzed. Their function is little known but might have importance in the arbuscule development and function (Fester et al., 2002). This observation indicated that *Iris germanica* plants were colonized during spring. Root samples were collected shortly after the flowering season. Between inoculated and non-inoculated plants was identified only a small difference regarding arbuscules abundance in mycorrhizal parts of root fragments.

A direction for future investigation would be to investigate the seasonal variation of root colonization in order to establish the link between plant phenology and mycorrhiza life cycle.

In five out of six studied *Iris germanica* cultivars, a decrease in stomata density was accompanied by increase in stomata length. These findings are in accordance with previous patterns observed in *Iris* stomata. In two taxa from Turkey, *Iris masia* subsp. *dumaniana Iris masia* subsp. *masica* higher number of stomata was linked to smaller length of stomata (Kandemir et Çelik, 2017). Similarly, Ohsumi et al., 2007 found a negative correlation

between stomata density and stomata length in *Oryza sativa* plants, another monocot.

All *Iris germanica* cultivars inoculated with arbuscular mycorrhizae presented increased potential stomatal conductance index (Figure 4). Previous studies showed that *Glomus mosseae* inoculation had a stimulating effect on photosynthesis of *Iris* plants (Chen et al., 2014). Also, leaves of AMF inoculated *Vigna unguiculata* had higher stomatal conductance than those of non-mycorrhizal plants before and after lowering soil water potential (Augé et al., 1992).

In contrast with the results of this study on *Iris* plants, in controlled conditions *Rhizophagus intraradices*-inoculated tomato plants presented significantly increased number of stomata in mature leaves; stomatal density was almost twice that of control tomato plants or *Funneliformis mosseae*-inoculated plants (Chitarra et al., 2016). By comparison, in this study the inoculated *Iris* plants grown in field conditions experienced a reduction in stomata density, maybe as a strategy to reduce water loss. Previous experiments showed that a route towards improving drought tolerance and water use efficiency without significantly affecting photosynthetic capacity or nutrient accumulation by mass flow is a slight reduction in stomata density (Hepworth et al., 2015).

In conclusion can be said that environmental factors influence stomata parameters, but plant genotype also has a strong influence as well. This can be exemplified by two observed phenomena from this study.

First, different *Iris germanica* cultivars did not responded identical to inoculation with arbuscular mycorrhizae, although in all cases there was an increased potential stomatal conductance index. Secondly, different rhizomatous species grown in Agro-Botanical Garden of Cluj retain their ancestral characteristics from adaptation to their habitat of origin. This is most simply explained by the known fact that each species maintains specific growing requirements similar with the environment they evolved in, just as stomata density in different *Iris* species mentioned above can be easily linked to the way their physiological processes were adapted to ecological niche they preferentially occupied. This suggests there might be a certain threshold up until a certain growing factor (like inoculation) can be used to enhance plant response and its effect cannot guarantee an equal response among plants.

Expression of genes homologous to those involved in the regulation of stomatal development in *Arabidopsis* known as *STOMAGEN* and genes encoding two intercellular signaling factors that act as negative regulators for stomatal development antagonistic to the first one was investigated in developing leaves of AMF inoculated tomato plants.

It was showed that genes EPF1 and EPF2 were significantly modulated only in the presence of AM symbiosis, while *LeEPFL9* transcript levels were correlated with changes in stomata density of *Rhizophagus intraradices*-inoculated plants (Chitarra et al., 2016).

According to the results of this study, supplementary inoculation has influence on two key stomata parameters.

In inoculated plants, on average stomatal density decreased but the length of guard cells increased, possibly hinting to a tendency to balance water use efficiency and increased assimilation rate that ultimately lead to potential stomatal conductance index to increase in all inoculated cultivars.

CONCLUSIONS

On average, stomata density decreased in arbuscular mycorrhizae-inoculated plants with about 10 stomata/mm^2, at the same time guard cell length increased on average with 3.56 µm. The changes in stomata parameters following inoculation lead to an increase of potential stomatal conductance observed in all studied cultivars.

Both inoculated and non-inoculated plants presented root colonization, with some close values for arbuscule abundance in mycorrhizal parts of root fragments between the two sets of plants.

More studies should be conducted to assess the physiological response of plants to mycorrhiza inoculation in field conditions, in order to further define the practicality of its application for ornamental irises as well as irises destined to obtaining orris oil.

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PRELIMINARY TESTING OF SRAP PRIMERS IN FOUR *RANUNCULACEAE* SPECIES FROM ROMANIA

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Abstract

The main aim of this paper was to identify the most reliable primer pairs out of the 64 used in order to investigate the genetic diversity of four medicinal Ranunculaceae species as follow: Aconitum moldavicum, Anemone transsilvanica, Ranunculus carpaticus and R. platanifolius. Amplified products were separated on 1.6% agarose gel and a total number of 886 DNA fragments were visualized by EtBr staining. After primer combinations screening, 27 primer pairs were selected based on clarity, reproducibility of the amplified bands and high rates of polymorphism for further research. The selected SRAP primer pairs resulted in 553 informative fragments with an average number of 20.5 per primer combination. The most efficient primer combinations were Me1/Em1, Me3/Em3, Me7/Em4, Me7/Em5, Me7/Em8, Me8/Em1 and Me8/Em5 which generated a total number of 201 visible DNA fragments. The current research represents a preliminary study for further conservation perspectives regarding the genetic diversity of several medicinal plants belonging to the Ranunculaceae family.

Keywords*: Ranunculaceae, genetic diversity, primer testing, SRAP.*

INTRODUCTION

Ranunculaceae Juss. (buttercup family) is one of the early lineages in flowering plants distributed worldwide, most commonly in the temperate and cold areas of the northern hemisphere (Heywood et al., 2007). The family including 56 genera and approximately 2,500 species used as medicinals, spices, and ornamentals, however, most of them are highly poisonous (Nyirimigabo et al., 2014; Aslam et al., 2012). The most representative genera of the buttercup family are *Aconitum*, *Anemone*, *Clematis*, *Delphinium*, *Ranunculus,* and *Thalictrum* which account approximately 90% of the species (Heywood et al., 2007). From genetic point of view this family has a great chromosomal variation in numbers, sizes and structures, including species with 2n=14 (e.g. *Hepatica asiatica* Nakai, *Ranunculu sceleratus* L.), 2n=16 (e.g. *Ranunculus cantoniensis* DC), 2n=18 (e.g. *Clematis brachyura* Maxim), and 2n=42 (e.g. *Thalictrum aquilegiifolium* var. *sibiricum* Regel & Tiling) (Chung et al., 2013).

In Romania, the *Ranunculaceae* family is represented by 23 genera and approximately 110 species including endemic plants such as *Anemone transsilvanica* Fuss (Săvulescu, 2007). Many local plants belonging to *Ranunculaceae* family (*Aconitum moldavicum* Hacq.*, Anemone transsilvanica*, *Ranunculus carpaticus* Herbich, *R. platanifolius* L.) have been used as human and veterinary medicine to treat various ailments such as bronchitis, cough, diarrhea, fever, hepatitis, gout, rheumatism, and skin diseases (Tămaş, 2005). However, little is known concerning their genetical diversity and conservation.

The main purpose of this work is to assess the genetic diversity at molecular level of *A. moldavicum, A. transsilvanica, R. carpaticus* and *R. platanifolius* using sequence-related amplified polymorphism (SRAP) markers in order to establish some conservation strategies for these species. SRAP primers are considering to be more suitable to revealing genetic diversity among related species than amplified fragment length polymorphism

(AFLP), simple sequence repeats (SSR), inter-simple sequence repeat (ISSR) or even random amplified polymorphic DNA (RAPD) markers (Budak et al., 2004). Therefore, preliminary testing of SRAP primers was necessary to be able to select the appropriate primer combinations on a large-scale use to characterize the genetic diversity of the studied species. Although, previous scientific reports show intra- and interpopulation genetic diversity of various *Ranunculaceae* species [*Ranunculus acris* (Odat et al., 2004), *R. cabrerensis* (Cires et al., 2013), *R. kuepferi* (Cosendai et al., 2013) and *R. reptans* (Fischer et al., 2000; Prati et al., 2016)] employing AFLP or RAPD primers, this is the first report regarding to assessment of genetic variation in this family using SRAP markers.

MATERIALS AND METHODS

Plant samples were collected from their natural habitats in two districts from Romania (Brașov and Hunedoara) namely at Mt. Postăvaru (45°35.215′ N, 25°433.146′ E) and Mt. Stâmba (46°12.508′ N, 22°51.354′ E). The voucher specimens have been stored in the Herbarium collection at the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania. The location and the voucher specimen numbers of the species are shown in Table 1.

Table 1. Voucher specimen numbers of the *Ranunculaceae* species and their geographical origins

Species name	VSN[b)]	Longitude E	Latitude N
Aconitum moldavicum Hacq.	CLA30049	22°51.354′	46°12.508′
Anemone transsilvanica FUSS.	CLA30047	25°433.146	45°35.215′
Ranunculus carpaticus Herbich.	CLA30044	25°433.146	45°35.215′
Ranunculus platanifolius L.	CLA30040	25°433.146	45°35.215′

Note: [b)]Voucher specimen number

The harvesting leaves were air dried at room temperature and stored at -20°C until processing. To find out the most suitable SRAP primer combinations four randomly selected samples were used one of each species.

In order to extract the genomic DNA, its isolation was made by applying the CTAB method as described by Lodhi et al. (1994) and improved by Pop et al. (2003) and Szabo et al. (2015). Concentration and purity of the extracted DNA were determined using NanoDrop1000 spectrophotometer. The SRAP analysis was carried out according to previously established protocols by Li and Quiros (2001). In order to select the most reliable primer combinations for further analyses, 64 different primer combinations were employed in this assay using eight forward and eight reverse primers (Table 2).

Table 2. SRAP primers sequence

Forward primers	
Me1	F: TGA GTC CAA ACC GGA TA
Me2	F: TGA GTC CAA ACC GGA GC
Me3	F: TGA GTC CAA ACC GGA AT
Me4	F: TGA GTC CAA ACC GGA CC
Me5	F: TGA GTC CAA ACC GGA AG
Me6	F: TGA GTC CAA ACC GGA CA
Me7	F: TGA GTC CAA ACC GGA CG
Me8	F: TGA GTC CAA ACC GGA CT
Reverse primers	
Em1	R: GAC TGC GTA CGA ATT AAT
Em2	R: GAC TGC GTA CGA ATT TGC
Em3	R: GAC TGC GTA CGA ATT GAC
Em4	R: GAC TGC GTA CGA ATT TGA
Em5	R: GAC TGC GTA CGA ATT AAC
Em6	R: GAC TGC GTA CGA ATT GCA
Em7	R: GAC TGC GTA CGA ATT CAA
Em8	R: GAC TGC GTA CGA ATT CAC

The polymerase chain reaction was performed in 15 µL reaction mixture containing 1 × Green Buffer, 1.5 mM $MgCl_2$, 200 µM of dNTPs, 0.3 µM of both forward and reverse primers (Generi Biotech), 1 U of Taq DNA polymerase (Promega) and approximately 50 ng of template DNA. Amplifications were made in a Corbette Research PalmCycler with an initial step at 94°C for 5 min and five cycles of 1 min. at 94°C, 1 min. at 35°C, and 1 min. at 75°C. The following 35 cycles consisted of 94°C for 1 min., 50°C for 1 min., and 72°C for 1 min., with a final extension at 72°C for 10 min. The PCR reactions were repeated to ensure the reproducibility of the DNA. Amplified products were separated on 1.6 % (w/v) agarose gels in 1.0 × TAE buffer at 115 V, for approximately 2 hours. A 100-bp DNA ladder was used as molecular weight marker in order to confirm the appropriate SRAP markers. The electrophoretic profiles were stained with 1µg/mL EtBr form 20 to 30 minutes and photographed with Biospectrum AC

The obtained images were processed with Total Lab 120 software (Figure 1).

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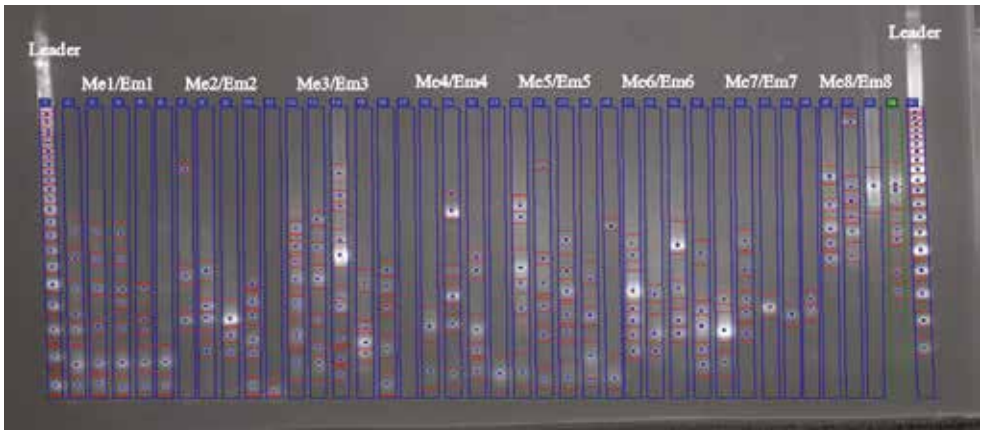

Figure 1. Sequence-related amplified polymorphism (SRAP) profiles obtained from 16 primer combinations of four species

RESULTS AND DISCUSSIONS

The screening of 64 SRAP primer pairs (eight forward and eight reverse) generated a total number of 886 DNA fragments ranging from 1 to 35 bands per primer combination. The average number band per primer set recorded was 13.9. The size of amplification products ranged from 400-3,200 bp.

In order to select the most efficient primer combinations for subsequent analyses, the main criteria taken into consideration were the clarity and reproducibility of the amplified bands together with the rate of polymorphism.

Therefore, after the screening of the amplified products, 37 primer pairs were discarded out of the 64 tested, because they either yielded no amplification or no polymorphic patterns.

The remaining 27 primer combinations produced a total number of 553 DNA fragments with an average number of 20.5 clear bands per primer pairs (Table 3).

Table 3. The remaining 27 primer combinations

Primer combination	Number of amplified fragments				Total number of bands	Average number of bands
	A. moldavicum	*A. transsilvanica*	*R. carpaticus*	*R. platanifolius*		
Me1/Em1	7	6	7	5	25	6.3
Me1/ Em3	3	6	5	3	17	4.3
Me1/ Em4	5	6	6	4	21	5.3
Me1/Em6	4	4	6	1	15	3.8
Me1/ Em7	5	6	6	1	18	4.5
Me2/Em2	3	4	3	6	16	4.0
Me2/ Em3	6	1	4	3	14	3.5
Me3/ Em2	6	2	1	2	11	2.8
Me3/Em3	7	6	10	4	27	6.8
Me3/ Em4	6	6	1	1	14	3.5
Me3/ Em5	3	8	4	4	19	4.8
Me4/ Em1	2	5	8	3	18	4.5
Me4/Em4	0	2	6	5	13	3.3
Me4/ Em6	3	4	7	4	18	4.5
Me4/ Em7	2	5	7	7	21	5.3
Me5/Em5	6	6	6	4	22	5.5
Me6/ Em4	3	6	7	3	19	4.8

Primer combination	Number of amplified fragments				Total number of bands	Average number of bands
	A. moldavicum	*A. transsilvanica*	*R. carpaticus*	*R. platanifolius*		
Me6/ Em5	4	8	6	5	23	5.8
Me6/Em6	7	3	4	5	19	4.8
Me7/ Em4	7	6	6	9	28	7.0
Me7/ Em5	6	4	8	7	25	6.3
Me7/ Em6	5	3	5	8	21	5.3
Me7/ Em8	10	7	6	8	31	7.8
Me8/ Em1	9	4	8	9	30	7.5
Me8/ Em6	3	2	5	10	20	5.0
Me8/ Em5	7	10	10	8	35	8.8
Me8/Em8	6	1	6	0	13	3.3
Total number of bands	**135**	**131**	**158**	**129**	**553**	
Average number of bands	**10.4**	**10.1**	**8.8**	**9.9**	**20.5**	

The most representative SRAP profiles were obtained with the primer combinations *Me1/Em1, Me3/Em3*, *Me7/Em4*, *Me7/Em5*, *Me7/Em8*, *Me8/Em1* and *Me8/Em5* which generated a total number of 201 visible DNA fragments with a mean value of. 28.7 clear bands per primer set (Figure 2).

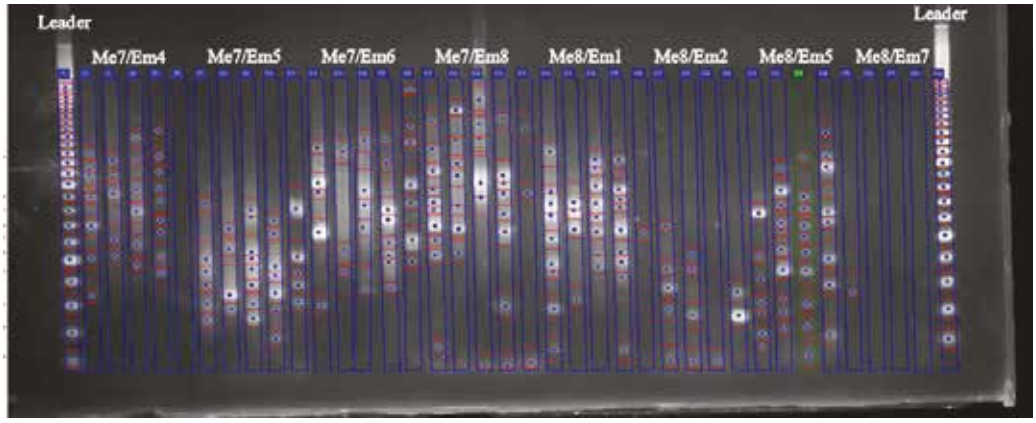

Figure 2. Electrophoretic profiles of four species obtained with eight primer combinations

A. moldavicum samples show good results with the primer combinations: *Me7/Em8, Me8/Em1, Me8/Em5, Me7/Em4*, *Me6/Em6*, while for *A. transsilvanica* the most effective primers pairs were founded to be: *Me3/Em5, Me6/Em5, Me7/Em8, Me8/Em5*. For *R. carpaticus* the following combinations were the most informative: *Me3/Em3, Me4/Em1, Me7/Em5, Me8/Em1, Me8/Em5*. Besides, the most effective primer combinations for *R. platanifolius* were *Me7/Em4, Me7/Em6, Me7/Em8, Me8/Em1, Me8/Em6*.

Regarding the number of amplified bands per primer pair, 13 primer combinations were selected as the most promising for *A. moldavicum*, *A. transsilvanica* and *R. platanifolius,* respectively, while for *R. carpaticus* 18 combinations were the most representative (Figure 3).

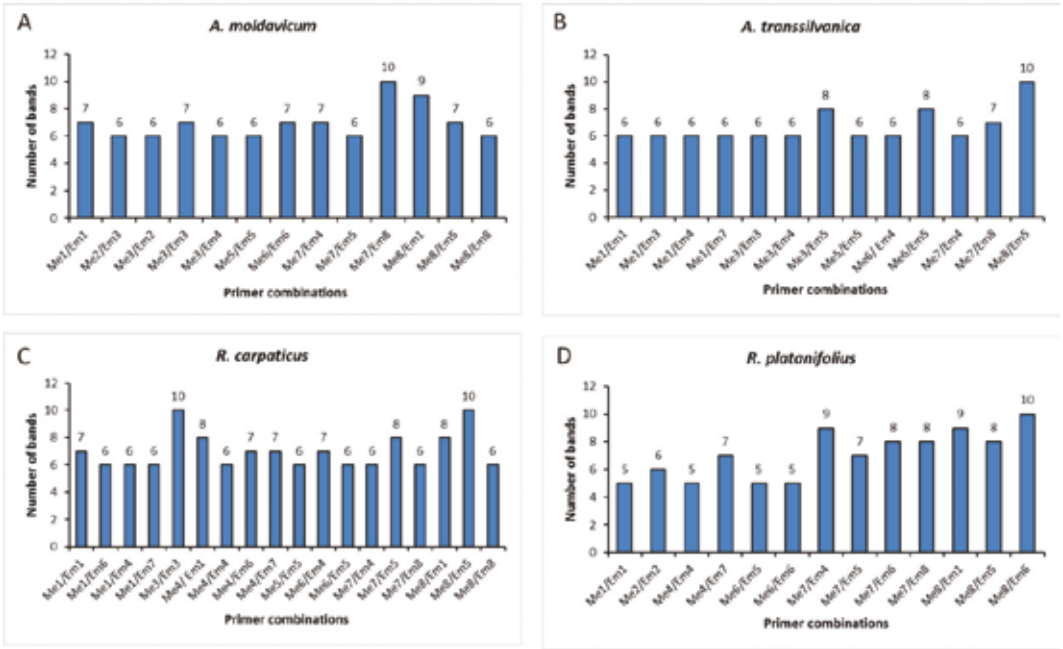

Figure 3. The representative combinations of *A. moldavicum*, *A. transsilvanica, R. carpaticus* and *R. platanifolius*

Similar studies were carried out on *Carthamus* species as reported by Mokhtari et al., 2013, who found that the most informative SRAP primer combinations were *Me4/Em1* and *Me5/Em2*.

Another report published on *Citrus* species show, that the most effective primers pairs that worked, were *Me3/Em2* and *Me6/Em4* (Uzun et al, 2009).

Moreover, based on the number of polymorphic fragments the most representative SRAP primer combinations reported for 76 *Vitis* species were *Me1/Em7, Me3/Em2, Me5/Em6* (Guo et al., 2012). Likewise, Huang et al, 2017 reported that for *Stylosanthes* species the best working SRAP primer pairs for genetic variation analysis were *Me1/Em2, Me6/Em7, Me8/Em1* and *Me9/Em2* (Huang et al., 2017).

Therefore, it can be concluded that the screening of SRAP primer combinations is needed to ensure a high polymorphic content for further analyses.

CONCLUSIONS

As a conclusion, these results provide useful information of the most efficient SRAP marker combinations of four medicinal species namely *A. moldavicum*, *A. transsilvanica*, *R. carpaticus* and *R. platanifolius* also for *in situ* and *ex situ* conservation perspective. Furthermore, in this study have been shown that the selected SRAP markers combinations represent a powerful tool and highly contribute to perform future analyze intra/interspecies and intra/interpopulation genetic diversity.

To the best of our knowledge, SRAP markers were used for the first time to analyze genetic diversity of species belonging to the *Ranunculaceae* family.

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POSSIBILITIES FOR SMALL-SCALE COMPOSTING OF HORTICULTURAL PLANT WASTES

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Abstract

The aim of the study was to investigate the possibilities for composting in a small suburban farm, where also vineyards, orchards and vegetable crops are grown. The experiment was carried out in the period 2016-2017 in the experimental field on University of Forestry - Sofia. In the spring of 2016 were selected plant wastes from viticulture and horticulture and were built two compost piles – one only with plant residues (grape vine canes, fruit twigs and grass windrow) and one with rabbit manure,(grape vine canes, fruit twigs and grass windrow+ rabbit manure). In the autumn of 2016 was built a compost pile only with plant residues from vegetable field (frostbitten tomato and pepper stems and fresh leek residues). During composting period it was monitored the temperature regime in the compost piles. They achieved high temperature (60 and above 60 °C). The active phase of composting, in the three compost piles lasted for about a month. At the beginning and after the 6 months period were measured pH and C/N ratio in the compost piles and changes were established. After the 6 months period the C/N ratio was below 20/1, which is an indicator of mature compost. The pH in the spring piles dropped to 7.5-7.6 while the autumn pile remained alkaline - 8.4.

Key words*: composting, grape vine canes, fruit twigs, rabbit manure, vegetable residues.*

INTRODUCTION

Composting is a natural process of decomposition of organic waste using microorganisms and under strictly controlled conditions, at the end of the process to give a stable humus-like organic product called compost. (Misra R.V. et al., 2003).

Organic waste from agriculture - from pruning, harvesting, manure, etc. can be transformed into material that is used to improve soil structure and provide nutrients. (Misra R.V. et al., 2003; Román P. et al., 2015)

Most of these materials can easily be harvested on each farm. In the presence of animals, it is well that plant waste materials to be composted are not those used as fodder (Inckel M. et al., 2005; Schuchardt F., 2005).

When testing various combinations of plant residues from tomatoes, cucumbers, eggplants and pepper (composted alone or with olive tree branches and vine rods), it has been found that the compost piles made by mixing vegetable crops with twigs or grapes rods have developed high temperatures and the composting process has been beneficial (Maniadakis K. et al., 2004).

In aerobic composting, one of the most used methods is the ordering layers by layers of the various materials for the construction of the compost piles, alternating layers of carbon and layers of nitrogen material, the different authors indicating a different thickness of the layers. (Misra R.V. et al., 2003; Inckel M. et al., 2005).

Aerobic composting is a dynamic process. Several main factors are favoring composting processes: temperature, pH, oxygen level and C/N ratios.

These factors are a key point in building sustainable practices for organic waste management (Maheshwari D.K. et al., 2014).

Studies on the carbon, nitrogen, pH content are key to tracking composting processes (López-González et al., 2013).

When testing a different initial ratio C/N of 20/1, 30/1 and 40/1, compost with an initial C/N ratio of 30/1 has developed the highest maximum temperature (63.5°C) (Azim K. et al., 2014).

To determine if the compost is mature, ie. whether it is ready for use, different methods

are available. Changes in temperature, pH, C/N ratio, microorganisms are observed. The C/N ratio gradually decreases and when it reaches less than 20, the temperature also decreases (Karadag D. et al., 2013).
Many authors point out that the C/N ratio can be taken as an indicator of maturation of compost (Goyal S. et al., 2005). Other authors have the opposite view because of the variety of materials that can be composted - with their structure and accessibility.
Others point out that the ratio is not important, but rather how much it decreases during the composting process. Ratio C/N=12 is indicated for the ideal but also a C/N=15-20 ratio is also acceptable (Estévez-Schvarz et al., 2012).
The aim of the study was to investigate the possibilities for composting in a small suburban farm, where also vineyards, orchards and vegetable crops are grown.

MATERIALS AND METHODS

The experiment was carried out in the period 2016-2017 in the experimental field on University of Forestry - Sofia.
In the spring of 2016 were selected plant wastes from viticulture and horticulture and rabbit manure. In the autumn of 2016 were selected plant residues from vegetable field.
Three compost piles were built from the crushed plant materials - two in the spring and one in the autumn:

- V1 - a combination of grape vine canes, fruit twigs and grass windrow;
- V2 - a combination of grape vine canes, fruit twigs and grass windrow + rabbit manure;
- V3 - a combination of frostbitten tomato and pepper stems and fresh leek residues.

It was made a pre-preparation of the starting materials - they were shredded into smaller part by garden shredder. Shredded material contained finely divided particles and larger ones. Larger parts were also inserted into the compost piles to aid natural aeration inside the pile.
To individual compost piles were incorporated starters - materials, making it easier to start the microbiological processes in the compost piles (large waste from last year's compost of plant waste and a thin layer of soil).
The first compost uses a combination of brown materials in a ratio of 2.4/1 (grape vine canes/fruit twigs). To them was added a grass windrow as a green material. The weight ratio of brown/green materials was 1.6/1 (brown/green).
The second one also uses a combination of the above-mentioned brown materials at a ratio of 2.2/1 (grape vine canes/fruit twigs). A combination of green materials was added to them - a grass windrow and a rabbit manure in a ratio of 1.1/1. The total weight ratio between brown and green materials is 1.8/1.
The compost bundle in autumn is dominated by green materials in order to develop a higher temperature in the compost bundle under conditions of low air temperatures.
During composting period it was monitored the temperature regime in the compost piles. With compost thermometers in the middle of the compost pile. The temperature was measured at noon hours.
Laboratory analyzes of the content of C, N, K, P, Mg, in starting materialsand in mature compost to track changes in compost and to establish its quality.
Calculate the ratio of C/N in the starting materials and in mature compost.
The data have been statistically processed and interpreted.

RESULTS AND DISCUSSIONS

The selection of waste materials is based on two criteria: to be available in sufficient quantity and not to be used in animal husbandry (such as bedding or fodder). Manure samples were taken for analysis from all groups of animals that are kept.
The total weight of materials used to build the first compost pile was 59,500 kg and for the second compost pile was 56,050 kg.
The total weight of the materials used to build the third compost pile was 218 kg.
Together with the preparation of the composting materials were taken samples to analyze the content of chemical elements. (Tables 1 and 2).

Table 1. Available plant waste and their chemical characteristics

Type of material	Season	Chemical elements				
		C %	N %	P mg/kg	K mg/kg	Mg mg/kg
Grape vine canes	Sping	26.56	0.753	1.02	4631	1176
Fruit twigs	Sping	25.09	0.670	0.89	2856	725
Corn leaves	Autumn	26.93	0.973	52.92	5619	1057
*Grass windrow	Sping/ Autumn	26.01	3.355	3.62	30622	2807
Tomato stems frostbitten	Autumn	24.11	2.225	301.28	3671	5150
Pepper stems frostbitten	Autumn	25.15	2.454	127.60	5796	7322
Fresh leek residues	Autumn	18.46	1.737	138.43	27490	3201

* Note: grass windrow can be used for composting and for fodder.

The C/N ratio was highest in the waste material from fruit (37.45) and grape vine canes (35.27), as they are carbon materials and the grass windrow had the lowest ratio (7.75), since it was high in total nitrogen. The remaining three macroelements were also the highest in grass windrow (P 3.62 mg/kg; K 30622 mg/kg; Mg 2807 mg/kg).

The rabbit manure had the highest nitrogen content (3.92%) compared to other manure, and the other chemical elements in it were also high in content.

Horse manure was low in nitrogen (1%) and respectively low C/N ratio (44.50%), probably due to the rough feed fed to the horses.

Table 2. Available animal waste and their chemical characteristics

Type of material	Season	pH (H_2O)	Chemical elements			
			C %	N %	P mg/kg	K mg/kg
Horse manure	All year round	7.55	44.50	1.00	0.68	1.45
Cattle manure	All year round	7.45	35.56	2.08	0.88	0.65
Pig manure	All year round	8.02	37.24	3.38	2.31	1.17
Mixed manure (sheep and goat)	All year round	7.30	37.78	2.70	2.77	4.26
Rabbit manure	All year round	8.05	40.46	3.92	2.56	3.24

Meteorological conditions during composting period were characterized by precipitation during the first and second ten days of June (06-08 and 13-15.06.2016), with the sum of precipitation being 49.4 and 23.1 l/m^2 respectively. Rainfall was also reported in July, but they were not heavy (Figure 1).

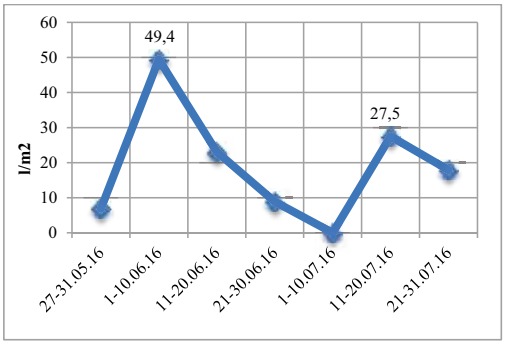

Figure 1. Amount of rainfall in l/m^2 at ten-day periods

Wet periods also led to a decrease in the average daily temperature at the end of May and in the first ten days of June (Figure 2). The precipitations recorded in the second ten days did not lead to a decrease in the air temperature (Figure 2).

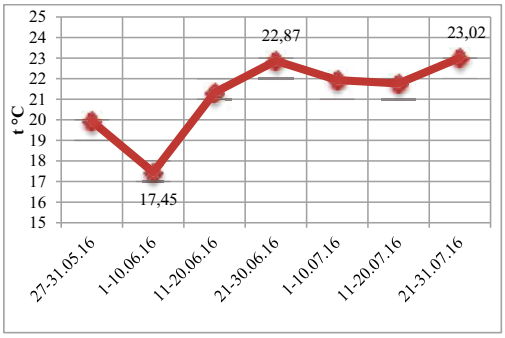

Figure 2. Average daily temperature at ten days periods

Weather conditions affected the temperature of the compost piles. The temperature graph of the spring compost piles shows several peaks, which outlined the turning of the compost from an initial mesophilic to a thermophilic and then again into the mesophilic phase (Figure 3).

