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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARHITECTURE

MISCELLANEOUS


EX·TERRA·AVRVM

FRUIT GROWING


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STUDIES REGARDING THE INFLUENCE OF THE STORAGE CONDITION UPON THE SHELF LIFE OF SOME APPLE CULTIVARS

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Abstract

In this study, few aspects regarding the influence of different storage condition upon the shelf life of some apple cultivars were highlighted. In the experiment following apple cultivars were used: 'Redix', 'Rubinola' and 'Golden Delicious'. All fruits were harvested at the harvesting ripeness time. Afterwards, the apple fruits were stored in different environments, which consists the experimental variants: natural ventilation, cooling storage, cooling storage by using micro perforated bag pre-package and cooling storage by using semipermeable plastic bag package introduced into plastic material boxes. The obtained results show that the fruit storage period ranges from 80 days for 'Golden Delicious', 95 days for 'Redix' and 'Rubinola' in natural ventilation conditions to 130 days for 'Golden Delicious' and 140 days for 'Redix' and 'Rubinola', in conditions of refrigerating storage and fruit semipermeable plastic bags package. Regarding the rotting process, the main pathogens involved were Gloeosporium album for 'Golden Delicious' and Penicillium sp. for 'Rubinola'.

Key words*: shelf life, losses, semipermeable plastic bag.*

INTRODUCTION

One of the most important aspects of a product is its quality, both during trading and on reaching the consumer or final buyer. For non – perishable products, only the influence of production on quality is of any importance. During trade and distribution this quality will hardly change. If no calamities occur, these products remain in the same quality state during distribution.

For perishable products this picture changes compatibility. During trading and distribution up to the moment the product arrives at the final consumer, the quality may change quite rapidly, depending on the circumstances during storage and transport, thereby affecting both the possibilities of selling the product and the price that can be obtained (Viškelis P. et al., 2011).

For both types of product, perishable and non – perishable, the quality is of utmost importance (Ahmadi Afzadi M., 2012). In addition to the quality resulting from production, for the second type sufficient care should be taken to conserve quality during trade.

MATERIALS AND METHODS

The apple varieties 'Redix', 'Rubinola' and 'Golden Delicious' were harvested at the harvesting ripeness. At this moment, the main quality as physico-chemical properties were analyzed, afterwards apple fruits being stored in different conditions (Chira L. et al., 2014), which represent the experimental variants:

V1 = natural ventilation storage

V2 = refrigerating storage

V3 = refrigerating storage and micro perforated bag pre-package

V4 = refrigerating storage and semipermeable plastic bag package introduced into plastic material boxes.

The fruits have been weighed both before storage and at the end of the storage period. The fruit firmness was determined immediately after harvesting by using Effegi penetrometer.
The depreciation due to rottenness, the origin of main pathogen agents and the main fruit physic - chemical proprieties of the best variant was evaluated.

RESULTS AND DISCUSSIONS

As we can observe in Table 1, the storage period in natural ventilation conditions was shorter. The longest period was for V4, when the fruits were stored in a refrigerating storage and semipermeable plastic bags. In this way, a higher relative air humidity was ensured, a modified gaseous composition, enriched with CO_2 (5-6%) and rarefied on O_2. This environment preserved the fruit quality very well.
The losses of weight were greater at the fruits stored in natural ventilation storage conditions. This happened due to the higher temperature and the lower relative air humidity.
The least losses were registered at V4 as the fruit transpiration has been diminished.
We can point out rather similar values after loss of weigh at the three apple varieties, but the best results were registered for the 'Rubinola' cultivar. For Golden Delicious', the dropping was more significant than in the case of the other cultivars, because of the thin epidermal trait.
The depreciation due to rottenness and physiological disturbs presented higher values in V1 case, while in V4 we can find the lowest values. Different varieties registered different reactions. Thus for 'Golden Delicious', the percentage of rotten apples was of 16.2 %, after a 80 days storage period (V1) in comparison with 'Rubinola' – 11.4 % after a 95 days storage period (V1).
In the case of 'Golden Delicious', the presence of the pathogen agents that caused fruit depreciation was influenced by the storage as follows: the *Gleosporium album* has developed better in low temperature conditions and high relative humidity (V2, V3, V4) in comparison with V1. *Botrytis cinerea* has manifested itself stronger at a high temperature (V1). The physiological disturb (such as interval decay and fruit dehydration) was more significant in V1 as compared to V2, V3 and V4.
It has been observed that at the 'Rubinola' cultivar, in V4 the *Penicillium* sp. the attack was stronger. Actually, in all cases for 'Rubinola' the main pathogen agent involved was *Penicillium* sp.
The results achieved demonstrate the advantage of storing apples in a cooling environment, using micro perforated plastic bags and semipermeable plastic bags that can assure the best air humidity level and a modified gaseous composition, favorable for the apple fruits longer shelf life.
The quality of the apples was tested both during their harvesting and at the end of the storage period.

Table 1. The apple fruits shelf life and storage losses

Cultivar	Storage conditions	Storage duration (days)	Weight losses (%)	Rot losses (%)	Total losses (%)
'Redix'	V1	95	12.5	13.7	26.2
	V2	110	8.3	7.2	15.5
	V3	130	5.5	5.5	11.0
	V4	140	3.2	3.5	5.7
'Rubinola'	V1	95	10.2	11.4	21.6
	V2	110	7.5	9.3	16.8
	V3	130	5.0	7.8	12.8
	V4	140	2.5	5.5	8.0
'Golden Delicious'	V1	80	14.5	16.2	30.7
	V2	95	10.4	11.6	22.0
	V3	115	7.5	8.5	16.0
	V4	130	4.5	7.0	11.5

In the Table 2, only the results for the V4 variant are shown, which has proven to be the best – from the storage capacity point of view.

Regarding the average weight of the apples at harvesting, the 'Rubinola' cultivar was the biggest (180 g), 'Golden Delicious' weighted 175 g and 'Redix' 155 g. During the storage, these values diminished because of the transpiration water loss.

The fruit firmness determined immediately after harvesting by using Effegi penetrometer, registered the following values: Redix–6.4 kgf/cm^2, Rubinola–6.2 kgf/cm^2 and Golden Delicious–5.6 kgf/cm^2. These values decreased during the period due to pectin substance solubilization and to the transformation of the substances into soluble pectines under the action of the pectinmetilesterase enzyme. The values at the end of the storage period were: 'Redix'–4.8 kgf/cm^2, 'Rubinola'–5.8 kgf/cm^2 and 'Golden Delicious'–4.0 kgf/cm^2.

The soluble dry matter and the titratable total acidity were two biochemical indicators of great interest. The soluble dry matter content had the following values at harvesting: 'Redix'–11.8%, 'Rubinola'–11.2% and 'Golden delicious' 12.2%. The hydrolysis process of the starch and the accumulation of the soluble sugars continued during the storage period. Part of the carbohydrates served as an energetic basis in the respiration process during the storage period. At the end of the period, the values increased, arriving at 13.4% -'Redix', 12.8% -'Rubinola' and 13.8% -'Golden Delicious'.

The titratable total acidity expressed in malic acid values was as follows: at harvesting for 'Redix' –0.27%, for 'Rubinola' –0.24% and for 'Golden Delicious' –0.20%. At the end of the storage period, the values were lower, arriving at 0.18% to 'Redix', 0.17% to 'Rubinola' and 0,14% to 'Golden Delicious'.

The other analyzed biochemical components are also presented in the Table 2.

Table 2. The evolution of mainly fruit quality characteristics during storage

Cultivar	The moment of the analysis	Average Weight (g)	The firmness (kgf/cm^2	Water content (%)	Total dry matter (%)	Soluble dry matter (%)	Titratable acidity (% malic acid)	Ascorbic acid (mg/100g)	Mineral subst. (%)
'Redix'	At harvest	155	6.4	82.6	17.4	11.8	0.27	13.60	0.40
	After storage V4	150	4.8	80.0	20.0	13.4	0.18	11.35	0.34
'Rubinola'	At harvest	180	6.2	83.4	16.6	11.2	0.24	12.40	0.38
	After storage V4	175.5	5.8	81.4	19.6	12.8	0.17	10.80	0.32
'Golden Delicious'	At harvest	175	5.6	84.0	16.0	12.2	0.20	11.8	0.30
	After storage V4	167	4.0	81.2	18.8	13.8	0.14	10.2	0.24

CONCLUSIONS

The fruit storage period ranges from 80 days for 'Golden Delicious', 95 days for 'Redix' and 'Rubinola' in natural ventilation conditions to 130 days for 'Golden Delicious' variety and 140 days for 'Redix' and 'Rubinola', in conditions of refrigerating storage and fruit semipermeable plastic bags package.

The weight losses during the storage period were greater in natural ventilation conditions to 'Golden Delicious' cultivar – 14.5 %. The least losses of weight were registered in refrigerating storage environment by using semipermeable plastic bag package, at the 'Rubinola cultivar – 2.5%.

The main pathogen involved in rotteness was *Gloeosporium album* for 'Golden Delicious' and *Penicillium* sp. for 'Rubinola'.

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

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STUDY REGARDING THE BLOSSOM PHENOPHASE OF CHERRY TREE CULTIVARS GROWN IN DOBROGEA

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Abstract

Cherries are among the first fruits that appear in late spring, their fresh consumption being a very important source of minerals, vitamins and sugars easily assimilated, of a great value for the human body. Period and duration of flowering is a genetic characteristic of cherry cultivar. The study on phenophase development of 31 cherry cultivars (Romanian and foreign origin), grown in the seaside area, demonstrates a strong variability in beginning, finish and duration of flowering. The beginning of the flowering took place in a long time, lasting 16 days, between April 10th ('Amara' and 'Catalina') and April 26th (NY 13272). Blossoming mass (optimal) occurred between April 19th to April 20th ('Catalina' and 'Amara') and May 4th to May 5th (NY 13272, 'Summit', 'Lambert', 'Sam', 'Izverna'). A group of 20 cultivars out of 31, bloomed in the same time, between May 2nd to May 5th, providing cross pollination. End of flowering (falling petals) are recorded between April 30th ('Catalina') and May 9th (NY 13272, H 15/25, 'Cristina', NY 7690, 'Sam', 'Izverna', 'Lambert', 'Summit'). In climatic conditions in Valu lui Traian flowering phenophase lasts 29 days and could be divided into two types: the early cultivars between 10th of April and 29th of April and late cultivars till 9th of May. Free cherry pollination was done extensively, but with significant variations depending on the cultivar, from 8.23% (NY 13272) to 83.3% ('Altenburger'). The only cultivars that can be considered partially self-compatible is NY 13272, 'Summit' and 'New Star', with more than 11% resulted fruits by self-pollination, the rest of them act as self-sterile cultivars.

Key words*: self-fertility, period and duration of flowering, pollination.*

INTRODUCTION

Cherry is a fruit tree species, which demand specific climate and soil. So, it requires average moisture conditions but doesn't like the water excess in the soil; also requires sufficient heat, but does not support very hot days and dryness, nor frosts either; it grows well in the hills region but does not go too high in the mountains (Constantinescu N., 1957).

Sweet cherry cultivars introduced in the new orchards, must be adapted to the climate and soil conditions specific for each cultural areas, with increased tolerance to stress factors (Parnia C. et al, 1985; Amzar Gh., 1990). Indicating the assortment for some geographical areas, but limited within the perimeters territorial administration perimeters, was the subject of some valuable works (Cociu V., 1971, 1988, 1990; 1981, 1999; Gozob T., 1986; Gozob T. and Micu C., 1987; Ivan I., 1981).

The study of cherry flowering allow to be drawn up a proper planning of artificial pollination and the knowledge of the period and duration of the flowering which helps for a proper selection of cultivars in the goal of new orchards establishment, knowing that the simultaneous flowering ensures optimal conditions for pollination.

Self-fertility presents a special importance for breeding and the technique of the artificial hybridization process is closely linked to pollination and productivity respectively. It is not conceivable that the future cultivars will not be self-fertile. However, Cociu V. (1971) believes that self-fertility is an involution process of cultivars, self-incompatibility being an evolutionary process in thus undertakes the pollination with stranger pollen, resulting

offspring with a broad genetic base and being an invaluable reservoir for creating genetic diversity. But in practice, self-fertile cultivars are preferred by growers.