Composte pile V1 continuously passes from thermophilic to mesophilic phase after about 25 days, and after 27 days the temperature steadily dropped below 30°C.

The thermophilic phase in compost V2 passes after about 21 days, and after 24 days it dropped permanently below 30°C.

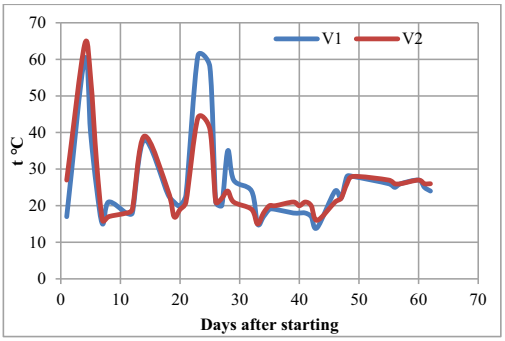

Figure 3. Dynamics of temperature in compost piles

The fall compost was built in the second half of November. The weather conditions were appropriate - sunny weather without precipitation and positive temperatures. During the first ten days of December, the air temperature dropped several times below zero, creating a risk of cooling the compost (Figure 4)

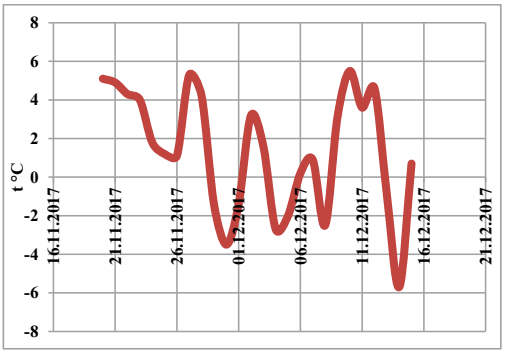

Figure 4. Average daily air temperature for the period 20.11.-15.12.2017

The temperature graph of the autumn compost pile showed that the compost was self-heating well and reached a high temperature of 61°C.
The temperature curve clearly indicates that the compost passed through the mesophilic and thermophilic phases (Figure 5).
With the fall of the compost temperature near 10°C, which coincided with the negative temperatures during the first ten days of December, there was a danger of a permanent cooling of the compost bundle. But the followed favorable daytime temperatures, along with the large mass of plant mass, helped to pass through (Figure 5).

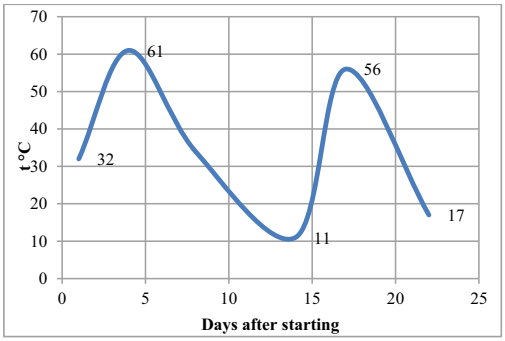

Figure 5. Changing the temperature in compost pile V3, bild in autumn

The last ten days of December, along with the fall of the snow, the temperature of the compost has dropped permanently and the temperature measuring has been stopped (Figure 5).

Table 3. Chemical composition of mature compost

Variants	pH (H_2O)	Chemical elements				
		C (%)	N (%)	P mg/kg	K mg/kg	Mg mg/kg
V1	7.6	16.33	1,028	2311	4970	2451
V2	7.5	12.65	1,005	2636	4434	2518
V3	8.3	4.86	1,070	2029	3592	2301

At the end of May 2017, were taken samples from the three compost piles for chemical analysis and tracking of composting processes (Table 3).
The highest level of pH is compost V3. The content of phosphorus, potassium and magnesium had lower values than the other two composts. Compost V1 had the highest C and K content, while compost V2 had the highest P content, which may be due to the presence of rabbit fertilizer in the compost. In the calculations of the ratio C/N at the beginning and end of the composting were found differences in the ratio C/Nin the three compost piles.
There is a decrease in the C/N ratio, and for spring compost pilesV1 and V2, this ratio has changed in the range of 11-13:1, while in autumn compost (V3) which at the beginning of the composting process is low , at the end of composting is reduced to about 4.5:1.
At this compost, all the basic materials showed low C/N ratios, where brown materials were virtually absent (Figure 6)

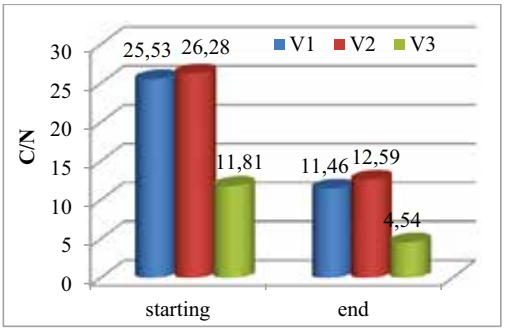

Figure 6. The C/N ratio of the compost piles at the beginning of the composting process and in the mature compost

A ratio of less than 20:1 at the end of composting is considered to be an indicator of mature compost (Hartz T.K. et al., 1996).

The obtained amounts of the three compost cups are as follows:

From V1 (compost 1) 17 kg of compost was sieved, from V2 (compost 2) 19.5 kg of compost was sieved and from V3 (compost 3) 72.5 kg of compost was sieved.

Visually, at the first two composts, there was more waste from vine branches and fruit rods, although the composting process was one year, while the third compost produced better composting of vegetable waste for only 6 months.

CONCLUSIONS

Small urban farms have the possibility of small-scale composting of plant and animal waste. The starting of the compost piles can be done seasonally and year-round, with sufficient waste materials.

In the spring, brown waste is in sufficient quantity, while the green is in short supply, especially if it is also used for fodder. In the presence of animals, then manure can also be used for green material. Rabbit manure is a good alternative to livestock manure.

In autumn, when cleaning the areas, there is a sufficient amount of plant waste that can be composted. In non-animal farming, there is no shortage of planting waste for autumn composting when we have a favorable mix of vegetable and food crops.

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MORPHOLOGICAL AND ANATOMICAL INVESTIGATION OF *ALOYSIA CITRODORA* PALAU - NEW MEDICINAL PLANT INTRODUCED IN ROMANIA

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Abstract

Lemon verbena - Aloysia citrodora Palau (Verbenaceae) is an aromati plant used for the medicinal properties of itsleaves and essential oils. The species is native to Argentina, Paraguay, Brazil, Uruguay, Chile, Bolivia and Peru. It is cultivated and commercialised as an aromatic plant for its lemon-like scent of its leaves and flowers. The dried plant and its extracts are valuable for medicinal preparations, in the perfumery industry and as an ingredient for the gourmet cuisine.It is often used in phytotherapiessuch as a digestive and diuretic, antispasmodic, carminative and sedative, antimicrobial and local analgesic. In 2015 Aloysia citrodora Palau was studied at theUniversity of Agronomic Sciences and Veterinary Medicine of Bucharest. The study targeted the stems and leaves and it shows the presence of glandular and non-glandular trichomeson all of its aerial parts. Morphological and anatomical analyses of the leaves and stems were made with the plant material cultivated in our University at the Research Center for Studies of Food Quality and Agricultural Products Greenhouse.

Key words*: Aloysia citrodora, morphological, leaf structure.*

INTRODUCTION

Aloysia citrodora, commonly called lemon verbena is a native plant - to Argentina and Chile. *Aloysia citrodora* Palau is synonymous with *Verbena triphylla* L'Hér., *Aloysia triphylla* L'Hér., *Lippia citriodora* Kunth, and *Lippia triphylla* (L'Hér.) Kuntze (Erikson, 2006). It is commonly known as "lemon verbena", "cedrón", "cidron", "hierba Luisa" or "verveineodorante", according to the country. The genus *Aloysia* Ortega ex Jussieu belongs to the *Verbenaceae*. This genus comprises about 30 species, distributed on the American continent from the south of the United States down to Chile and Argentina. Spanish explorers brought this plant to Spain in the 17th century at which point it was named after Princess Louisa of Parma, genus name honors Maria Louisa, princess of Parma and wife of King Carlos IV of Spain. Lemon verbena isan evergreen plant in tropical or warm winter locations, but deciduous in areas where freezing temperatures occur. In colder climates it is grown in containers and overwintered indoors, a period in which the plant is leafless and requires low amounts of water. In spring, the plant recovers with leaves. *Aloysia citrodora* has been a popular garden plant in warm southern and western parts of the U.S. The plant has a gentle sedative action and areputation for soothing abdominal discomfort, has amild tonic effect upon the nervous system and helps to depression（Gattuso et al., 2008).

The leaves of lemon verbena confer a refreshing lemon flavor to the teas and infusions isused for digestive problems (as flatulence, indigestion and acidity). The leaves and flowers are used for culinary purposes (fruit salads and jams, teas, desserts), cosmetics, for potpourris and as herbal medicines (colds, fevers, dyspepsia and diarrhea). In addition, the essential oil of lemon verbena has anti-bacterial and anti-fungal properties (Hanna et al., 2011), while the essential oils, tea and tinctures proved an antibacterial and antioxidant activity (Cowan 1999; Valentão et al., 2002; Sartoratto et al., 2004; Pereira and Meireles, 2007). Traditionally, lemon verbena is used as folk

remedy in treatments of spasms, cold and fever (Carnat et al., 1999), insomnia and anxiety (Van Hellemon, 1986; Newal et al., 1996; Wanmacher et al., 1990 a, 1990 b) and as source of analgesic, antiinflammatory and/or antipyretic remedies (Pascual et al., 2001). It is also often included in phytomedicines such as digestive and diuretic (Duke, 1985; Torrent Martí, 1985), antispasmodic (Torrent Martí, 1985), carminative and sedative (Alonso Paz et al., 1992; Mors et al., 2000), antimicrobial and local analgesic (Hieronymus, 1882; Dellacasa and Bandoni, 2003). Used alone or in combination with other herbs, the leaves make an excellent herbaltea. The essential oil is used in aromatherapy, extensively used in perfumery, especially in making eau de toilette and eau de cologne. The plants repels midges, flies and other insects (Benzi et al., 2009). The essential oil is an effective insecticide in 1-2% concentration. *Aloysia citrodora* is codified by the Argentine Pharmacopeia (1978), Francaise Pharmacopeia (1996), Argentine Alimentary Codex (1969-2007) and European Pharmacopoeia (2007). It is included in the FDA's GRAS list, i.e. the list of food additives which are Generally Regarded As Safe (Newall et al., 1996).

MATERIALS AND METHODS

Plant material

The plant material is a species and not a cultivar. The plant material to start the study was bought from plant-shop.ro as a 15 cm plant (initially in a jiffy peat pellet) in a 9 cm pot.

In the spring of 2015 Paduraru Jorj began the study of multiplying *Aloysia citrodora*. The activity took place in the Hortinvest Greenhouses and in Domnesti village (44° 24′ 1.5″ N, 25° 54′ 55.1″ E) Ilfov, 14 km of Bucharest. The crop was fertilised with BioHumusSol.

Being the first experimental study of having *Aloysia citrodora* in Romania's climate, the study began with 200 plants in the field. Considering the results of testing the planting distances of *Aloysia citrodora* in other temperate climates, such as in the experimental fields in Zalec, Slovenia, 1997, it was chosen the 40/40 cm as planting distance. At planting distances in the field of 40 x 40 cm, the average yield was 1050-1150 kg of dry leaves per hectare.

The fresh material, leaves and stems, whichwas used for the macroscopic and micro-morphologic studies, was collected in 2017, in the summer period, from the greenhouse of the Research Center for Studies of Food Quality and Agricultural Products, located in Bucharest, Romania.

The cross sections intended to illustrate the morphological and anatomical aspect were made on the same material, in Botanics laboratory. The sections were clarified with chloral-hydrate for 24 hours, and stained with Alaun-Carmine and Iodine Green suitable to optical microscopy techniques. Observations and images of the anatomical structures in stem and leaves were made with the optical microscope Leica DM1000 LED, Camera video Leica DFC295 and the Stereomicroscop Leica S8 APO, belonging to the Laboratory of microscopy and plant anatomy of the same research center.

RESULTS AND DISCUSSIONS

Macroscopical characteristics. The lemon verbena is a woody shrub, with lanceolate green leaves, with strong aroma and lemon-taste. The analysis of the foliar architecture shows simple pinnate, leaves, 5-10 cm long, lanceolate, with short petioles, in whorls of three. The color of lamina is pale green, white to pale lilac flowers, usually assembled in terminal racemose inflorescences.

Microscopical characteristics. The blades of the leaves exhibit anomocytic stomata on the abaxial side (Figure 1) and: two types of trichomes present on both sides of the leaf surfaces, non-glandular and glandular trichomes (Figure 2, Figure 4). The dorsiventral mesophyll, epidermic abaxial cells present striate cuticule around of the stomata. Metcalfe and Chalk (1972) mentioned for the genus *Aloysia* the occurrence of anomocyticstomata. Mesophyll is dorsiventral (Figure 3), with a 2-3 layer of palisade parenchyma and spongy parenchyma cells, this tissue is located next to the abaxial epidermis, the midrib consists of a collateral vascular bundle and the lower epidermis is unstratified and presents stomata. Transverse sections of the leaf measuring 10.297 to 12.318 μm and palisade parenchyma measuring 3.610 to 4.404 μm (Table 1).

Table 1. Leaf and palisade parenchyma thicknesses

Thick of leaf (µm)	Palisade parenchyma (µm)
12.065	3.770
12.206	4.304
11.306	4.292
10.638	3.859
11.306	3,398
12.318	4.404
11.430	3.705
10,297	3.610
10.915	4.224
10.407	3.871

Figure 1. Abaxial epidermis with anomocytic stomata and glandular capitata trichomes

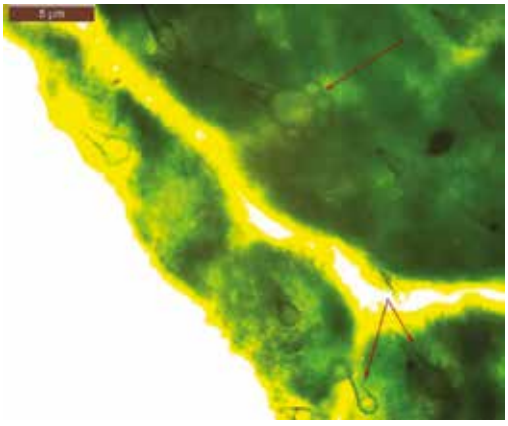

Figure 2. Adaxial epidermis with non-glandular trichomes

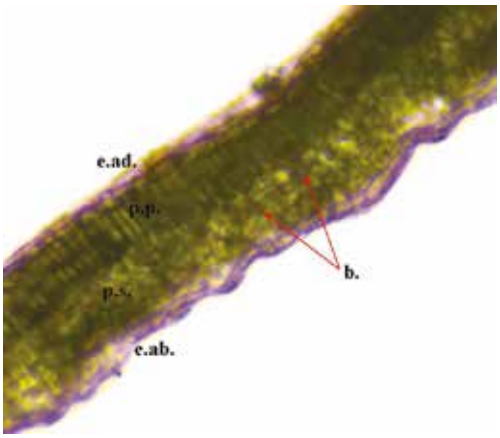

Figure 3. Leaf (cross section): e.ad. - adaxial epidermis; p.p. - palisade parenchyma; b. - colateral vascular bundle; p.s. - spongy parenchima; e.ab - abaxial epidermis

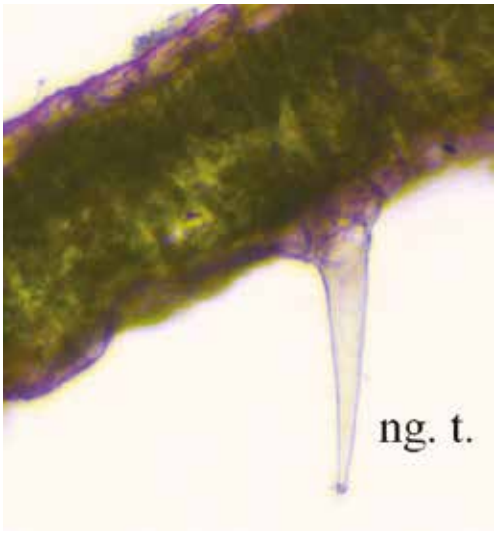

Figure 4. Detail of abaxial epidermis with non-glandular trichomes

Stem anatomy: the stem, in incipient secondary growth, presents epidermis, angular collenchyma and the vascular bundles which are open collaterally; transverse sections show a circular margin with 6 ribs, the epidermis is unstratified, with stomata.

At the level of the ribs and in a subepidermal position, 3-4 layers of angular collenchyma can be observed and cork usually appears near the phloem. Vascular bundles are open collaterally (Figures 5 and 6).

Nonglandular and glandular trichomes were observed in the unistratified epidermis as described for the leaf.

For the *Verbenaceae* family several descriptions of trichomes exist (Cantino, 1990; Yashodhara et al., 2001). Specifically for *A. citrodora*, Casadoro and Rascio (1982) in their ultrastructural study of its leaf trichomes, reported the presence of three types of glandular and one typeof non-glandular trichomes.

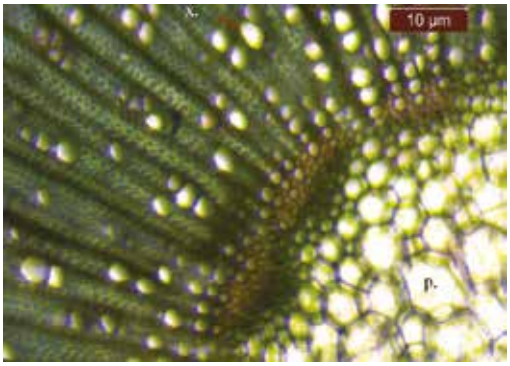

Figure 5. Stem (cross section):
x. - xylem; p. - parenchyma

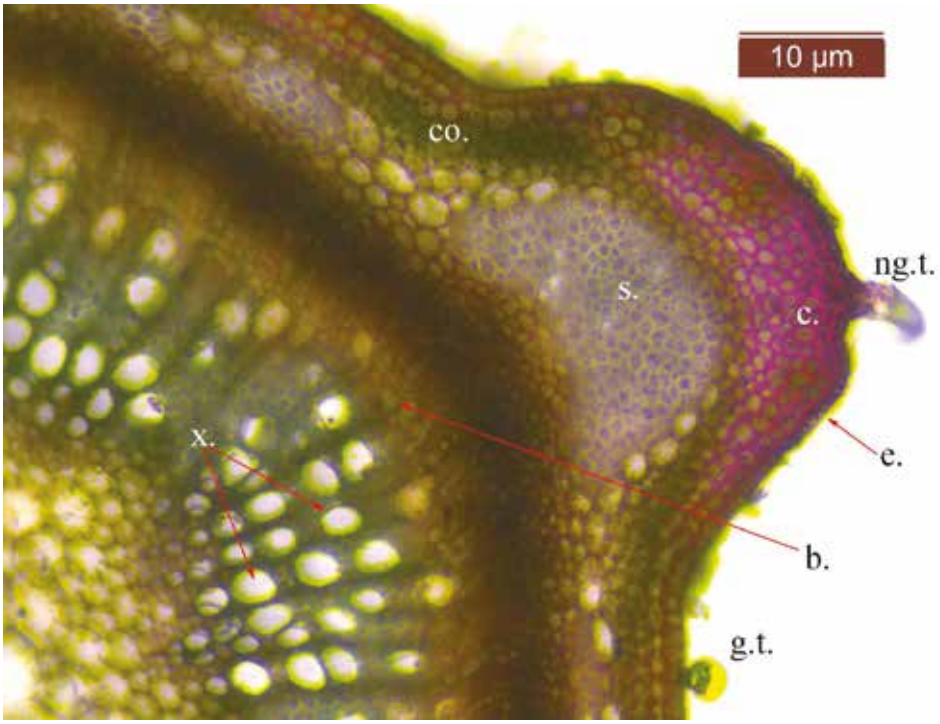

Figure 6. Stem (cross section): e. – epidermis; ng.t. - non-glandular trichome; g.t. – glandular trichome; co. - cork; c - colenchyma; s. – sclerenchyma; b. – bundle; x. - xylem

OBSERVATIONS IN THE FIELD. REVIEW

The species does well in Romania mainly as an annual crop. It showed that it reaches it's biological potential, producing leaves above the the parameters mentioned in the morphology of the species (20.0 cm^2): 10/2.5cm, 21.75 cm^2 leaf.

For mechanized harvesting, it can be planted in wide strips. The recommended plant material is vigorous rooted cuttings in a perlite and peat mix substrate, using rooting hormones from parent plants grown in the greenhouse.

The species can be cultivated successfully in Romania as an annual crop, where it has demonstrated that it reaches its biological vegetative potential, producing leaves beyond the parameters of the morphology of the species. It also blooms, but sporadically.

On the first harvest at Domnesti, after harvesting plant material on August 1st 2015, 45 days after planting, the plants continued to grow by giving vigorous shoots that grew equally fast. This fact, corroborated with the data from the literature, shows that two crops per year can be carried out, manually or mechanically cutting all leaf shoots from the entire cultivated area, in the temperate zone with scented verbena cultivated as annual.

It shows horticultural interest for taking into commercial, possibly ecological crops, a statement based on the fact that in the Domnesti field the disease attack was zero and pests only by chance and without reaching an economic threshold of harm.

The species yields higher yield when fertilized with organic fertilizer.

50 of 2 years of age plants were left outside over winter as bushes cut to 20 cm over the ground level, covered with nothing but soil as winter protection and they survived winter well 3 years in a row. However the winters were atypical and this shows that the species even hardy at only about minus 10 Celsius degrees, sometimes it can pass winters outside just well even in temperate climates depending overall conditions.

CONCLUSIONS

The following macroscopic and micro morphological parameters were established after the analysis of the studied plant material cultivated in our University.

From the morphological point of view there were determined: simple, entire, lanceolate, petiolate, pinnate leaves. The stem presented many ribs.

After this study it can be mentioned the following anatomical characteristics of the leaves: adaxial epidermis with anticlinal thin walls and strains, abaxial epidermis with anticlinal thin and sinuous walls, anomocytic stomata, non glandular and glandular trichomes, hypostomatic dorsiventral mesophyll. The above described elements should be useful for corect botanical identification of *A. citrodora* species.

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DYNAMICS OF MICROBIOLOGICAL INDICATORS FOR COMPARATIVE STUDY OF COMPOST VARIANTS

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Abstract

The microbiological dynamics of different starting vegetable and animal waste was investigated. There is a 5 variants scheme of compost bunches (4 spring and 1 autumn) with different starters - two spring composts containing a combination of grape vine canes, fruit twigs and grass swath, in addition to one rabbit manure; starter: last year`s compost, two spring composts of mulberry twigs with leaves, and when the compost are turned, a different amount of clean litter and twigs is introduced; starter: soil and one autumn compost containing pepper and tomato stems and leaves, roots of tomatoes and pepper with soil, green leaves of leeks, corn and leek waste. A 6-fold microbiological analysis was started on the 7th day of the compost materials and repeated in 7-10 days during the compost reversal period. Main groups of heterotrophic microflora - ammonifying bacteria (non-spore and bacilli), actinomycetes and micromycetes are defined. The study was carried out by the method of dilution and culture of solid nutrient media with determination of cfy (colony forming units) in 1 g abs. dry substrate. The data from the microbiological analysis show differences in the course of the individual stages of composting by microbiological indicators. These differences are expressed in terms of both the total amount of microorganisms in the substrates and the dominant physiological and systematic groups of microorganisms in the microbiocenosis. The different microbiological composition of the compost materials determines a different rate of decay of the separate raw materials, which is reflected in the duration of the composting process itself.

Key words*: compost, grape vine canes, horticulture wastes, microbiological dynamics, rabbit manure.*

INTRODUCTION

Microorganisms play an important role in the composting process. They use organic matter as a source of nutrients (Rynk et al., 1992; Borken et al., 2002), as a result of the development and the activity in the formation of compost removed heat, CO2, water vapor and forming a humus (Epstein, 1997). Composting involves different types of microorganisms - bacteria, fungi and actinomycetes. They have different characteristics and functions that are vital to the process of composting (Lee, 2016). According Tiquai (2005) in addition to studies of microbial biomass, respiration rate and content of ATP, the enzyme activity is also one of the most effective methods that can be used to monitor the stability and maturity of compost.

In a typical composting process both bacteria and fungi are present (Gray et al., 1971). Earlier studies have shown that the main bacterial groups at the start of the composting process are mesophilic bacteria producing organic acids such as *Lactobacillus* spp. and *Acetobacter* spp., and later, in the thermophilic stage dominated Gram-positive bacteria such as *Bacillus* spp. and *Actinobacteria* (De Bertolli et al., 1980). According to Abu-Bakar (2015), exactly bacteria provide the fastest and most efficient composting. In his study, Partanen et al. (2010) found that bacteria from *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Deinococcus-Thermus* and from over 2000 different philotypes participated in the composting process. Fungi develop both in mesophilic and thermophilic phase of the composting, in an amount between 0.01 and 1 million per gram compost (Kowalik, 2015), as are present on the surface of the compost, when the temperature is higher. Some types of actinomycetes develop during the thermophilic phase, while others develop in

subsequent stages of maturation and ripening, by degrading resistant compounds in the final stages of humification (Lee, 2016; Adani et al., 1997; Andrews and Hirano, 1991).
Successful composting depends on a number of factors that have a direct and indirect impact on the activity of microorganisms. Tiquia et al. (2000); Fracchia et al. (2006) indicate as important factors the type of composite raw material, its nutrient composition and its physical characteristics such as bulk density, pH, moisture content, etc. According to Garcia et al. (1993) and Serra-Wittling et al. (1996), composting results in homogeneity in the composition of the product, irrespective of the starting material and the composting process itself.
Biddlestone and Gray (1985) report that the complexity of the degraded plant materials and the quality of the final product may depend on the type of biomass. In composting, the C: N ratio is considered the most important parameter as it reflects the degree of biotransformations that have occurred in compost from a chemical point of view (Saber et al., 2011). A ratio of C: N of less than 12 during the solid phase is an indicator of maturity of the compost (Bernal et al., 1998; Iglesias-Jimenez, 1993).
The purpose of the present study is to trace the dynamics of different compost variants on the quantity and quality composition of microorganisms developing in composts.

MATERIALS AND METHODS

There has been developed a 4 variants scheme of compost piles (V1, V2, V3, V4) with different row materials:
V1-Brown materials: grape vine canes and fruit twigs; Green materials: grass swath; Starter: last year compost residues.
V2-Brown materials: grape vine canes and fruit twigs; Green materials: grass swath and rabbit manure; Starter: last year compost residues.
V3-Mulberry twigs with leaves; Starter - soil. On the 7th and 13th day of building the compost pile, when it was turning, were added bedding for silkworm growing and twigs.
V4-Mulberry twigs with leaves (from a contaminated area with heavy metals); Starter - soil. On the 7th and 13th day of building the compost pile, when it was turning, were added bedding for silkworm growing and twigs from the same polluted area.
Microbiological studies include determination of non-sprouting bacteria, bacilli and micromycetes by method of selective plating and direct viable counts. They were used two solid nutrient media (meat-peptone agar for determination of non-sprouting bacteria and bacilli, and medium of Chapek-Dox for determination of micromycetes) and counting of colony forming units, recalculated to 1 g of absolute dry substrate.
The statistical processing of microbiological data includes calculation of an average of three replicates and a coefficient of variation.

RESULTS AND DISCUSSIONS

Dynamics of compost temperature and pH

For the determination of the composting phases, the temperature measurement started with the building of the compost piles and continued until their last turning (Figure 1).

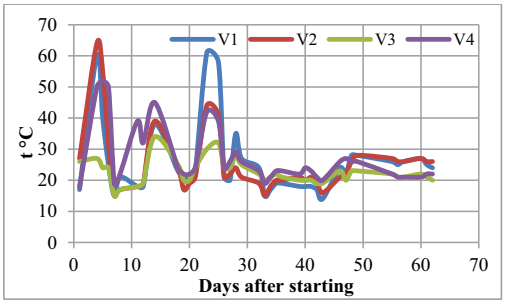

Figure 1. Dynamics of temperature in compost piles

The thermophilic phase of compost pile V1 lasts for about 25 days, and after 27 days the temperature steadily drops below 30°C. The thermophilic phase of compost pile V2 lasts for about 21 days, and after 24 days it drops permanently below 30°C. For compost pile V4 the thermophilic lasts for about 13 days, and after about 26 days, the temperature is continuously dropping below 30°C.
The compost pile V3 did not switch to a thermophilic phase. The highest measured temperature in this pile is 34°C, reached around the 13th day and, similar to V4 compost, after about 26 days, the temperature steadily drops below 30°C.

By making a comparison of the temperature at different compost piles, with the amount of material used for their building, it can be seen that from the four composting piles, with the highest temperatures and longest lasting thermophilic phase is compost pile V1, which is with the most greater mass of starting material (59,500 kg). In descending order, for this indicator are arranged compost pile V2 (56,050 kg) and compost pile V4 (32,200 kg). Compost pile V3, which has the smaller mass of the starting material (24,330 kg), did not reach the thermophilic phase.

Throughout the composting process, the pH of the compost piles was monitored and it was. between 7.8 and 8.0. During the thermophilic phase and the maturation phase, normally the medium is alkaline. Probably because of the rapid passage from mesophilic to thermophilic phases, no acidification of the medium from the microbial extraction of organic acids in the mesophilic phase was established. According to some authors, low initial pH limits microbial activity and slows temperature rise (Sundberg et al., 2004; Romanschuk et al., 2005).

Microbiological analyses

The microbiological analyses were started one week after the building of compost piles V1, V2 and V4, and on the 2nd day after the building of compost pile V3. The analyzes were repeated at each compost turn (between 7-10 days).

The results of the dynamics of the total microflora (total number of microbes in 1 g of substrate) give an idea of the degree of development, resp. settlement of the compost with microbes. This indicator is important for assessing the degree of destruction of organic wastes as far as the microorganisms carry out the mineralization of the organic compounds in them. Data from the general microflora dynamics are presented in Figure 2.

The data show a different dynamics of the total microflora in the four composting variants. It is also possible to note the different start for the presence of microorganisms in the individual variants provided by the various formulations. On the 7th day of experimentation, the highest amount of total microflora was found at V1. This amount is about 1 times higher than the same in the other variants. For this variant (V1), plant wastes have the highest microbial presence, whereas variants with a different amount of mulberry leaf and twigs (V3 and V4) have a lower microbial diversity and presence. Reducing the amount of started plant waste materials and adding the rabbit fertilizer to V2 slows the growth of microorganisms at the start of the experiment (Day 7), but in the next days of reporting, their activation is determined, most preferably on the 13th day.

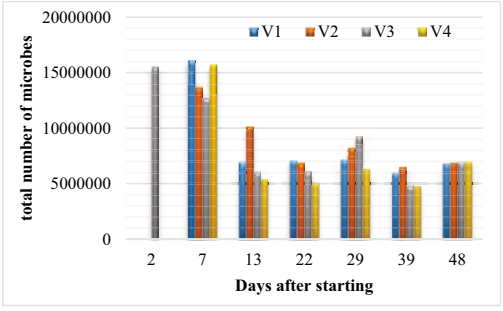

Figure 2. Dynamics of total microflora (number of microbes/g substrate)

In V2, the amount of the total microflora decreases more gradually, whereas in the other variants, the multiplication of microorganisms decreases sharply to the 13th day - 2 times at V1 and V3, and 3 times at V4, after which the breeding process gradually decreases to the end of the composting, with a slight increase in microbial growth on the 29th day after the trial. In all four composting variants it was found that on the 39th and 48th day the amount of microbes was the lowest. At the end of composting, it decreased about 2 times for variants V1, V2 and V3, and 3 times for V4 compared to the amount at the beginning of the experiment. By the seventh day of the experiment, mesophils and thermophiles are grown at V1, V2 and V4, whereas at V3 were found only mesophils, as this compost does not pass through the thermophilic phase. In the period from 22 to 29 days, temperatures rise above 60°C at V1 and above 40°C at V2 and V4, i.e. these variants pass through the thermophilic phase again. The most active is the development of microorganisms up to the 7th day of experiment. Activation of thermophiles and increase of total microflora from Day 22 to Day 29 of experimental was set-up - 1 time at V1, 1, 2 times at V2, 1, 3

times at V4. The most active is the development of mesophils in V3 during this period - the total amount of microorganisms is increased by 1, 5 times. After 29 days to 48 days, compost temperatures are below 30°C, i.e., mesophilic microorganisms develop, the composts are aged and ripened.

In the different phases of composting, different quantitative development of the studied microbial groups - non-sprouting bacteria, bacilli and micromycetes is also reported. The results for the composition of the microflora for 7 days (passage through the mesophilic and thermophilic phase) after the experimental assay in all variants and on the 2nd day (mesophilic phase) at V3 are presented in Table 1.

Table 1. Composition of the microflora 7 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V3 (2-ри ден)	13760 ± 0.262 (88.3)	1720 ± 0.291 (11.0)	100 ± 0.400 (0.6)
V1	13120 ± 0.202 (81.4)	2940 ± 0.340 (18.2)	60 ± 0.333 (0.4)
V2	11200 ± 0.179 (81.9)	2400 ± 0.300 (17.5)	80 ± 0.125 (0.6)
V3	10880 ± 0.243 (85.4)	1720 ± 0.116 (13.5)	140 ± 0.357 (1.1)
V4	13600 ± 0.074 (86.3)	2060 ± 0.243 (13.1)	90 ± 0.222 (0.6)

Mesophils and thermophiles develop in these two phases - mesophilic and thermophilic, with the highest contribution to the composition of the general microflora as non-sprouting bacteria, followed by the bacilli, which are actively involved in the initial stages of destruction of organic matter.

The microbiological analyses at the 13th day (mesophilic phase) after experimentation show a different quantitative development of the different groups of microbes (Table 2):

Table 2. Composition of the microflora 13 days after the experiment (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	5120 ± 0.195 (73.6)	820 ± 0.244 (11.8)	1020 ± 0.196 (14.7)
V2	5200 ± 0.254 (51.2)	960 ± 0.417 (9.4)	4000 ± 0.150 (39.4)
V3	4940 ± 0.202 (81.5)	880 ± 0.341 (14.5)	240 ± 0.417 (4.0)
V4	4040 ± 0.248 (74.8)	880 ± 0.455 (16.3)	480 ± 0.208 (8.9)

The lower temperature with 7°C (V1), 13°C (V2), 5°C (V3) and 18°C (V4) for 1 week is a stress factor for the development of microorganisms which limits their development and changes the role of the different groups of microorganisms in compost 1 and 3. A major share in the composition of the total microflora again occupies non-sprouting bacteria and bacilli, with the exception of composts 1 and 3, where the development of micromycetes is more active than that of the bacilli. Compost variants are in the mesophilic phase, decomposition of organic matter occurs with the development of mesophils.

The composition of the microflora 22 days after the trial of the experiment is presented on Table 3:

Table 3. Microflora composition 22 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	5600 ± 0.089 (79.1)	520 ± 0.385 (7.3)	960 ± 0.208 (13.6)
V2	4800 ± 0.208 (70.0)	500 ± 0.400 (7.3)	1560 ± 0.385 (22.7)
V3	4000 ± 0.250 (65.6)	1900 ± 0.316 (31.1)	200 ± 0.250 (3.3)
V4	3040 ± 0.164 (60.8)	1460 ± 0.342 (29.2)	500 ± 0.100 (10.0)

The composition of the total microflora follows the same trend on the 29th day of the experiment - a higher amount of ammonifiable bacteria and lower micromucites (Table 4).

Table 4. Microflora composition 29 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	6000 ± 0.200 (83.6)	920 ± 0.217 (12.8)	260 ± 0.385 (3.6)
V2	5880 ± 0.307 (71.4)	1940 ± 0.258 (23.5)	420 ± 0.238 (5.1)
V3	7920 ± 0.126 (85.5)	780 ± 0.256 (8.4)	560 ± 0.107 (6.1)
V4	5200 ± 0.192 (82.3)	520 ± 0.288 (8.2)	600 ± 0.167 (9.5)

The temperatures of the compost piles in the two last samples are close - ranging from 20°C to 26°C.

On the 39th day after experimentation, non-sprouting bacteria and bacilli dominate, with the exception of compost 2, where the amount of micromycetes is higher than that of bacilli (Table 5).

As on the 39th day and 48th day of experimentation, the temperature range suggests the development of mesophilic microorganisms.

For all compost materials, the amount of non-sprouting bacteria and bacilli is higher than that of micromycetes (Table 6).

Table 5. Composition of the microflora 39 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	5160 ± 0.194 (86.3)	800 ± 0.250 (13.4)	20 ± 0.500 (0.3)
V2	4840 ± 0.179 (74.5)	500 ± 0.200 (7.7)	1160 ± 0.086 (17.8)
V3	3960 ± 0.253 (82.2)	460 ± 0.326 (9.5)	400 ± 0.125 (8.3)
V4	3760 ± 0.266 (78.7)	760 ± 0.066 (15.9)	260 ± 0.154 (5.4)

Table 6. Microflora composition 48 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	6020 ± 0.332 (88.3)	500 ± 0.120 (7.3)	300 ± 0.167 (4.4)
V2	5220 ± 0.096 (75.7)	1080 ± 0.093 (15.7)	600 ± 0.100 (8.7)
V3	5460 ± 0.317 (78.4)	1100 ± 0.182 (15.8)	400 ± 0.150 (5.7)
V4	5940 ± 0.168 (85.1)	820 ± 0.122 (11.7)	220 ± 0.182 (3.2)

CONCLUSIONS

The studied aerobically active composting variants pass through all phases of typical compost: mesophilic, thermophilic, aging and maturing. Only one of the composts with mulberry leaf branches (V3) does not pass into a thermophilic phase.

According to the common microflora indicator, the most active composting process starts with the highest starting material at the first variant (V1). In variants with a different amount of mulberry leaf and twigs (V3 and V4), the microbial diversity and presence is less, due to the unity of the material used. Reducing the amount of green and brown materials and adding fertilizer (V2) slows down the development of microorganisms at the beginning of the experiment, but in the next period they are activated.

Throughout the process, all the studied microbes groups have a prominent role in the composting process, with the dominant role of ammonifier bacteria - non-sprouting bacteria and bacilli. These groups of microbes, such as highly plastic, are among the most active disruptors of organic compounds in compostable materials. The lowest in the composition of the total microflora is the micromycetes.

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CONTRIBUTIONS TO HALOPHILIC FLORA AND VEGETATION IN OLTENIA (ROMANIA)

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Abstract

The halophilic flora from Oltenia is known only from few localities: Gighera, Seaca de Câmp, Bratovoiești, Sadova, Tâmburești, Piscu Sadovei, Murta, Dobrești, Afumați (Dolj County), Ocnele Mari - Ocnița (Vâlcea County), Osica de Sus, Gura Padinii (Olt County).
By identification of new surfaces with halophilic plant species inside Oltenia region (Rastu Vechi - Dolj County) are brought important contributions regarding the chorology of these plant species, associations and habitats. The halophilic vegetation is fragmentary present near springs and small streams with salt water. From the habitats with halophilic vegetation in Romania on the area investigated by us we find: R1511 West-Pontic communities with Crypsis aculeata, R1514 West-Pontic communities with Trifolium fragiferum, Cynodon dactylon and Ranunculus sardous, R1521 Pontic-Sarmatic communities with Puccinellia limosa and Plantago maritima, R1529 Pontic-Pannonian meadows with Hordeum hystrix and R1507 Pontic-Pannonian meadows with Carex distans, Taraxacum bessarabicum and Aster tripolium ssp. pannonicus. Some of these habitats have a high conservative value (ex. R1521) while others do not know area in this country side (ex. R1507).

Key words: *flora, Oltenia, Romania, salty area, vegetation.*

INTRODUCTION

The origin of the salty fields from Oltenia are either from salt deposits (in case of those from Ocnele Mari - Ocnița area - Vâlcea County) or climate consequences, in which the efflorescence processes or slightly soluble salt exudation, predominates over leaching processes (Țopa, 1954).
The salty lands are easily distinguished from distance by the lighter color compared to the normal soil in that area. The most widespread salty lands in our area belong to solonceac or soloneț types without sodium carbonate (Țopa, 1954; Popescu et al., 2000).
The presence of salty lands lacking vegetation is due to missing air from soil.
The classification of halophilic plant species is different realized from one author to another. Some herbalists (Țopa, 1954; Popescu et al., 2000) are grouping the plant species in 4 big categories: mandatory, preferably, tolerant and accidental, others (Prodan, 1939) in first category, second and third.

Following the consultation of specialty literature which has as study object the halophilic flora and vegetation inside Oltenia area (Buia et al., 1959, 1960, 1961; Șerbănescu, 1963; Păun et al. 1971; Popescu et al., 2000; Răduțoiu, 2013, 2014) and from country (Doltu et al., 1979; Mihai, 1969; Pop, 1968; Prodan, 1922; Sanda and Ciobanu, 1967; Șerbănescu, 1963; Țopa, 1939, 1954, 1965; Dihoru, 1990) we found out that in this side of country are few data (Popescu et al., 2000; Răduțoiu, 2013, 2014, 2017).
Almost 40 years ago, Al. Buia et al. (1961), reported in this area, in the meadows, on low salty soils, numerous species such as: *Trifolium ornithopodioides, T. subterraneum, T. echinatum, T. angulatum, Medicago arabica, M. hispida* and *Scorzonera cana* (*Podospermum canum*).
However, in the consulted research papers, were not found any data regarding the halophilic flora and vegetation from south part of country between Rastu Vechi and Rastu Nou localities. In the IX volume from Romania Flora is mentioned a point for *Aster tripolium*

L. ssp. *pannonicus* (Jacq.) Soó (between Negoiu and Catanele) (Morariu and Nyárády, 1964 in Săvulescu et al.). The identification of appreciable surfaces with this type of vegetation made us to take in study this for having a real situation and to propose the protection of these places being known that the surfaces occupied by this kind of flora and vegetation in Oltenia region are very few.

The territory with halophilic vegetation between Rastu Vechi and Rastu Nou is near the Danube (N43º54'690"; E23º17'161", Alt. 52 m.s.m.). We can say that is placed on the upper terraces of the Danube. It is a flat field with a length of almost 1875 m and a variable width, between 3.75 m and 40 m, totaling over 4 ha.

MATERIALS AND METHODS

For making this study were made numerous field trips starting from 2006, when these salty fields appeared for the first time, after the catastrophic floods from the spring of same year to the present. The collected data were analyzed and compared with those already present from other stations with halophilic vegetation from Oltenia.

The samples that cannot be identified in the field were collected using special material, specific to this study and subsequent identified using the specialty work papers from our country and abroad (Beldie, 1977, 1979; Ciocârlan, 2009; Sârbu et al., 2013; Săvulescu et al., 1952-1976; Tutin et al., 1964-1980). After identification, the material was preserved in the botanical lab to be included in the herbarium from University of Craiova (CRA).

The authors' abbreviations were done according to Brummitt and Powell (1992).

After establishing the existing vegetation type in the research area was made also a framing of it to the Romania habitats (Doniță et al., 2005; Gafta and Mountford, 2008).

The coordinates were noticed using a Garmin etrex 30 GPS.

RESULTS AND DISCUSSIONS

Following analysis of the surfaces occupied by this type of vegetation in the research territory, we can say that the salty lands from here are interesting not only because of plant species but also by the aesthetics given by the color of these plant species flowers. In the lilac sea offered by *Aster tripolium* ssp. *pannonicus* (Figure 1) we find a yellow winding carpet given by *Lotus tenuis* to which fixation participates species like *Trifolium fragiferum* ssp. *bonannii* and *T. repens*, being speckled by their reddish and white flowers.

Figure 1. *Aster tripolium* ssp. *pannonicus* between Rastu Vechi and Rastu Nou localities

On the background of these colors we meet clusters of grasses which diversifies the chromaticity of the place (*Puccinellia limosa*, *Crypsis aculeata*, *C. schoenoides* etc.).

The floral list of salty lands from Rastu Vechi-Rastu Nou area totals a number of 71 taxa:

Phylum Spermatophyta, Magnoliopsida Class, Ranunculaceae Family: *Ranunculus sardous* Cr., *R. sceleratus* L., *Consolida regalis* S.F. Gray, Caryophyllaceae Family: *Cerastium dubium* (Bast.) Guépin, *Gypsophila muralis* L., *Spergularia rubra* (L.) J&C Presl., Chenopodiaceae Family: *Atriplex patula* (L.), *A. prostrata* Boucher ex DC., *A. tatarica* L., *Chenopodium glaucum* L. Polygonaceae Family: *Polygonum aviculare* L., *P. arenastrum* Boreau, *Rumex conglomeratus* Murray, *R. crispus* L. Rosaceae Family: *Potentilla reptans* L., Fabaceae Family: *Lotus tenuis* Waldst. & Kit., *Medicago lupulina* L., *Melilotus albus* Medik., *M. dentatus* (Waldst. & Kit.) Pers., *Trifolium fragiferum* L. subsp. *bonannii* (C. Presl) Soják, *T. repens* L. Lythraceae Family: *Lythrum virgatum* L., Apiaceae Family: *Bupleurum tenuissimum* L., *Peucedanum latifolium* (Bieb.) DC., Malvaceae Family: *Althaea officinalis* L., Brassicaceae Family: *Cardaria draba* (L.) Desv., *Diplotaxis muralis* (L.) DC., *Lepidium perfoliatum* L., *L.*

ruderale L., *Rorippa austriaca* (Cr.) Bess., *R. sylvestris* (L.) Bess. ssp. *kerneri* (Menyh.) Soó, *Sisymbrium polymorphum* (Murray) Roth, Gentianaceae Family: *Centaurium pulchellum* (Sw.) Druce, Boraginaceae Family: *Heliotropium supinum* L., Scrophulariaceae Family: *Gratiola officinalis* L., *Verbascum blattaria* L., Lamiaceae Family: *Mentha pulegium* L., Plantaginaceae Family: *Plantago cornuti* Gouan, *P. uliginosa* F.W. Schmidt, Asteraceae Family: *Achillea collina* Becker ex Rchb., *Aster tripolium* L. ssp. *pannonicus* (Jacq.) Soó, *Bidens cernua* L., *Inula britannica* L., *Lactuca saligna* L., *Matricaria recutita* L., *Pulicaria vulgaris* Gaertn, *Scorzonera cana* (C.A. Mey.) Griseb., *Sonchus arvensis* L. ssp. *uliginosus* (M. Bieb.) Nyman, *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz., Liliopsida Class, Butomaceae Family: *Butomus umbellatus* L., Juncaceae Family: *Juncus bufonius* L., *J. compressus* Jacq., Cyperaceae Family: *Bolboschoenus maritimus* (L.) Palla, *Carex distans* L., *C. divisa* Huds., Poaceae Family: *Agrostis stolonifera* L., *Alopecurus pratensis* L., *Bromus commutatus* Schrader, *Crypsis aculeata* (L.) Aiton, *C. alopecuroides* (Pill. & Mitterp.) Schrad., *C. schoenoides* (L.) Lam., *Cynodon dactylon* (L.) Pers., *Elymus repens* (L.) Gould, *Festuca arundinacea* Schreb., *F. pulchra* Schur, *Hordeum geniculatum* All., *Lolium perenne* L. *Phragmites australis* (Cav.) Steudel ssp. *humilis* (De Not.) Asch. et Graebn., *Poa bulbosa* L., *Puccinellia limosa* (Schur) Holmb., *P. convoluta* (Horrnem.) Hayek ssp. *pseudobulbosa* (E.I. Nyárády) Borza.

The highlighting of ecological particularities of all plant species from the research area allows us to determine the ecological specificity of vegetation in accordance with the complex of local pedoclimatic factors.

The analysis was based on the moisture index because it is the only climatic factor that could be observed and appreciated in the field.

From its analysis it can be observed that first place is occupied by the mesohigrophilic plant species (Table 1, Figure 2) with almost 40%. If we add also the plant species that have an index of moisture close to that one specific to mesohigrophilic species, we can realize that these salty lands belong to the category of those with high humidity. The presence of some xerophilic and mesophilic species can be explained by the periods of drought present in this territory during the summer.

Table 1. The analysis of moisture index

Nr. crt.	Scale for moisture	Nr. taxa
	Mezohigr.	27
1.	Mez.	8
2.	Xeromez.	7
3.	Mezohigr.-higr.	6
4.	Higr.	6
5.	Mez.-mezohigr.	3
6.	Xeromez.-mez.	3
7.	Xeromez.-mezohigr.	2
8.	Mezoxer.	2
9.	Mez.-higr.	2
10.	Eurif.	2
11.	Higr.-hidr..	1
12.	Xer.-xeromez.	1
13.	Xer.	1

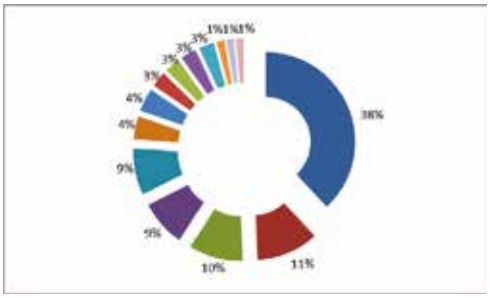

Figure 2. The ecological spectrum of salty lands plant species from the research area

As a general observation on these salty lands, is the lack of the typical species for salty lands: *Camphorosma annua* Pall. *Salicornia europaea* L. and *Suaeda maritima* (L.) Dumort., aspect mentioned also on the salty lands from Seaca de Câmp (Popescu et al., 2000).

From the habitats with halophilic vegetation in Romania on the territory researched by us we find: R1507 Pontic-sarmatic meadows of *Carex distans*, *Taraxacum bessarabicum* and *Aster tripolium* ssp. *pannonicus*, R1511 West-Pontic communities with *Crypsis aculeata*, R1514 West-Pontic communities with *Trifolium fragiferum*, *Cynodon dactylon* and *Ranunculus sardous*, R1521 Pontic-sarmatic communities with *Puccinellia limosa* and *Plantago maritima*, R1529 Pontic-Pannonian meadows of *Hordeum hystrix*.

The vegetation characteristic of these habitats is framed to the next associations: *Taraxaco bessarabici*, *Caricetum distantis* Wendelberger 1943; *Crypsidetum aculeatae* (Bojko 1932)

Ţopa, 1939; *Trifolio fragifero - Cynodontetum* Br.-Bl. et Balas 1958; *Puccinellietum limosae* Rapaics ex Soó 1933, 1936 and *Hordeetum hystricis* (Soó 1933) Wendelberger 1943.

Taraxaco bessarabici, *Caricetum distantis* Wendelberger 1943

This is the association to which the phytocoenosis are enlightened by the caespitose plant species called *Carex distans*.

Next to it, a good representation has *Aster tripolium* ssp. *pannonicus*. The cortege of species is completed by *Taraxacum bessarabicum*, *Festuca pulchra*, *Atriplex hastata*, *Trifolium repens*, *Trifolium fragiferum* ssp. *bonannii*, *Medicago lupulina* and *Cynodon dactylon*.

The vegetation framed to this association is characteristic to the R1507 habitat and has a moderate conservative value.

Crypsidetum aculeatae (Bojko 1932) Ţopa 1939

The phytocoenosis of these association have small plant species in composition. Next to the dominant one (*Crypsis aculeata* - Figure 3) we meet *Aster tripolium* ssp. *pannonicus*, *Taraxacum bessarabicum*, *Crypsis schoenoides*, *Trifolium fragiferum* ssp. *bonannii*, *Cynodon dactylon* etc.

Figure 3. *Crypsidetum aculeatae* from area investigated

These characterize the vegetation of R1511 habitat, that at national level has a moderate conservative value.

Puccinellietum limosae Rapaics ex Soó 1933, 1936 (Figure 4) - is found in those places with higher moisture. The most frequent halophilic plant species in these phytocoenosis are: *Lotus tenuis*, *Hordeum geniculatum*, *Trifolium fragiferum* ssp. *bonannii* and *Carex distans*.

Figure 4. *Puccinellietum limosae* - autumnal aspect

This is the vegetation characteristic to R1521 habitat, that at national level has a high conservative value (Doniţă et al., 2005).

Trifolio fragifero - Cynodontetum Br.-Bl. et Balas 1958

Is characteristic to R1514 habitat. Is recognized through the nucleus of subhalophilic species: *Cynodon dactylon*, *Lolium perenne*, *Gypsophila muralis*, *Ranunculus sardous*, *Consolida regalis* and *Atriplex prostrata*.

Has a low conservative value.

Hordeetum hystricis (Soó 1933) Wendelberger 1943

The surfaces occupied by the phytocoenosis of this association are small (few m^2) and placed in area that become dry during the summer. Next to the dominant species we find: *Cerastium dubium*, *Gypsophila muralis*, *Plantago uliginosa*, *Matricaria recutita*, *Lepidium ruderale*, *Achillea collina* and rare specimens of *Puccinellia limosa*.

It is characteristic to the R1529 habitat and has a moderate conservative value.

In the territory researched by us the salty vegetation in near the one framed to *Phragmitetea australis* because is placed close to a water channel flowing from Seaca de Câmp to Negoi, settlements neighboring to those in between are placed the researched salty lands.

To the west part of the land are present numerous specimens of *Phragmites australis* ssp. *humilis* what makes a cover of 60-70% (Figure 5). This thing let us to think that in the next future will be formed phytocoenosis of the association called *Astero tripolii - Phragmitetum humilis* Krisch (1972) 1974 association that characterizes the R5311 habitat. West-Pontic communities with

Phragmites australis ssp. *humilis* and *Aster tripolium*.

Figure 5. Areas where *Phragmites australis* ssp. *humilis* has a good representation

CONCLUSIONS

Comparing the flora from the territory researched by us with the one of salty lands from other areas from Oltenia region, we can say that the one from other stations is richer in species of *Trifolium* (ex. *T. angulatum*, *T. campestre*, *T. dubium*, *T. echinatum*, *T. hybridum*, *T. ornithopodioides*, *T. pratense*, *T. retusum*, *T. resupinatum* and *T. striatum*).

The influence of zoo-anthropogenic factor is visible on the salty lands vegetation from Rastu Vechi-Rastu Nou area by: their location in the vicinity of agricultural fields, collecting of some plant species by the locals (ex. *Taraxacum bessarabicum*) or by irrational grazing with sheep or goats (rarely horses), that affects the appreciated feed plant species: *Trifolium fragiferum*, *Lotus tenuis*, *Puccinellia limosa*, *Crypsis aculeata*, *C. schoenoides*, *Aster tripolium* ssp. *pannonicus*.

The need for protection of these surfaces with halophilic vegetation in Oltenia is justified not only by the reduced area in Oltenia, but also by the presence of some new habitats for this part of country (R1507) or with high conservative value (R1521).

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PLANT GROWTH PROMOTING BACTERIA WITH ANTIFUNGAL ACTIVITY

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Abstract

The aim of the present study was to analyse the potential of two plant beneficial bacteria, Cp.b4 and 75.1s, to prevent fungal pathogens proliferation and improve tomato seedlings growth. The bacterial strains were selected from the RDIPP microbial collection, due to their antifungal activity towards solanaceous plant pathogens. Both strains were previously identified as Bacillus cereus/thuringiensis based on their biochemical profile with the Biolog GEN III system. Growth chamber tests on tomato seedlings have shown that biological treatments, with the above-mentioned bacterial strains, induced a better vegetative growth to the seedlings, and increased the photosynthetic capacity of the plants. To prevent early blight caused by Alternaria sp. in solanaceous plants, the ability to inhibit conidia germination was analysed. Biological treatments with bacterial suspension reduced the number of germinated conidia by 4.98 fold (when using 75.1s strain) and by 2.57 fold (when using Cp.b4 strain). The study also revealed that mixed treatments of bacteria suspension along with a low dose of fungicide inhibit conidia germination, being comparable with the chemical control, Mycoguard 500 SC, applied at the recommended dose of 0.2%.

Key words*: PGPB, tomatoes, early blight prevention.*

INTRODUCTION

Tomato culture is an important vegetable grown in greenhouses and open fields. The culture has numerous pests and diseases in all stages of vegetation and post-harvesting. Moreover, new races of pathogens and emerging pests make plant protection more difficult. Therefore, there is a continuous interest in preventing pathogenic infections and increasing the productive potential of the plant.
Among plant pathogens, *Alternaria* spp. (like *A. solani*, *A. alternata* or *A. tomatophila*) are ubiquitous fungal phytopathogens, which can induce early blight in various plant species, including tomatoes and other edible plants (Thomma, 2003; Woudenberg et al., 2015). These pathogens infect plants in various growth stages, including vegetable fruits during ripening, causing fruit rot. It survives and overwinters as saprophyte on plant debris. The leaf infections occur on cool, wet weather, and fruit rot symptoms are usually associated with a predisposing injury due to sunscald, frost, or blossom end rot (calcium deficiency) (Chaerani and Voorrips, 2006; Kennelly et al., 2012). It is, therefore, important to maintain a good physiological status during plant growth in order to prevent infections. Moreover, microbial inoculation with plant growth promoting bacteria having biocontrol activity could improve plants health and vigour in order to prevent pathogenic infections (Wang et al., 2008; Jagadeesh and Jagadeesh, 2009; Zahoor et al., 2017).
Considering these, we studied two plant beneficial bacteria, Cp.b4 and 75.1s, regarding their biocontrol potential in preventing *Alternaria* sp. and other fungal pathogens proliferation. Microbial ability to improve tomato seedlings growth was also evaluated.

MATERIALS AND METHODS

Bacterial strains

Two bacterial strains were used in this study, 75.1s and Cp.b4, both from the RDIPP microbial collection. These two strains were

isolated from soil of Bărăgan area (75.1s) and onion rhizosphere from Dolj county (Cp.b4). Bacteria were stored at -80°C, in Luria Bertani (LB) medium supplemented with 30% glycerol. Routinely, they were grown on LB agar, at 28°C.

Bacterial identification procedure

Bacteria were identified with the Biolog GENIII system, using protocol B for Gram-positive, spore forming, fast growing bacilli, according to the manufacturer's guidelines (US Patent 5,627,045). Therefore, each bacterial strain was grown on Biolog Universal Growth medium, at 33°C, for not more than 18 hours. Isolated colonies were then homogenised in B type inoculation fluid, at 97% turbidity, detected in 590 nm wavelength light. Biolog GEN III Microplates, having 96 wells, were filled with bacterial suspension, disposing 100 µl in each well. These special plates are preloaded with 71 different carbon sources and 23 chemicals, which allow several biochemical reactions a single test. Inoculated plates were maintained at 33°C. Within 20 to 48 hours of incubation, plates were spectrophotometrically analysed at 590 nm and 750 nm wavelengths, at the Biolog Microstation Reader. The readings were processed with the MicroLog3 software, which identifies the microbial strains based on their phenotypic pattern.

Enzymatic assays

Bacterial ability to produce hydrolytic enzymes was tested on different growth substrates (Sicuia et al, 2015). Bacterial ability to solubilize tricalcium phosphate was tested on Pikovskaya agar medium. Chitinase production was determined on Roberts and Selitrennikoff (1988) medium with colloidal chitin from crab shell. Cellulase activity was tested on carboxymethil cellulose containing medium, and revealed with 0.1% Congo red. Lipase activity was evaluated on Tween 80 supplemented medium (mTMB), and protease production was tested on slim milk agar.

Antifungal assay

The biocontrol activity of the studied/ mentioned bacterial strains was evaluated *in vitro*, using the dual culture technique, on Potato-Dextrose-Agar medium. The antifungal assay was performed against five plant pathogens, *Alternaria* sp. (new isolate from tomato plant), *Botrytis cinerea* (new isolate from tomato fruit), *Fusarium oxysporum* f. sp. *radicis lycopersici* ZUM 2407, *Pythium debaryanum* DSM 62946 and *Rhizoctonia solani* DSM 63002.

Bacterial ability to inhibit conidia germination was also studied against *Alternaria* sp. Tests were performed *in vitro*, in 2 ml tubes, on Potato-Dextrose-Broth. Fungal spore suspension was prepared in 10^5 conidia/ml, and bacteria in 10^8 cfu/ml. A chemical control was also included within the study. Mycoguard® 500SC commercial product, based on chlorothalonil 500 g/l, was used at the recommended concentration (0.2%) for *Alternaria* spp. control (according to the pesticide factsheet). Bacterial treatments were tested individually and in combination with half of the chemical dose. This was carried out in order to see if there is any possibility in reducing the dose of pesticides for *Alternaria* spp. prevention. Samples were incubated at 25°C, optimal for conidia germination (Troncoso-Rojas and Tiznado-Hernández, 2014). The observations were made after 72 hours of incubations, using a light microscope. The test was performed in triplicates and repeated twice.

Plant growth promotion

This test was performed on tomato seedlings. Three experimental variants were studied, two biological treatments, 75.1s and Cp.b4 respectively, comparing to an untreated control. Bacterial suspensions of 10^8 cfu/ml were first applied as seed treatment, by immersion, for 20 minutes. The second treatment was applied to the soil, after transplantation, with 2 ml of bacterial suspension near each plant. Seedlings were transferred in 10 cm diameter pots. Plants were grown in controlled conditions using a Sanyo MLR351H growth chamber, in 16 hours photoperiods, at 24ºC/light, 18ºC/dark, 14000 lx and 70% RH.

Biometric observations were made six weeks after transplantation. From each experimental variant, eight plants were analysed. Root length was determined through direct measurement. Fresh weight of total aerial growth and roots were separately weighted. Dry weight was determined after 3 hours of soaking at 105°C.

Assimilatory pigments were quantified through specific analytical methods. One gram of freshly harvested leaves, from different plants of each experimental variant, were used in order to quantify chlorophyll a (chl a), chlorophyll b (chl b), xanthophyll and carotenoids. Pigments were extracted in acetone (99.99%) at 4°C, overnight. The chlorophyll, xanthophyll and carotenoids content were quantified according to Lichtenthaler and Welburn (1987). Thereafter, the filtered extracts were spectrophotometrically analysed at 662 nm, 645 nm and 470 nm, respectively. The absorbance measurements were used for assimilatory pigments quantification. Their concentration was calculated, in mg/g of fresh weight, using Lichtenthaler's equations.

RESULTS AND DISCUSSIONS

Bacterial identification

The identification was made based on bacterial phenotypic fingerprint obtained in the GEN III Microplates. These plates started out colourless. However, during incubation, an increased respiration, due to carbon source utilization or bacterial growth, reduced the tetrazolium redox dye, forming a purple color in specific wells, giving a phenotypic fingerprint for each bacterial strain (Figure 1). The color intensity was compared with the negative control for carbon source utilization assays, and positive control for chemical sensitivity assays. The phenotypic pattern was compared to Biolog's extensive species library, and the identification was given at specie level. Both strains were identified as *Bacillus cereus*/*thuringiensis*.

Figure 1. Phenotypic profile of *Bacillus cereus*/*thuringiensis* Cp.b4 on Biolog GEN III microplate under B type protocol (after 20 hours of incubation at 33°C)

Enzymatic characterisation

The bacterial strains were able to produce chitinase, carboxymethil cellulase (CMC-ase), caseinase, and lipase enzymes (Figure 2). Some of these enzymes are involved in the biocontrol strategy, degrading insect and fungal cell wall structure (Veliz et al., 2017).

Chitinase activity was detected on colloidal chitin media containing bromocresol purple as pH indicator dye (pH 4.7). Therefore, the breakdown of chitin into N-acetyl glucosamine is modifying the pH from acid (4.7) towards alkaline, thus changing the colour of the pH indicator dye, from yellow to purple. As a result of chitinase activity, a purple zone was generated around the bacterial colonies after 5 days of incubation on the specified medium (Figure 2 a). Regarding CMC and casein hydrolysis, the enzyme producing strains were surrounded by a clear hallo due to the cellulase and protease activity. The CMC-ase production was revealed by flooding the plates with 0.1% Congo red solution for 15 minutes, followed by 1M sodium chloride rinses (Figure 2 b, c). The hydrolysis of Tween 80 from mTMB medium, due to lipase activity, generated a white precipitate around bacterial colonies as a result of calcium soap production (Figure 2 d).