Considering the eco-biological requests of cherry trees and the proper climatic conditions, Budan S. (1995) proposed eight areas of culture of this species in Romania, Dobrogea (eighth area) being evaluated as less favourable one.

Our researches were conducted in the centre of Dobrogea, which offers some cultural micro-zones and technical possibilities that allow the obtaining of a harvest at least at the level and the quality of the average favourably zones (zones IV and V).

MATERIALS AND METHODS

The study targeted 31 cherry cultivars and selections of Romanian origin (Research Institute for Fruit Growing Pitesti, Research Station for Fruit Growing Iaşi, Research Station for Fruit Growing Bistriţa), and foreign origin, grafted on the rootstock *Prunus mahaleb*: 'Amara', NY 13971, 'Simbol', 'Catalina', 'Bing', 'Ponoare', 'Scorospelka', 'Viscount', 'Altenburger', Ny 7690, 'Amar Galata', 'Van', 'Cerna', 'Rivan', 'Rainier', 'New Star', 'Boambe de Cotnari', 'Stella', 'Severin', 'Jubileu', 'Big Dönissen', 'Colina', H 15/25, NY 9295, 'Kristin', 'Izverna', 'Sam', 'Lambert', 'Summit', 'Hedelfinger' and NY 13272.

Experiments began at the beginning of 2008 by grafting the respective varieties in the nursery of the Research Station for Fruit Growing (R.S.F.G) Constanta and were continued in the orchard, until 2014, on 5 trees of each variety.

There were made observations on the ongoing phenological phases of growth and fructification and were determined compatibility coefficients by open and directed pollination after Fleckinger method.

The observations and determination had the final aim to identify the varieties with the best ability to adapt in area, e.g. normal course of phenological phases of growth and fruiting, age-appropriate production, good quality of the fruit, specific to each variety and to establish a conveyer of varieties from early to the late ripening ones.

RESULTS AND DISCUSSIONS

1. Flowering

Data on the period and duration of the flowering concerning all the 31 studied varieties are presented in Table 1.

The beginning of the flowering took place in a fairly long period, of 16 days, between April 10th and April 26th.

Table 1. Flowering period of sweet cherry varieties

Crt. no.	Variety	Begi-nning	Opti-mal	End	Duration (days)
1	Amara	10 IV	20 IV	26 IV	16
2	NY 13971	12 IV	26 IV	2 V	20
3	Simbol	14 IV	25 IV	1 V	17
4	Cătălina	10 IV	19 IV	24 IV	14
5	Bing	15 IV	24 IV	28 IV	13
6	Ponoare	15 IV	25 IV	29 IV	14
7	Scorospelka	15 IV	25 IV	29 IV	14
8	Viscount	16 IV	27 IV	2 V	16
9	Altenburger	16 IV	29 IV	5 V	19
10	NY7690	16 IV	29 IV	7 V	21
11	Amar Galata	16 IV	28 IV	2 V	16
12	Van	16 IV	26 IV	30 IV	14
13	Cerna	16 IV	26 IV	30 IV	14
14	Rivan	16 IV	28 IV	2 V	16
15	Rainier	16 IV	26 IV	30 IV	17
16	New Star	17 IV	29 IV	4 V	17
17	Boambe de Cotnari	17 IV	29 IV	2 V	15
18	Stella	17 IV	29 IV	2 V	15
19	Severin	17 IV	26 IV	30 IV	13
20	Jubileu	17 IV	26 IV	30 IV	13
21	Big Dönissen	18 IV	29 IV	3 V	15
22	Colina	21 IV	2 V	7 V	16
23	H 15/25	21 IV	3 V	7 V	16
24	NY 9295	21 IV	30 IV	4 V	13
25	Kristin	21 IV	2 V	7 V	16
26	Izverna	21 IV	2 V	5 V	14
27	Sam	22 IV	3 V	7 V	15
28	Lambert	22 IV	3 V	6 V	14
29	Summit	22 IV	3 V	6 V	14
30	Hedelfinger	23 IV	30 IV	4 V	11
31	NY 13272	26 IV	5 V	9 V	13
	Limite	10.IV-26.IV	19.IV-05V	24 IV-09 V	11-21

Earliness in flowering is a genetic attribute, specific to each variety. The earlier blooming varieties were 'Amara' and 'Catalina' (April 10th), followed by varieties NY 13971, 'Symbol', 'Bing', 'Ponoare' and 'Scorospelka' (15th of April). In some areas these varieties are vulnerable to spring low temperature (Figure 1). The later start of flowering occurs at variety NY 13272 (April 26th), followed by cultivars 'Hedelfinger', 'Summit', 'Sam', 'Lambert' and 'Izverna'. The late flowering varieties are recommended for breeding, as sources of genes for the late flowering and, of course, in production, being protected from the temperature decreases.

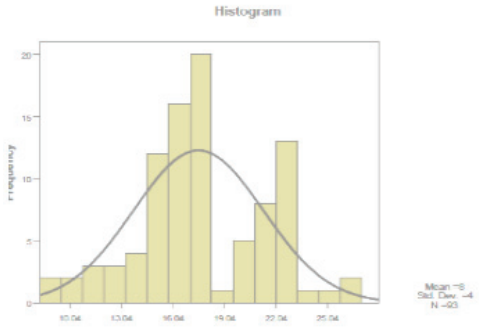

Figure 1. Beginning of flowering

Full bloom (optimal) took place between April 19th to 20th of April ('Catalina' and 'Amara') and 4th – 5th of May (NY 13272, 'Summit', 'Lambert', 'Sam', 'Izverna'). A group of 20 varieties, out of the 31 varieties studied, bloomed actually at the same time, on 2nd to 5th of May, which provides cross pollination (Figure 2).

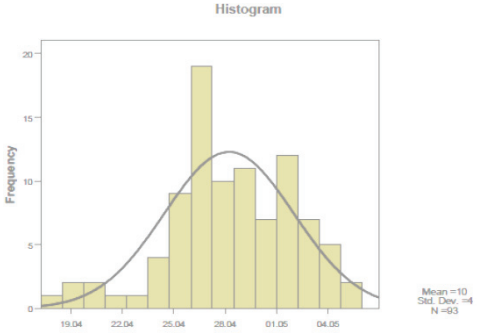

Figure 2. Full bloom

End of flowering (falling petals) occurred between April 24th ('Catalina') and 9th of May (NY13272, H 15/25, NY7690, 'Sam', 'Izverna', 'Lambert', 'Summit'), as shown in Figure 3.

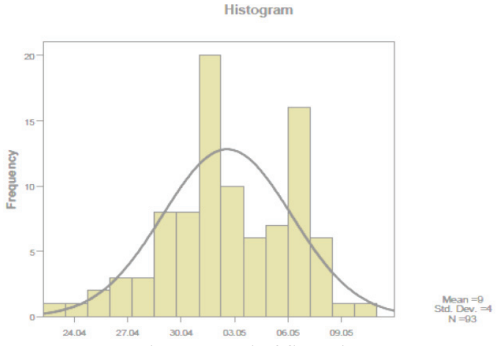

Figure 3. End of flowering

Some of these varieties have an important practical interest, short duration of flowering ensuring an earlier growth and maturation of the fruits.

It appears that in Valu lui Traian area, in case of all 31 varieties tested, the phenophase of the flowering lasts between 11 to 21 days (Table 1 and Figure 4) and could be divided into two: for the early varieties between 10th and 29th April and for the late varieties to 9th of May.

In both cases the simultaneous flowering of the majority varieties ensures cross pollination.

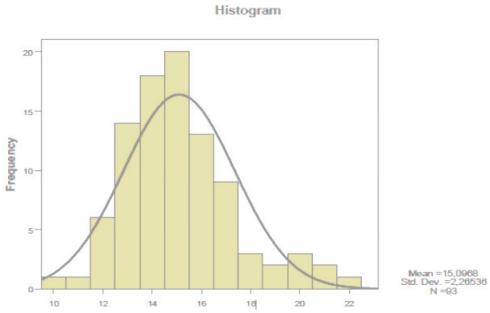

Figure 4. Duration of flowering

2. Pollination

a. Coefficient of fertility by free pollination

Depending on the existing flowers on trees, between 12 flowers – variety 'Viscount' and 53 flower-variety 'Severin' were counted and labelled (table2).

Table 2. Resulted fruits by free pollination

Crt.No.	Variety	No. counted flowers	Resulted fruits (%)
1	Altenburger	16	83.3
2	Kristin	15	82.2
3	Izverna	30	78.9
4	Summit	14	78.5
5	Rainier	36	78.7
6	Viscount	12	75.0
7	NY 9295	32	72.2
8	Stella	18	70.9
9	Sam	41	70.9
10	Scorospelka	28	63.0
11	H 15/25	27	62.9
12	Van	18	57.7
13	Severin	53	57.7
14	Simbol	31	57.1
15	Jubileu	52	51.1
16	Rivan	27	50.9
17	Cătălina	21	42.8
18	Cerna	43	42.2
19	Lambert	25	40.0
20	Big Dönissen	50	35.8
21	Amara	25	35.1
22	Colina	45	33.3
23	New Star	16	24.0
24	Ponoare	15	23.4
25	NY 7690	27	14.8
26	Ny 13272	18	8.23
	MEDIA	29.8	58.6
	LIMITE	12-52	8.23-83.3

In favourable climatic conditions, the cherry free pollination was done in large or even very large proportion, the percentage of fruits set at varieties 'Altenburger' and 'Kristin' exceeding 80% (Figure 5).

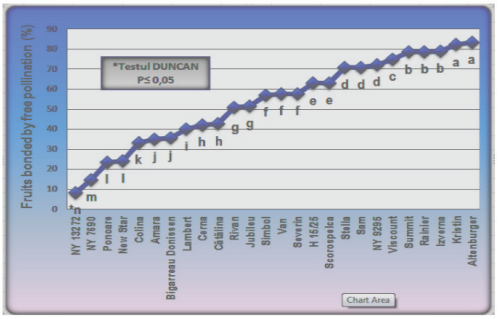

Figure 5. Fruits set by free pollination

The variation between varieties was significant, from 8.23% (NY 13272) to 83.3% ('Altenburger').

At varieties Izverna', 'Rainier', 'Summit', NY 9295, 'Viscount', 'Stella' and 'Sam the percentage of bonded fruits varies between 78.9 and 70.2%, and for the other seven varieties this percentage exceeds 50%. Only cultivars 'Ponoare', 'New Star', 'Big Dönissen', 'Amara', 'Colina' and 'Lambert' have a percentage of fertile flowers below 50%.

The percentage of only 8.23% for the variety NY 13272 can be attributed to the late flowering of this variety, when the most varieties flowering had finished and could not assure pollen .

b. Coefficient of fertility at self-pollination

Observations on self-compatibility (self-pollination) of 27 of sweet cherry varieties (Table 3), confirmed literature data, synthesized by Budan S and Grădinariu G. 2000), regarding the auto-sterility of the majority cherry varieties. In our case, the only varieties that can be considered partially auto compatible are NY 13272, 'Summit' and 'New Star', with more than11% resulted fruit.

We mention that being the first year of flowering, a small number of flower were bagged, between 10 flowers ('Bing') and 357 flowers ('Severin'), the average for the studied flower being of 90.

Table 3. Resulted fruits by self-pollination

Crt.No	Variety	No. bagged flowers	Resulted fruits (%)
1	NyY13272	42	11.9
2	Summit	50	11.7
3	New Star	44	11.3
4	Amara	82	8.5
5	Sam	96	7.2
6	Severin	357	5.8
7	Cerna	196	5.6
8	Viscount	100	4.0
9	Rainier	95	2.5
10	Simbol	45	2.2
11	H 15/25	52	1.9
12	Colina	27	0.0
13	Altenburger	45	0.0
14	NY 7690	29	0.0
15	NY 9295	135	0.0
16	Kristin	64	0.0
17	Amar Galata	86	0.0
18	Big Dönissen	42	0.0
19	Boambe de Cotnari	42	0.0
20	Bing	10	0.0
21	Cătălina	186	0.0
22	Van	181	0.0
23	Jubileu	206	0.0
24	Izverna	39	0.0
25	Ponoare	37	0.0
26	Scorospelka	138	0.0
27	Rivan	43	0.0

CONCLUSIONS

In terms of Valu lui Traian's area, the phenophase of flowering lasts 29 days. The phase starts earlier at: 'Amara', 'Catalina' (10th of April), NY 13971, 'Symbol', 'Bing', 'Ponoare' and 'Scorospelka' (15th of April). These varieties can be vulnerable to decreases of the spring temperature in some areas. The later start of flowering (April 26th) occurs at variety NY 13272, 'Hedelfinger', 'Summit', 'Sam', 'Lambert' and 'Izverna'. These varieties have produced fruits every year. They can also serve as sources of genes for late flowering cultivars in breeding programs of cherries varieties.

A group of 20 varieties, out of the 31 varieties studied, blooms in the same period, on 2nd to - 5th of May.

In favorable climatic conditions during flowering, free pollination of cherry occur out at a rate of minimum 20-40% ('Ponoare', 'New Star', 'Bigarreau', 'Dönissen', 'Amara', 'Colina', 'Lambert') and maximum 83.3-70.2% ('Altenburger', 'Kristin', 'Izverna', 'Rainier', 'Summit, NY 9295, 'Viscount', 'Stella' and 'Sam'). It is considered that a coefficient of free pollination of 30% of the total cherry flower/tree provides a normal crop.

The vast majority of cherry varieties known worldwide are self-incompatible, so will not set fruits by pollination with own pollen. This "*handicap*" of cherry varieties began to be overcome by breeding of self-compatible cultivars. The test of self-pollination, performed at Valu lui Traian, confirmed the self-incompatibility of the studied varieties. It might be considered partially self-compatible only varieties: NY 13272, 'Summit' and 'New Star', with more than 11% obtained fruits by pollinated with own pollen. Further research is needed in the coming years.

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DETERMINATION OF SOME PHYSICAL AND CHEMICAL PROPERTIES OF NATIVE CORNELIAN CHERRY (*CORNUS MAS* L.) DISTRICT OF ALMUS (TOKAT)

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Abstract

The present study was realized in 2013-2014 on different type of cornelian cherry grown from seed, in the district of Tokat Almus, Turkey. The determination of some physical and chemical properties was studied. For this purpose, in this region, 40 cornelian cherry trees have been identified and were recorded by GPS. The evaluation was based on the observations and particular type selection criteria. In this regard, nine genotypes of cornelian cherry were subjected to the analysis. The fruit width ranged between values of 8.41 and 10.67 mm, the average fruit height between 13.51 to 18.84 mm, and the average fruit weight between 0.78 to 1.73 g were determined. As results provided by physico-chemical analysis were the pH between 2.60 and 4.02, soluble solids (TSS) 11.4-15.5 % and the titratable acidity were up to 0.37.

Key words: *Almus, Cornelian Cherry, pH, pomological, soluble solids.*

INTRODUCTION

Thanks to its geographical location, our country is the home of many plant species. Also, the climate is also the homeland of cornelian cherry. Therefore, Anatolia has a very rich cornelian population (Ülkümen, 1973). In 2013, the cornelian cherry production was 11.838 tons, and the production area was 1.675 decares (Anonymous, 2014). The cornelian cherry populate Belgium and Southern Europe, especially Germany. Thereby, the cornelian cherry appear like tall shrubs and small trees and can be found in the form of sorting between 5 to 8 meters (Ercişli, 2004a; Tetera, 2006). Usually, in our country, cornelian is found in the wild form in mountainous areas and riverbeds (Mert and Soylu, 2006). Unlike Tokat Almus, the dogwood populations are scattered in other counties (Gerçekçioğlu, 1998). Quality is a hard core of the fruit species with very different families, the cornelian cherry being a fruit with shape and coloration. Fruit varies from elliptical cylinder and 2-2.5 cm long and is dark red in color. The fruit varies from elliptical cylinder and 2 – 2.5 cm long and is dark red in color. The fruit contain high amounts of sugar and vitamin C between 7-9 % (Feyzioğlu and Ayan, 2002). Cornelian fruit is very rich in phenolic compounds, anthocyanins and total flavonoids. Although the antioxidant capacity varies according to the maturity period, is very high, as in all red fruits. Therefore the cornelian cherry fruit is a natural source of antioxidants (Gündüz et al., 2013).

In recent years, the cornelian cherry is grown by consumers both for ornamental and its health value, facing a demand from the pharmaceutical industry (Bijelik et al., 2012). Cornelian cherry shows diversity with regard to the fruit shape, massiveness, color, taste and nutritional value due to open pollination. Therefore genotype is a potential genetic resource for breeding programs (Ercişli, 2004a). Antioxidant properties of cornelian cherry fruit with outstanding antimicrobial, antiallergenic and in terms of human health is a very valuable fruit characteristics regarding antihistamines (Çelik et al., 2006). However, herbal preparations are used for patients with diabetes (Jayaprakasa et al., 2005).

Cornelian cherry is used in wood industry because of its powerful and flexible features. It is also an important source of nectar and honey in terms of the flowering period. Cornelian cherry has high levels of air pollution tolerance, protects from erosion and is used as a hedge plant in landscaping in urban areas. In this way it is able to tolerate the high levels of air pollution (Bijelic, 2008). Although it is widely known, cornelian cherry is a fruit species neglected in our country. A large part of the cornelian cherries are grown either in mixed gardens or in breeding places. This constitutes a major problem in reaching the fruit harvest (Ercişli, 2004a). This problem can only be solved successfully with the selection of promising genotypes and their controlled propagation. Because cornelian cherry pollination of the plant, over the centuries many different characteristics occurred. In terms of ensuring the standardization of type, cornelian cherry with desired properties should be selected within populations (Gerçekçioğlu, 1998). Selection is made in our country working in different regions (Access et al., 1992; Turkoglu et al., 1999; Yalcinkaya and Eti, 2000; Karadeniz, 2002; Pırlak et al., 2003). Naturally-grown cornelian cherry promising genotypes in Tokat (Almus) were identified some physical and chemical properties have been investigated in this study.

MATERIALS AND METHODS

This study was conducted to determine the physical and chemical properties of 9 different genotypes of cornelian cherry in Tokat (Almus). Coordinates and altitude of the genotypes are presented in Table 1.

In our research in terms of physical properties the fruit, fruit size and fruit weight was examined. Chemical properties as the juice pH, soluble solids (TSS) have been examined and titratable acidity ratio (Smiley, 1977; Karaçalı, 1990; Cemeroğlu, 2007) also. Neck fruit shape index was determined by the proportion of fruit transverse (Gundogdu, 2006). Measuring and weighing fruit from trees was made for 30-60 fruits randomized taken.

Table 1. Promising genotypes of Cornelian cherry coordinates and elevations

Genotypes Number	East Coordinates	North coordinates	Altitude
60 ALM 01	37318787	4469704	1069
60 ALM 02	37319061	4469802	1065
60 ALM 03	37319150	4469836	1052
60 ALM 04	37319049	4469806	1041
60 ALM 05	37319060	4469796	1035
60 ALM 06	37318775	4469704	1058
60 ALM 07	37318788	4469737	1060
60 ALM 08	37319009	4469833	1032
60 ALM 09	37319112	4469834	1047

Fruit digital calipers were used in the measurement, with a sensitivity of 0.01, while the precision of the weighing was 0,001 grams. The amount of fruit soluble solids (TSS) was determined using a hand refractometer, and the pH of the juice was identified using a pH-meter. The titratable acidity (TEA) was determined using a titration method (Yarılgaç and Yıldız, 2001).

RESULTS AND DISCUSSIONS

The results of the weighted ratings were determined as genotypes mentioned above. Physical and chemical properties of those genotypes which are presented in tables 2 and 3. The average fruit weight of the genotype is seen as the most promising low 0.78g (60 ALM 04), and the highest 1.73 (60 ALM 01), respectively. The average width of the fruit was between 8,41mm (60 ALM 04) and 10.67 mm (ranged from 60 ALM06). The lowest average fruit size values were 13.51 mm (13 ALM 05) and highest average was 18.84mm (13 ALM 01). Previous research studies on cornelian cherry fruit weight were differing regarding the length and width, the major effect on the pomological characteristics being due by environmental factors as well as genotype (Güleryüz et al., 1998; Demir and Kalyoncu 2003).

We obtained values that are supported by earlier work selection. Another study which was conducted by Erzincan, fruit weight ranged between 1.44 – 4.24 g, most of the fruit size being between 9.6 – 15.8 mm and 14.1 – 22.8 mm (Selçuk and Özrenk, 2011). Oblak (1980), presented that the average fruit weight in the studied population, which grows naturally in Slovenia was 1.78 grams. Bolu, Zonguldak, Karabük and Bartin grown in natural population between 1996-1998 in the provinces and in the manufacturer's garden were conducted to determine the best cornelian cherry. Such studies were found that the average fruit weight was between 1.02 and 4.07 grams (Yalcinkaya and Eti, 1999). Derebucak district of Konya in a study of ten different types of cornelian cherry, naturally grown, showed that the weights were between 3.65 – 4.57 g (Turkoglu et al., 1999). In another study, in Konya the selection of the fruit weight was determined being between 1.496 g and 4.116 g (Demir and Kalyoncu, 2003). Another study, realised by Rural and Koca (2008) in Samsun shows that the weight of the fruit that grows naturally was between 0.39 - 1.03 g, the fruit length between 14.24 mm – 22.20 mm, and most of the fruit varies between 9.59-13.21mm

Table 2. Some physical properties of promising cornelian cherry genotype (2013-2014)

Genotype number	Weight (g)	Width (mm)	Height (mm)	Shape index
60 ALM 01	1.73 ± 0.14	10.64 ± 0.14	18.84 ± 1.36	1.77 ± 0.19
60 ALM 02	1.32 ± 0.10	10.52 ± 1.01	14.94 ± 1.24	1.42 ± 0.06
60 ALM 03	1.02 ± 0.08	09.55 ± 0.96	14.64 ± 1.85	1.53 ± 0.43
60 ALM 04	0.78 ± 0.05	08.41 ± 0.29	13.96 ± 2.06	1.66 ± 0.28
60 ALM 05	1.13 ± 0.07	09.83 ± 0.35	13.51 ± 0.53	1.37 ± 0.17
60 ALM 06	1.46 ± 0.16	10.67 ± 0.44	17.47 ± 1.61	1.64 ± 0.32
60 ALM 07	1.24 ± 0.21	10.24 ± 1.06	15.08 ± 0.80	1.47 ± 0.02
60 ALM 08	1.17 ± 0.11	10.29 ± 0.83	14.44 ± 0.72	1.40 ± 0.41
60 ALM 09	1.01 ± 0.13	09.69 ± 0.18	14.09 ± 1.40	1.45 ± 0.75

Table 3. Promising chemical properties of the cornelian cherry genotype (2013-2014)

Genotype number	pH	TSS (%)	TEA
60 ALM 01	3.12 ± 0.35	11.4 ± 0.24	0.35 ± 0.04
60 ALM 02	3.56 ± 0.40	11.8 ± 0.65	0.37 ± 0.08
60 ALM 03	4.02 ± 0.15	13.8 ± 0.32	0.35 ± 0.03
60 ALM 04	3.88 ± 0.66	14.9 ± 1.28	0.28 ± 0.07
60 ALM 05	3.80 ± 0.10	17.2 ± 0.87	0.30 ± 0.08
60 ALM 06	3.60 ± 0.24	15.2 ± 0.37	0.26 ± 0.15
60 ALM 07	3.92 ± 0.32	15.0 ± 0.20	0.36 ± 0.10
60 ALM 08	2.60 ± 0.18	15.5 ± 1.24	0.33 ± 0.03
60 ALM 09	3.28 ± 0.25	15.1 ± 2.49	0.32 ± 0.06

The results of this study show closeness with our obtained results, the highest values in terms of the weight of the fruit being low in both studies. We consider that the results are dependent on the genotype and the environmental conditions, the shape of the fruit affecting the quality of the fruit. The evaluation showed that the lowest index value was 1.37 for 13 ALM 05 genotype. 60 ALM 01 genotype had the longest length, with the highest value of shape index 1.77. This value is lower than 2.50 found in cornelian cherry genotype from Serbia (Bijelic, 2012).