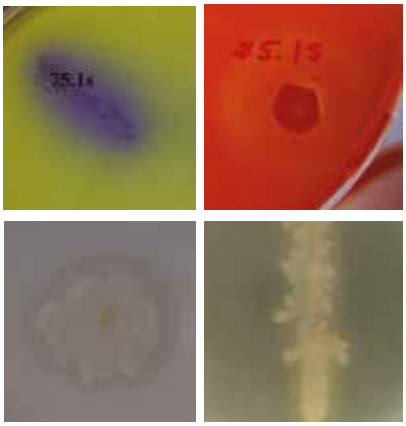

Figure 2. Bacterial hydrolytic activity:
a. chitinase, b. CMC-ase, c. protease, d. lipase

The bacterial ability to solubilize tricalcium phosphate from Pikovskaya agar medium indicates the bacterial potential in increasing plant growth through nutrient availability. Secondarily, an improved phosphorus uptake is also improving plant resistance to the phytopathogenic attacks.

Antifungal activity

The antifungal potential of the studied bacterial strains was revealed, by dual culture assay, against (new isolate from tomato plant), *Botrytis cinerea* (new isolate from tomato fruit), *Fusarium oxysporum* f. sp. *radicis lycopersici* ZUM 2407, *Pythium debaryanum* DSM 62946 and *Rhizoctonia solani* DSM 63002 (Table 1).

We consider that the antifungal potential of these strains is related with the lytic enzyme production and direct competition for nutrients and niche.

Table 1. Antifungal spectrum of the studied *Bacillus cereus/thuringiensis* strains

Microbial strains	75.1s	Cp.b4
Alternaria sp.	+ + +	+ + +
Botrytis cinerea	+ +	+ + +
Fusarium oxysporum f. sp. *radicis lycopersici*	+ +	+
Pythium debaryanum	+	+
Rhizoctonia solani	+ + +	+ + +

Where: + + + = strong inhibition of the fungal growth; + + = moderate inhibition of the fungal growth; + = slight inhibition of the fungal growth; – = no inhibition of the fungi

Fungal cell lysis associated with protoplasmatic content likings were seen at the microbial interaction zone between *Botrytis cinerea* and both bacterial strains (figure 3 a, c). Hyphal growth deformations were also seen in *Fusarium oxysporum* and *Rhizoctonia solani* at the interaction zone with the biocontrol bacteria (figure 3 b). The bacterial strains induced swelling of the fungal cells of *Fusarium oxysporum* (figure 3 c), and mycelia dehydration in *Botrytis cinerea* after prolonged incubation (figure 3 d). Similar aspects were previously described on plant pathogenic fungi when *Bacillus* spp. treatments were used for biological control (Huang et al., 2012; Boiu-Sicuia et al., 2017a, b).

Regarding the bacterial potential to inhibit conidia germination, we noticed that all tested treatments reduced the number of germinated conidia of *Alternaria* sp. (Table 2). The most efficient treatments were the chemical control and the mixed applications of biocontrol bacteria and chemical pesticide in half dose. Among bacterial treatments, 75.1s had a better biocontrol effect than Cp.b4 strain.

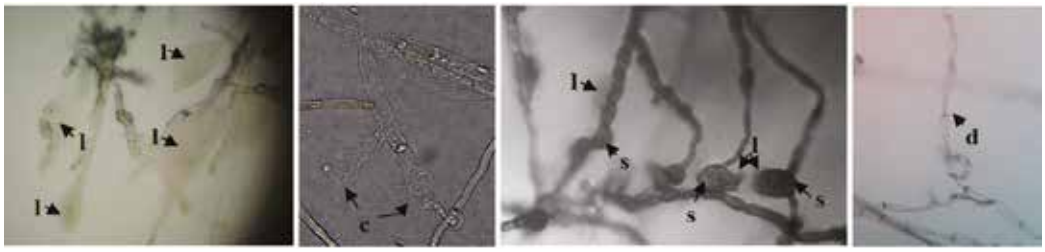

Figure 3. Fungal growth deformations and lysis due to biocontrol bacteria:
A, D = *Botrytis cinerea*, B = *Rhizoctonia solani*, C = *Furarium oxysporum* f.sp. *radicis lycopersici*
l = cell lysis and cytoplasm likings, c = mycelial curling, s = fungal cell swelling, d = mycelia dehydration

Table 2. Inhibition of *Alternaria* sp. conidia germination

Experimental variant	Germinated conidia	Inhibition of germinated conidia	Inhibition of germinating filaments
	(%)		
Alternaria sp. - Untreated control	32.14	-	-
Chemical control 0.2% Mycoguard	2.7	91.6	86.7
Cp.b4 biocontrol treatment	12.5	61.1	98.7
Mixed treatment Cp.b4 & 0.1% Mycoguard	2.33	92.8	99.1
75.1s biocontrol treatment	6.46	79.2	98.7
Mixed treatment 75.1s & 0.1% Mycoguard	3.33	89.6	99.1

The germination filaments were measured using the APS Assess 2.0 software, and the filaments length was quantified compared to conidia dimensions. Concerning this, the

biocontrol and mixt treatments had a better influence to inhibit the growth of germination filaments in *Alternaria* sp. Although the chemical control reduced the number of germinated conidia, the germination filaments were longer than the other treatments (Table 2).

Plant growth promoting activity

Tomato seedlings were grown in controlled conditions. Six weeks after transplantation, several biometric observations were made (Table 3). Root length, in cm, was measured, fresh and dry weight of total aerial growth and roots were separately determined as grams of plant tissue per single plant.

Growth chamber tests on tomato seedlings have shown that biological treatments increase plant vigour. Plant growth promotion was observed both on aerial parts and root systems when seedlings were treated with beneficial bacteria.

Table 3. Biometric parameters of bacterial inoculated tomato seedlings

Biometric parameters	Untreated control	Bacterial inoculated plants	
		Cp.b4	75.1s
Root length (cm)	13.6	17.9	15.7
Aerial fresh weight (g/plant)	5.7675	9.1043	7.2463
Aerial dry weight (g/plant)	0.5391	0.9630	0.7778
Root fresh weight (g/plant)	0.6345	1.0500	0.7952
Root dry weight (g/plant)	0.0380	0.0819	0.0639

An increase in root length and weight was achieved for the bacterial inoculated plants (Figure 4), and a positive correlation of the root system with the shoots was revealed. Between the two tested bacterial strains, Cp.b4 was more efficient than 75.1s in plant growth promotion (Table 3).

Figure 4. Tomato roots: a) untreated control; b) 75.1s treated seedlings; c) Cp.b4 treated seedlings

A positive influence of the bacterial treatments on tomato seedlings was also seen when the assimilatory pigments were quantified (Table 4).

Table 4. Assimilatory pigments in the leaves of tomato seedlings

Biometric parameters	Untreated control	Bacterial inoculated plants	
		Cp.b4	75.1s
	mg/ g of fresh weight		
Chlorophyll a	2.435	3.041	3.052
Chlorophyll b	0.949	1.231	1.168
Xanthophyll and Carotenoids	0.487	0.580	0.610

Regarding the chlorophyll content, bacterial treatments slightly increased chl a and chl b content compared to the untreated control, with no significant differences among the applied treatments. Moreover, for xanthophyll and carotenoids, the pigments content was a little higher in treated plants. These results showed an increased photosynthetic capacity in bacterial inoculated seedlings.

CONCLUSIONS

Two bacterial strains of *Bacillus cereus*/ *thuringiensis*, Cp.b4 and 75.1 s, were analysed in this study. Both strains demonstrated *in vitro* biocontrol activity and plant growth promotion properties on tomato seedlings.

Both strains produced chitinase, carboxymethil cellulase, caseinase, and lipase enzymes. Due to their high metabolic activity this biocontrol strains expressed a wide antimicrobial action against *Alternaria* sp. (new isolate from tomato plant), *Botrytis cinerea* (new isolate from tomato fruit), *Fusarium oxysporum* f. sp. *radicis lycopersici* ZUM 2407, *Pythium debaryanum* DSM 62946 and *Rhizoctonia solani* DSM 63002. The biocontrol strains also inhibited conidia germination in *Alternaria* sp., showing a high potential in prevent early blight infections. Biological treatments with bacterial suspension reduced the number of germinated conidia by 4.98 fold (when using 75.1s strain) and by 2.57 fold (when using Cp.b4 strain) than the untreated control. Mixed treatments of bacteria suspension and low dose (0.1%) of chemical fungicide strongly inhibit conidia germination, being comparable with the

chemical control, Mycoguard 500SC, applied at the recommended dose of 0.2%.

Regarding plant growth promotion activity, the mentioned strains, applied as seed and soil treatment, increased tomato root length, biomass fresh and dry weight, and plant photosynthetic capacity. The bacteria also revealed the ability to solubilize tricalcium phosphate from Pikovskaya agar medium indicating an increasing potential in improving nutrient availability for the plants.

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ANTIMICROBIAL POTENTIAL OF ROMANIAN SPONTANEOUS FLORA - A MINIREVIEW

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Abstract

In the past decades, clinical microbiologists, practitioners and professionals in food safety, are facing new challenges related to new born microbial pathogens as well as to the phenomenon of the antibiotic and biocide resistance developed by the pathogens. Meanwhile, in Romania has been noticed an increase in scientific publications dealing with the potential of Romanian aromatic and medicinal plants and their therapeutic use. The paper proposes a mini-review on scientifically proved antimicrobial activity of aerial and underground parts of some spontaneous plant from Romanian flora. The review approaches annual and perennial plants, from herbaceous species to bushes and trees. In our search we have identified a total of 64 species from autochthonous flora involved in studies on antimicrobial activity, belonging to 21 botanical families. Among these species, 28.1% are annual plants, 46.9% are herbaceous perennial plants and the rest (25%) are woody perennial species (bushes and trees). Almost 50% of the active species belongs to Asteraceae and Lamiaceae botanical families. For 89% of the species have been reported antibacterial activity, while only 57.8% of the species have proven antifungal activity.

Key words: *Romanian spontaneous flora, antibacterial, antifungal, phytopharmaceutical use.*

INTRODUCTION

In the past decades, clinical microbiologists, practitioners and professionals in food safety, are facing new challenges related to new born microbial pathogens as well as to the phenomenon of the antibiotic and biocide resistance developed by the pathogens, of which the most studied is methicillin-resistant *Staphyloccous aureus* MRSA (Lee et al., 2015). The antibiotic resistance crisis has been attributed to the overuse and misuse of the medications, as well as the lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements (Ventola, 2015). In ancient times, microbial infections were treated empirically, by the use of different natural solutions, including the use of different plants prepared under different formulations. Starting with the discovery of penicillin by Sir Alexander Fleming in 1928, antibiotics have transformed modern medicine. Over the past decades, starting with 1950, it has been proven that bacteria have developed resistance to different antibiotics; as a consequence, new synthesis substances have been developed to treat the infections (Ventola, 2015); because of different mechanisms, more or less elucidated, the microorganisms became over and over resistance to the new antibiotics and biocids. Meanwhile, with the growing consumer demand for natural preservatives to replace chemical compounds, plant antimicrobial compounds must be thoroughly investigated for their potential to serve as natural preservatives (Hintz et al, 2015); on this side, very recently, an exhaustive overview on natural food preservatives with antimicrobial properties has been reported by Pisoschi et al. (2018).In this context, the “return to the origins”, meaning the use of the spontaneous flora as antimicrobial tool may be a solution of the antibiotic “crisis” and the replacement of chemical preservatives. A lot of studies have been published in the past two decades and it has been proven that spontaneous flora of each continent, hemisphere, country or region has an immense potential in obtaining different products with antimicrobial and anti-inflammatory impact. The repository of the whole information is quite difficult because of the huge volume of data, but there are some reports related to different countries or regions. For example, in Balkan regions (Southeast Europe), where Romania belongs, an ethnobotanical analysis

showed that 128 plant species (105 wild, 22 cultivated and 1 wild/cultivated) are used in the treatment of wounds. Their application is external, in the form of infusions, decoctions, tinctures, syrups, oils, ointments, and balms, or direct to the skin. Among those plants recorded, the most commonly used in Balkans are *Plantago major, Hypericum perforatum, Plantago lanceolata, Achillea millefolium, Calendula officinalis, Sambucus nigra, Tussilago farfara* and *Prunus domestica* (Jaric et al., 2018). As in all the other countries or regions, on the ancient lands of the actual Romania, it was a vivant interest in the use of different plants to treat human, animal or even plants' infections. Jaric et al. (2018) make references to different studies and report that in Romania, out of more than 3600 species of plants, over 700 are medicinal plants. Nowadays the interest has been resuscitated and more complex studies have been performed related to the chemical composition of the popular plants, as well as on testing their antimicrobial effect by the use of a standardized methodological approach. On our knowledge, there is no comprehensive review on the published studies developed on Romanian level in relation to our local flora and its antimicrobial properties. However, Amarioarei et al. (2016) has published a scientometric analysis performed on data collected from Scopus, during 2000-2015; the review contains information on number of papers, citations, affiliation and number of authors dealing with the potential of Romanian aromatic and medicinal plants and their therapeutic use. The authors have reported an increasing trend for such publications in Romania starting with 2007 which we could rely with the variety of funding available through the national and regional funding programs. Also, there is some information provided from some Romanian geographical regions on plants having different phytopharmaceutical effects; for example, in Banat region were identified about 140 plant species with antioxidant potential (Antal, 2010). Generally, antioxidant activity is due to polyphenol content, especially flavonoids compounds, and it is assumed that same compounds are partially responsible for the antimicrobial activity. The present paper proposes to present an overview of Romanian spontaneous flora proved by experimental approach to have antimicrobial effect.

MATERIALS AND METHODS

Online information research was conducted by the use of different database collections and on-searching engines (Google Academic, Web of Knowledge, PubMed, ScienceDirect and Embase, InTech and Hindawi databases). The information has been structured in relation to the plants type (annual/biennial and perennial plants, from herbaceous species to bushes and trees).Where information was available, the district or the county of the plant origin have been specified.

RESULTS AND DISCUSSIONS

The database screening has led to a collection of over sixty scientific publications, dated from 2007 to 2018. The authors have reported the use of different types of extracts (aqueous, alcoholic or PEG extracts), as well as the use essential oils of different plants in testing the antimicrobial activity. Different methods have been used to test the antimicrobial activity of different plants products. The most usual method is the agar diffusion method described by different authors or clinical standards in USA or Europe (Brown, 1978; Das et al., 2010). The findings are described in the following, grouped in annual or perennial plants, herbaceous or woody groups.

1) Annual / Biennial plants

The reported antimicrobial activity of annual/biennial plants from Romanian flora are synthetized in Table 1 and their appurtenance to botanical family is clearly specified.

There are different studies targeting the botanical family Lamiaceae, which includes most of the aromatic plants like *Origanum vulgare* L., *Melissa officinalis* L. or *Ocimum basilicum* L.; ethanolic extracts of aerial parts of these plants have been proven to have some antibacterial activity on *Listeria monocytogenes, Stahpylococcus aureus* (Benedec et al., 2015), as well as antifungal activity on *Candida albicans* (Tuchila et al., 2008; Benedec et al., 2015).

Table 1. Romanian annual herbaceous plants with antimicrobial activity

Plant species	Botanical family	Plant part	Antimicrobial activity		Reference
			Bacteria	Fungi	
Origanum vulgare L.	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus* *E. coli* *Salmonella enteridis*	*Candida albicans*	Benedec et al., 2015 Dobre et al., 2011 Sandru et al., 2015
Melissa officinalis L.	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus*	*Candida albicans*	Benedec et al., 2015 Hancianu et al., 2008
Ocimum basilicum L.	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Staphylococcus aureus* *E. coli* *Streptococcus cricetus*	*Candida albicans*	Benedec et al., 2015 Stefan et al., 2011 Tuchila et al., 2008 Vlase et al., 2014
Thymus vulgaris L.	Lamiaceae	hb; fl; fs	*Staphylococcus aureus* *Klebsiella pneumoniae* *Salmonella typhimurium* *E. coli* *Enterococcus faecalis* *Salmonella enteridis* *Pseudomonas aeruginosa* *Listeria innocua* *Streptococcus pyogenes*	*Candida albicans* *Aspergillus niger*	Boruga et al., 2014. Dobre et al., 2011 Grigore Armatu et al., 2012 Varga et al., 2015
Thymus pulegioides	Lamiaceae	hb	*E. coli* *Enterobacter cloacae* *Proteus mirabilis* *Bacillus subtilis* *Micrococcus flavus* *Staphylococcus aureus* *Streptococcus faecalis* *Pseudomonas aeruginosa* *Listeria innocua* *Streptococcus pyogenes*	*Candida albicans*	Pavel et al., 2010 Varga et al., 2015
Thymus glabrescens	Lamiaceae	hb	*Salmonella typhimurium* *Pseudomonas aeruginosa* *Proteus mirabilis* *Listeria innocua* *Streptococcus pyogenes*	*Candida albicans*	Pavel et al., 2010 Varga et al., 2015
Satureja hortesis	Lamiaceae	hb	*Streptococcus cricetus* *Staphylococcus aureus*	*Botrityis cinerea* *Candida albicans*	Sesan et al., 2015 Tuchila et al., 2008
Anethum graveolens	Apiaceae	hb; fl; fs; sm	*Shigella flexneri* *Klebsiella pneumoniae* *Salmonella typhimurium* *E. coli*		Jianu et al., 2012
Tropaeolum majus	Tropaeolaceae	hb	*Pseudomonas aeruginosa* *Salmonella* sp. *Bacillus* sp.	*Candida albicans*	Butnariu et Bostan, 2011
Veronica officinalis	Plantaginaceae	hb	*Listeria monocytogenes* *Listeria ivanovii*	-	Mocan et al., 2015a Mocan et al., 2015b
Veronica teucrium	Plantaginaceae	hb	*Staphylococcus aureus* *Bacillus cereus* *Enterococcus faecalis* *Peptostreptococcus anaerobius*	-	Mocan et al., 2015a Mocan et al., 2015b
Veronica orchidea	Plantaginaceae	hb	*Listeria monocytogenes* *Listeria ivanovii* *Peptostreptococcus anaerobius*	-	Mocan et al., 2015a Mocan et al., 2015b
Veronica persica Poiret	Plantaginaceae	hb; fl; fs		*Aspergillus niger* *Penicillium hirsutum*	Fierascu et al., 2018
Arctium lappa	Asteraceae	rx fl	*E. coli* *Salmonella abony* *Staphylococcus aureus* *Staphylococcus epidermidis*	*Aspergillus niger* *Penicillium hirsutum*	Fierascu et al., 2018 Ionescu et al., 2013 Pirvu et al., 2017
Xanthium strumarium	Asteraceae	hb	-	*Phytophthora infestans*	Rodino et al., 2013
Cnicus benedictus	Asteraceae	fs	*Salmonella typhimurium* *Salmonella enteritidis*, *Shigella sonnei* *Staphylococcus aureus* *Streptococcus pyogenes* *Proteus vulgaris*, *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*	-	Szabo et al., 2009
Calendula officinalis	Asteraceae	hb	*Klebsiella penumoniae* *S. aureus* *E. coli*	*Candida albicans*	Jianu et al, 2016
Tagetes patula	Asteraceae	fs		*Pythium* sp. *Botrytis cinerea*	Rodino et al., 2015 a Sesean et al., 2015

Legend: hb: herba (flowering aerial parts); fl: folium (leaves); fs: flos (flowers); nd: needles; fr: fructus (fruits); cx: cortex (bark); sm: semen (grains); rx: radix (roots); rh: rhizoma (rhizome); st: stipites (branches).

Similar results on essential oil of *Melissa officinalis* L. have been reported by Hancianu et al. (2008). Regarding *Origanum vulgare*, Sandru et al. (2015) have proven that essential oils made of the aerial parts have strong inhibitory effect on *E.coli*, same as other different species of *Ocimum* used as essential oils (Stefan et al., 2011). *Ocimum* sp. spectrum is completed by *Streptococcus cricetus* which is inhibited by the alcoholic extract (Tuchila et al., 2008).

In the same family, essential oils of *Thymus vulgaris* aerial parts, harvested in Mehedinţi County, have moderate to strong inhibition on *Staphylococcus aureus, Klebsiella pneumoniae*, *Salmonella typhimurium*, *E. coli, Enterococcus faecalis* and *Candida albicans* (Boruga et al, 2014); the authors correlate this activity with the presence of phenolic compounds (thymol) and terpene hydrocarbons (γ-terpinene). Some other authors reported higher antibacterial activity of thyme extracts originated in Southern Romania (Dobre et al., 2011), as well as anti-fungal effect (Grigore Armatu et al., 2012).

Other *Thymus* species have been investigated. Aerial parts of *Thymus pulegioides* collected at the flowering stage from two areas of the Bucegi Mountains at different altitudes (1000 and 1800 m above sea level) and aerial parts of *T. glabrescens* from the district of Gorj have been used for essential oils extraction (Pavel et al., 2010). *Escherichia coli*, *Enterobacter cloacae*, *Proteus mirabilis*, *Bacillus subtilis* and *Micrococcus flavus* were the strains most susceptible to *T. pulegioides* essential oil. *T.glabrescens* essential oil inhibited the growth of *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The authors have related the inhibitory activity tothe presence of monoterpenoid alcohols in this sample, especially of geraniol (55.5%), which manifests an antiseptic activity comparable to that of thymol, often against *Pseudomonas*. All the tested samples showed antifungal effects by inhibiting the growth of *Candida albicans*.

The *Thymus* sp. spectrum of antibacterial activity is completed with data reported by Varga et al. (2015). Essential oils of four different *Thymus* species (*T. vulgaris, T. serpyllum, T. pulegioides,* and *T. glabrescens*) harvested in Mures county have been proven to inhibit the growth of *Pseudomonas aeruginosa*, *Listeria innocua* and *Streptococcus pyogenes*.

In the same family (Lamiaceae), *Satureja hortensis* harvested in Southern Romania has been reported to have antifungal effect on *Botrytis cinerea* (Sesan et al., 2015). Aqueous extracts of *Satureja hortensis* from Banat county have inhibited *Streptococcus cricetus*, while in alcoholic extract inhibited *Staphylococcus aureus* and *Candida albicans* (Tuchila et al., 2008).

The list of aromatic plants with antimicrobial activity is also completed by the dill. Essential oils from inflorescences, stems, immature and mature seeds of *Anethum graveolens* L. grown in Western Romania (Timis county) were isolated by steam distillation and tested on different bacteria. Significant antimicrobial activity was recorded against *Shigella flexneri, Klebsiella pneumoniae, Salmonella typhimurium* and *E. coli,* while no inhibitory effects were observed against *Streptococcus pyogenes* and *Staphylococcus aureus*, results which is partially in contradiction with other reported results (Jianu et al., 2012).

From a plant mainly cultivated as ornamental plant, *Tropaeolum majus*, Butanriu and Bistan (2011) have extracted essential oils starting from dehydrated leaves and flowers harvested in Timis county. The authors assumed that the antimicrobial action is determined by the phenols and metil-ethers identified in the *T. majus* extracts, but also by the tymol and carvacrol present in the volatile oil; the volatile oils tested presented a wide range of action over both Gram-positive and Gram–negative species. The most sensitive microorganism to the action of the tested natural compounds of *T. majus* proved to be *P. aeruginosa* and *C. albicans*, followed by *Salmonella* sp. and *Bacillus* sp, while the most resistant is the *E. coli* stem.

A plant considered annual, but sometime being an over winter specie, is *Veronica persica* Poiret. Crude hydroalcoholic extracts of its aerial parts originated in Pitesti hills showed important inhibitory effect on two pathogenic fungal species, *Aspergillus niger* and *Penicillium hirsutum* (Fierascu et al., 2018). This results have completed the image of antimicrobial effect of *Veronica* sp. described by Mocan et al. (2015), which have proven that

V. officinalis, V. teucrium and *V. orchidea* have inhibitory effect on *Staphylococcus aureus*, *Listeria monocytogenes* and Listeria *ivanovii*.
Other species of *Veronica* genus have been studied by Mocan et al. (2015 a, b). Hydroalcoholic extracts of aerial parts harvested in Cluj county shows that in the case of *V. officinalis*, the most sensitive bacterial strains were *Listeria monocytogenes* and *Listeria ivanovii*; regarding *V. teucrium* antibacterial activity, the strains of *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* have been the most sensitive. Referring to the *V. orchidea* extract, the most sensitive strains were *Listeria monocytogenes* and *Listeria ivanovii*. Also, the extracts of *V. teucrium* and *V. orchidea* have been proven to have antibacterial activity, on *Peptostreptococcus anaerobius*, an anaerobic Gram-positive bacteria responsible for clinical infections. The authors come with the assumption that the activity of *Veronica* ethanolic extracts against Gram-positive bacteria like *L. monocytogenes*, *L. ivanovii* and *S. aureus* could be attributed at least in part to their high β-sitosterol content but also to the presence of campesterol and stigmasterol and may be might be influenced also by the presence of hispidulin. Relatively recently, have been given special attention to the biennial *Arctium lappa*. Crude roots hydroalcoholic extract proved inhibitory effect on *Escherichia coli, Salmonella abony* (Ionescu et al., 2013), as well as on fungi as *Aspergillus niger* and *Penicillium hirsutum* (Fierascu et al., 2018). Authors have attributed the antimicrobial properties to the phenolic acids content (such as chlorogenic acid, rutin, quercitrin, luteolin, p-coumaric acid, caffeic acid and quercetin). In 2017, results obtained by Pirvu et al. suggest the potential uses of *Arctii folium* whole (70%, v/v) ethanol extract in restoring the activity of the antibiotics affected by microbial resistance, as well as inhibitory effect on *Stapyloccous epidermidis.*
A thistle-like plant from Asteraceae family, *Cnicus benedictus* in different extracts of immature capitulum harvested in North-Western Romania during prebloom period, have been proven to have a very large antibacterial spectrum, from *Salmonella typhimurium*, *Salmonella enteritidis* to *Shigella sonnei*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* (Szabo et al., 2009).
From Asteraceae family have been taken into account also a species growing as weed, *Xanthium strumarium*. Ethanolic extracts of aerial parts of the plant have been demonstrated to have inhibitory effect on the growth of a phytopathogenic fungi, *Phytophthora infestans*, the causative agent of late blight in tomatoes and potatoes (Rodino et al., 2013). Further, Rodino et al. (2015 a) have tested another Asteraceae representative, *Tagetes patula* (marigold) on the phytopathogenic fungi *Pythium* sp., which can cause serious diseases such as damping off, seed rot, root rot and soft rot in wheat, maize, soybean, peppers, bean, cucumber, tomato. Ethanolic extracts of marigold flowers harvested from Southern Romanian from non-polluted sites, exhibited moderate to high inhibition on the fungal specie. Other authors (Sesan et al., 2015) reported *Tagetes* sp. extracts as having good antifungal activity on *Botrytis cinerea.*
Another annual member of Asteraceae family, *Calendula officinalis*, rarely studied for its antimicrobial activity, as essential oils of aerial parts has inhibited the growth of *Klebsiella penumoniae, S. aureus, E. coli* and of the fungus *Candida albicans* (Jianu et al., 2016).
There are some other annual plants studied for their antimicrobial activity, activity which have been proven to be weak on Romanian extracts, even some other reports are opposite. An example is *Agrimoniae herba* ethanolic extract which has only a weak inhibitory effect on *Pseudomonas aeuroginosa* (Pirvu et al., 2016).

2) Perennial plants

The reported antimicrobial activity of perrenial plants from Romanian flora are synthetized in Table 2 (herbaceous plant) and Table 3 (shrubs and trees), including their appurtenance to botanical family and the plants' part tested for the inhibitory activity.

Herbaceous plants

Different species from Asteraceae family have been proven to have inhibitory activity on pathogenic microorganisms. *Achillea* sp.

Table 2. Romanian perennial herbaceous plants with antimicrobial activity

Plant species	Botanical family	Plant part	Antimicrobial activity		Reference
			Bacteria	Fungi	
Achillea distans	Asteraceae	fs	*Listeria monocytogenes* *Stahpylococcus aureus*		Benedec et al., 2013
Achillea shurii	Asteraceae	fs	*Listeria monocytogenes* *Staphylococcus aureus* *Salmonella typhimurium*		Benedec et al., 2016
Achillea millefolium	Asteraceae	hb	*Klebsiella pneumoniae* *Salmonella typhimurium* *Staphylococcus aureus*	*Candida albicans*	Jianu et al., 2016
Achillea collina	Asteraceae	fs	*E. coli*, *Shigella flexneri* *Klebsiella pneumoniae* *Salmonella typhimurium* *Staphylococcus aureus*		Jianu et al., 2015
Artemisia spp.	Asteraceae	fs		*Sclerotinia sclerotiorum*	Badea and Delian, 2014
Tanacetum vulgare	Asteraceae	fs	*Bacillus cereus* *Staphylococcus aureus*		Muresan, 2015 Muresan et al., 2015
Inula helenium	Asteraceae	rx	*E. coli, Enterococcus faecalis* *Bacillus cereus* *Staphylococcus aureus*	*Candida albicans* *Candida parapsilosis*	Diguta et al., 2014
Santolina rosmarinifolia	Asteraceae	hb; fl; fs	*Staphylococcus aureus*	*Candida albicans*	Ioannou et al., 2007
Cynara scolymus	Asteraceae	fl	*E. coli, Salmonella abony* *Listeria innocua, Bacillus cereus*		Ionescu et al., 2013 Vamanu et al., 2011
Taraxacum officinale	Asteraceae	fl	*Escherichia coli* *Salmonella abony*		Ionescu et al., 2013
Eupatorium cannabium	Asteraceae	fl	*Escherichia coli* *Bacillus subtilis*	*Candida albicans*	Purcaru et al., 2015
Salvia officinalis	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus* *Klebsiella pneumoniae*	*Candida albicans*	Benedec et al., 2015 Ilie et al., 2016
Rosmarinus officinalis	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus*	*Candida albicans* *Aspergillus flavus* *Aspergillus ochraceus*	Benedec et al., 2015
Hyssopus officinalis	Lamiaceae	hb; fl; fs	*Listeria monocytogenes* *Stahpylococcus aureus* *P. aeruginosa*	*Candida albicans*	Benedec et al., 2015 Jianu et al., 2016 Mihai and Popa, 2015 Vlase et al., 2014
Mentha piperita	Lamiaceae	hb	-	*Candida albicans* *Botrytis cinerea*	Jianu et al., 2016 Sesan et al., 2015
Mentha smithiana	Lamiaceae	hb	-	*Candida albicans*	Jianu et al., 2016
Mentha spicata	Lamiaceae	hb	*Listeria monocytogenes*	*Candida albicans*	Moldovan et al., 2014
Mentha rotundifolia	Lamiaceae	hb	*Listeria monocytogenes*	*Candida albicans*	Moldovan et al., 2014
Ajuga genevensis	Lamiaceae	hb	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Listeria monocytogenes,* *E. coli, Salmonella typhimurium*		Toiu et al., 2016
Teucrium chamaedrys	Lamiaceae	hb	*Staphylococcus aureus*	*Candida albicans*	Vlase et al., 2014
Hypericum perforatum	Hypericaceae	hb	*S. aureus, S. typhimurium* *E. coli*	*Candida albicans*	Jianu et al., 2016
Eryngium campestre	Apiaceae	hb; fl; fs	*Staphylococcus aureus* *Stahpylococcus epidermidis* *Pseudomonas aeruginosa*		Conea et al., 2016
Humulus lupulus	Cannabaceae	fs	*Bacillus subtilis*, *E. coli* *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus, Enterobacter cloacae,* *Pseudomonas fluorescens,*	-	Arsene et al., 2015
Hedera helix	Araliaceae	fs; fr	*Stahpylococcus aureus* *Listeria monocytogenes*		Pop et al., 2017
Allium ursinum *Allium sativum*	Amaryllidaceae		*Bacillus subtilis* *Staphylococcus aureus* *Streptococcus pyogenes* *E.coli*	*Aspergillus glaucus* *Geotrichum candidum* *Candida albicans* *Botrytis cinerea*	Lupoae et al., 2013 Sesan et al., 2015
Helianthemum nummularium	Cistaceae	hb	*E. coli, Staphylococcus aureus* *Salmonella typhimurium* *Salmonella enterritidis* *Pseudomonas aerugionsa*	*Candida albicans*	Pirvu et al., 2017a
Epilobium hirsutum	Onagraceae	hb	*Staphylococcus aureus* *E. coli*		Pirvu et al., 2014 Pirvu et al., 2015
Chelidonium majus	Papaveraceae	hb		*Botrytyis cinerea*	Parvu et al, 2011
Glycyrrhiza glabra	Fabaceae	rx		*Pythium* sp.	Rodino et al., 2015a
Paeonia officinalis	Paeoniaceae		*E. coli, Pseudomonas aeruginosa* *Salmonella abony*, *Staphylococcus aureus* *Enterococcus faecalis* *Brevibacterium flavum* *Sarcina* sp., *Bacillus cereus*	*Aspergillus niger*	Soare et al., 2012

Legend: hb: herba (flowering aerial parts); fl: folium (leaves); fs: flos (flowers); nd: needles; fr: fructus (fruits); cx: cortex (bark); sm: semen (grains); rx: radix (roots); rh: rhizoma (rhizome); st: stipites (branches).