As well as fresh, the cornelian cherry are consumed and processed in fruit industry, due to their physical and chemical properties. The pH of the promising occurring kızılcık genotypes soluble solids (TSS) and titratable acidity (TA) is given in percentage in Table 3. PH 2.60 in the genotype (60 ALM 08) to 4.02 (60 ALM 03) in between, while the soluble solids ratio of 11.4 (60 ALM 01) to 15.5 (60 ALM 08) ranged. The lowest rate in the amount of titratable acidity 0.28 (60 ALM 04), and the highest ratio 0.37 (60 ALM 02) respectively. Selcuk and Özrenk (2011), the pH 2.9-5.7 in a similar study conducted in cornelian cherry have found in the water soluble dry matter

content of 9.0-17.7. In a population study carried out in Slovenia, the average amount of TSS in the cornelian cherry grown naturally was 20.6 %, total sugar was found to be 7.42 % and the pH 3.38 (Oblak, 1980). Another study which was conducted in Trabzon, has been reported to range between 8% and 13.5% of the total dry matter (Karadeniz et al., 2001). In another study conducted in Zonguldak amount of soluble solids it has been reported to be between 12.1 to 16.9%. Tural and Koca (2008), the amount of soluble solids in chemical analysis they have found in cornelian cherry have been obtained through selection between 28.19% and 15.88%. The same researchers have found the total amount of 1.10 to 2.53% for acidity. Total acidity between 4.69% and 1.24% was found in similar studies (Smiley et al., 1998; Strain, et al., 2000; Demir and Kalyoncu 2003). In particular, the same kinds of chemical compounds (or type) of the year even though the ecological differences are greatly influenced by the environment and maintenance requirements (Gerçekçioğlu, 1998).

CONCLUSIONS

Our results have shown that it is consistent with the results provided by other genotypes grown both inside and outside the country. However, it is expected to give better results with a different cultivation technology. Also, characterıstıcs such as phenolic compounds, the antioxidant capacity, the pollen biology and others can provide more information about the genotype's value. Research like this will shed light our study and the genetic material can be considered as the promising genotypes.

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SOME PHYSICO-CHEMICAL CHARACTERISTICS OF BLACK MULBERRY (*MORUS NIGRA* L.) IN BITLIS

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Abstract

In this study, physico-chemical properties (total soluble solid contents, pH, titratable acidity, vitamin C, antioxidant activity, total phenolic and total anthocyanins) of black mulberry (Morus nigra L.) fruits grown in the Bitlis province of Turkey were investigated. The total soluble solids content of black mulberry varies between 15.65 % (13-BIT-2) and 22.1 % (13-BIT-6), titratable acidity between 1.45 % (13-BIT-1) and 1.85 % (13-BIT-4), pH between 3.65 (13-BIT-2) and 4.12 (13-BIT-5), respectively. Ascorbic acid (vitamin C) was in the range from 18.40 (13-BIT-3) to 23.67 (13-BIT-6) mg/100 g fresh weight (FW). The highest total phenolic contents were found 1920 (13-BIT-2) to 2575 (13-BIT-7) mg of gallic acid equivalent (GAE) 100 g^{-1} fresh weight. Antioxidant capacity (DPPH) was in the range from 18.24 (13-BIT-1) to 23.18 (13-BIT-6) % and total anthocyanin content between 643 (13-BIT-4) and 826 (13-BIT-8) mg/100 g.

Key words: *antioxidant, phenolic, Morus nigra, mulberry, selection.*

INTRODUCTİON

Morus nigra L., called black mulberry is a species of flowering plant in the family *Moraceae,* native to in a wide area of tropical, subtropical, and temperate zones in Asia, Europe, North America, South America, and Africa.

The trees historically have been used for sericulture especially in east, central, and south Asia. There are at least 24 species with more than 100 known cultivars. Farmers cultivate mulberry for silkworms in the China and India but european farmers cultivate for fruit. (Pawlowska et al., 2008).

Anatolia is one of the important diversity centers of mulberries with a long cultivation history dating back to 400- 500 years ago. The most popular mulberry species with edible fruits grown in Turkey are black, white and red mulberry (Ercisli and Orhan, 2007).

Traditionally, the fruits have been processing into several products like mulberry juice, molasses, jam, vinegar and some very special products such as 'mulberry pestil', 'mulberry kome' in Turkey. All of these have significant marketing value due to its nutritive and distinct characteristics (Erturk and Gecer, 2012).

Black mulberry fruits have also been effectively used in folk medicine in Turkey for a long time to treat fever, protect liver, strengthen the joints, facilitate discharge of urine and lower blood pressure (Baytop, 1984).

Dark-coloured fruits, particularly berries (currant, honeyberry, aronia, blackberry, blueberry, mulberry, etc.) are recognized as contributors to the human health. In addition, there is increasing interest in pigment components of this group of fruits that may improve human health or lower the risk of disease (Lin and Tang, 2007). Black mulberry contains bioflavonoids that are important natural antioxidants and also contains non anthocyanin phenolics that are known to have many bioactive functions, including neuroprotective effects, which may be responsible for their medicinal properties (Ercisli and Orhan, 2007).

The aim of this research was to determine the some physico-chemical characteristics of local *Morus nigra* L. cultivars grown in Bitlis region in Eastern part of Turkey. The obtained results can be used in the registration process of these local cultivars and may be taken into consideration in the selection of parents in future breeding programs.

MATERIALS AND METHODS

Fruit material

Eight local black mulberry cultivars were harvested in different region of Bitlis, East Anatolia, Turkey, in August 2014. The harvested fruits were then transported to the laboratory for analysis.

Methods

Total soluble solid content (TSS) was measured with a digital refractometer (Model HI-96801 Hanna, German) at 20 ºC. pH measurements were done by using Hanna-HI 98103. pH meter calibrated with pH 4.0 and 7.0 buffers. Titratable acidity was determined potentiometrically by titrating the sample with 0.1 NaOH until the pH reached 8.01 and expressed as citric acid.

Determination of total phenolics

Total phenolic content were determined with Folin-Ciocalteu assay (Singleton and Rossi, 1965). For this, flesh and peels (10 g) were centrifuged at 6000 rpm after homogenized in 40 ml ethanol solution. After, diluted (1/10) 1000 µl Folin-Ciocalteu and 800 µl Na_2CO_3 solution was added upon supernatant. After 2 hours of incubation, the samples were read spectrophotometrically at the wavelength 750 nm. Water-ethanol mixture was used as blank. Gallic acid was used as standard in the calculation.

Determination of total anthocyanins

For total anthocyanin analysis, 10 g flesh and peels were homogenized in methanol solution that included HCl 1 %. After a night of standing, the samples were filtered using a filter paper. The samples were read against the blank at the wavelengths of 530 and 700 nm (Giusti and Wrolstad, 2001).

Determination of vitamin C

After pureeing and filtering, the fruit juices samples were obtained. The juices were used for vitamin C analysis. The samples were homogenized by centrifuge and 400 µL oxalic acid (0.4 %) and 4.5 ml 2,6 - diclorofenolindofenol solution were added upon supernatant. The data were read spectrophotometrically at the wavelength of 520 nm against the blank.

Determination of radical – scavenging activity

In the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) assay, antioxidants were capable to reduce the stable radical DPPH• to the yellow coloured diphenylpicrylhydrazine (DPPH-H). The test is based on the reduction of an alcoholic solution of DPPH• in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H (Gulcin, 2007). The DPPH• radical-scavenging activity was estimated after Blois (1958). Briefly, 0.1 mL of each sample extract was mixed with 0.9 mL of 0.04 mg/mL methanolic solution of DPPH•. The mixtures were left for 20 min at room temperature and its absorbance then measured at 517 nm against a blank. All measurements were carried out in triplicate. The percentage of DPPH• scavenging activity was calculated using the following equation:

$$\% \text{ DPPH} = [(A_c - A_s)/A_c] \times 100$$

where A_c was the absorbance of the negative control (contained extraction solvent instead of the sample), and A_s was the absorbance of the samples.

Statistical analysis

Five replicates including 20 fruits per replicate were used. Descriptive statistics of total soluble solid contents, pH, titratable acidity, vitamin C, antioxidant activitiy, total phenolic and total anthocyanins extracted from eight *Morus nigra* cultivars were expressed as mean ± standard error (SE). The phytochemical characteristics were statistically analyzed with One-way ANOVA with five replicates. Duncan Test determined significant differences between the evaluated cultivars. All statistical evaluations were performed using SPSS 20 program.

RESULTS AND DISCUSSIONS

Table 1 shows the results of pH, TSS (%) and titratable acidity composition. In Table 2 are presented the results regarding the analysis of vitamin C, free radical scavenging activity, total phenolic and antocyanin content of eight mulberry cultivars collected from Hizan district in Bitlis province. The analysis of variance indicated that the cultivar had a major influence on all parameters under evaluation ($p < 0.05$).

pH, total soluble solids and titratable acidity in black mulberry cultivars

pH value in black mulberry cultivars was between 3.65 (13-BIT-2) and 4.12 (13-BIT-5). The average pH of cultivars was 3.85. The pH

contents in different mulberries ranged from 3.3 to 3.8 (Uzun ve Bayir, 2009) and our pH results are generally within limits of these studies.

Total soluble solid content (TSS) in black mulberry cultivars varied from 15.65 (13-BIT-2) to 22.10 % (13-BIT-6) with an average of 18.94 %. Previous studies had shown that soluble solid content of mulberry fruits grown in different agroclimatic regions of Turkey is between 15.27–30.80 % (Lale and Ozcagiran, 1996; Aslan, 1988), and our SSC results are generally within limits of these studies.

The titratable acidity of black mulberry cultivars was between 1.45 % (13-BIT-1) and 1.85 % (13-BIT-4).

The average titratable acidity of black mulberry cultivars was 1.63 %, which is a little higher than those reported for red and white mulberries (Ercisli et al., 2010) and black mulberry (Iqbal et al., 2010).

Table 1. pH, TSS (%) and titratable acidity composition of black mulberry cultivars

Cultivars	pH	TSS (%)	TA
13-BIT-1	3.82 b	17.20 bc	1.45 d
13-BIT-2	3.65 c	22.10 a	1.75 b
13-BIT-3	3.90 ab	16.70 c	1.56 c
13-BIT-4	3.70 bc	20.50 a	1.85 a
13-BIT-5	4.12 a	18.40 b	1.62 bc
13-BIT-6	4.06 a	15.65 d	1.80 a
13-BIT-7	3.92ab	21.40 a	1.55 c
13-BIT-8	3.66 c	19.55 a	1.48 c
Mean value	3.85	18.94	1.63

Vitamin C, antioxidant activity, total phenolic and total anthocyanins content in black mulberry fruits

We found vitamin C content between 18.40 (13-BIT-3) and 23.67 (13-BIT-6) mg/100 g for black mulberry cultivars under the investigation. In the earlier work conducted on the northeast Anatolia region of Turkey, Ercisli and Orhan (2008) reported that vitamin C contents of black mulberry cultivars varied from 14.9 to 18.8 mg/100 mL. Ercisli et al. (2010) reported the average vitamin C content in black and purple mulberries as 20.79 and 18.87 mg per 100 mL extract, respectively. Lale and Ozcagiran (1996) reported that vitamin C content in black and purple mulberries was 16.6 and 11.9 mg/100 ml extract.

Table 2. Vitamin C, DPPH, total phenolic and anthocyanins content of black mulberry cultivars

Cultivars	Vitamin C	DPPH %	Total phenolic content	Total anthocyanins content
13-BIT-1	22.65 a	18.24	2125 c	815 a
13-BIT-2	19.50 b	21.56	1920 c	793 ab
13-BIT-3	18.40 bc	20.44	2330 ab	710 c
13-BIT-4	20.28 ab	19.86	2255 ab	826 a
13-BIT-5	21.15 b	22.38	1970 d	674 d
13-BIT-6	23.67 a	23.18	2345 ab	808 a
13-BIT-7	18.87 c	20.42	2575 a	756 b
13-BIT-8	22.36 b	18.66	2215 b	643 d
Mean value	20.86	20.59	2217	753.13

The antiradical activity of black mulberry cultivars were 18.24 (13-BIT-1) to 23.18 % (13-BIT-6) (DPPH assay).

Ozkaya (2015) reported that antioxidant activity in black mulberry were 15.037-24.443 μM TE/g. The results for total phenolics content between 1920 (13-BIT-2) and 2575 (13-BIT-7) GAE mg/g. Earlier reports had shown that the total phenolic content in mulberry fruits was between 1515–2570 GAE mg/g (Lin and Tang, 2007; Bae and Suh, 2007*)*. The difference between mulberry cultivars and between species in terms of phenolics is supposed to be a genetic characteristic because all plants were grown under the same agroclimatic conditions.

The effect of cultivar within the same fruit species on total phenolic content is well documented by several researchers on apples and strawberries (Scalzo et al., 2005; Voča et al., 2008), sea buckthorns (Ercisli et al., 2007) and cornelian cherries (Yilmaz et al., 2009). The total anthocyanin content per fresh weight of black mulberry (*Morus nigra*) cultivars ranged from 643 (13-BIT-4) and 826 (13-BIT-8) Cy 3-glu mg/g. According to earlier reports, total anthocyanin content in purple and black mulberries was 99 and 571 Cy 3-glu mg/g (Ozgen et al., 2009).

CONCLUSIONS

The results clearly indicate the difference between the cultivars used grown in the same conditions. Antioxidant activity also varies among the different cultivars of black mulberry, and this is a reflection of the phytochemical differences between cultivars.
These local black mulberry cultivars have high vitamin C, total phenolic, anthocyanin and antioxidant capacity in fruit. It is known, positive effect on human health of these substances.
This cultivars can be used for future breeding activities to obtain more healthier black mulberry.

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RESEARCH CONCERNING THE INFLUENCE OF CLIMATE ON EVOLUTION OF PHENOLOGICAL STAGES IN SWEET CHERRY TREE

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Abstract

Carrying out the phenological stages of vegetation and fructification in sweet cherry cultivars is determined by the cumulative action of rainfall registered and daily average temperatures exceeding 5°C, value considered as biological limit of this species. Research was conducted during 2011-2014 at six Romanian cultivars 'Cetăţuia', 'Cătălina', 'Maria', 'Andreias', 'Margonia' and 'George' and three introduced sweet cherry cultivars 'Van' (Canada), 'Bigarreau Burlat' (France) and 'Bigarreau Dönissen' (Germany). Were recorded number of days, active thermal balance (°C) and rainfall quantity (mm) on periods between phenological stages: from swelling buds to blooming, flowering period, from end of flowering to ripening of fruits and from ripening of fruits to leaves fall. The number of days recorded in periods between phenological stages from swelling buds to start of blooming was between 20-26 days, the active thermal balance ranged between 192 to 269 °C and rainfall quantity ranged between 41 mm to 63 mm. In the blossom period number of days ranged between 8-12 days, with the active thermal balance ranged between 97°C to 151°C and rainfall quantity ranged between 6.5mm to 30 mm. The number of days from swelling buds to start of blooming, from the end of blossom to fruit maturity and from ripening of fruits to leaves fall is positive correlated with the active thermal balance. The number of days from swelling buds to start of blooming and from ripening fruit to leaves fall is positive correlated with the rainfall quantity registered.

Key words: *Prunus avium* L.*, temperature, rainfall, cultivars, blooming.*

INTRODUCTION

Sweet cherry is an important specie in Romania and occupies an area of 7,760 ha (Coman and Chitu, 2014) with the great extension in the following years by new plantations established.
The period between phenological stage of swelling buds and fruit maturation is very short for sweet cherry cultivars compared with other tree species, excepting strawberry (Budan and Grădinariu, 2000). Global climate change are subject to the recent research on plants having great influence in the development of phenological stages (Ansari and Davarynejad, 2008; Balaci et al., 2008; Chmielewski et al., 2004; Inouye et al, 2003). The previous research showed that phenological stages carrying the sweet cherry are determined by the cumulative action of daily average temperatures that exceed the value of 5°C (Sîrbu et al., 2013; Istrate, 2007). Average daily temperature has a direct influence in flowering plants (Radicevic et al., 2011; Tooke and Battey, 2010; Roversi & Ughini, 1996). Temperature is very important during ripening fruit, but from the end of flowering to strengthen of the kernel, the influence of these climatic parameter is low (Budan and Grădinariu, 2000). Sparks et al. (2000), shows that climate change affects the starting time of flowering but Darbyshire et al. (2012) show that every Celsius degree increased of temperature advancing phenophases with 4 to 7 days. Also, rainfall in the period of fruit maturity induced fruit cracking in sweet cherries and can cause heavy losses in yields and returns (Meland et al., 2014).
This paper aims to determine the number of days of active heat balance and rainfall necessary to conduct phenological stages at different sweet cherry cultivars in terms of climate change and establish correlations between the studied parameters.

MATERIALS AND METHODS

In this study during 2011-2014, six Romanian sweet cherry cultivars: 'Cetăţuia', 'Cătălina', 'Maria', 'Andreiaș', 'Margonia' and 'George' and three introduced sweet cherry cultivars

'Van' (Canada), 'Bigarreau Burlat' (France) and 'Bigarreau Dönissen' (Germany) were evaluated. All cultivars were cultivated on *P. mahaleb* L. seedlings rootstock.

Three trees presented each cultivar and were planted at spacing of 4 x 5 m, with free palmette crown shape with support system. The orchard was located on a medium sandy clay loam with medium (6%) humus content.

Herbicide spraying were mantained along trees rows and grass was cut three times during summer in alleyways. No irrigation, rainfall, frost or birds protection system provided.

Phenological data were determined through the Fleckinger system (Fleckinger, 1960): B_1 - the bud swelling: the bud rounds delicate and gains a green light at the top; F_1 – start of blooming: the flowers are open for 5%; G - the end of the flowering: the petal of flowers have fallen for 90%. The data of the fruit ripening was established in the time of marketing quality traits (colour, the content of dry matter) specific to each cultivar.

The climatic data were recorded with the AgroExpert system by the station located on the perimeter of the experimental plot of the Research Station for Fruit Growing, Iaşi - Romania. The active thermal balance (Σt°a) is provided by the sum of average daily temperature degree, which exceeds the biological limit characteristic to the sweet cherry tree, considered to be 5°C (Istrate, 2007).

$$\Sigma t°a = \Sigma T \text{ atd} - BL, \text{ in which:}$$

ΣT atd = sum of average temperature of days between two subsequent phenological stages;

BL = the biological limit of fruit tree species.

The statistical analysis was performed with the XLSTAT programme (ProAcademic, 2011, Addinsoft). The differences between cultivars were determined by the Duncan's test ($p \leq 0.05$). The Pearson correlation coefficient has been calculated between the variables measured ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

For studied sweet cherry cultivars, the number days during the swelling buds to start of blooming ranged between 20 ('Cetăţuia') and 26 ('Margonia') (Table 1).

Table 1. Number of ffdays, active themal balance and rainfall quantity registered during the swelling buds to start of blooming at sweet cherry cultivars (2011-2014).

Cultivar	Number days[1]			Active themal balance (°C)			Rainfall quantity (mm)		
	Av	Min	Max	Av	Min	Max	Av	Min	Max
Cetăţuia	20[b2]	11	26	191.6[b]	114.6	242.8	43.9[ab]	7.6	85.4
Cătălina	22[b]	12	28	197.7[b]	123.2	245.7	44.9[ab]	7.6	85.4
Bigarreau Burlat	22[b]	14	28	208.5[b]	148.7	253	46.0[ab]	7.6	85.4
Maria	22[b]	14	29	217.4[b]	151.4	261.2	47.4[ab]	7.6	85.4
Van	21[b]	15	23	204.7[b]	166.2	225.8	46.3[ab]	4.6	85.4
Andreias	21[b]	15	26	213.9[b]	160	261.6	41.3[b]	9.2	85.4
Bigarreau Dönissen	22[b]	17	27	222.4[ab]	183.3	255.05	48.9[a]	7.6	85.4
Margonia	26[a]	21	33	269.1[a]	233.2	309.1	62.9[a]	33.4	85.4
George	21[b]	15	26	214.2[b]	171.1	254.8	41.9[b]	9.2	85.4
LSD $_{0.05}$	3.1			25.6			20.8		

[1] Av - average; Min - minimum; Max – maximum;

[2] - Different letters after the number corresponds with statistically significant differences for P 5% - Duncan test.

Also, 'Bigarreau Burlat' is a control cultivar for other cultivars as start of blossom, according with other studies (Kazantzis et al., 2011), but 'Cetăţuia' was earlier.

Minimum days for this phenological stage was 11 ('Cetăţuia') and maximum was 33 ('Margonia'). The active termal balance as average on period 2011-2014 ranged between 191.6°C ('Cetăţuia') and 269.1°C ('Margonia'). Except 'Margonia' all others sweet cherry cultivars have not differed statistically significant among them as active termal balance during the swelling buds to start of blooming.

Rainfall quantity ranged between 41.3 mm ('Andreias') and 62.9 mm ('Margonia') but significant statistical differences registered just 'Andreias' and 'George' (table 1). For period 2011-2014, the minimum values was 4.6 mm at 'Van' but the maximum value was the same for all studied cultivars as 85.4 mm.

Number of days for entire blossom time as average during 2011-2014 ranged between 8 ('Van' and 'George') to 12 ('Cătălina') (Table 2). The minimum value was 4 at 'Cetăţuia' and 'Andreiaş' but the maximum value was at 'Cătălina' with 16 days. Active thermal balance ranged between 97.3ºC ('Cetăţuia') and 145.7 ('Margonia') but significant statistical differences registered only 'Cetăţuia' and 'Van'. Minimum value was at 'Cetăţuia' with 46.2ºC but the maximum value was 174.7ºC at 'Bigarreau Dönissen' (Table 2).

Table 2. Number of days, active themal balance and rainfall quantity registered during the blossom time at sweet cherry cultivars (2011-2014).

Cultivar	Number days[1]			Active themal balance (°C)			Rainfall quantity (mm)		
	Av	Min	Max	Av	Min	Max	Av	Min	Max
Cetăţuia	9^{ab2}	4	15	97.3^{b}	46.2	131.3	28.2^{ab}	0	66.6
Cătălina	12^{a}	8	16	151.0^{a}	147.8	153.4	29.8^{a}	0	67.8
Bigarreau Burlat	9^{ab}	5	15	105.9^{ab}	68.3	155.1	29.3^{ab}	0	72.6
Maria	9^{ab}	8	11	119.1^{ab}	94.6	147.8	26.4^{ab}	0	67.8
Van	8^{b}	5	11	99.9^{b}	82.2	125	25.4^{ab}	0	67.8
Andreias	10^{ab}	4	12	123.9^{ab}	80.2	161.3	28.7^{ab}	0	71.2
Bigarreau Dönissen	11^{ab}	6	15	143.2^{ab}	114.5	174.7	27.6^{ab}	2.6	72.6
Margonia	9^{ab}	6	13	145.7^{ab}	114.5	174.1	6.5^{c}	0	14.8
George	8^{b}	5	9	107.2^{ab}	65.1	166.4	23.5^{abc}	0	65.0
$LSD_{0.05}$	3.3			48.1			15.9		

[1] Av - average; Min - minimum; Max – maximum;

[2] - Different letters after the number corresponds with statistically significant differences for P 5% - Duncan test.

For studied sweet cherry cultivars, the rainfall quantity registered values between 6.5 mm to 29.8 mm as an average for 2011-2014 period with a minimum as 0 mm to 72.6 mm. Number of days recorded from the end of blossom to fruit maturity at the studied sweet cherry cultivars ranged between 32 ('Cătălina') to 67 ('George') with a minimum value at 'Cătălina' with 26 days and a maximum value at 'George' with 81 days. These results are according with other studies for sweet cherry cultivars (Sîrbu et al., 2011) which show that from the blossom to fruit ripening are needed 70 - 98 days.