Is one of the most studied in the family. *Achillea distans* Waldst. et Kit. ex Willd., found in the Rodna Mountains (a subdivision of the Eastern Carpathians in Northern Romania), is confirmed as a native species of the Romanian flora; its flowers hydroalcoholic extract showed inhibitory activity on Gram-positive bacteria as reported by Benedec et al (2013). From the same family, hydroalcoholic extract of *Achillea schurii* Sch.-Bip., an endemic species from Romania, has revealed a remarkable inhibitory effect on *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella typhimurium* (Benedec et al., 2016). Essential oil of inflorescence harvested from *Achillea millefolium* and its hybrid *Achillea collina* Becker growing wild in Western Romania inhibited most strongly the growth of *E. coli*, followed by *Shigella flexneri, Klebsiella pneumoniae*, *Salmonella typhimurium* and *Staphylococcus aureus*. No effects were observed against *Clostridium perfringens* and *Streptococcus pyogenes* (Jianu et al., 2015; Jianu et al., 2016). The authors assumed this could be the results of the inhibitory effects exhibited by the major constituents of the analyzed essential oils, respectively chamazulene, caryophyllene and β-pinene; also they noticed the presence of certain minor components, known for their strong antimicrobial activity, such as limonene, *α*-pinene or 1.8-cineole.

Essentila oils obtained by hydro distillation, from *Artemisia* spp growth in different Romanian areas, as spontaneous flora or as cultivated species have been tested against fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary, from carrots roots stored in the refrigerator (Badea and Delian, 2014); minimum inhibitory concentration (MIC) was found to be 2400 µL L-1 for *A. santonica, A. pontica, A. annua, A. austriaca, A. dracunculus, A. lerchiana, A. vulgaris* and *A. vulgaris* var. *pilosa*.

Tanacetum vulgare is known mainly for its toxicity and insect repelent properties. Essential oils and ethanolic extracts of this plant, harvested in Transylvania (Sibiu and Alba county) exhibited moderate inhibition on *Staphylococcus aureus and Bacillus subtilis*, but low activity on *E. coli* and *Pseudomonas aeruginosa* (Muresan, 2015; Muresan et al., 2015).

The Asteraceae list is completed by *Inula helenium*; the ethanolic extracts were obtained from the roots of plants harvested in Brasov county (Transilvania); moderate to high bacterial inhibition have been shown on *Escherichia coli, Enterococcus faecalis, Bacillus cereus* and *Staphylococcus aureus*; meanwhile, moderate anti-*Candida* effects have been proven (Diguta et al., 2014).

Essential oils of the flower heads and leaves of *Santolina rosmarinifolia* L. were obtained through hydrodistillation and tested against Gram-positive and Gram-negative bacteria strains and the fungus *Candida albicans* (Ioannou et al., 2007). The highest inhibitory potential has been shown on *Staphylococcus aureus* and *Candida albicans.*

In the same family (Asteraceae) two other species, *Cynara scolymus* and *Taraxacum officinale* have proven antibacterial activity in hydroalcoholic leaves extracts against *Escherichia coli* and *Salmonella abony* (Ionescu et al., 2013). Meanwhile, Vamanu et al. (2011) has reported that freeze-dried ethanolic extracts of *Cynara scolymus* harvested in Hunedoara county (Transylvania) have significant inhibitory effect on *Listeria innocua and Bacillus cereus.*

Purcaru et al. (2015) have tested different dried leaves *Eupatorium cannabium* extracts made of a Romanian cultivar from Brasov county. In the case of the chloroformic extract and hydro-alcoholic extract the inhibitory activity has been noticed only in the case of *Escherichia coli* and *Bacillus cereus*, as well as on the dimorphic yeast *Candida albicans*. No clear inhibition has been noticed in the case of *Staphylococcus aureus, Enterococcus faecalis* and *Aspergillus niger*.

Lamiaceae family is on the top list of plants tested for their antimicrobial activity. Benedec et al. (2015) has proven that the rosmarinic acid from *Salvia officinalis* L. and *Rosmarinus officinalis* L. has strong antibacterial effect on Gram positive bacteria, even higher than gentamicin; similarly, strong effect has been noticed against *Candida albicans*, higher than fluconazole. Meanwhile, essential oils from aerial part of *Salvia officinalis* originated in Arad county showed strong inhibitory effect of

Staphyloccus aureus and *Klebsiella pneumoniae* (Ilie et al., 2016). The antimicrobial activity recorded have been attributed mainly to the major components of *S. officinalis* essential oils, i.e., camphor, alpha-thujone and alpha-humulene, recognized for their biological activities. Also, essential oils and terpens extracted from *Rosamarinus officinalis* have been proven to have antifugal effects, both on growth and sporulation of *Aspergillus flavus* and *Aspergillus ochraceus*; lower effect have been registered on *Aspergillus niger* (Mihai and Popa, 2015).

Remaining in the same Lamiaceae family, in Romania has been reported for the first time antimicrobial activity of essential oils of *Mentha smithiana* (Jianu et al., 2016). Aside *Mentha piperita,* their essential oils inhibited mainly the Gram-positive bacteria, as well as the fungus *Candida albicans.* Similar results have been obtained by the same authors in the case of essential oils of *Hypericum perforatum*. Also. *Mentha* sp. has been reported to have significant antifungal activity on *Botrytis cinerea* (Şesan et al., 2015). Other species of *Mentha* genus have been reported for anti-*Candida* activity by Moldovan et al. (2014), respectively extracts of *M. spicata* subsp. *crispata* and *M. x rotundifolia.* Same research group reported that other *Mentha* sp. extracts have strong inhibitory activity on *Listeria monocytogenes.*

Aerial part of *Ajuga genevensis* harvested from wild populations from Cluj county at full flowering stage, in alcoholic extracts showed high inhibitory activity against *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa, Listeria monocytogenes, Escherichia coli* and *Salmonella typhimurium* (Toiu et al., 2016).

Lamiaceae family list of plants with animicorbial activity is completed by the ornamental *Teucrium chamaedrys*. Aerial parts harvested during summer in Sibiu county (Transilvania) have been prepared as ethanolic extract; the extract showed stronger antibacterial activity against *S. aureus* than gentamicin used as reference antibiotic, as well as antifungal activity against *Candida albicans,* higher than fluconazole(Vlase et al., 2014).

From Apiaceae family, different species of *Erymgium* have been tested for their antimicrobial activity. Tincture of aerial plants from *E. planum* and *E. campestre* from Cluj county and *E. maritimum* from Constanta county have proven to have moderate antibacterial activity on *Stahpylococcus aureus* and *Stahpylococcus epidermidis* and high inhibitory activity on *Pseudomonas aeruginosa*, especially in the case of *E. campestre* (Conea et al., 2016); authors assumed that the activity of *Eryngium* tinctures probably results from the synergistic effect of triterpene saponins, polyphenols, sterols, pectin and other active compounds.

Humulus lupulus (common hop) from Southern Romania, an herbaceous climbing plant, as hydroalcoholic extracts of female inflorescences, has been proven to have antagonistic effect on both Gram-positive and negative bacteria (Aresene et al., 2015). In the case of hope the substances associated with antibacterial activity are humulone, lupulone and xanthohumol (Cermak et al., 2017).

Another climbing plant, *Hedera helix* harvested in Cluj county as leaves, flower and immature fruits has been tested by Pop et al. (2017). They have arrived to the conclusion that the immature fruits extract showed a significant activityagainst *S. aureus*, followed by the flower extract with a good growth inhibitory effect against the same bacterial strain. Both immature fruits and flowers extracts possess appropriate antibacterialcapacity against *L. monocytogenes*.

Among perennial bulbous of Romanian wild flora has been tested *Allium ursinum* in hydroalcoholic or acetic acid extracts obtained from different parts (leaves, roots, bulbs). The extracts inhibited the growth of different altering or pathogen fungi like *Aspergillus glaucus*, *Geotrichum candidum* and *Candida albicans*, as well as on different Gram-positive and Gram-negative bacteria (Lupoae et al., 2013). The authors recommend their use in the food industry as additive. Şesan et al. (2015) have also demonstrated that *Allium sativum* extracts have inhibitory effects on *Botrytis cinerea* (grey mould)affecting cultures of *Ribes nigrum*.

The hydroalcoholic extract of *Chelidonium majus* (Papaveraceae) obtained from powder of dried aerial plant organs collected from a private homegarden in Cluj county had antifungal effect against *B. cinerea* (Pârvu et

al., 2011). Another phytopathogenic fungi, *Pythium* sp., have been proven to be inhibited by ethanolic extracts of *Glycyrrhiza glabra* (Fabaceae) roots harvested in Southern Romania from non-polluted sites (Rodino et al., 2015a).

A novelty may be considered the studies conducted by Pîrvu et al. (2017 a) regarding the antimicrobial activities of extracts from rock rose (*Helianthemum nummularium* Mill.) harvested in July from Romanian Carpathian Mountains. These extracts show certain antimicrobial activity on *E. coli*, as well as weak to moderate activity on *S. aureus, S. typhimurium* and *S. enterritidis*; the list is completed by *Pseudomonas aueriginosa* and *Candida albicans.*

Among perennial herbaceous plants tested for antimicrobial activity an ornamental plant was in the research attention. Red petals of *Paeonia*

Table 3. Romanian perennial woody plants with antimicrobial activity

Plant species	Botanical family	Plant part	Antimicrobial activity		Reference
			Bacteria	Fungi	
BUSHES/SHRUBS					
Lavandula angustifolia *Lavandula x intermedia*	Lamiaceae	fs	*Shigella flexneri* *Staphylococcus aureus* *E. coli*	*Candida albicans*	Jianu et al., 2013 Robu et al., 2016
Viburnum opulus	Caprifoliaceae	hb; fl; fs	*Staphylococcus aureus* *Staphylococcus epidermidis*		Bubulica et al., 2012
Lonicera tatarica	Caprifoliaceae	hb; fl; fs	*Staphylococcus aureus* *Staphylococcus epidermidis*		Bubulica et al., 2012
Aronia melanocarpa	Rosaceae	fr; fl	*Vibrio vulnificus,* *V. cholera, V. mimicus* *E. coli* *Enterococcus faecalis*		Giupana et al., 2016
Lycium barbarum	Solanaceae	fl; fs	*Staphylococcus aureus* *Listeris monocytogenes* *Bacillus subtilis*		Mocanu et al., 2014 Mocanu et al., 2015c
Lycium chinense	Solanaceae	fl	*Staphylococcus aureus* *Bacillus subtilis* *Listeria monocytogenes* *Salmonella thyphimurium*		Mocanu et al., 2014
Sambucus ebulus	Adoxaceae	fr	*Pseudomonas fluorescens* *Enterococcus faecalis*		Rodino et al., 2015b
TREE					
Juniperus communis	Cupressaceae	fr	*Bacillus subtilis* *Streptococcus luteus* *Staphylococcus aureus* *Escherichia coli*		Ivopol et al., 2016
Abies alba	Pinaceae	nd; cx	*Bacillus subtilis* *Streptococcus luteus* *Staphylococcus aureus* *Escherichia coli*		Ivopol et al., 2016 Sandru et al., 2015
Picea abies	Pinaceae	nd	*Staphylococcus aureus* *Bacillus cereus* *Proteus vulgaris*	*Candida albicans* *Aspergillus niger*	Radulescu et al., 2011
Pinus sylvestris	Pinaceae	nd; cx	*Bacillus subtilis* *Streptococcus luteus* *Staphylococcus aureus* *Escherichia coli*		Ivopol et al., 2016
Pinus cembra L.	Pinaceae	nd; cx	*Staphylococcus aureus* *Sarcina lutea* *Bacillus cereus* *Escherichia coli* *Pseudomonas aeruginosa*	*Candida albicans*	Apetrei et al., 2011 Apetrei et al., 2013
Fagus sylvatica	Fagaceae	fl	*Staphylococcus aureus*		Pirvu et al., 2014
Robinia pseudoacacia	Fabaceae	fs; sm cx; fl	*Staphyloccous* sp., *Streptococcus* sp. *E coli., Pseudomonas aeruginosa,* *Proteus* sp., *Salmonella enterica*	*Candida albicans*	Rosu et al., 2012
Cydonia oblonga	Rosaceae	fl	*Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa*		Cerempei et al., 2016

Legend: hb: herba (flowering aerial parts); fl: folium (leaves); fs: flos (flowers); nd: needles; fr: fructus (fruits); cx: cortex (bark); sm: semen (grains); rx: radix (roots); rh: rhizoma (rhizome); st: stipites (branches).

officinalis, in ethanolic and methanolic extracts have shown strong inhibitory activity on bacteria and fungi, respectively *Escherichia coli, Pseudomonas aeruginosa, Salmonella abony*, *Staphylococcus aureus, Enterococcus faecalis, Brevibacterium flavum, Sarcina* sp.,

Bacillus cereus and *Aspergillus niger* (Soare et al., 2012).

Bushes/Shrubs

Lavender (*L. angustifolia* Miller) and lavandin (*Lavandula x intermedia*) are well known for their medical and cosmetics applications. Essential oils obtained by steam distillation from fresh inflorescences harvested in Western Romania showed antimicrobial activity against *Shigella flexneri*, *Staphylococcus aureus*, *E. coli* and *Salmonella typhimurium*, while *Streptococcus pyogenes* was not sensitive to their action (Jianu et al., 2013). The authors emphasize the fact that even in the absence of active principles like linalool and linalyl acetate, considered responsible for the antibacterial and antifungal properties of essential oils obtained from different species of *Lavandula.* This results looks to be in contrast with results reported by Robu et al. (2016) on essential oils of lavandin (*Lavandula hybrida* Reverchon) harvested in North-Eastern Romania (Neamt county); these oils showed no activity against Gram-negative strains; also the results showed that the antistaphylococcal activity is reduced, while there is a moderate antifungal activity.

Two bushes belonging to Caprifoliaceae family, *Viburnum opulus* and *Lonicera tatarica* from Craiova, Dolj county have been tested by Bubulica et al. (2012); aqueous extracts of aerial parts (stem, flower buds, fruit pulp) have been tested on *Staphylococcus aureus* and *Staphylococcus epidermidis*. The results showed a higher inhibition in the case of *Lonicera tatarica* extracts.

Studies on bacterial strains isolated from wild birds captured in Danube Delta Biosphere Reservation proved that extracts of fresh fruits of *Aronia melanocarpa* has important inhibition on *Vibrio* spp. (*V. vulnificus, V. cholera, V. mimicus*), *E. coli, Enterococcus faecalis* (Giupana et al., 2016).

Ethanolic extracts of *Lycium* sp. (Solanaceae) leaves originated in Cluj county, have been tested for antimicrobial activity (Mocan et al., 2014). The authors reported that *L. chinense* extract was more active than *L. barbarum* against both Gram-positive and Gram-negative bacterial strains and that these species as important sources of flavonoids and chlorogenic acid. The best antibacterial activity was shown by *L. chinense* extract against *Bacillus subtilis*. Meanwhile, extract made of *L. barbarum* flowers was found to be more active on the Gram-positive bacterial strains; the best antibacterial activity was shown against *Staphylococcus aureus* (Mocan et al., 2015 c).

The perennial herbaceous extracts of *Epilobium hirsutum* harvested in Prahova county inhibited both *Stapyloccous aureus* and *E. coli* (Pirvu et al., 2014). Same group (Pîrvu et al., 2015) suggested an augmented antimicrobial potency on *Stapyloccous aureus* of the combination kaempferol-caffeic acid derivates (aqueous fraction) than myricetin-gallic acid derivate (ethyl acetic fraction).

The dwarf elderberry (*Sambucus ebulus*) used in traditional medicine, has proven to have antibacterial effects on *Pseudomonas fluorescens* and *Enterococcus faecalis* when used as ethanolic extract made of fruits (Rodino et al., 2015 b).

Trees

Conifers are widely used for the extraction of essential oils and their volatile oils contain mainly monoterpene (Ivopol et al., 2016). Among the conifers *Pinus* sp. (Pinaceae) has been widely studied. *Pinus cembra* L. from Carpahtian Mountains, bark and needles, have antimicrobial effects against *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Apetrei et al., 2011; Apetrei et al., 2013). Common *Pinus sylvestris* essential oils from needles and sprouts showed inhibitory effects on *Bacillus subtilis, Streptococcus luteus, Staphylococcus aureus* and *Escherichia coli*; similar results have been obtained by the use of essential oils from *Juniperus communis* berries and *Abies alba* needles and sprouts (Ivopol et al., 2016). Sandru et al. (2015) also proved inhibition on *E. coli* by the use of *Abies alba* essential oils.

Antimicrobial properties of volatile oil is olated from sprouts of *Picea abies* growing wild in Romanian Carpathian Mountains (Prahova Valley) have been tested by Radulescu et al. (2011). The most evident inhibitory effect was noticed against the Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*), Gram-negative (*Proteus vulgaris*) and fungal strains (*Candida albicans*, *Aspergillus niger*).

There are authors which have tested more species from a specific Romanian region for their antimicrobial activity. For example, Pirvu et al. (2014) have focused on herbaceous and woody plants from Prahova county in propylene glycol solutions or in separate aqueous, ethyl acetate and chloroform fractions. Among the trees, extracts from leaves of *Fagus sylvatica* exhibited moderate inhibitory effect on *Stapyloccous aureus*, ethanolic extracts from flowers and seeds of *Robinia pseudoacacia* have inhibitory activity mainly on Gram-positive coci (*Staphyloccous* sp., *Streptococcus* sp.), while same extracts from bark and leafs inhibited *E coli, Pseudomonas aeruginosa, Proteus* sp., *Salmonella enterica* and *Candida albicans.*

As a novelty can be mentioned the use of fall quince (*Cydonia oblonga*) leavesoriginated in North-Eastern Romania for the production of natural dye; it has been proven (Cerempei et al., 2016) that such dye with mordant (silver nitrate) have a good antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *Ps. aeruginosa*); the authors assumed that a possible explanation can be that the wool-Ag-flavonoid complex has a larger surface area that gives antibacterial effect.

CONCLUSIONS

In our tentative to find out the interest of Romanian researchers to prove the antimicrobial activity of autochthonous flora from different regions and counties in our country, we have identified over sixty articles published in the time frame 2007-2018. We are aware that some other authors may have published in the subject and have escaped to our search.

The tested plants have been harvested from different geographical regions of Romania, from fields, hills and mountains; we have noticed more abundant information coming from Transylvania and Banat region, followed by Southern counties and Moldavia. In our search we have identified a total of 64 species from autochthonous flora taken into account for studies on antimicrobial activity, belonging to 21 botanical families. Among these species, 28.1% are annual species, 46.9% are herbaceous perennial and the rest (25%) are woody perennial species (bushes and trees). The antimicrobial studies have been mainly focused on species belonging to two botanical families, Asteracea and Lamiaceae, which represents 50% of the total studies species.

In terms of microbial species can be noticed an intensive focus on pathogenic Gram-positive and Gram-negative bacteria, responsible for clinical infections or food contamination. For 89% of the species have been reported antibacterial activity, while only 57.8% of the species have proven antifungal activity. The most reported susceptible fungus was *Candida albicans*; few reports are focused on filamentous fungi like *Aspergillus* sp., *Penicillium* sp, *Botrytis cinerea* or *Pythium* sp.

It has been noticed that some of the reports are novelty in the subject and the researchers have approached some spontaneous species little or not ever reported in the international databases (*e.g. Helianthemum nummularium, Cydonia oblonga, Paeonia officinalis*). This trend may be a solution for further research in the topic, as well as enlarging the studies on filamentous fungi, even if they are of medical or feed/food interest.

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TRICHODERMA SPP. SOURCE OF HYDROLASE ENZYMES WITH ROLE IN *F. OXYSPORUM* INHIBITION

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Abstract

The fungi from Trichoderma genus are a very large group of microorganisms that are present in nearly all agricultural soils and play a significant role in plant protection. Enzymes produced by species of genus Trichoderma constitute an important group of biotechnologically enzymes because of the versatility of its properties and ease of mass production. This study was focused on the effect different factors of antagonistic T. harzianum (ICCF 417) and T. koningii (ICCF 418) against pathogenic fungus F. oxysporum (ZUM 2407) by microbiologic and biochemical tests. There was different patterns in cell wall degrading enzymes production by Trichoderma isolates so production of chitinase was 113.93 % by T. harzianum comparative to T. koningii, the lipase was 38.53% in T.harzianum comparative to T. koningii while the protease was 91.43% in T. harzianum comparative to T. koningii. Both Trichoderma strains studied showed maximum lipase activity at pH 9, which was significantly higher than those at other pH levels. On the other hand, optimum pH for producing highest activity of protease was pH 6. A high level of chitinase activity was observed in the culture medium with pH 6. Our results showed that hydrolase activities studied in this experiment play an important role in pathogenic fungus F. oxysporum inhibition and the degree of effect is different.

Key words*: Trichoderma harzianum, Trichoderma koningii, lipase, protease.*

INTRODUCTION

The use of *Trichoderma* fungus species against many fungal phytopathogens has seen significant progress in recent years because their high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, strong aggressiveness and efficiency in defense mechanism (Harman, 2006; Harman et al., 2004). Due to their ability to protect plants and because they are commonly found in almost all soil types, these fungi have been widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments. *Trichoderma* spp. were shown to be very efficient producers of extracellular enzymes like chitinases, β-1.3-glucanases, cellulases, lipases, proteases some of these have been implicated in antifungal effect on different pathogens (Gajera et al, 2012; Vinale et al., 2007; Harman, 2006; Markovich and Kononova, 2003; Viterbo et al., 2002; Monte, 2001). Several reports have been given to explain the role of lytic enzymes produced by *Trichoderma* spp. during plant defense. *Fusarium oxysporum*, a well-known species of soilborne fungus, causes significant losses to horticultural and ornamental crops throughout the temperate climatic regions (Chen et al., 2017). The fungus *Fusarium* spp. is a fungal species that have adapted to different range of environmental from worldwide, in addition this types was described as the important pathogen of the different crops. Lobna et al. (2017) and Houssein et al (2010) found that the use of the *T. harzianum* as strategy to improve the response of the tomato resistance against

Fusarium wilt under greenhouse conditions caused biochemical changes in plants. The correlation between the enzyme level and resistance to the pathogens was increased, especially under conditions of stress and attack pathogens. Enzymes activity plays an important role in plant resistance against attacking pathogens (Zhang et al., 2008; Cherif et al., 2007; Mohammadi and Karr, 2002). The inhibitory effect of *Trichoderma* on the phytopathogenic *F. oxysporum* could be due to the secretion of lipolytic and proteolytic enzymes that inactivate enzyme of *F. oxysporum* thereby decreasing its pathogenity (Elad and Kapat, 1999).

The aim of this study was the evaluation of different factors from live and dead cells of *T. harzianum*, (ICCF 417) and *T. koningii* strain (ICCF 418) on hidrolytic enzyme production that influence virulence against pathogenic fungus *F. oxysporum* strain (ZUM 2407).

MATERIALS AND METHODS

The ability of fungi *T. koningii* and *T. harzianum* to produce enzymes were evaluate in synthetic medium. The fungus was grown in 250 ml Erlenmeyer flask that contained 100 ml of synthetic medium (SM) then we add the specific substrates for each enzyme separately, then cultures were incubated at 28°C and the ability of enzymes production was determined after 1, 2, 3 and 4 weeks of incubation. Also enzymes were used diluted to 100%, 50% and 10%. The fungi weight was determined by weighting an empty tube, then put (1 mL) of the fungi from each period of culture into the weighted tube after that drying and centrifuging and taking the precipitate which was dried at 50^0C for 3 days, then weighted again. Preparation of cell lysate was done by taking 10 ml of each culture separately and centrifuged. The precipitate obtained was re-suspended in 5 mL of phosphate buffer (pH 7.4) with vortex, then adding sand to the tube, put it in IKA® ULTRA TURRAX device at 6000 rpm for 30 s then put it at ice for 1 min., replay for three times and centrifuged at 10000 rpm for 10 min. at 4°C. Then the solution obtained filtered by Millipore 0.22 µm, and aliquots of the supernatant were used for next assays. All cultures were put at 100^0C for 15 min., filtered by micro filter, aliquots of the supernatant were used for next assays. Characterization of enzymes produced by *T. koningii* and *T. harzianum* that had effect on *F. oxysporum* was done in PDA medium using a Petri dishes divided into 6 sections. At the first one we put 10 µL synthetic medium SM, the second 10 µL of dead cell of enzymes culture, the third one 10 µL of living cell of enzymes culture, the forth section 10 µL of dead cell from fungi culture, the fifth with 10 µL of dead lysate, the last one with 10 µL living lysate.

For control, we put a Petri dish of *T. koningii*, *T. harzianum* and *F. oxysporum* alone and a Petri dish divided into two parts, one with *Trichoderma* and the other with *F. oxysporum.* All experiments were made in triplicate.

Chitinase activity assay was performed according to Miller (1959). Lipase activity was measured spectrophotometrically using an assay based on the hydrolysis of p-nitrophenyl palmitate as substrate, according to Gupta et al. (2002) while for determination of protease activity has been used the method of Saad (1995). The protein quantity of the crude enzyme extract was determined by the Lowry method using bovine serum albumin as standard (Lowry et al., 1951). The enzymes activity and protein concentration were measured after 1, 2, 3 and 4 weeks incubation of the culture incubation. In order to determine the optimum pH value for the enzyme produced by *Trichoderma* obtained after fermentation, the activity of the enzyme was assayed between the pH values of 3.0-9.0.

RESULTS AND DISCUSSIONS

Figure 1 shows the influence of pH on chitinase, lipase and protease production by *T. harzianum*, *T. koningii* and *F. oxysporum*. According to the results, the optimal pH for lipase was 9. The results are similar with Ulker et al., 2014, which showed the optimum pH value for lipase activity produced by *T. harzianum* was 8.5. Also has been establish that optimum pH for maximum chitinase and protease activity in *T. harzianum* and *T. koningii* was pH = 6. This agrees with results obtained by Cirano et al., 1991, and Kredics et al., 2004.

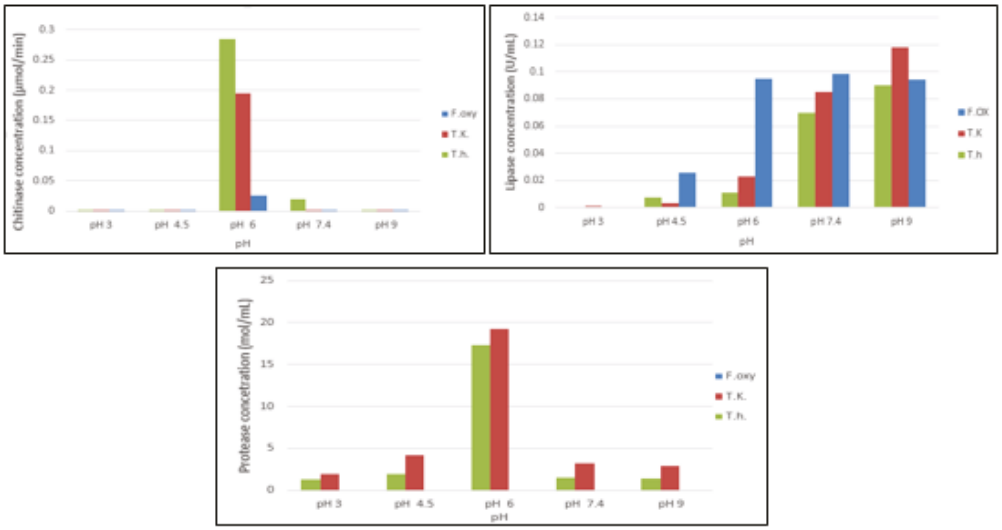

Figure 1. Determination of optimum pH for chitinase, lipase and protease enzymes production by *T. harzianum*, *T. koningii* and *F. oxysporum*

The results of chitinase enzyme showed that the two fungi can produce the chitinase but its level on *T. harzianum* was higher than *T. koningii*, and for the two fungi the period of 14 days was the highest production, also the dilution ratio 100% was the highest, as shown in figure 2.

The levels of chitinase enzyme was 0.42 and 0.48 µmol/min in *T. koningii* and *T. harzianum*, respectively, after 14 days.

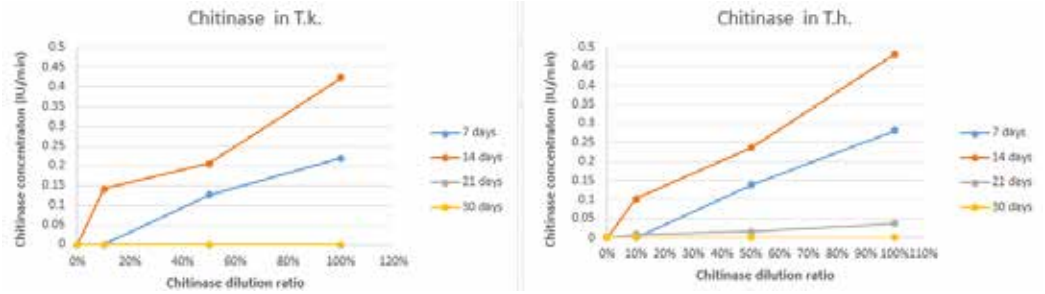

Figure 2. Chitinase production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The production of lipase enzyme in synthetic medium for *T. koningii* was higher than *T. harzianum* and the second week was the highest period of production, as shown in figure 3.

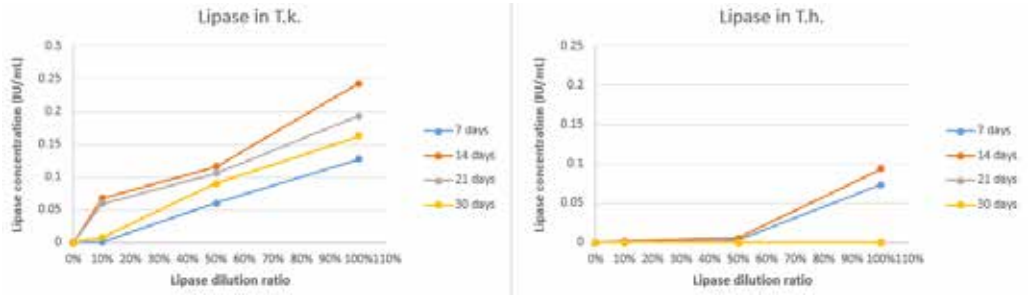

Figure 3. Lipase production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The results of protease production by *T. koningii* and *T. harzianum* are presented in figure 4. The protease levels in *T. koningii* was higher than in *T. harzianum*, and the 30 days incubation period was with the highest production, also the dilution ratio 100% was the highest increasing.