Also, for this stage the studied cultivars required an active thermal balance ranged between 539.2ºC ('Cătălina') to 1,226.1ºC ('George') with a minimum value to 'Cetăţuia' with 485ºC and a maximum value at 'George' with 1,381.6ºC (Table 3).

Number of days registered from the ripening fruit to leaves fall at the studied sweet cherry cultivars ranged between 112 ('George') to 152 ('Cetăţuia') with a minimum value at 'Maria' with 99 days and a maximum value at 'Cetăţuia' with 157 days (Table 4).

Table 3. Number of days, active themal balance and rainfall quantity registered during the end of blossom to fruit maturity at sweet cherry cultivars (2011-2014).

Cultivar	Number days[1]			Active themal balance (°C)			Rainfall quantity (mm)		
	Av	Min	Max	Av	Min	Max	Av	Min	Max
Cetăţuia	33[c]	28	38	541.3[c]	485	586.6	67.7[b]	41	104
Cătălina	32[c]	26	41	539.2[c]	489	600.3	75.9[b]	54.6	101.4
Bigarreau Burlat	36[c]	31	41	602.3[c]	554.9	667.4	89.8[b]	54.6	113.8
Maria	52[b]	42	60	911.5[b]	763.9	1027.6	114.6[b]	72	148.2
Van	54[b]	45	58	955.1[b]	770.5	1092.5	120.8[b]	62.2	182.8
Andreias	50[b]	44	57	897.7[b]	804.7	1006.2	113.1[b]	63.4	150.8
Bigarreau Dönissen	56[b]	47	65	1006.6[b]	845.4	1074.7	123.6[ab]	56	192.8
Margonia	52[b]	47	59	930.5[b]	845.4	1002.5	313.5[a]	56	892.8
George	67[a]	49	81	1226.1[a]	858.7	1381.6	167.4[ab]	62.2	345.6
$LSD_{0.05}$	8.2			155.1			190.9		

[1] Av - average; Min - minimum; Max – maximum;
[2] - Different letters after the number corresponds with statistically significant differences for P 5% - Duncan test.

Table 4. Number days, active themal balance and rainfall quantity registered during the ripening fruit to leaves fall at sweet cherry cultivars (2011-2014).

Cultivar	Number days[1]			Active themal balance (°C)			Rainfall quantity (mm)		
	Av	Min	Max	Av	Min	Max	Av	Min	Max
Cetăţuia	152[a2]	146	157	2,767.2[a]	2,537.3	3,031.8	271.7[a]	177.0	415.4
Cătălina	149[a]	146	152	2,701.9[a]	2,537.3	3,031.8	261.8[ab]	177.0	415.4
Bigarreau Burlat	146[a]	142	150	2,654.9[a]	2,466.1	2,983.9	240.6[ab]	139.8	406.0
Maria	120[bcd]	99	132	2,338.2[b]	2,248.9	2,514.0	223.2[abc]	131.8	368.6
Van	126[bc]	115	138	2,300.8[b]	2,058.6	2,432.0	215.6[bc]	123.2	336.6
Andreias	128[b]	119	132	2,337.5[b]	2,262.4	2,497.8	223.3[abc]	132.0	368.6
Bigarreau Dönissen	121[bcd]	116	133	2,209.0[b]	1,946	2,433.3	210.8[bc]	131.8	324.0
Margonia	114[cd]	102	121	2,229.9[b]	1,934.6	2,528.4	210.9[bc]	132.0	324.0
George	112[d]	102	134	2,021.9[c]	1,670.1	2,343.8	170.8[c]	129.2	240.2
$LSD_{0.05}$	11.3			153.2			49.6		

[1] Av - average; Min - minimum; Max – maximum;
[2] - Different letters after the number corresponds with statistically significant differences for P 5% - Duncan test.

Also, for this stage the studied cultivars required an active thermal balance ranged between 2,021.9ºC (‘George’) to 2,767.2ºC (‘Cetăţuia’) with a minimum value to ‘George’ with 1,670.1ºC and maximum value at ‘Cetăţuia’ and ‘Cătălina’ with 3,031.8ºC. Correlating the number of days with the active thermal balance (Table 5) we observed distict significant positive correlation in the period between swelling buds to start of blooming (r=0.9139), in the period between end of blossom to fruit maturity (r=0.9995) and between ripening fruit to leaves fall (r= 0.9730).

Table 5. Correlation coefficient (r) between number days and active thermal balance and number days and rainfall quantity

Period	Number of days - active thermal balance[1]	Number of days – rainfall quantity
I - Swelling buds -start of blooming	0.9139**	0.9353**
II - Blossom time	0.7702*	0.3137^{ns}
III - End of blossom - fruit maturity	0.9995**	0.4961^{ns}
IV - Ripening fruit - leaves fall	0.9730**	0.9158**

[1]-*-significant correlation; **- distinct significant correlation; [ns]-non-significant correlation.

CONCLUSIONS

The climate change from recent years have influenced the duration of the phenological phases of different sweet cherry cultivars.
The number of days with the rainfall quantity was distict significant positive correlated in the period between swelling buds to start of blooming (r=0.9353) and between ripening of fruits to leaves fall (r=0.9158). The action of daily average temperatures determines different blooming periods in different year conditions.
The number of days from swelling buds to start of blooming, from end of blossom to fruit maturity and from ripening fruit to leaves fall are positive correlated with the active thermal balance.
The number of days from swelling buds to the start of blooming and from ripening of fruits to leaves fall are positive correlated with the the rainfall quantity registered.

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THE EFFECT OF FROST AND HALE ON THE PEACH TREE CULTIVARS FROM R.S.F.G. CONSTANȚA

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Abstract

One of the problems which occurred during the last years concerning all fruit-growing species is determined by climate changes. Some phenomena related to climate stress occur in a chronic manner (low fertility, weak structuring of soils, etc.) or periodically (droughts, excess of humidity in the soil, etc.) or occasionally (early or late frosts, hale, etc.); their unfavourable influence depends both on the intensity and the duration of the stress as well as on the specific phenophase of crop plants. Due to the climate changes which occurred during the last couple of years, it was observed that the resistance of peach tree cultivars differs greatly from one year to the next. The present studies were carried out over a period of three years on plantations of ripe peach trees and nectarine trees from R.S.F.G. Constanța. Branch samples belonging to 7 peach tree cultivars ('Springcrest', 'Springold', 'Collins', 'Cardinal', 'Redhaven', 'Southland' and 'Jerseyland') and 3 nectarine tree cultivars ('Cora', 'Delta' and 'Romamer2') were harvested and analysed three days after the frost occurred. The paper presents the manner in which certain peach tree and nectarine tree cultivars reacted to the effect of the frost which occurred in 2012, 2013 and 2014 and the effect of hale (July 11th, 2014) on the peach tree production. The greatest losses caused by frost were recorded in the winter of 2012: 90% fruit buds affected at the 'Springold' cultivar, 94% fruit buds affected at the 'Springcrest' cultivar and 62% fruit buds affected at the 'Redhaven' cultivar. The losses caused by the hale which occurred on the 11th of July 2014 reduced the production of the 'Redhaven' cultivar by 40% and that of the 'Southland' cultivar by 80%. The carried out studies and the obtained results demonstrate both the importance of choosing the assortment of cultivars according to favourable areas as well as the importance of placing anti-hale nets upon establishing fruit-growing plantations.

Key words: *climate changes, late frosts, Prunus persica, 'Redhaven', 'Southland'.*

INTRODUCTION

In this paper is presented the manner in which the frost and the hail influenced the fruit production of certain peach tree and nectarine tree cultivars cultivated in Dobrogea between 2014-2014.

The frosts which occur in March and April after a relatively warm period are more dangerous than those which occur during the obligatory resting period (December-January). The fruit buds in the pink button stage can resist to temperatures as low as -3.9°C for 2-3 hours; the opened flowers can tolerate a temperature of -2.8°C, while the newly tied fruits can resist to temperatures as low as -1.1°C (Chira et al., 2005). Nevertheless, the major climatic changes which have taken place during the last few years have had a significant negative influence over the triggering of the flowering, the tying of the fruit and, evidently, over the peach tree and nectarine tree production. The climate changes problems should not be ignored in this case and might be a relevant subject for further researches.

Previous research papers have revealed that the impact of climatic changes upon fruit-growing species can already be felt. For instance, by the end of the 90's, the flowering of the trees in Germany occur several days earlier (Chmielewschi et al., 2004 and 2005). The vegetative season in Europe became longer by 10 days in the past 10 years (Chmielewschi and Rotzer, 2002).

Due to the early flowering of the trees, in certain regions of Europe there was an increase in the risk of damage caused by late frosts (Anconelli et al., 2004; Sunley et al., 2006; Legave and Clazel, 2006; Legave et al., 2008; Chitu et al., 2004 and 2008) or by the disorders

in the pollination and fruit setting processes (Zavalloni et al., 2006).

According to the estimations of the weather forecasts, there have been presented in the frame of the 4th report of the International Committee for Climatic Changes in 2007, the whole Europe and implicit Romania will face in future with a process of global warming, characterized by increasing of temperatures with -0.5 - 1.5° C for the period 2020 – 2029 and with -2 – 5° C for the period 2029 – 2099. In the 2090-2099 periods, Romania will confront with pronounced drought during the summer time. Researches from many countries, in the frame of climatic research methodology have the approached aspects regarding climatic changes effects on growth and development of some fruit tree species (Chmielewski and Rotzer et al., 2002; Olensen 2002; Sunley et al.2006, Chitu et al., 2010; Sumedrea et al, 2009). Climatic changes occurred also in Romania, they have determined meteorological phenomena, which are manifesting with augmented amplitude and intense frequency (severe drought, intense flooding, tornados and hail).

MATERIALS AND METHODS

The research was carried out in the period 2012-2014 at R.S.F.G. Constanţa in Valu lui Traian. The studied material was represented by the experimental plots from R.S.F.G. Constanţa, where the peach tree and nectarine tree cultivars can be found. A number of 10 such cultivars were studied (with a different ripening period), out of which 7 were peach tree cultivars – 'Springold', 'Springcrest', 'Cardinal', 'Collins', 'Redhaven', 'Southland', 'Jerseyland' and 3 were nectarine tree cultivars – 'Cora', 'Delta' and 'Romamer 2'. The trees were planted in 1986, the utilised parent stock being *Prunus persica*; the planting density is of 625 trees/ha (planting scheme 4m x 4m) and the trees' shape of the head is that of a free palmette. As far as the soil concern on which the plantation is situated is a calcareous cernoziom with a claylike texture and only slightly alkaline pH (8.2) throughout its entire profile. In addition, the overall climatic conditions were favourable to the growth and fructification of the trees, with exception of the years 2012 – 2014, when a very strong frost was registered in both January and February, leading to the loss of some of the fruit buds, while the hail on July 11th, 2014 affected the production of the 'Redhaven' and 'Southland' cultivars. With regard to these cultivars we observed the main fructification phenophases: the beginning of the blossoming, upon the appearance of the pink button; the beginning of the flowering, upon the appearance of the first open flowers; the ending of the flowering, when most of the flowers have lost their petals. The duration of the flowering phenophase at a certain cultivar can vary according to the action of the maximum temperatures during the day and the intensity of the wind, correlated with the degree of differentiation of the trees (i.e. the amount of flowers per tree). The intensity of the flowering was ranked on scale from 0 to 5, 0 being used when the cultivars displays no flowers at all, while 5 is used when the cultivar displays a plethora of flowers. The hardening of the core was determined by means of piercing it with a needle at regular intervals, usually 2 days. The process was carried out progressively, in the same day for all the observed cultivars. The harvesting maturity is largely influenced by a series of climatic and agro-technical factors, such as: temperature, drought, quantity of fruit per tree, shape of the head, density of the trees, etc. The observations and determinations were carried out 3-5 days after the climatic accidents recorded in 2012, 2013 and 2014, respectively and the production was assessed after the hail occurrence on July 11th, 2014. The hail, with a dimension of approximately 5-20 mm, seriously damaged the fruit production of some of the peach tree cultivars, more exactly those who had not been harvested until July 11th, 2014. The climatic data were recorded with the aid of an automatic meteorological station (the WatchDog type) and were processed as daily averages. We observed the manner in which certain peach tree and nectarine tree cultivars reacted to the change in the climatic conditions recorded during the winter of the previously mentioned years. We noticed that the resistance of peach tree cultivars differs from one year to the next because of the climatic changes that have occurred during the past few years and it depends on the gravity of climatic accidents.