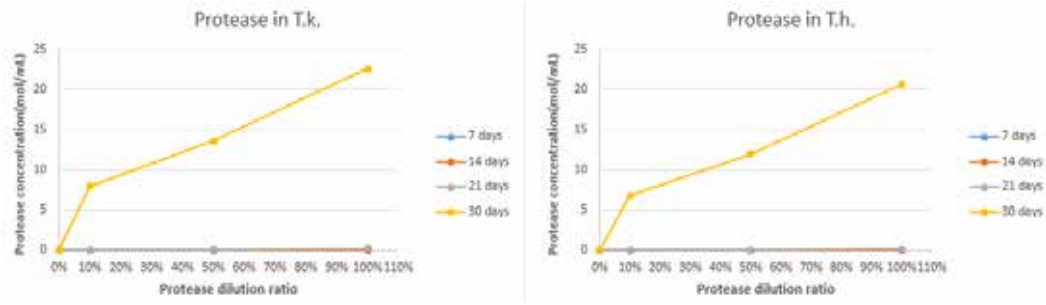

Figure 4. Protease production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The protein levels in chitinase enzyme were in *T. harzianum* higher than the *T. koningii*, and the second week had the highest levels of protein in the two fungi, as shown in figure 5.

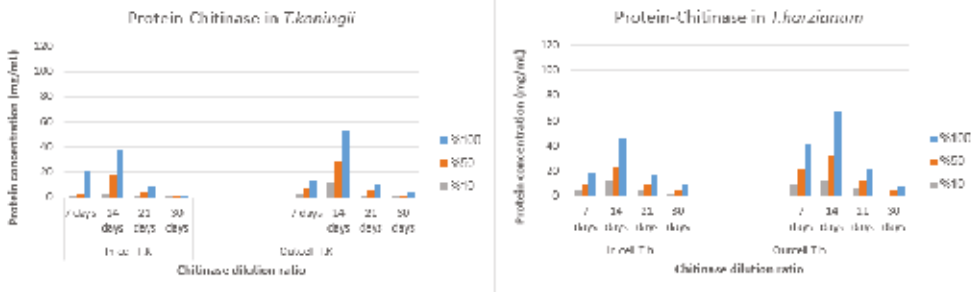

Figure 5. Protein levels in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The highest protein level in the lipase enzyme medium was in *T. koningii* in comparison with its level in *T. harzianum*, but also the period of 14 days was the highest level of protein production in comparison with the rest periods, as shown in figure 6.

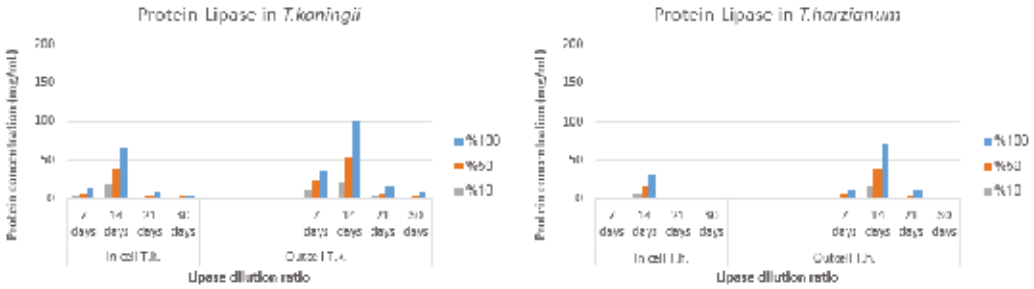

Figure 6**.** Protein levels in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

In figure 7 are presented the levels of proteins in synthetic medium for protease production by in *T. koningii* and *T. harzianum* respectively, protein levels on *T. koningii* was higher than *T. harzianum*, and for the two fungi the period of 30 days was the highest production.

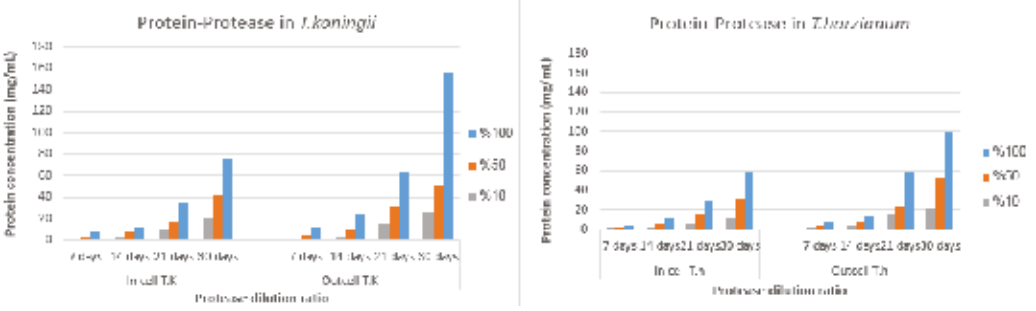

Figure. 7. Protein levels in *T. koning*ii and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The levels of protein in *T. koningii* were 52.97, 101.85 and 155.23 mg/mL for chitinase, lipase and protease, respectively, in out of cell, while in cell lysate were in chitinase 36.98 mg/mL, lipase 65.69 mg/mL and protease 75.68 mg/mL. In *T. harzianum* the protein levels were 67.85, 72.02 and 99.25 mg/mL for chitinase, lipase and protease, respectively, in out of cell, while in cell lysate were in chitinase 45.26 mg/mL, lipase 31.68 mg/mL and protease 58.61 mg/mL. The results of the effect of *T. harzianum* and *T. koningii* on *F. oxysporum* showed that these fungi have a high ability against the pathogenic fungi *F. oxysporum*, the ratio reached to 1, according to the scale of Bell et al. (1982). The lipase and the protease enzymes were effective on *F. oxysporum* in the second and forth weeks, respectively. These results are in accordance with those obtained by Elad et Kapat, 1999, which supported that some proteases and lipases secreted by *Trichoderma* spp. may be involved in inactivating extracellular enzymes of *F. oxysporum*.

CONCLUSIONS

The results obtained in this study show that both strains of *Trichoderma* studied produced chitinases, proteases and lipases in synthetic medium but the concentration depends on the strain and the incubation period.

There is an inhibitory effect of *T. harzianum*, strain ICCF 417 and *T. koningii* strain ICCF 418 against pathogen *F. oxysporum* strain ZUM 2407 by production of protein and extracellular enzymes which may effect on the action *F. oxysporum*.

The lipase has the highest weight in comparison with the others enzymes, while the chitinase enzyme was not effective on *F. oxysporum* for all period of incubation.

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EFFECT OF PERIODS AND REPETITION OF THE BURNING METHOD IN THE CONTROL OF WATER HYACINTH (*EICHHORNIA CRASSPIES*) PLANT

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Abstract

The experiment carried out using basins under field conditions of the Faculty of Agriculture and Forestry, University of Mosul in the season 2010-2011. The aim of study is using the mechanical method (burning method) for controlling water hyacinth and decrease the pollution on aquatic environment. The study included a two factors, influence burning repetition and periods of the burning in the control of water hyacinth weed. The experiment was applied in randomized completed design it was applied in three repeaters. The results showed the no influence on studied traits in the case of an incineration process one time while the increase in the number of times the incineration twice to a negative impact on the studied traits of the water hyacinth plant this effect was more severe in the event of an increase in the replicates of burning to three times, with low in the all studied traits (length of plant, the number of leaves, length of spike, number of flowers and weight wet and dry weight the total vegetation and roots).

Key words*: water hyacinth, burning, repetition burning.*

INTRODUCTION

Water hyacinth of floating plants on the surface of the water and usually floats by rafts publish on the water surface, which consists of a roots group under the water's surface and total vegetative floats above the surface of the water, there are several names for this plant, including the flower Nile, yanst water or herb Nile (*Eichhornia crassipes* (Mart.) Solms), belongs to the family Pontederiaceae, also called family Pickerel weed family. Belong to this family six genera and about 20 species, and the plants of this family are either floating on the surface of the water or floating and flourish in the warmer regions of the globe. There are several species of the genus *Eichhornia* most important (*E. crassipes*) for being a dangerous weed, which occupies the eighth spot (Holm et al., 1977). The ornamental plant enter in Irak in mid 1980s because it have purple flowers and some civil nurseries selling this plants as ornamental plant and they spread to the rest of the other areas currently present in separate regions in Mosul, and speed spread and growth of it led to more problems impeding the flow of water in irrigation channels and drainage and also reduced quantities of water consumption and impeded the work of the hydro power and pumping stations as well as a haven for many of the diseases and insects in the depletion of oxygen dissolved in the water, which negatively affects life's bountiful fish and some of the people who depend on fishing. In order to reduce the pollution caused by the excessive use of herbicides and non-compliance with the control programs and the recommended concentrations used for control, the burning is the one of mechanical methods used to combat weed widespread in agricultural and non-agricultural land. There are several methods that have been used to combat this weeds in many countries where preventive, mechanical, biological and chemical methods have been deployed. So researchers and studies in this field are interested in working hard in the preparation of control programs in order to limit the spread and control of the weed, its money has an impact on the human and its sources of livelihood and other resources. Due to the lack of sources on the use of the method of burning in the control against the water hyacinth, it was pointed out that this method was used with the reed plant, Common reed, *Phragmites australis* (Cav.)Trin., because it is in an environment similar to the environment of

the water hyacinth plant. Burning in general does not completely reduce the ability of wild reed to grow because it is difficult to burn roots and rhizomes which are often covered with a thick layer of soil or soil and water (Beall, 1984). Of course, this result applies to the water hyacinth, where if burned green parts and leaves, it may be difficult to reach the burning to some of the leaves and roots because they are covered with a layer of water, which leads to the re-growth of these parts. Several studies have indicated that the date of burning of cane has an effect on plant density and growth. Burning at the end of summer is better than burning in winter and spring (Cross and Fleming, 1989). In an experiment conducted by (Thompson and Shay, 1989) in the Marsh Marshes (Delta March Manitoba), he found that burning in spring, summer and fall leads to a higher growth rate in the vegetative growth but shorter and thicker but the biomass of the vegetative group was greater for the plants burned In the spring and fall while they are lower in plants that were burned in the summer. This is due to the fact that plants whose growth season was in the spring and fall gave them the opportunity to grow well. Burning leads to a doubling of the size of the biomass on the surface of the earth due to the availability of light and soil ventilation around the rhizomes (Graneli, 1989). The same researcher recommended that the burning of cane plants in winter causes slight damage to plants, while the date of burning in the period of emergence of plants leads to the killing of the total vegetative almost completely. (Uchytil, 1992) found that burning of wild reed plants when growing well and intensely is more efficient, the high heat resulting from burning vegetation also causes damage or death to reed rhizomes found beneath the surface of the soil. The objective of the research is to study the effect of the recurrence of the date of burning in the fight against the water hyacinth, as well as reduce the percentage of pollution caused by the use of chemical herbicides in control.

MATERIALS AND METHODS

Effect of repeated burning at different dates in the controlling of water hyacinth

This experiment was carried out in basins where it was placed under the conditions of the field of the Faculty of Agriculture and Forestry, University of Mosul on 3.03.2010, three plants were taken from the basin, which was prepared in the field length of 15 m and width 6 m in each pot, which number 30 and dimensions 73 cm length and width 32 cm and depth of 33 cm. This element was covered with thermal insulated edges of aluminum to preserve the element of heat consisting of the burning process and the burn tool is a regulator + gas tube + burner.

Where the factors of the experiment included one factor is the factor of burning times and repetition as follows:

1. Burning one after month of comparison in day 12.07.010.
2. Burn one after a month + burn again after 10 days of the first burn.
3. Burn one after a month of comparison + burn three after 20 days of the first burn.
4. Burning one after two months of comparison on day 12.08.2010.
5. Burn one after two months of comparison + burn the second after 10 days of the first burn.
6. One burn after two months of comparison + burning three after 20 days of the first burn.
7. Burning one after three months of comparison on day 12.09.2010.
8. Burn one after three months of comparison + burn second after the 10 days of the first burn.
9. Burn one after three months of comparison + burn the second after 20 days of the first burn.
10. Comparison (without burning).

Basins were distributed at random within the design (CRD) and with three replicates. The water was added twice a week to complete the water volume in the basin to 77088 cm^3, using the same water source used in the first experiment. A basin was randomly selected for each treatment and burned by the burner for one minute and repeated three times. The experiment was analyzed according to the random complete design of simple experiments (CRD). I used the computer according to the (SAS) program and used the Dunkin Multi-Range Test to compare (Al-Rawi and Abdul-Aziz, 1980).

RESULTS AND DISCUSSION

Plant height (cm)

Table 1 indicates that the height of the Nile flower plants was not significantly affected by the one-time burning process and its various dates (12.07., 12.08. and 12.09.2010). In the case of repeated burning two times, the same table indicated that the process of burning at the first date of the plant life (22.07.2010) and twice did not significantly affect the attribute of plant height, but the progress of the plant age, after the process of burning after a month or two months and the repeat of burning (55.72 and 52.18%) treatment of burn twice and for the second and third seasons, respectively, compared to the plants that were not treated by burning. This is due to the fact that the burning process was carried out at the stage where the plant during this period in the stage of active growth and despite the influence of this process in total vegetative the effect of burning was temporary, as the stolon and daughter plants were able to form new growths and daughter plants new where they were about the same height as those plants that were not treated with burn. The results were taken at the end of the experiment, after about eight months. This period is enough for a plant to give these growths. As for the plants treated late in the treatment, it seems that the plants treated with burning were in a good growth stage and so the burning process led to significant damage in the vegetative group and when the plant returned to grow again there was a second burning, which led to the weakening of the plant, negatively affect the height of these plants during the time period between the treatment procedure and the period of termination of the experiment compared with the comparison treatment. In the case of repeated burning of three times and their different dates, all these factors have resulted in a significant decrease in plant height (61.34, 77.87 and 76.64%) for the burning processes (2.08., 2.09. and 2.10.2010) which were burned three times respectively.

Length of inflorescence (cm) and number of flowers / plant

The process of burning one time and its different dates (12.07. and 12.08. and 12.09.2010) and also when the burning (22.07.2010 + 10 days of the first heartburn) The length of the inflorescence (cm) and the number of flowers/plants of the water hyacinth plants were not significantly affected compared with the comparison treatment (Table 1). In the case of plant age progress after the burning process after a month or two months and repeated burning twice resulted in a significant decrease for two adjective (61.80, 63.87, 54.72 and 54%) for both the two adjective after a month or two months of burning twice compared to Comparative Plants. In the case of repeated burning three times and their different dates, it all these treatments significantly reduced the length of the inflorescence (cm) and the number of flowers/plant of the water hyacinth (37.57, 75.77, 100, 45.45, 72.73 and 100%) for the third burn with their different dates and the two adjective, respectively, compared with non-treated plants.

Number of leaves/plant, leaves area (m^2/m^2)

It is noted from the same table that the process of burning one time at the date (12.07.2010) did not significantly affect the number of leaves/plant as well as the of leaves area (m^2/m^2) compared with the treatment of comparison, but it was observed there is a significant effect of the two adjectival on the burning date (12.08. and 12.09.2010). Morally decline was observed of these two adjectival (43.5, 38.83, 35.97 and 23.17%) for both the two dates respectively. In the case of repeated burning of two times, the same table indicated that the process of burning on the first date (22.07.2010) and twice did not significantly affect the number of leaves/plant of the water hyacinth compared with the comparison treatment, but it was observed there is a significant effect of the leave's area in this date, where the decrease was 29.27% compared to the unburned plants. In the increase plant age, after burning after a month or two months and repeated burning twice, resulted in a significant decrease in the number of leaves/plant and the leaves area (m^2/m^2) of the water hyacinth by 64.67, 79.5, 75 and 84.15% after month or two months after burning twice compared to the Plants that did not burn. This may be due to the fact that the plants that were burned by a late date were in the growth and total good vegetative and when treated with burning, this process led to significant damage to the vegetative group and when the plant to

compensate the growth again, there was a second burning, which led to the depletion of food stored in places Plant storage, which led to the weakening of the plant, which adversely affected the number of leaves/plant, which in turn affected the leaves area (m^2/m^2) of this plant. the case of repeated burning of the three times and their different dates, all these treatments resulted in a decrease in the number of leaves/plant as well as the leaves area of the plant (m^2/m^2) by (37.17, 71.17, 97 and 51.22%) to burn three times.

Table 1. Effect of the date and repeated of burning in the traits studied Plant water hyacinth growing in the site of the Faculty of Agriculture and Forestry University of Mosul for the season

Thoughtful adjective									
	Plant height / cm	Number of leaves	Leaves area (m^2/m^2)	Length of Inflorescence (cm)	Flowers number/ plant	Fresh weight of vegetative total (kg/m^2)	Dry weight of the vegetative total (kg/m^2)	Fresh eight of root (kg/m^2)	Dry weight of the root (kg/m^2)
Comparison (without burning).	38.10a	600 a	1.64 a	15.97 a	11 a	22.59 a	2.40 a	8.69 a	1.23 a
Burning one after month of comparison in day 12.07.2010.	37.63 a	615 a	1.84 a	14.80 ab	9 ab	19.63 ab	2.41 a	7.62 ab	1.40 a
Burn one after a month + burn again after 10 days of the first burn.	30.00 a	454 ab	1.16 b	13.17 ab	8 ab	13.79 c	1.74 b	7.95 ab	0.98 a
Burn one after a month of comparison + burn three after 20 days of the first burn.	14.73 b	377 bc	0.80 e	9.97 bc	6 cd	9.27 d	0.83 cd	5.94 b	0.77 a
Burning one after two months of comparison on day 12.08.2010.	32.63 a	339 bc	1.05 de	13.50 ab	9 ab	13.73 c	1.33 bc	6.80 ab	19.44 a
Burn one after two months of comparison + burn the second after 10 days of the first burn.	16.87 b	212 cd	0.41 f	6.10 cd	3 cd	4.61 ef	0.27 de	2.53 c	0.23 a
One burn after two months of comparison + burning three after 20 days of the first burn.	8.43 b	173 cd	0.47 f	3.87 de	3 cd	2.52 f	0.15 e	1.35 c	0.12 a
Burning one after three months of comparison on day 12.09.2010.	28.87 a	367 bc	1.26 b	13.97 ab	11 a	18.05 b	1.86 ab	7.73 ab	0.90 a
Burn one after three months of comparison + burn second after the 10 days of the first burn.	8.90 b	123 d	0.26 f	5.77 cd	5 bc	6.73 de	0.54 de	2.21 c	0.27 a
Burn one after three months of comparison + burn the second after 20 days of the first burn.	18.22 b	18 d	0.00 g	0.00 e	0.00 d	0.45 d	0.03 e	0.32 c	0.04 a

The values followed by similar letters are not significantly different from each other at the 5% probability level in each of the study factors and their interference.

Fresh and dry weight of vegetative total (kg/m^2)

The adjective of fresh and dry weight of vegetative total (kg/m^2) Plants of the water hyacinth table (1) were not significantly affected when you make the burning process once the deadline (12.07.2010) as well as at the date (12.09.2010) for the weight dry for vegetative total, the reason is that a burning process in the early stages of the experiment may cause damage to the vegetative total, but these plants soon quickly re-growth as a result of the growing vegetative by daughter plant or stolon in this period the plant active in produce many new growth Therefore it was able to reach the fresh weights of almost equal or equal to those plants not treated by burning. In the case of progress of the plant age and after one or two months of this burn, there is a significant effect for the two adjective and decrease by (39.22, 20.10 and 44.58%) for both the two adjective and both others time respectively. It may be because the burning process was carried out in the case of the plant is very activity where it had a large number of vegetative leaves so many, the process of burning in this period leads to significant damage to the total vegetation so that some of these plants, which was able to re-growth Were not able to form a total vegetative equal in these treatments were not treated by burn during the period of time the

experiment was harvested, which was less than the period during which the burning process was carried out at the beginning of the experiment. In the case of repeated burning of two times, three times and their different dates, all the treatments resulted in a significant decrease in the fresh and dry weight of the total vegetation of the water hyacinth plant (38.95, 79.59, 70.21, 27.5, 88.75, 77.5, 58.96, 88.84, 98.01 and 65.42, 93.75 and 98.75%) the burn two time and three time, for both the different s dates respectively, compared to non-burning plants.
Table 1 shows that the one-time burning process (12.07., 12.08. and 12.09.2010) did not significantly affect the adjective fresh weight of root total of the water hyacinth plant. In the case of repeated burning two times, the same table indicated that the process of burning at the date (22.07.2010) and twice did not significantly affect the fresh weight of the total root of the plants of the water hyacinth plant, but in the case increase plant age after burning after a month or two months and In the case of repeated burning, this process resulted in a significant decrease in the fresh weight of root of the plant by (70.89 and 74.57%) for both the two times respectively, compared to the plants that did not burn. While when the repeated burning three times, for different times in all the treatments, there was a significant decrease in the fresh weight of the root group of the Nile flower plants by (31.64, 84.46 and 96.32%) for the burning three times and different dates, respectively compared with the natural plants. It was also observed that there was no significant difference between recurrence of burning two and three times when compared between them. The dry weight of the root plant of the water hyacinth was not significantly affected by the various incineration factors and their different dates compared with the plants that were not treated with burning.

CONCLUSIONS

Delaying the time of burning is favoring for the control of water hyacinth plant.
More cover the results of present study confirmed that increasing the frequency of burning more than twice gave an excellent results for the controlling of this weed which reflexed in the reduction of all characteristics studied.

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BAKERY PRODUCTS FORTIFIED WITH DRIED FRUITS OF *ARONIA MELANOCARPA*

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Abstract

Aronia (Aronia melanocarpa) has gained a huge interest due to its complex biochemical composition that gives it various beneficial effects on health. Polyphenols (anthocyanins and procyanidins, especially), represent the most important group of biologically active compounds, which give to these fruits their therapeutical properties and antioxidant potential. In this study it was evaluated the quality of bakery (bread, minibaguette and biscuits) products fortified with dried fruits of Aronia melanocarpa. Products made have superior sensory quality, high nutritional value and antioxidant potential. Thus, products are characterized by the content in polyphenols (193.34 ... 263.22 mg GAE/100g), proteins (11.92 ... 12.95%), fibres (9.13 ... 16.60%) and mineral elements (potassium, calcium, magnesium, iron and zinc). Antioxidant capacity of the achieved products varied in the range 1.91 ... 3.42 mg Trolox Equivalents/g. Biscuits with Aronia and ginger recorded the highest value of antioxidant capacity, and Bread with Aronia had the minimum value. Shelf-life of products studied is quite long, possibly due to the antioxidant and antibacterial properties of fruits of Aronia melanocarpa. In addition, in case of bread and minibaguette, lactic acid bacteria from sourdough used for fermentation have an important role to ensure the preservation of these products. This study has practical value; dried fruits of Aronia melanocarpa can be a functional ingredient to increase the nutritional value and antioxidant capacity of bakery products.

Key words*: Aronia melanocarpa, polyphenols, antioxidant capacity, bakery products.*

INTRODUCTION

There are scientific evidences that a diet rich in fruits and vegetables may reduce the risk to have different chronic diseases (Borges et al., 2010). Berries are recommended in a healthy diet as it provides protection against degenerative diseases, cardiovascular diseases and cancer (Howard et al., 2012). This protective role is given by some biologically active compounds they contain, like: phenolic acids, anthocyanins and flavonoids (De Pascual-Teresa et al., 2008). Among berries, fruits of *Aronia melanocarpa*, they have gaine recently attention due to the health claims associated with their consumption (Chrubasik et al., 2010; Kokotkiewicz et al., 2010).

Fruits of *Aronia melanocarpa* (Michx.) Elliott are rich sources of biologically active compounds, polyphenols (anthocyanins and procyanidins, especially) representing the most important group. Polyphenols are the main substances which give the antioxidant potential of black chokeberry fruits (Kokotkiewicz et al., 2010). Total polyphenolic content varies in the range 2-8 mg/100 d.m. and depends on the cultivar, growing conditions and harvesting time (Kähkönen et al., 1999; Hudec et al., 2006; Oszmiański and Wojdyło, 2005; Benvenuti et al., 2004; Sueiro et al., 2006; Hakkinen et al., 1999). Jakobek et al. (2012) determined total polyphenolic content in case of three cultivars ('Viking', 'Nero', 'Galicianka') of fruits of chokeberry (*Aronia melanocarpa*) and wild chokeberries, in Croatia, region Slavonia during two consecutive years (2010 and 2011). Cultivars 'Viking', 'Nero' and wild chokeberries had a similar total polyphenolic content (9012–10,804 mg kg^{-1} in the first year, 9361–12,055 mg GAE/ kg FW in the second year). Cultivar 'Galicianka' had a lower total polyphenolic content (8564 mg GAE/kg^{-}FW first year, 8600 mg GAE/kg FW second year).

Besides polyphenols, fruits of *Aronia melanocarpa* are sources of sugar (10-18%), pectins (0.6-0.7%), the sugar alcohol sorbitol, and parasorboside (Wolski et al., 2007; Niedworok and Brzozowski, 2001; Weinges et al., 1998; Kulling and Rawel, 2008).

Fresh fruits of *Aronia melanocarpa* can be consumed a short period time and thus to benefit by their nutritional qualities and antioxidant potential these fruits are processed under various forms: dried fruits, puree, juice, liqueur, syrup, jam, wine, compote, tea, powder (Chrubasik et al., 2010; Ochmian et al., 2012; Kapci et al., 2013; Šnebergrová et al., 2014). On the other side, fresh fruits of *Aronia melanocarpa*, have sour and astringent taste and therefore consumers prefer juice of *Aronia melanocarpa*, in combination with other fruits, such as, apples, pears and blackcurrant (Lehmann, 1990; Ara, 2002).

Kapci et al. (2013) investigated the antioxidant potential of fruits of *Aronia melanocarpa* and of their derivate products (Chokeberry juice, Chokeberry pomace, Chokeberry concentrate, Chokeberry syrup, Chokeberry compote, Chokeberry jam, Raspberry-chokeberry syrup, Sour cherry-chokeberry syrup). Total polyphenolic content varied in the range: 0.78 ± 0.02 g GAE/kg ... 63.1 ± 0.5 g GAE/kg (minimum value was recorded by Raspberry-chokeberry syrup and the maximum one by Chokeberry pomace, due to the fact that it contains skin and seeds of chokeberry). It should be noted that dried chokeberry have a high total polyphenolic content (39.9 ± 0.3 g GAE/kg, respectively, 50.1 ± 0.4 g GAE/kg). Antioxidant capacity of chokeberry fruit determined by ABTS, DPPH, and CUPRAC were 10.9 $g \cdot kg^{-1}$, 11.3 $g \cdot kg^{-1}$ and 67.7 $g \cdot kg^{-1}$, respectively. By all methods, the highest antioxidant capacity was recorded in case of dried chokeberries (54.4 ± 1 $g \cdot kg^{-1}$ by ABTS, 30.5 ± 1 $g \cdot kg^{-1}$ by DPPH and 233.2 ± 1.3 $g \cdot kg^{-1}$ by CUPRAC). The lowest antioxidant capacity was recorded in case of Raspberry-chokeberry syrup (0.7-1.2 $g \cdot kg^{-1}$), the obtained results correlated with content in total polyphenols, total flavonoids, total anthocyanins.

Taking into account the high content of biologically active compounds and antioxidant capacity of dried fruits of *Aronia melanocarpa,* their use to fortify food products has a real interest.

In this paper are presented bakery products („Bread with *Aronia*", „Minibaguette with *Aronia*", „Biscuits with *Aronia* and cinnamon", „Biscuits with *Aronia* and ginger") fortified with dried fruits of *Aronia melanocarpa.* Products quality was evaluated through sensory, physico-chemical and microbiological analyses. Also the antioxidant potential was evaluated by determination of total polyphenolic content and of antioxidant capacity.

MATERIALS AND METHODS

Materials

Dried fruits of *Aronia melanocarpa* used within experiments were obtained from organic culture (Figure 1). These fruits were ground with a Retsch mill within the performed experiments. The raw materials and materials used for making bakery products were purchased from the market.

Figure 1. Dried fruits of *Aronia melanocarpa*

Methods

Sensory analysis

Sensory evaluation of the bakery products fortified with dried fruits of *Aronia melanocarpa* was performed 12 hours after baking, using *„Comparison method with unitary score scales"*. 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 - qualyfing „very good"; 15.1 ... 18 - qualyfing „good"; 11.1 ... 15 - „satisfactory"; 7.1 ... 11 - „unsatisfactory"; 0 ... 7 - „inadequate").

For measurement of colour parameters dried fruits of *Aronia melanocarpa* were ground with Retsch mill, and bakery products fortified were liophylised and then grounded with a Retsch mill. Measurement of the colour parameters of samples was performed at room temperature, using a HunterLab colorimeter, equipped with Universal Software V4.01 Miniscan XE Plus

programme, to register CIELab parameters (the Commission Internationale de l'Eclairage - CIE), *L**, *a** and *b**: *L** - colour luminance (0= black, 100 = white); *a** - red-green coordinate (-a = green, +a = red); *b** - yellow-blue coordinate (-b = blue, +b = yellow).
The texture properties of the bakery products fortified with dried fruits of *Aronia melanocarpa* were measured through a compression test using an Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA).

Physic-chemical analysis
The moisture content was determined according to the AACC 44-15A method. Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005). Fat content was determined according to AOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005). Mineral elements content was determined by atomic absorption spectrophotometer (type *AAnalyst* 400, Perkin-Elmer, Waltham, MA, USA) from HCl mineralized sample.
Total dietary fiber (TDF) was determined by enzymatic method using the assay kits: K-TDFR „Total dietary fiber" (AOAC Method 991.43).
Total sugar and reducing sugar content was determined according to Schoorl method. For total sugar, the method is applied after an acid hydrolysis (20% HCl solution) at 70ºC, for 27 minutes.
Calorie contents were calculated using the following conversion factors: 9 for fat, 4 for carbohydrates, 4 for protein and 2 for fibre, according to the Commission Regulation no. 1169/2011 (European Commission, 2011).
Joule contents were calculated using the following conversion factors: 37 for fat, 17 for carbohydrates, 17 for protein and 8 for fibre, according to the Commission regulation no. 1169/2011 (European Commission, 2011).
Total polyphenol content
Total polyphenol content was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The extraction of phenolic compounds was performed in methanol: water = 50:50 and the absorbance of the extracts were determined at a wavelenght λ = 755 nm. Results were expressed as mg of Gallic Acid Equivalents (GAE) per g product.

Antioxidant capacity
The DPPH scavenging radical assay was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range 0-0.4375 mmol/L and the results were expressed as mg Trolox Equivalents per g product.

Microbiological analysis
The water activity (*A*w) was determined by an instrument Aquaspector AQS-2-TC, Nagy. The measurements were performed at 25ºC. Yeasts and molds were determined by the method SR ISO 21527-1: 2009. *Enterobacteriaceae* were determined according to the SR ISO 21528-2: 2008 method and *Escherichia coli* by SR ISO 16649-2: 2007 method. *Salmonella* was determined by the method SR EN ISO 6579:2003/AC: 2006.

RESULTS AND DISCUSSIONS

Sensory analysis
Sensory analysis plays an important role in characterizing the quality of food products. Results of sensory analysis of bakery products showed that the addition of powder of *Aronia melanocarpa,* in their composition, has not a negative effect on sensory characteristics (Figure 2).
So, the analyzed products were tested by an expert panel receiving qualifying „very good", with scores in the range 19.44-19.92, as follows: Biscuits with *Aronia* and cinnamon-19.44; Biscuits with *Aronia* and ginger-19.52; Bread with *Aronia* and seeds-19.76; Minibaguette with *Aronia* and seeds-19.92 (Figure 3).

Using of sourdough for fermentation and final proofing, in case of bread and minibaguette, give them an elastic and dense crumb, proper texture and in the same time intense and pleasant flavour.

Figure 2. Bakery products fortified with *Aronia*

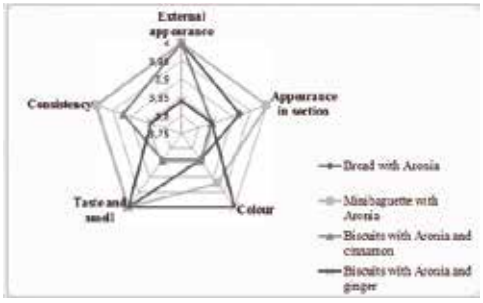

Figure 3. Sensory evaluation of bakery products fortified with *Aronia*

According to the results obtained, the darkest colour was recorded for the product „Biscuits with *Aronia* and ginger" (L* = 29.6), and the least intense for the product „Bread with *Aronia*" (L* = 40.18). In comparison with the achieved products, *Aronia* fruits have the most intense colour (L* = 18.55) (Figure 4).

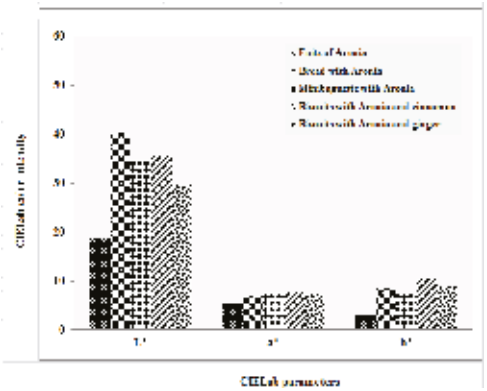

Figure 4. Colour parameters of *Aronia* fruits and bakery products fortified with *Aronia*

In the case of the four bakery products, the colour parameter *a** recorded close values (7.10 ... 7.84), while the colour parameter *b** varied in the range 7.45 ... 10.58 (the minimum value was recorded for the product „Minibaguette with *Aronia*" and the maximum one for the product „Biscuits with *Aronia* and cinnamon").

Texture properties of bakery products fortified with dried fruits of *Aronia melanocarpa,* during the shelf-life, packed in polypropylene film are presented in Tables 1 and 2.

Table 1. Texture properties of products Bread with *Aronia* and Minibaguette with *Aronia*

Product	Period, days	Firmness, N	Elasticity	Cohesiveness	Gumminess, N
Bread with *Aronia*	1	4.23 ± 0.27	0.91 ± 0.01	0.57 ± 0.07	2.41 ± 0.15
	2	5.11 ± 0.32	0.89 ± 0.04	0.48 ± 0.05	2.45 ± 0.16
	3	6.09 ± 0.59	0.85 ± 0.04	0.40 ± 0.05	2.44 ± 0.16
	4	7.08 ± 0.80	0.80 ± 0.04	0.35 ± 0.05	2.48 ± 0.19
	5	7.96 ± 1.07	1.00 ± 0.06	0.30 ± 0.02	2.35 ± 0.43
	6	8.83 ± 1.37	0.77 ± 0.23	0.25 ± 0.05	2.35 ± 0.74
	7	10.38 ± 2.29	0.76 ± 0.14	0.29 ± 0.03	2.96 ± 0.67
Minibaguette with *Aronia*	1	9.08 ± 0.88	0.91 ± 0.01	0.59 ± 0.03	5.90 ± 0.64
	2	15.46 ± 0.72	0.71 ± 0.15	0.36 ± 0.01	5.55 ± 0.15
	3	17.5 ± 0.75	0.65 ± 0.05	0.31 ± 0.03	5.43 ± 0.15
	4	20.73 ± 0.77	0.62 ± 0.02	0.29 ± 0.03	6.01 ± 0.17
	5	23.97 ± 0.80	0.59 ± 0.04	0.26 ± 0.03	6.23 ± 0.17
	6	27.2 ± 0.82	0.58 ± 0.01	0.28 ± 0.05	7.62 ± 0.19
	7	30.44 ± 0.84	0.56 ± 0.01	0.25 ± 0.03	7.61 ± 0.19

Table 2. *Texture* properties of products Biscuits fortified with *Aronia*

Biscuits with *Aronia* and cinnamon			Biscuits with *Aronia* and ginger		
Period, days	Firmness, N	Brittleness, MPa	Period, days	Firmness, N	Brittleness, MPa
1	11.26 ± 5.31	27.29 ± 6.09	1	14.91 ± 3.97	28.47 ± 8.08
8	20.48 ± 9.70	95.09 ± 11.06	8	32.91 ± 3.49	72.11 ± 16.94
16	27.49 ± 9.41	55.12 ± 24.89	16	36.06 ± 3.25	69.57 ± 4.21
23	28.43 ± 2.19	56.58 ± 9.35	23	41.87 ± 1.43	52.06 ± 4.92
29	29.60 ± 4.58	60.42 ± 6.87	29	47.81 ± 0.92	85.51 ± 12.81
36	32.35 ± 1.28	105.73 ± 9.43	36	55.10 ± 2.73	101.74 ± 4.27
43	35.71 ± 4.31	125.02 ± 49.36	43	62.36 ± 3.08	95.86 ± 9.22
51	35.83 ± 2.02	82.97 ± 4.58	51	71.37 ± 1.16	155.61 ± 22.26
58	37.20 ± 2.68	113.72 ± 27.20	58	74.78 ± 5.48	139.63 ± 6.83
65	41.13 ± 0.70	117.74 ± 34.33	65	78.10 ± 6.23	158.62 ± 12.99
72	45.25 ± 0.85	122.66 ± 19.46	72	85.22 ± 6.90	157.31 ± 0.63

Bread with *Aronia* had the lowest firmness, in comparison with those of Minibaguette with *Aronia*, due to the highest moisture content (41.69 ± 0.30%). During the 7 days firmness varied in the range 4.23 ... 10.38 N, in case of Bread with *Aronia*, and, respectively, 9.98 ... 30.44 N, in case of Minibaguette with *Aronia*. Elasticity and, respectively, cohesiveness, have values relatively close in case of those two bakery products. Although initially those two assortments of biscuits with *Aronia* have close values of firmness and brittleness, after 72 days of storage, Biscuits with *Aronia* and ginger have firmness of 1.84 times higher in comparison with Biscuits with *Aronia* and cinnamon, and brittleness of 1.28 times. This can be explained by difference in lipid content, water content and, in the same time, by difference in composition between those two biscuit assortments.

Physic-chemical analysis

Dried fruits of *Aronia melanocarpa,* used to fortify bakery and pastry products, have a complex biochemical composition, by total polyphenolic content, mineral elements and fibre content, especially. Their total polyphenolic content was 3180.90 ± 84.67 mg GAE/100 g, comparable with those reported by Kapci et al. (2013): 3990 ± 30 mg GAE/100 g, 5010 ± 50mg GAE/100 g, respectively. Also, fruits used within experiments had antioxidant capacity of 67.29 ± 2.82 mg Trolox/g, higher than those reported by Kapci et al. (2013): 36.3 ± 1.2 mg Trolox/g, respectively, 30.5 ± 1.0 mg Trolox/g. In the same time, dried fruits of *Aronia* are an important source of potassium (9693.3 ± 1095.34 mg/kg), calcium (1563.7 ± 161.22 mg/kg), magnesium (694.1 ± 109.95 mg/kg), iron (36.8 ± 1.10 mg/kg), zinc (6.36 ± 0.36 mg/kg) and fibres (16.50 g/100 g). Moisture of *Aronia* fruits was 10.2%, and total sugar content 30.25%.

Chemical composition of bakery products fortified with *Aronia* is presented in Table 3.

It is noteworthy that these products have high protein content (11.92 ... 12.95%), fibres (9.13 ... 16.60%) and mineral elements, ash varying between 1.51 ... 2.03%.

Due to the high fibres content and low sugar content (4.33 ... 5.79%), bakery products with *Aronia* can be beneficial in diet of peoples with type 2 diabetes and obesity.

Table 3. Chemical composition (%) of bakery products fortified with *Aronia*

Component	Bread with Aronia	Minibaguette with Aronia	Biscuits with Aronia and cinnamon	Biscuits with Aronia and ginger
Moisture	41.69±0.30	30.49±0.30	14.26±0.30	12.7±0.30
Protein	11.92±0.11	12.95±0.11	12.42±0.11	12.30±0.11
Fat	8.85±0.10	6.91±0.10	18.23±0.10	17.95±0.10
Total sugar	5.70±0.11	4.52±0.11	4.33±0.11	5.79±0.11
Reducing sugar	5.01±0.08	3.35±0.08	3.37±0.08	4.56±0.08
Carbohydrates	26.81±0.14	38.9*±0.14	38.60±0.14	39.44±0.14
Ash	1.60±0.01	1.51±0.01	2.03±0.01	1.91±0.01
Total dietary fiber	9.13±0.17	9.17±0.17	14.46±0.17	16.60±0.17
Energy[a]	252.83	268.21	397.07	393.61
Energy[b]	1058.90	1211.45	1665.85	1648.23

[a] Expressed as kcal/100 g product.
[b] Expressed as kJ/100 g product.

Also biscuits can have a beneficial effect on diabetes because they contain ingredients with hypoglicemiant effect: fruits of *Aronia melanocarpa,* ginger and cinnamon (Simeonov et al., 2002; Valcheva-Kuzmanova et al., 2007; Li et al., 2012; Lu et al., 2012).

Due to the used raw materials, the bakery products fortified with *Aronia* have high mineral element content (Table 4). The most abundant among investigated elements was potassium, its concentration varying in the range 4784,60 … 10547 mg/kg (maximum concentration was recorded for Minibaguette with *Aronia*).

Table 4. Mineral element content of bakery products fortified with *Aronia*

Component	Bread with Aronia	Minibaguette with Aronia	Biscuits with Aronia and cinnamon	Biscuits with Aronia and ginger
K, mg/kg	4784.60±540.07	10547.0±1191.81	5921.6±669.14	6513.7±735.91
Ca, mg/kg	1100.29±113.44	1598.57±164.81	1504.3±155.14	1691.4±174.38
Mg, mg/kg	839.2±112.91	1088.6±172.62	1020.3±162.99	1196.6±189.53
Fe, mg/kg	10.9*±0.33	16.1±0.42	57.29±1.75	65.27±1.96

Also, the achieved products have high calcium content, which varied between 1100.29 mg/kg (for Bread with *Aronia)* and 1691.4 mg/kg (for Biscuits with *Aronia* and ginger), being significantly higher than those obtained by Ajibola et al. (2015) in case of biscuits prepared from different blends of whole-wheat flour, *Moringa oleifera* leaves and cocoa powder (291.7 … 524.7 mg/kg) and, respectively, Mahmoud et al. (2012), in case of fenugreek supplemented biscuits (465.10 mg/kg d.w., respectively, 561.7 mg/kg d.w.). Magnesium content of products varied in the range 839.20 ... 1196.6 mg/kg, being comparable with those of the achieved biscuits by Vitali et al. (2009) with inulin added and one of the following raw materials: soy flour, amaranth, carob, apple fibre or oat fibre. The higher iron content was recorded for Biscuits with *Aronia* and ginger (65.27 mg/kg, higher than those reported by Vitali et al., 2009), and the lower, in case of Bread with *Aronia* (10.97 mg/kg).

Total polyphenol content

Fortification of bakery products with dried fruits of *Aronia melanocarpa* had a positive effect on total polyphenol content and their antioxidant properties. The biscuits present the highest total polyphenol content: 263.22 mg GAE/100g in case of Biscuits with *Aronia* and ginger and 221.58 mg GAE/100 g, respectively, in case of Biscuits with *Aronia* and cinnamon (Figure 5).

Total polyphenol content is higher than those reported by Mildner-Szkudlarz et al. (2013), in case of biscuits made from wheat flour and addition of 10% white grape pomace (211 mg GAE/100 g). Also, total polyphenol content of biscuits with *Aronia* exceeds those of biscuits supplemented with 10% germinated fenugreek (*Trigonella Foenum Graecum*) seeds flour: 196.58 mg GAE /100 g (Mahmoud et al., 2012).

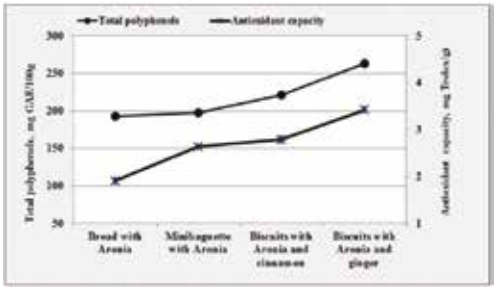

Figure 5. Total polyphenol content and antioxidant capacity of bakery products fortified with *Aronia*

Bread with *Aronia* and Minibaguette with *Aronia* had close values of total polyphenol content (193.34 mg GAE/100 g, respectively, 197.91 mg GAE/100 g), but small in comparison with biscuits. This fact can be explained by difference in composition (lower percentage of powder obtained from dried fruits of *Aronia melanocarpa*) and by higher value of moisture.

Total polyphenol content of those two bakery products is superior to those recorded in case of bread prepared with 10% of grape pomace powder (89.43 mg GAE/100 g; Hayta et al., 2014). Grape pomace presents high antioxidant capacity, due to high content in phenolic compounds, such as proanthocyanidins (Özkan et al., 2004).

It should be noted that bread prepared with sourdough mixed rye and four different levels: 4%, 6%, 8% and 10% of grape by-products has a significantly higher polyphenol content (334.32 ... 613.77 mg GAE/100 g d.m.), in comparison with bread assortments with *Aronia* (Mildner-Szkudlarz et al., 2011). These results were achieved mainly due to the high content of polyphenols of grape by-products (5895 ± 150 mg GAE/100 g d.m.), in comparison with those of the dried fruits of *Aronia*, and the difference in composition of products.

Bread with *Aronia* and Minibaguette with *Aronia* have a higher content of polyphenols than those recorded for bread with adding of 15% amaranth flour (173 ± 9 mg GAE/100 g d.m.) and of 15% quinoa flour (188 ± 7 mg GAE/100 g d.m.), respectively, and increase of dose of these pseudocereal flours, to 30%, determines an increase of total polyphenol content of the achieved breads: 261 ± 4 mg GAE/100 g d.m. in case of amaranth flour and 254 ± 10mg GAE/100 g d.m. in case of quinoa flour (Chlopicka et al., 2012). Also, in case of bakery with *Aronia*, there is an inversion relationship between total polyphenol content and colour parameter L*, demonstrating that presence of these compounds in composition of products determines dark colour of them (Figure 6).

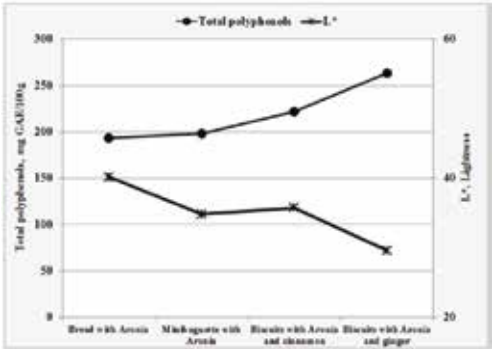

Figure 6. Correlation between total polyphenols and colour parameter L* in case of bakery products fortified with *Aronia*

Antioxidant capacity

Due, mainly, to phenolic compounds contained in powder of *Aronia*, bakery products fortified with it have antioxidant capacity which varied in the range: 1.91 ... 3.42 mg Trolox Equivalents/g (minimum value was recorded for Bread with *Aronia*, and the maximum one in case of Biscuits with *Aronia* and ginger).

Between total polyphenol content and antioxidant capacity there is a directly proportional relationship. The obtained results are consistent with those obtained by Zheng and Wang (2003), mentioning that content of polyphenols of fruits of *Aronia* was strongly correlates with their antioxidant capacity. In case of bakery products fortified with *Aronia* in this study, between total polyphenol content and antioxidant capacity there is a linear correlation, $R^2 = 0.8126$. Antioxidant capacity of biscuits with *Aronia* is higher than those recorded in case of biscuits enriched with grape marc extract: 0.79 ± 0.045 µmol Trolox/g (Pasqualone et al., 2014). Antioxidant capacity of those two assortments bread with *Aronia* (7.64 ± 0.73 µmol Trolox/g for bread and 11.16 ± 0.45 µmol Trolox/g, respectively, for minibaguette) is superior to bread which contains 2.5 ... 7.5% grape seed and has antioxidant capacity in the range 4.15 ... 6.28 µmol Trolox/g d.m. (Meral and Dogăn, 2013).

Microbiological analysis

Based on microbiological, sensory and peroxide index analyses it was established shelf-life of products with *Aronia*, as follows: Bread with *Aronia* - 7 days; Minibaguette with *Aronia* - 7 days; Biscuits with *Aronia* and cinnamon - 72 days; Biscuits with *Aronia* and ginger - 72 days.

Microbiological analysis of bakery and pastry products fortified with *Aronia*, at the end of shelf-life is presented in Table 5.

Table 5. Microbiological analysis of bakery products fortified with *Aronia*

Microbiological indicator	Bread with Aronia	Minibaguette with Aronia	Biscuits with Aronia and cinnamon	Biscuits with Aronia and ginger
Yeasts and molds, CFU/g	< 10	< 10	< 10	< 10
Enterobacteriaceae, CFU/g	< 10	< 10	< 10	< 10
Escherichia coli, CFU/g	< 10	< 10	< 10	< 10
Salmonella	absent/25 g	absent/25 g	absent/25 g	absent/25 g

Microbiological indicators determined come under provisions of the Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs.

During shelf-life water activity varied differentiated depending on product, as follows: Bread with *Aronia* - 0.938 ... 0.945; Minibaguette with *Aronia* - 0.926 ... 0.938; Biscuits with *Aronia* and cinnamon - 0.615 ... 0.834; Biscuits with *Aronia* and ginger - 0.694 ... 0.849.

Shelf-life relative high of bakery products fortified with *Aronia* can be explained mainly by antioxidant and antibacterial potential of fruits of *Aronia melanocarpa*. Bräunlich et al. (2013) have shown that *Aronia melanocarpa* extracts can inhibit bacterial growth of *Escherichia coli* and *Bacillus cereus in vitro*. Also, Liepiņa et al. (2013) have shown that extracts from fruits of *Aronia melanocarpa* and wild rowan (*Sorbus aucuparia* L.) inhibited the growth of Gram-negative bacterium *Pseudomonas aeruginosa* but did not have influence on *Escherichia coli.* However, in case of bread and minibaguette, lactic acid bacteria from sourdough used for fermentation and final proofing, act as a natural antibiotic, thereby increasing the shelf-life of these products.

CONCLUSIONS

This study showed that dried fruits of *Aronia melanocarpa* are an important source for fortification of bakery products. Bakery products achieved with adding of powder of dried fruits of *Aronia melanocarpa* have a complex biochemical composition and antioxidant potential.

Bakery products fortified with dried fruits of *Aronia melanocarpa* have a high polyphenol content (193.34 ... 263.22 mg GAE/100 g), proteins (11.92 ... 12.95%), fibres (9.13 … 16.60%), potassium (4784,60 … 10547 mg/kg), calcium (1100.2 ... 1691.4 mg/kg), magnesium (839.20 ... 1196.6 mg/kg) and iron (10.97 ... 65.27 mg/kg). Due to high content of fibres and low content of sugars (4.33 ... 5.79%), bakery products with *Aronia* can be included in diet of people with type 2 diabetes and obesity. Also, noteworthy is that those two assortments of biscuits with *Aronia*, contain ingredients with hypoglicemiant effect (fruits of *Aronia melanocarpa,* ginger and cinnamon) could have beneficial effects on glycemic equilibrium of consumers with type 2 diabetes.

Antioxidant capacity of bakery products fortified with dried fruits of *Aronia melanocarpa* varied in the range 1.91 to 3.42 mg Trolox Equivalents/g. Biscuits with *Aronia* and ginger recorded the higher value of

antioxidant capacity and Bread with *Aronia* had the minimum value.

Products achieved with *Aronia* received qualifying „very good" at sensory analysis, recording scores in the range: 19.44-19.92. Due to use of sourdough in composition, bread and minibaguette have crumb elastic and dense, proper texture and, in the same time, pleasant and intense flavour. Colour of products with *Aronia* was apreciated by expert panel receiving 4 or 5 points after evaluation. Biscuits with *Aronia* and ginger had the darker colour (L* = 29.6),

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INTEGRATING SOIL PH MEASUREMENT INTO AN INTERNET OF THINGS APPLICATION

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Abstract

The paper presents a device for soil pH determination on-site and remote transmission of the result. Soil pH is an indicator of the soil quality because it affects plant growing. Normally, soil samples are taken from the field and tested in-door in aqueous solutions using ISO 10390: 2005 specification. However, it was an increasing demand for on-site measurements materialized in a plethora of stand-alone devices. Most of them offer a narrow range of pH values, e.g. 3 to 8, because the majority of plants has an optimum range between 5.5 and 7.5. The Internet of Things exigencies have led to design a node of multiple sensors including soil pH measurement with an increased precision. Therefore, it was used a sensor for hydrogen ion activity measurement based on glass membrane as a junction between the soil solution and a reference solution. The probe was calibrated using pH known solutions and then tested on different soil samples - all data being remotely collected. The results emphasized the capability of the device to measure the soil pH on-site and to send it to remote.

Key words*: environmental monitoring, internet of things, precision agriculture, soil pH, wireless communication.*

INTRODUCTION

Precision agriculture and Agriculture 4.0 concepts imply, among other things, more data to the farmer. These require a lot of sensors that will generate information. One of the most important parameters is soil pH. As known, soil pH can change during the year due to rainfall, quality of the water used in irrigation, fertilizers, pesticides, root respiration and decomposition of organic matter by microorganisms. Also, soil pH depends on temperature and moisture conditions and can vary to with the soil depth. Temperature changes the chemical activity of the hydrogen ions (H^+), so the measurements of pH must include the temperature correction to a standard temperature of 25°C. The whole variation of a soil can vary up to a unit of pH within a year, which is quite a lot, since, for example pH 6.4 is a slightly acidic soil, while pH 7.4 is a slightly alkaline one. Therefore, the information must arrive in time to the farmer in order to take corrective measures.

Currently, soil pH determination is done in laboratories on samples of mixed soil in an aqueous solution; although very accurate, the method is costly, takes time, and the distance from authorized laboratories which could be several tens of kilometers reach a price that farmers will not pay. It is an increased demand for a competitive solution for continuously soil pH monitoring from remote. Practical solutions have emerged on the market based on indicators, colorimeters or different types of electrodes sensitive to H^+ activity (Plotog et al., 2016), but the lack of these methods is that data is available randomly and it is eventually inserted manually in a database. The accuracy of these cheap devices is poor.

As the Internet of Things (IoT) technology emerges, all sensors are required to be automatically controlled from the distance and integrated in environmental monitoring systems. There are mentioned remote sensing (RS) methods based on bare-soil images and spectroscopic reflectance of soil samples for soil survey, mapping, and quantitative soil-property characterization (Yufeng et al., 2011), one work claiming that for pH, the soil map and the RS estimate were nearly as accurate (Roelofsen et al., 2015). The methods, which imply satellite and aerial imagery or other non-contact nature of measurement, are costly.

There are mentioned real time and maped-based approaches using vehicle-based on-the-

go soil sensors for measuring soil properties including electromagnetic and even electrochemical sensors for pH determination (Adamchuk et al., 1999). Another work states that automated soil sampling system could be used to estimate soil pH on-the-go (Adamchuk et al., 1999). The data can be sent to a remote center through the vehicle communication station. The disadvantages of the method: the values could not be as accurate as a laboratory test, the vehicle could not access any place and the data is available discontinuously.
In this context, the paper presents the way the authors have solved the objectives they have proposed: in-field soil pH determination with incresed accuracy and the reception of data at a remote center using wireless communication technology.
The pH sensing is part of a more complex device for monitoring environmental parameters such as air (temperature, relative humidity, harmful substance concentration, wind speed and direction), soil (temperature, relative humidity, pH, macronutrient content) and lighting level. The goal was to build an automatic system dedicated to coordinate and streamline all resources and factors involved in optimizing agricultural crops.

MATERIALS AND METHODS

In order to meet the proposed objectives it was designed an electronic module which interface a pH sensor to a microcontroller to read the data and to send it to remote by means of a radio-frequency (RF) block (Figure 1). This approach allowed to implement a complete IoT application for agriculture. Since the main requirement for an IoT application is the low power, the circuitry was designed around a low power 8-bit AVR RISC-based microcontroller, ATmega2560, Microchip Technology. Generally, the largest power consumption in an IoT application is the RF block, therefore, it was selected the long-range low-power technology wireless platform that is the top technology choice for building IoT networks, LoRa. In addition, it uses a license-free spectrum.
The pH probe was a silver/silver chloride sensor, Atlas Scientific LLC, which can measure pH of solutions in the range 0-14 with the resolution +/- 0.0001 (Device #1). It has been chosen this type of sensor because the manufacturer states that the pH probe may have in the soil indefinite placement. The life expectancy of the probe is more than 2.5 years without maintenance.
The Signal conditioning block adapts the analog signal from the output of the pH sensor into a digital signal to be applied to any microprocessor that supports UART, or I2C protocol.
The power supply block assures the voltages for the electronic circuits from a solar cell panel and a rechargeable battery.

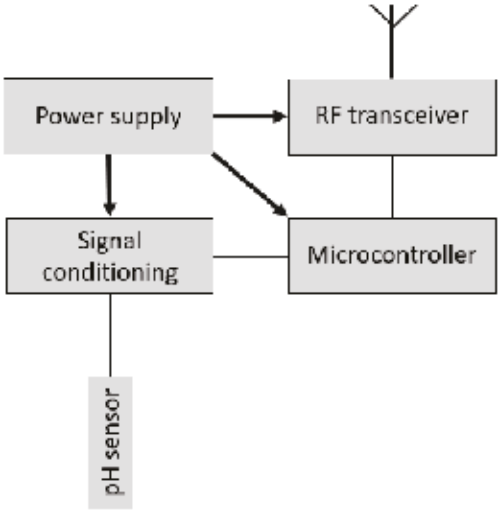

Figure 1. The block diagram of the electronic module for in-filed pH measurement and data transmission to remote

On the reception side of the data it has to be used a Wi-Fi gateway connected to a laptop to store and process the information.
The goal of the experiment was to collect the data regarding the soil pH from a remote center in real time and to analyze the accuracy of the measurement.
The soil pH determination shall be compared with the measurements made by other devices: I) EC500, a pH - Conductivity meter from the EC510 kit, Extech Instruments/FLIR Systems, which has a pH range between 0.00 to 14.00, as benchmark ((Device #2);
II) A cheap 3-in-1 moisture meter with light and pH test function for gardening, which offer a pH range between 3.5 to 8, Shenzhen Xing Ying Da Industry Co (Device #3).

Before starting the experiments the pH sensor was calibrated. The measurements were performed in the laboratory area on flower soil mixed with water at room temperature (25°C). According to the manufacturer's recommendations the measurement of the soil pH should be preformed with a delay of 25 minutes after the immersion in the mud.
In the Figures 2 and 3 there are presented the pH devices used for comparision.

Figure 2. EC510 conductivity testing kit including: EC500 meter, 3 calibration standards, 3 pH buffer pouches plus rinse solution

Figure 3. 3-in-1 plant flowers soil tester pH tester/ moisture meter/light meter

The experiments were performed in the laboratory of the company; the temperature was around 25°C ± 3°C.
Since the rezult of the measurements performed with the Device #1 are available only on a remote display where the data can be stored and processed, while the other two devices needed a local operator for readings, the following method was proposed:
- three equal amount of aqueous solutions were prepared and measured with all three types of pH meters; each solution had a different pH covering most of its scale: acidic, neutral and alkaline;
- three equal amount of mixed soil and aqueous solution were prepared and measured with all three pH meters;
- it is supposed that samples equal amount of the same soil mixed with three different aqueous solutions change their properties regardless the measuring instrument;
- the recorded data from the Device #1 will be analyzed and discussed.
The following three solutions were prepared: alkaline (pH 13.2 on both devices, ALKALINE indication on Device #3), approximately neutral using running water (pH 7.82, pH 7.88 and slightly over 7 on Device #3) and acidic (pH 4.5 on both devices, 7 on Device #3).
The solutions were poured into equal pots with soil and the devices were infiltrated into the created mud. The automatic reading period of the pH values for the Device #1 was set to 10 minutes; at the same time the values of the other devices were noticed by an operator.

RESULTS AND DISCUSSIONS

The data from the pH sensor could be read on the monitor of a laptop (Figure 4). The pH sensor and the laptop were placed in two different rooms of the company.

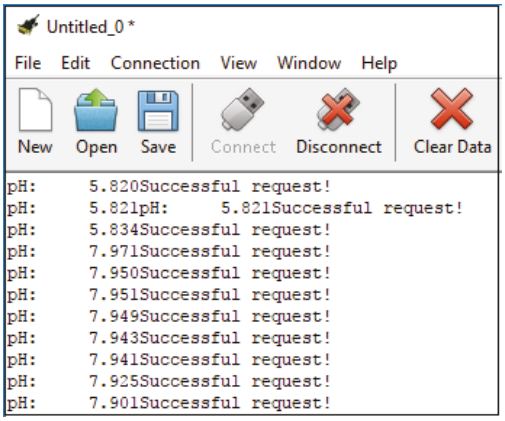

Figure 4. An extract from the print screen on the laptop monitor

This prove the main parts of the designed electronic module - microcontroller and LoRa transmission - work correctly.
The interconnection between the computer and the pH sensor device offer the possibility to

transmit several commands: the period of continuous readings, the single reading mode, start/stop of readings in order to save power, mathematical compensation of pH readings with temperature.
The results for neutral domain are displaying a slow variation for Devices #1 and #2, while Device #3 shows always a value between 7 and 8 (Table 1).

Table 1. Determination of soil pH with three different devices for neutral domain

Time [min.]	Soil pH		
	Device #1	Device #2	Device #3
0	6.939	7.27	7 - 8
10	6.960	7.20	7 - 8
20	6.958	7.22	7 - 8
30	6.945	7.14	7 - 8
40	6.942	7.16	7 - 8
50	6.958	7.08	7 - 8
60	6.864	7.07	7 - 8
70	6.849	7.01	7 - 8
80	6.850	7.03	7 - 8
90	6.850	7.02	7 - 8
100	6.855	7.01	7 - 8
110	6.854	7.12	7 - 8

Their graphical representation is presented in Figure 5. The measurements performed with the EC500 meter have a greater margin of variation when it is immersed in the mud. The difference between the maximum and the minimum values of soil pH is 0.260 while for the Device #1 is 0.111. However, this is an improper use of the probe it has to be used only for aqueous solutions.

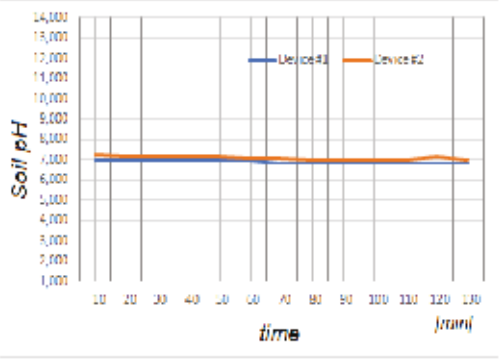

Figure 5. Graphical representation of soil pH measurement for near neutral domain

For both very strongly alkaline solution and the moderately acidic solution the measurements have shown that the soil pH has decreasing values. The Device #3 indications were ALKALINE for the first case and 7 for acidic.
A graphical representation of the cumulative measurements using the Device #1 is presented in Figure 6. The measurements were performed during a period of more than 3.5 hours. The slowly decreasing trend of the measurements is due to the gravitational leakage of the water from the created solution into the soil. This is a situation that is also encountered in practice.

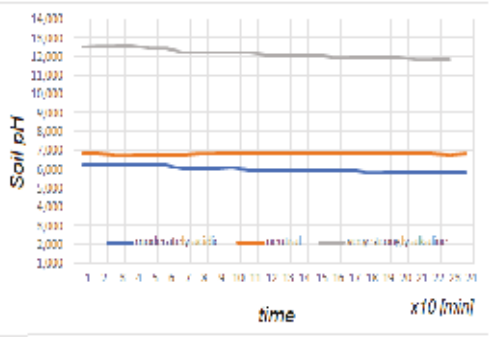

Figure 6. Graphical representation of soil pH cumulative measurements for Device #1

It is supposed that during the laboratory tests the pH values will tend to fixed value due to the limitation of the container with the soil.
In order to test the repeatability of measurements it was recorded the variation of the pH values received from the Device #1 during a longer period. As it can be seen in the Figure 7, during 9 hours the soil pH values were practically constant. It is a real situation because the solution was almost neutral and during the period of test the conditions in the laboratory were unchanged and no other material was added.
The lack of extremely values proves the high quality of the pH sensor.
Although it is supposed that the pH sensor will stay in the soil much longer time than tested above, in order to make correct determinations it will be created around it a mixed solution using pure water (pH 7) every time when desired.