The minimum and maximum temperatures during winter alternate and together with the gravity of climatic accidents lead to the weakening of the trees.

RESULTS AND DISCUSSIONS

The triggering of the main fructification phenophases in the years 2012-2014 occurred between rather wide limits, according to the characteristics of the cultivar and the climatic characteristics of the studied years.

In the period 2012-2014, the blossoming of the fruit buds of the peach trees occurred between the following limits: between 18.03 and 29.03 for the 'Springold' cultivar, between 21.03 and 27.03 at the 'Springcrest' cultivar, between 24.03 and 30.03 at the 'Collins' cultivar, between 24.03 and 29.03 at the 'Cardinal' cultivar, between 28.03 and 03.04 at the 'Redhaven' cultivar, between 24.03 and 03.04 at the 'Jerseyland' cultivar and between 27.03 and 04.04 at the 'Southland' cultivar. The blossoming at the peach tree occurred between 18.03 and 04.04 (17 days) in the studied years 2012-2014. (Table 1).

The beginning of the flowering. For all the studied cultivars the beginning of the flowering in the period 2012-2014 was recorded; however, the cultivars entered this phenophases at different times, albeit not necessarily significant (a few days from one cultivar to the next), so that cross pollination was fully ensured. The limits for this phenophase were 26.03 and 21.04.

Table 1. The main stages of peach fructification in the 2012-2014 periods

No.	CULTIVAR	Year	The swelling of the flowering buds	The flowering			Intensity	The hardening of the stone	Harvesting maturity
				Beginning	Ending	Duration (days)			
1	SPRINGOLD	2012	18.03	26.03	16.04	20	2	04.06	26.06
		2013	25.03	06.04	21.04	15	2	10.06	27.06
		2014	29.03	03.04	12.04	9	4	07.06	01.07
		Limits	**18.03-29.03**	**26.03-06.04**	**12.04-21.04**	**9-20**	**2-4**	**04.06-10.06**	**26.06-01.07**
2	SPRINGCREST	2012	21.03	29.03	16.04	19	2	04.06	28.06
		2013	27.03	08.04	24.04	22	4	08.06	07.07
		2014	22.03	05.04	16.04	12	3	10.06	**09.07**
		Limits	**21.03-27.03**	**29.03-08.04**	**16.04-24.04**	**12-22**	**2-4**	**04.06-10.06**	**28.06-09.07**
3	COLLINS	2012	24.03	30.03	11.04	12	3	02.06	18.07
		2013	29.03	08.04	21.04	13	4	10.06	16.07
		2014	30.03	09.04	20.04	11	3	12.06	27.07
		Limits	**24.03-30.03**	**30.03-09.04**	**12.04-30.04**	**11-13**	**3-4**	**02.06-12.06**	**16.07-27.07**
4	CARDINAL	2012	26.03	04.04	17.04	13	2	06.06	13.07
		2013	29.03	09.04	23.04	14	3	10.06	18.07
		2014	24.03	20.04	28.04	8	2	08.06	25.07
		Limits	**24.03-29.03**	**04.04-20.04**	**10.04-28.04**	**8-14**	**2-3**	**06.06-10.06**	**13.07-25.07**
5	REDHAVEN	2012	02.04	05.04	20.04	12	4	08.06	29.07
		2013	28.03	11.04	19.04	8	5	10.06	02.08
		2014	03.04	20.04	30.04	10	4	07.06	12.07
		Limits	**28.03-03.04**	**05.04-20.04**	**19.04-30.04**	**8-12**	**4-5**	**07.06-10.06**	**12.07-02.08**
6	JERSEYLAND	2012	24.03	05.04	18.04	13	4	07.06	17.07
		2013	29.03	09.04	16.04	7	5	09.06	15.07
		2014	03.04	18.04	27.04	9	4	10.06	19.07
		Limits	**24.03-03.04**	**05.04-18.04**	**16.04-27.04**	**7-13**	**4-5**	**07.06-10.06**	**15.07-19.07**
7	SOUTHLAND	2012	27.03	06.04	13.04	7	5	09.06	04.08
		2013	29.03	08.04	17.04	9	5	11.06	30.07
		2014	04.04	21.04	27.04	6	5	07.06	06.08
		Limits	**27.03-04.04**	**06.04-21.04**	**13.04-27.04**	**6-9**	**5**	**07.06-11.06**	**30.07-06.08**

The ending of the flowering. In the studied period 2012-2014 the ending of the flowering occurred between 12.04 and 21.04 for the 'Springold' cultivar, between 16.04 and 24.04 for the 'Springcrest' cultivar, between 12.04 and 30.04 for the 'Collins' cultivar, between 10.04 and 28.04 for the 'Cardinal' cultivar, between 19.04 and 30.04 for the 'Redhaven' cultivar, between 16.04 and 27.04 for the 'Jerseyland' cultivar, between 13.04 and 27.04 for the 'Southland' cultivar. The dates were recorded as the days when the flowers lost their

last petals. The duration of the flowering at the peach tree (average for the three studied years) expressed in number of days varied between 6 days (the 'Southland' cultivar in 2014) and 22 days (the 'Springcrest' cultivar in 2013).

The intensity of the flowering. In 2012 the following cultivars displayed a weak intensity of the flowering: 'Springold' - 2, 'Springcrest' - 2, 'Cardinal' - 2 and 'Collins' - 3.

The hardening of the core. This phenophase occurred in the first half of the month of June (between the 6th and the 11th) in the years 2012, 2013 and 2014.

The harvesting maturity. Each ripening period had large variation limits from one year to another, depending on how the climatic factors determine the type of vegetation in a specific year: early, late or extra late. The harvesting maturity of the fruit had as variation limits the 26th of June and the 6th of August.

At the nectarine trees, the blossoming occurred between 16.03 and 04.04 (Table 2).

Table 2. The main stages of nectarine fructification in the 2012-2014 periods

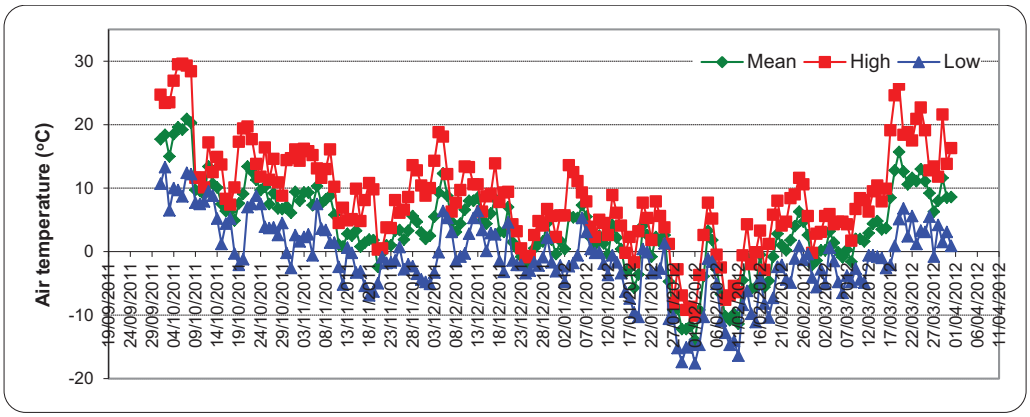
No.	CULTIVAR	Year	The swelling of the flowering buds	The flowering			Intensity	The hardening of the stone	Harvesting maturity
				Beginn-ing	Ending	Duration (days)			
1	CORA	2012	16.03	29.03	14.04	15	5	05.06	19.06
		2013	27.03	06.04	23.04	17	5	11.06	27.06
		2014	25.03	10.04	28.04	18	5	07.06	28.06
		Limits	**16.03-27.03**	**29.03-10.04**	**14.04-28.04**	**15-18**	**5**	**05.06-11.06**	**19.06-28.06**
2	DELTA	2012	20.03	29.03	16.04	19	5	04.06	23.06
		2013	29.03	08.04	30.04	22	5	08.06	20.06
		2014	21.03	05.04	16.04	12	5	10.06	06.07
		Limits	**20.03-29.03**	**29.03-08.04**	**16.04-30.04**	**12-22**	**5**	**04.06-10.06**	**20.06-06.07**
3	ROMAMER 2	2012	24.03	28.03	11.04	13	5	04.06	08.07
		2013	04.04	06.04	30.04	24	5	10.06	11.07
		2014	30.03	02.04	24.04	22	5	14.06	13.07
		Limits	**24.03-04.04**	**28.03-06.04**	**11.04-30.04**	**13-24**	**5**	**04.06-14.06**	**08.07-13.07**

The limits for the beginning of the flowering in the studied years 2012-2014 were 29.03 and 08.04. The duration of the flowering (average for the three analysed years) expressed in number of days varied between 12 days (the 'Delta' cultivar in 2014) and 24 days (the 'Romamer 2' cultivar in 2013). All the studied cultivars displayed a large abundance of flowers and obtained the grade 5 in all the three studied years. The hardening of the kernel occurred in the first half of the month of June (between the 4th and the 14th). The harvesting maturity of the fruit had as variation limits the 19th of June and the 13th of July, period in which there are no other nectarine types on the market. This constitutes a great advantage for retailers through the income that can be realised. The cultivars become ripe at a difference of 3-5 days one from the other.

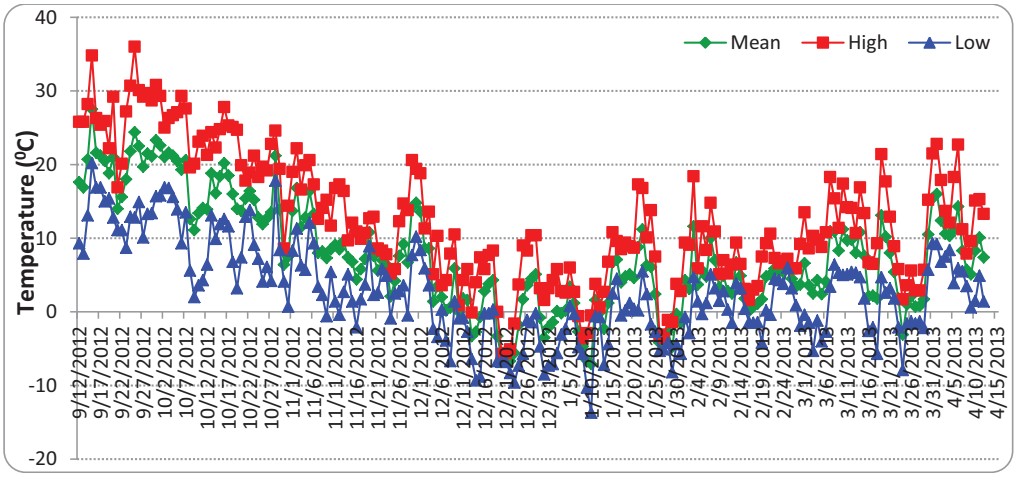
As we can notice in Figure 1a, January of 2012 was the coldest month, during which 9 days recorded daily average temperatures ranging from -10.2 °C and -17.6 °C. These values, together with those that were extremely varied in February (7 days with daily average temperatures -10.4 -16.4 °C la °C) and 8 consecutive days of hoarfrost, the ice on the branches caused the loss of 19% - 94% of the fruit buds at the studied cultivars**.**

Figure 1b. reveals the fact that the coldest month in the period September 2012 - April 2013 was January 2013, when the recorded values were as low as -13.7°C (January 10th, 2013). These values did not significantly influence the loss of fruit buds at the peach tree cultivars (local observations).