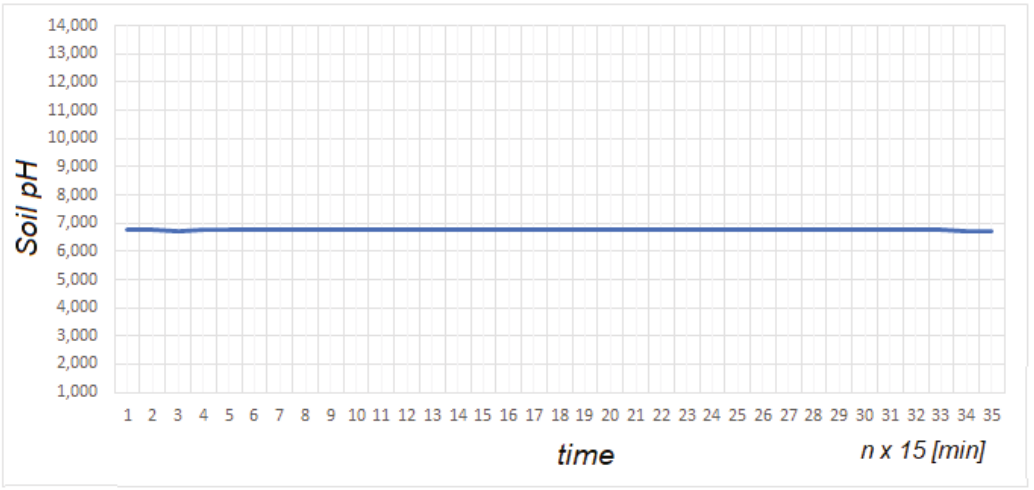

Figure 7. The graphical representation of pH measurement performed with Device #1 during 9 hours

The practical implementation of the pH measurement and the remote transmission of the data is presented in Figure 8.

Figure 8. View of the multi sensors device with autonomous energy and wireless communication including soil pH measurement

CONCLUSIONS

In-field soil pH determination and remote transmission of the values in real time was proved.

The data can be stored and processed on a computer placed anywhere in the cloud.

The numerical values of the pH measured by the proposed device were similar compared to a standard pH instrument for different aqueous solutions. The extrapolation of the measurements to the soil mixed with water has to be confirmed by a specialized laboratory.

The pH sensor device could be programmed to enter single reading mode, standby mode and multiple readings with different periods of readings.

The measurements using inexpensive 3-in-1 device did not prove to be realistic.

The designed pH device can be interconnected to other systems for parameter monitoring in agriculture that have UART or I2C protocol capability.

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ADVANTAGES AND DISADVANTAGES OF PESTICIDE ANALYSIS METHODS USED IN AGRICULTURAL SAMPLES

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Abstract

Pesticides are substances (herbicides, fungicides, insecticides, plant growth regulators etc.) used primarily for pest control that can occur in both animals and plants. Unfortunately, besides beneficial effects, their use also has many disadvantages, these being toxic to humans and environment. For this reason, it is very important to have precise and accurate analytical methods for pesticide determination and quantification. The main purpose of this paper was to provide a description of the most commonly used methods of analysis and sample preparation for qualitative and quantitative determination of pesticides. The field of agriculture was analysed as a field of use of pesticides. Thus it has been highlighted that current analysis methods heavily rely on the use of gas chromatography analysis tools and in regard to the methods of agricultural samples preparation, these are in general extraction methods. The paper presents the advantages and disadvantages of the mentioned methods, in qualitative and quantitative assessment of pesticide present in agricultural biomass.

Key words*: pesticides, agriculture, analytical method, chromatography.*

INTRODUCTION

Pesticides are substances used primarily for pest control (US Environmental, 2007) that can occur in both animals and plants.
There are several classes of pesticides, the most common ones being:
- Organochlorine pesticides: DDT is the most used pesticide from this class and although its use has been restricted, there are countries that are thinking of reintroducing it (Turusov et al., 2002; Van den Berg, 2009);
- Organophosphorus pesticides: although they are said to be a more ecological option, many of the substances from this class have been associated with an endocrine disrupting potential (Mnif et al., 2011; Karami-Mohajeri et al., 2011);
- Carbamate pesticides: unfortunately the use of this class of pesticide can lead to a series of negative activity on the human body such as: possible reproductive disorders (Jamal et al., 2015), genotoxic effects in hamster ovarian cells (Soloneski et al., 2015) and last but not least increased risk for dementia (Lin et al., 2016).

There are three ways in which humans can be affected by pesticides (Yusà, Coscollà and Millet, 2014) and the most important source is through diet or ingestion.
Another way is through dermal contact, a way that it's more and more encountered due to household use of pesticides.
And a third way is by inhalation of contaminated air, particularly for those are staying nearby the agriculture areas.
Pesticides besides activities that have a negative impact on the health of people, affect on long term all type of water (surface and underground), air, soil and also soil organisms (Sarfraz et al., 2009)
To understand better the importance of pesticide use a short history of these compounds have been summarised in Table 1.

Table 1. Brief history of pesticide use (Jojiya et al., 2017)

Period	Type of pesticide used
Ancient time	Ashes, common salts and bitters
1st century	Arsenic, suggestion of soda and olive oil for treatment of legumes (Pliny the Elder, a Roman naturalist-Historia naturalis)
16th century	Arsenicals and nicotine in the form of tobacco extracts (Chinese farmers)
1850	Pyrethrum, soap and a wash of tobacco, sulfur and lime also used
1867	The pigment Paris green (impure form of copper arsenite), Paris green and kerosene oil emulsion
1896	Bordeaux mixture ($CuSO_4$ and $Ca(OH)_2$) and selective chemical herbicides
1900	Dilute sulfuric acid, copper nitrates and potassium salts
1900-1950	Sodium arsenite solutions become the standard herbicides and are used in large quantities
1913	Organomercury seed dressing
1913-1939	Dithiocarbamates fungicides used in US
1939	Insecticidal potential of DDT discovered in Switzerland. Chlorinated hydrocarbons (DDT, BHC, dieldrin, aldrin and chlordane)
1950s	Fungicides captan, glyodin and organophosphorus insecticide: malathion
1961	DDT registered for use on 34 different crops as pesticide usage dramatically increases
1962	Bio accumulation and long-term toxicity and pest resistance became evident. Stoppage of DDT usage and other chlorinated compounds by farmers. Favor of the use of Organophosphates and Carbamates
1972	Environmental Protection Agency revoked the use of DDT on all food sources in the United States. The World Health Organization, however, still reserves the right to use DDT on particularly virulent outbreaks of malaria
1972-1980	Herbicidal sulfonylureas, neonicotinoids, glyphosate, synthetic fungicides such as metaxyl and triadimefron and light-stable pyrethroid pesticides are introduced
1990s	Integrated pest management, intensified research on biological pest control methods and other alternatives to pesticides
1990-1995	Increased interest in Integrated Pest Management (IPM) programs
2000	Wide spread usage of IPM techniques organic farming excluding the usage of synthetic pesticides.
2010-2015	Involvement of genetic engineering and biotechnological methods to control the usage of pesticides eg. baculoviruses

EXTRACTION METHODS OF PESTICIDES

A novel method for sample extraction that it is used more and more is QuEChERS (Sherish et al., 2017).

One study use QuEChERS for the determination of seven pesticides from Okavango Delta water samples and present a detection limit situated between 0.102 µg/L-1.693 µg/L and a recovery value situated between 61% and 95% (V.C. Obuseng et al., 2013). Correia-Sa et al. have analysed soil samples with organic carbon over 2.3% and obtained 3.42-23.77 µg/kg limit detection and 70%-120% recovery (Correia-Sa et al., 2012).

Fresh peppermint samples were analysed by Magdalena Slowik-Borowiec et al. using this type of extraction method and obtain 0.01 mg/kg limit of detection and 100% recovery (Slowick-Borowiec et al., 2012). This method has several advantages such as: environmentally friendly and is simple and fast.

Another type known and used extraction method is Supercritical Fluid Extraction (SFE) which can be used for both solid and semi-solid samples. It is a recently developed method. The main advantage is that SFE is simple and less time consuming. Teresa Castelo-Grande et al. developed a SFE method for pesticides from soil and obtain a recovery of atrazine higher that 96% (Teresa Catselo-Grande et al., 2005). Tatsuo Yoshida et al. obtain a value for recovery of Isotianil extracted from rice and rice cultivation soil between 95.1% and 99.3% (T. Yoshida et al., 2013).

Hiroaki Chikushi et al. (H. Chikushi et al., 2009) evaluated the presence in water sample and proposed a method with low limit of detection: 0.002-2.3 µg/l.

A quite new extraction method is accelerated solvent extraction (ASE). Important points in

this technique are temperature and pressure and also have advantages like speed and simplicity. Another plus is represented by the volume of reactive which is relatively low. Michelle L. Hladik et al. have analysed environmental sediment samples and obtain 81-101% recovery and 0.6-3.1 µg/kg limit of detection (Michelle L. Hladik et al., 2012).

Beside the extraction methods mentioned above, there are presented in Table 2 other data obtained by using different types of extractions.

Table 2. Extraction method of pesticide

Extraction method	Recovery/Limit of detection (LOD)	Sample	Advantages/Disadvantages	Reference
Headspace Single-Drop Micro-extraction (HS-SDME)	LOD = 0.07-12.54 µg/kg Recovery = 74-102%	Honey	Advantages: Possibility of using various solvents; Very good for extraction of diazinon; Possibility of extracting volatile and water-soluble analytes.	Amvrazi et al., 2012
Solid Phase Extraction (SPE)	LOD = 0.01-0.088 µg/L Recovery = 74.2-116.4%	Water samples	Advantages: Can be used to determine may types of pesticides; Present very good limit of detection; Rapid and efficient method.	Lopez-Mesas et al., 2007
Dispersive liquid–liquid micro-extraction (DLLME)	LOD = 0.0032-0.0174 µg/L Recovery = 84-108 %	Water samples	Advantages: Fast; Sensitive; Multi-residue method; Very good recovery. Disadvantages: Limited solvent choice; It's not suitable when the matrix composition is complex.	Abdullash et al., 2017
Solvent-based de-emulsification dispersive liquid–liquid micro-extraction (SD-DLLME)	Recovery = 60-120%	Water samples	Advantages: Environmentally friendly; Less expensive than other techniques; Can be applied also for pharmaceuticals and personal care products. Disadvantages: same as DLLME.	Caldas et al., 2016
Accelerated solvent extraction (ASE)	LOD = 0.6-3.1 µg/kg Recovery = 81-101%	Sedimentation of agricultural drainage samples	Advantage: Small volumes of solvents; Fast, easy and simple. Disadvantages: Relatively high price of the equipment.	Hladik et al., 2012
	LOD = 0.8-3.4 µg/kg Recovery = 75-102%	Sediment samples from the estuary		
Solid-phase extraction (D-SPE) by quick, easy, cheap, effective, rugged and safe (QuEChERS)	LOQ = 0.1-100 µg/kg	Rice paddy soils	Advantages: Alternative materials are more effective and less expensive than traditional sorbents Disadvantages: Limited solvent choice for extraction.	Arias et al., 2014
QuEChERS	Recovery = 70-120%	Cereals (corn, wheat flour and rice)	Advantages: Multi-residue analysis; Simple and with satisfactory accuracy.	He et al., 2015
Direct immersion solid-phase micro-extraction (DI-SPME)	LOD = 0.015-0.13 µg/L	Aqueous samples	Advantages: Can be applied on all types of water samples; Multi-residue analysis. Disadvantages: Relatively expensive because of fiber cost; Matrix effects.	Tankiewicz et al., 2013
Microwave-assisted extraction (MAE)	Recovery = 81.5-108.4%	Grass samples; Vegetation from the contaminated industrial area of Torneiros	Both methods are suitable for chlorinated pesticides analysis. Advantages MAE: shorter extraction times, higher extraction rates.	Barriada-Pereira et al., 2003
Soxhlet extraction	Recovery = 75.5-132.7%			

DETERMINATION METHODS OF PESTICIDES

Javad Ghodsi and Amir Abbas Rafati have developed a method for the determination of diazinon made by use of a MWCNTs/TiO2NPs nanocomposite sensor (J. Gjodsi et al., 2017).
Comparative with other methods used such as gas chromatography, high-performance liquid chromatography (HPLC), mass spectrometry method, spectrophotometry, infrared spectroscopy and an enzyme immunoassay (M. Khadem et al., 2017; T.D. Lazarevic-Pasti et al., 2013; G. Erdogdu, 2003) that are expensive, time-consuming and with the need of trained employers, this method has shown to be sensitive, fast and use an easy fabricate sensor that is not so expensive. Also the method presents a good limit of detection of 3 nM. The real samples have included city piped and agricultural water.
Another determination method that uses HPLC/MS/MS was developed by Hwa-mi Lee et al. They analyse 56 residual pesticides from commercial crops and obtain a recovery value between 65-82% and a detection limit up to 11.54 µg/kg (Hwa-mi Lee et al., 2013).
Hirahara et al. described a screening method for determination of 200 pesticides using GC/MS/MS.
The method present a recovery value situated in 50-150% interval and a good limit of quantification of 0.01 mg/kg (Hirahara et al., 2006).
There are many methods for determination of pesticides, some of which are presented in the Table 3.

Table 3. Determination method of pesticide

Determination method	Recovery/ Limit of detection (LOD)	Sample	Advantages/Disadvantages	Reference
GC-iECD	LOD = 0.07-19 µg/kg	Honey	Very good for determination of organochlorines and organophosphorus pesticides; Highly sensitive; Low detection limit.	Amvrazi et al., 2012
GC/MS	LOD = 0.6-3.1 µg/kg Recovery = 81-101%	Sedimentation of agricultural drainage samples	Very good recovery value; Sensitive method.	Hladik et al., 2012
	LOD = 0.8-3.4 µg/kg Recovery = 75-102%	Sediment samples from the estuary		
Micellar electro-kinetic chromatography–electrospray–mass spectrometry (MEKC-ESI-MS/MS)	LOD = 0.001-0.144 µg/L Recovery = 83-101%	Environmental or drinking water samples	Advantages: Low limit of detection Disadvantages: Relatively high price of the equipment	Moreno-Gonzalez et al., 2012
Ultra-high-performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOF-MS)	Recovery = 74-111%	Vegetable and fruit samples	Multi-residue method; suitable for routine quantitative analyses of pesticide	Sicaperumal et al., 2015
GC/SQ-MS	LOD = 0.4-48.2 µg/kg Recovery = 70-110%	Grape, lemon, onion and tomatoes	Advantages: The method is repeatable; Can be used in many types of matrices Disadvantages: Lemon and onion showed poor recoveries	Lesueur et al., 2008
HPLC/IT-MS	LOD = 1-115 µg/kg Recovery = 70-110%			
GC-MS/MS	Recovery = 96 ± 9%	Cereal samples (wheat, rye, barley, oats, maze, buckwheat)	Improved analytical performance parameters Multi-residue method.	Walorczyk et al., 2012

CONCLUSIONS

Due to the numerous negative effects on human and environmental, it is important and there is still a need to develop precise, sensible and robust extraction and analysis methods to determine the amount of pesticides and to keep them in conformity with applicable laws. Taking into account all these aspects, in this paper we have briefly discussed the most commonly used extraction and determination method for pesticides mainly from agriculture domain and also from other fields.

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SCREENING OF MICROORGANISMS DISPLAYING ACETYL XYLAN ESTERASE ACTIVITY

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Abstract

Hemicellulose is a major component of the lignocellulosic biomass, located between lignin and cellulose fibers and is the second most abundant natural polymer on earth. The main constituent of hemicellulose is xylan, a polysaccharide comprised of β-1.4-linked xylopyranosyl residues. Due to its complex structure and heterogeneous nature, hydrolysis of xylan requires a synergic action of several enzymes generally named xylanases. Amongst them, acetyl xylan esterase (AXE) is an accessory enzyme that liberates acetyl groups from the side chains of the xylan backbone. These acetyl side-groups protect the backbone of xylan from the action of others enzymes, therefore their release facilitates the action of endoxylanases. Several microorganisms display acetyl xylan esterase activity, the main producers being fungi and bacteria. The aim of this study was to test different strains in regard to their ability to display acetyl xylan esterase activity. The microorganisms subjected to screening belonged to the genres: Aspergillus, Penicillium, Bacillus, Trichoderma and Fusarium. The strains subjected for screening were also studied for their xylanase activity, in order to compare their ability to produce both xylanase and acetyl xylan esterase. In addition, the microorganisms selected after the screening process were subjected to protein assay, in order to determine the specific enzymatic activities. The best acetyl xylan esterase activities were detected with Aspergillus brasiliensis ATCC 16404 UV 7, Aspergillus brasiliensis ATCC 16404 UV 5, Aspergillus niger UV 10 and Penicillium digitatum UV 11. These experimental results are significant for further studies related to hydrolysis of hemicellulose, regarding lignocellulosic biomass valorisation.

Key words*: acetyl, xylan, esterase, xylanase.*

INTRODUCTION

Lignocellulosic materials, such as forestry and agricultural wastes, are the major source for renewable organic matter (Pothiraj et al., 2006; Yoo et al., 2014).

Lignocellulose has three main components: cellulose, hemicellulose and lignin, with hemicellulose as the second most abundant natural heteropolymer on earth (Hendriks and Zeeman, 2009).

Xylan, as the main component of hemicellulose, consists of β-1.4-linked xylopyranosyl residues (Blum et al., 1999).

It can be degraded by the synergic action of several enzymes such as: β-1.4-endoxylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase and phenolic acid (ferulic and p-coumaric acid) esterase (Dhiman et al., 2008; Motta et al., 2013).

Several reports suggest that enzymatic removal of xylan enhances cellulose hydrolysis, therefore it is important to improve xylan enzymatic degradation in order to obtain an efficient enzymatic hydrolysis of lingocellulosic materials (Zhang et al., 2011).

Acetyl xylan esterases (AXE, E.C. 3.1.1.72) are key accessory enzymes involved in deacetylation of xylans and xylo-oligosachharides. They are able to hydrolyse acetyl groups from D-xylopyranosyl residues in xylan chains (Hou, 2005).

After the hydrolysis of the acetyl ester groups, xylan main chain has new ester-free regions targeted by β-1.4-endoxylanase. Acetylxylan hydrolysis by endoxylanases proceeds with a higher and faster rate when AXEs are involved. (Hou, 2005; Zhang et al., 2011)

Due to the lack of low-cost and well characterized acetylated xylo-oligosaccharides and xylans, screening of microorganisms displaying AXE activity is usually conducted by using chromogenic or fluorogenic acetylesterase substrates such as α-naphtyl acetate, β-naphtyl acetate, 4-nitrophenyl acetate or 4-methylumbelliferyl acetate (Biely et al., 2014; Johnson et al., 1988; Shao and Wiegel, 1995). In addition, some methods use as a substrate

chemically acetylated xylan, obtained usually by Johnson's method (1988) (Hou, 2005).
Some studies have concluded that supplementation of culture medium with Tween 80 could improve significantly AXE and xylanase activities, due to the fact that Tween 80 has a strong impact on the microorganism ability to synthesize the enzymes (Atta et al., 2011).
There are several AXE producing microorganisms studied over the years, including fungi and bacteria: *Penicillium purpurogenum, P. notatum, P. chrysogenum, Phanerochaete chrysosporium, Schizophylum commune, Aspergillus awamori, A. niger, Bacillus pumilus, B. subtilis, Thermoanarobacterium* sp.*, Streptomyces lividans, S. flavogriseus, Fusarium oxysporum, Trichoderma reesei, T. longibrachiatum, Aureobasidium pullulans* etc. (Atta et al., 2011; Bajpai, 1997; Biely et al., 1988; Degrassi et al., 1998; Halgasová et al., 1994; Hou, 2005; Yang et al., 2017).
The aim of this study was to test different microbial strains regarding their ability to display acetyl xylan esterase activity, under different cultivation conditions.

MATERIALS AND METHODS

Microorganisms

The bacterial and fungal isolates studied were provided by the Department of Genetics and genetic engineering of the Faculty of Biotechnologies from USAMV Bucharest.
For this study, several microbial isolates were subjected to screening: wild strains (*Bacillus amyloliquefaciens B4, B. amyloliquefaciens BN7, Trichoderma viride TV2, Fusarium oxysporum, Aspergillus niger, A. brasiliensis ATCC 16404, Penicillium digitatum)* and mutant strains *(A. niger UV 5, A. niger UV 10, A. niger UV 20, A. brasiliensis UV 5, A. brasiliensis UV 7, A. brasiliensis UV 14, Penicillium digitatum UV 6, Penicillium digitatum UV 11, Penicillium digitatum UV 12*).
The mutant strains were obtained after random mutagenesis through UV irradiation according to Ho and Ho method (2015). Briefly, the spore suspensions were kept at a distance of 10 cm at 254 nm in a vertical laminar flow cabinet for different time exposure: 30 minutes (*A. niger UV 5* and *A. brasiliensis UV 14)*, 40 minutes (A. *brasiliensis UV 7* and *P. digitatum UV 6)*, 50 minutes (*A. niger UV 10* and *Penicillium digitatum UV 11*) and 60 minutes (*A. niger UV 20, A. brasiliensis UV 5* and *Penicillium digitatum UV 12).*

Primary screening of microbial strains for AXE activity

The selected isolates were cultivated on two different liquid mediums containing 0.8% corncob xylan as the only carbon source; only one medium supplemented with 0.5% Tween 80. Other components of the cultivation medium were for fungi (g/L): 0.05 g $MgSO_4 \cdot 7H_2O$, 0.005 g $CaCl_2$, 0.005 g $NaNO_3$, 0.009 g $FeSO_4 \cdot 7H_2O$, 0.002 g $ZnSO_4$, 0.012 g $MnSO_4$, 0.23 g KCl, 0.23 g KH_2PO_4, 2 g peptone (Adesina and Onilude, 2013) and for bacteria (g/L): 0.05 g $MgSO_4 \cdot 7H_2O$, 0.05 g NaCl, 0.01 g $CaCl_2$, 0.2 g yeast extract, 0.5 g peptone (Mahilrajan et al., 2012).
The Erlenmeyer flasks containing the cultivation medium were inoculated with the selected microorganism and incubated at 30 ± 2°C in an incubator with shaker at 140 rpm for 5-9 days depending on the strain. Samples were taken at every 24 hours and centrifuged at 4500 rpm for 30 minutes. The supernatants were subjected to acetyl xylan esterase assay.

Secondary screening

The isolates that displayed high acetyl xylan esterase activity were subjected to a second screening being cultivated on the same liquid medium, but with different carbon sources: wheat bran (WB) and de-starched wheat bran (DSWB), supplemented with 0.5% Tween 80.
De-starched wheat bran was prepared according to Mukherjee (2007) and Huang (2013). Samples were taken at every 24 hours and were analysed for their acetyl xylan esterase activity and xylanase activity.

Acetyl xylan esterase assay

The enzymatic activity of AXE was measured by hydrolysis of p-nitrophenyl acetate (pNPA) to p-nitrophenol (pNP), according to a modified method by Johnson et al. (1988) (Atta et al, 2011). The assay mixture consisted of 1mL 0.1M sodium phosphate buffer (pH 7), 0.9 mL 10 mM pNPA and 0.1 mL enzyme sample and

was incubated at 37ºC for 10 minutes. The release of pNP was measured by reading the absorbance at 410 nm using a spectrophotometer. One unit of acetyl xylan esterase activity was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol per minute under the specified assay conditions.

Xylanase assay

Xylanase activity was determined according to the DNS (3.5-dinitrosalicylic acid) assay for reducing sugars (Bailey et al., 1992). Assay mixture consisted of 0.5 mL sample and 0.5 mL of 0.6% oat spelt xylan and was incubated at 40ºC for 10 min. The reaction was terminated by adding 1 mL of DNS and heating for 5 min at 80ºC. 3 mL of distilled water was added to the mixture and after 30 min the absorbance was read at 540 nm using a spectrophotometer to determine de amount of sugar released by the enzyme. One unit of xylanase was defined as the amount of enzyme that released 1 µmol reducing sugar as xylose equivalent per minute in the reaction mixture under the specified assay conditions.

Protein assay

The samples from the secondary screening were analysed for their soluble protein quantification using Lowry method (1951). This analysis was necessary to determine the specific enzymatic activity.

RESULTS AND DISCUSSIONS

Primary screening

The wild and mutant strains obtained with random mutagenesis through UV exposure were evaluated for their ability to produce acetyl xylan esterases on xylan medium with or without Tween 80.

From the bacterial strains, *B. amyoliquefaciens B4* was detected with the highest AXE activity of 1.20 µmol/ml/min. on xylan medium and 1.29 on xylan medium supplemented with Tween 80 (Table 1). The results were higher than other data regarding AXE activity of *Bacillus* sp. (Christov et al., 1993).

As shown in Table 1, for the bacterial strains, the addition of 0.5% Tween 80 had a low improvement on AXE production (7.5% and 13.8%) under this study experimental conditions, lower than the results of other studies (Atta et al., 2011).

The best AXE activity were recorded with fungal strains (Table 1), such as: *A. niger UV 10, A. brasiliensis UV 5, A. brasiliensis UV 7* and *P. digitatum UV 11*. Amongst them, *A. brasiliensis UV 7* had the best AXE activity on corncob xylan medium (2.68 µmol/ml/min.), while *A. brasiliensis UV 5* had the highest activity of 3.24 µmol/ml/min. on corncob xylan medium supplemented with Tween 80, comparable with other studies (Christov et al., 1993; Khan et al., 1990). The results obtained with the mutant strains were comparable or slightly higher than the activities recorded with the wild type fungal strains (Table 1).

Although other studies (Christov et al., 1993; Christakopoulos et al., 1999) suggest that *Fusarium oxysporum* is an important producer for AXEs, under this experimental conditions, the strain displayed a low AXE activity on both cultivation mediums.

Table 1. Acetyl xylan esterase activity on xylan medium with or without Tween 80

Microorganism	AXE activity (µmol/ml/min)	
	Xylan medium	Xylan + Tween 80 medium
B. amyloliquefaciens B4	1.01	1.21
B. amyloliquefaciens BN7	0.29	0.33
T. viride TV2	1.40	1.67
Fusarium oxysporum	0.32	0.41
A. niger - wild type	1.78	2.19
A. niger UV 5	1.71	2.14
A. niger UV 10	**2.31**	**2.93**
A. niger UV 20	1.68	1.99
A. brasiliensis ATCC 16404 - wild type	1.81	2.15
A. brasiliensis UV 5	**2.50**	**3.24**
A. brasiliensis UV 7	**2.68**	**3.19**
A. brasiliensis UV 14	1.92	2.37
P. digitatum - wild type	2.05	2.66
P. digitatum UV 6	2.02	2.59
P. digitatum UV 11	**2.34**	**3.03**
P. digitatum UV 12	1.92	2.25

For *P. digitatum* mutant strains (Table 1), the AXE activities were comparable with the wild strain, except for *P. digitatum UV 11*, that displayed a relatively high AXE activity (3.03 µmol/ml/min) on xylan medium supplemented with Tween 80. This can suggest that UV mutagenesis could be an important method for

improvement of microbial strains, regarding their AXE activities.
According to these results (Table 1), the addition of Tween 80 improved the enzymatic activity of the isolates with 20-30%, comparable with other similar studies (Atta et al., 2011).

Secondary screening
After the first screening, several fungal isolates detected with the highest AXE activity were subjected to a second screening in order to examine the influence of the selected carbon source upon AXE and xylanase activities.

Table 2. AXE and xylanase activity on wheat bran (WB) and de-starched wheat bran (DSWB)

Microorganism	AXE activity (µmol/ml/min)		Xylanase activity (µmol/ml/min)	
	WB	DSWB	WB	DSWB
B. amyloliquefaciens B4	1.05	1.14	1.72	1.89
A. niger - wild type	2.10	2.31	2.44	2.67
A. niger UV 10	2.72	2.99	2.68	2.90
A. brasiliensis ATCC 16404 - wild type	2.08	2.27	2.51	2.73
A. brasiliensis UV 5	3.09	**3.41**	**3.24**	**3.52**
A. brasiliensis UV 7	**3.10**	3.35	2.87	3.1
P. digitatum - wild type	2.48	2.72	2.63	2.89
P. digitatum UV 11	2.89	3.17	2.80	3.05

The best AXE activity was detected with *A. brasiliensis UV 7* on wheat bran medium (3.10 µmol/ml/min.) and with *A. brasiliensis UV 5* on de-starched wheat bran medium (3.41 µmol/ml/min.). AXE activities of the mutant strains were higher than the activities of the wild type strains with almost 50% (Table 2).
Comparing the results of AXE activity from table 1 and table 2, it can be observed that when using wheat bran the microbial strains show a slightly lower activities compared with the activities on corncob xylan medium, both supplemented with Tween 80. However, with de-starched wheat bran, there's an increase in enzymatic activity of 8-10%, in comparison with the results obtained with wheat bran (Table 2).
According to the xylanase assay, the results show that the highest enzymatic activity was detected with *A. brasiliensis UV 5* on both wheat bran medium (3.24 µmol/ml/min) and de-starched wheat bran medium (3.52 µmol/ml/min), higher than the activities of the wild type strains with cca 29% (Table 2).
In addition, the use of de-starched wheat bran instead of wheat bran as the only carbon source was correlated with an increase of enzymatic activities of 7-10% (Table 2).
Regarding *P. digitatum,* AXE activities of the mutant isolates were higher with almost 16.5% than the wild strain, while xylanase activities were higher with cca 6% than the wild type strain (Table 2).

Protein assay
After the secondary screening, the samples from the strains cultivated on de-starched wheat bran with Tween 80 were also analysed for their protein content in order to determine their specific AXE activity.
The highest specific enzymatic activity of 1.34 µmol/mg protein was recorded with *A. brasiliensis UV 5* (Table 3), higher than the wild type strain with cca 12%.

Table 3. Specific AXE activity

Microorganism	Specific enzymatic activity (µmol/mg protein)
B. amyloliquefaciens B4	1.05
A. niger - wild type	1.22
A. niger UV 10	1.43
A. brasiliensis ATCC 16404 - wild strain	1.20
A. brasiliensis UV 5	**1.34**
A. brasiliensis UV 7	1.27
P. digitatum - wild type	0.78
P. digitatum UV 11	0.81

P. digitatum had a low specific enzymatic activity with both wild and selected mutant strain (Table 3).

CONCLUSIONS

Acetyl xylan esterases (AXE) are key accessory enzymes necessary for the complete hydrolysis of xylan, their action increasing the activity of β-1.4-endoxylanases.
In this study, several microbial strains were subjected to a screening regarding their ability

to produce acetyl xylan esterases on both corncob medium and corncob medium supplemented with Tween 80. Amongst them, 4 mutant strains displayed the highest AXE activities: *Aspergillus niger UV 10, Aspergillus brasiliensis ATCC 16404 UV 5, Aspergillus brasiliensis ATCC 16404 UV 7* and *Penicillium digitatum UV 11*.

The best AXE activity of 3.24 µmol/ml/min was detected with *Aspergillus brasiliensis ATCC 16404 UV 5* on corncob xylan medium supplemented with 0.5% Tween 80. The mutant strain was obtained after random mutagenesis through UV irradiation for 60 minutes.

The highest specific enzymatic activity of 1.34 µmol/mg protein was displayed by *Aspergillus brasiliensis ATCC 16404 UV 5*, higher than the wild type strain with almost 12%.

The addition of Tween 80 was correlated to an improvement in enzymatic activities with 20-30% for all the isolates.

The cultivation of the selected strains on both wheat bran and de-starched wheat bran, allowed the observation that by using de-starched wheat bran instead of wheat bran, the enzymatic activities were higher with 7-10% for the majority of the microbial strains, under this experimental conditions.

A less studied strain for its AXE activity, *Penicillium digitatum* displayed a relatively high enzymatic activity of 3.03 µmol/ml/min. (corncob xylan + Tween 80) and 3.17 µmol/ml/min (de-starched wheat bran + Tween 80).

Considering the results obtained, UV mutagenesis could be an important tool for obtaining improved mutant strains with higher AXE activities.

These results are significant for further experimental studies related to hydrolysis of hemicellulose, regarding lignocellulosic biomass valorisation.

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