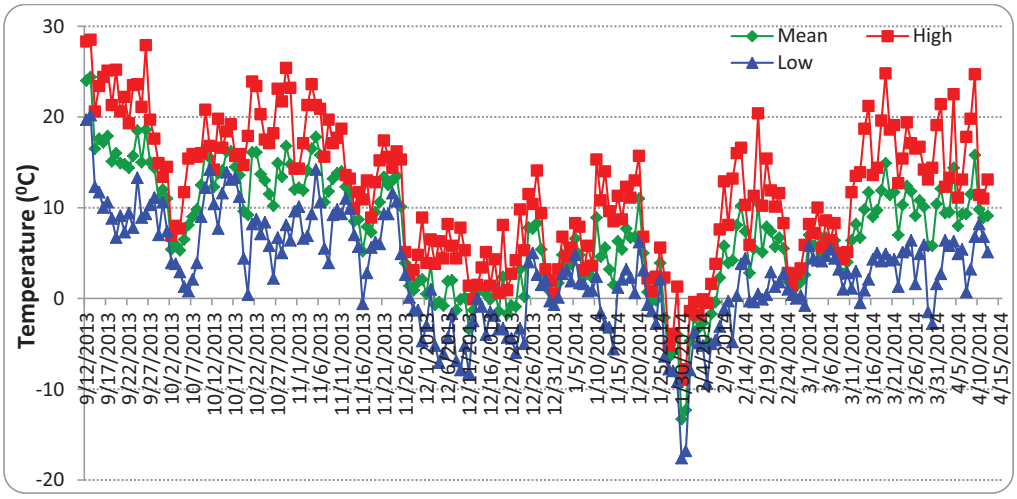
In the period October 2013 - March 2014 (Figure 1c.) the lowest temperature was recorded in January: -17.6 °C (January 30th, 2014); another day when the recorded temperature was low (-9.4° C) was February 5th, 2014. The low temperatures recorded during this period affected the 'Cardinal' cultivar (57%) and the 'Jerseyland' cultivar (70%).



1 a



1b



1c

Figure 1. a,b,c,. Air temperature (°C) in the cold period October 2011 – March 2012 (a), October 2012 – March 2013 (b), October 2013 – March 2014 (c) at Valu lui Traian, Constanţa

The observations were carried out with the aim of assessing the losses of fruit buds because of temperature variations during winter and the low temperatures during the day.

Thus, for the 'Springold' cultivar the losses recorded for 2012 were of approximately 90%, 14% for 2013 and 49% for 2014, there being difference from one cultivar to another. The winter frost caused losses for the 'Springcrest' cultivar of 94% in 2012, 21% in 2013 and 48% in 2014.

For the 'Cardinal' cultivar, the losses were of 66% in 2012, 19% in 2013 and 57% in 2014. We must bear in mind the fact that the losses caused by the winter frost of 2012, together with those caused by hoarfrosts and late frosts were very severe, taking also into account the surface of the Station's orchards cultivated with this cultivar.

These losses were also caused by the warm period before the frost – in the first three weeks of January 2012 the average temperature of the air was positive, of approximately 5 °C.

For the 'Collins' cultivar the losses were of 54% in 2012, 29% in 2013 and 53% in 2014. The 'Redhaven' cultivar recorded losses of 62% in 2012, 15% in 2013 and 56% in 2014. The 'Jerseyland' cultivar recorded losses of 63% in 2012, 27% in 2013 and 70% in 2014. For the 'Southland' cultivar the recorded losses were of 48% in 2012, 21% in 2013 and 49% in 2014.

The losses caused by frost recorded by the nectarine tree cultivars were rather small: for the 'Cora' cultivar they were of 23% in 2012, 19% in 2013 and 28% in 2014, for the 'Delta' cultivar, 21% in 2012, 17% in 2013 and 14% in 2014, while for the 'Romamer 2' cultivar, the losses were of 19% in 2012, 9% in 2013 and 29% in 2014 (Figure 2).

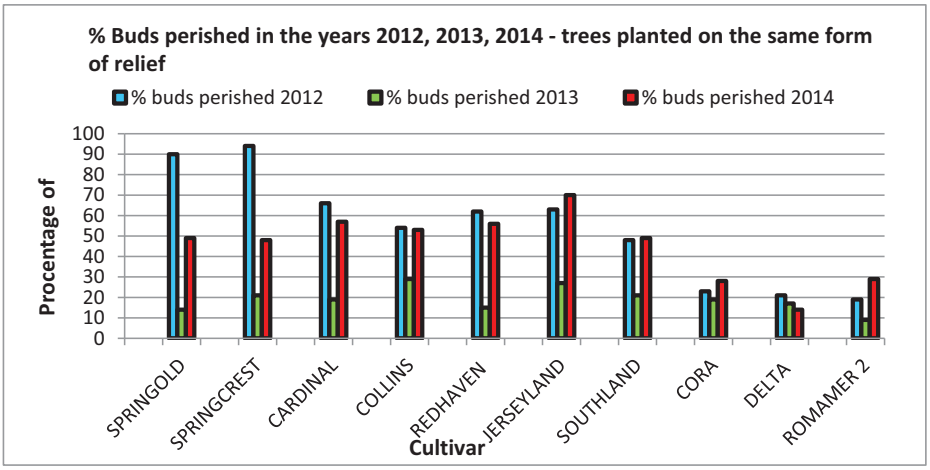


Figure 2. Procentage of peach and nectarine tree flowering buds perished due to frosts during the winter of 2012,2013 and 2014 at Valu lui Traian, Constanța

A good resistance to frost during the winter of the three studied years was remarked at the nectarine cultivars, with the following percentages: 'Cora' - 23%, 'Delta' - 17% and 'Romamer 2' - 19% (Figure 3).

In these conditions, the 'Springold' and 'Springcrest' cultivars were more than 50% damaged, while other cultivars such as 'Redhaven' and 'Southland' were less affected, the percentages being 44% and 39%, respectively. The climatic accidents recorded in January and February 2012 (sudden temperatures of -16.4°C, minimum temperature during the day) and 8 days of hoarfrost caused the damaging of the production for the early cultivars 'Springold', 'Springcrest' and 'Cardinal', while the 'Redhaven', 'Collins' and 'Southland' were only partially affected.

At R.S.F.G. Constanţa, in the second week of June 2014, more exactly on July 11th, the amount of precipitations was accompanied for 10 minutes by hail, which affected 80% of the fruit production for the 'Southland' cultivar (the fruit were just beginning to ripe) and 40% for the 'Redhaven' cultivar (Figures 4 and 5).

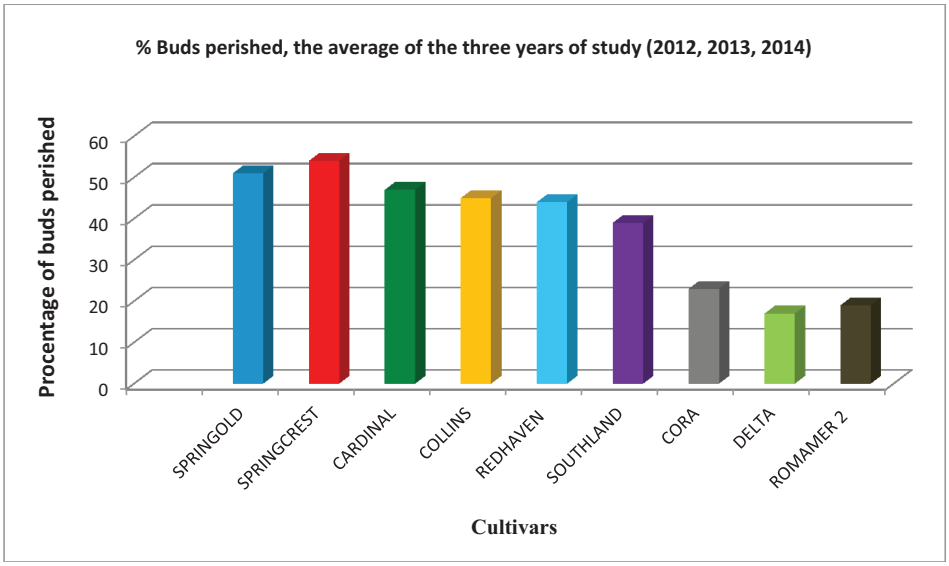


Figure 3. Procentage of peach and nectarine tree flowering buds affected by frosts (average over the three years), Valu lui Traian

Figure 4. Fruit of the 'Southland' cultivar affected by the hail on July 11th, 2014

Figure 5. The 'Redhaven' cultivar affected by the hail on July 11th, 2014 (full maturity)

The hail bruised the fruit, shoots and stems, thus creating an environment for future infections and diseases. The bruises on the fruits, despite some of them becoming scars, diminished the commercial aspect and the quality of the production.

Although the south-eastern part of Romania is generally considered favourable to the culture of the peach tree, the specie has suffered greatly over the past decade because of climatic variations which manifested themselves mainly through the aggressiveness of low temperature in alternation with maximum positive temperatures. The peach tree encountered considerable losses because of temperature variations which occurred during the dormand period, in the climatic conditions of 2012, 2013 and 2014; the losses were also caused by late hoar frosts in spring, especially in the second half of March and in April, as well as by hail occurrences.

CONCLUSIONS

The novelty brought forward by the results is represented by the fact that the winter frosts from 2012, 2013 and 2014 affected the peach tree and the nectarine tree to various extents, according to the cultivar (approximately 9-94%).

The greatest production losses were recorded in 2012 – 94% for the 'Springold' cultivar and 90% for the 'Springcrest' cultivar.

The smallest losses during the three studied years were recorded by the nectarine tree cultivars 'Cora', 'Delta' and 'Romamer 2'.

The hail from July 11th, 2014, which lasted for only 10 minutes, affected the 'Redhaven' cultivar (40%) and the 'Southland' cultivar (80%).

In order to protect the trees from hail occurrences we recommend that the orchards be equipped with anti-hail nets.

Moreover, when choosing the assortment of cultivars to be cultivated in a specific area one must make sure that particular area is favourable to the setting up of fruit-growing plantations.

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STUDY REGARDING THE PRODUCTIVITY OF SOME PEACH VARIETIES

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Abstract

The study describes the behavior of eight peach varieties, with high adaptability to the pedoclimatic conditions of the South-Eastern region of the country, with very good productivity, increased frost and disease strength and with very good quality of the fruit. The objective of the study was the improvement of the peach assortment related to productivity and the extension of the fresh fruit consumption period. The best average yield was recorded to 'Raluca' (24.55 t/ha), followed by 'Southland' (24.02 t/ha). The ripening season of the fresh peach is extended to 97 days.

Key words: *Prunus persica, assortment, yield, fruit quality.*

INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is the third most important temperate tree fruit species in terms of production quantity (http://faostat.fao.org). It is originated from China where it has been cultivated for more than 4000 years (Cepoiu, 2006).

Peach breeding programs are confronted with the need to find genetic solutions to ever changing posed by disease and pests, the ever-changing environment (e.g. drought, global warming, cold temperatures, etc), superior quality of fruit, as well as the widening of the ripening time of the peach varieties for fresh market (Bassi & Monet, 2008) .

At Research Station for Fruit Growing Constanta (RSFG Constanta) peach is growing since 1960-1965 (table 1). The present study had as aim to improve the peach assortment in direct relation with their productivity and with the extension of fresh fruit consumption.

Table 1. The evolution of peach assortment (1965-2014) at RSFG Constanta

Year	Species	Number of varieties	Number of days
1965	Peach	7	50
	Nectarine	-	-
	Clingstone	-	-
1970	Peach	10	65
	Nectarine	-	-
	Clingstone	-	-
1980	Peach	15	90
	Nectarine	4	30
	Clingstone	4	30
1990	Peach	14	95
	Nectarine	8	55
	Clingstone	8	50
1995	Peach	12	97
	Nectarine	10	70
	Clingstone	10	50
2014	Peach	11	97
	Nectarine	13	75
	Clingstone	5	60

MATERIALS AND METHODS

RSFG Constanta is located in the south-eastern part of Romania, in the area between the Danube River and the Black Sea, and has specific steppe climatic conditions, with a semi-arid character.

Frosts return is a quite often phenomena in spring and affect fruit trees with early blooming as nectarine. Absolute temperature beyond the limits of resistance of peach and nectarine species, e.g. -25°C or above +40°C is rare (1/20

or 1/30 years). Rainfall is deficient to the requirements of the trees; the average amount of rainfall is around 400 mm, with unequal time distribution in the active growing season (April 1 to September 30). Chernozem soil type is deep, well supplied with humus, showing proper conditions for water circulation.
Phenological observations and measurements, and physical and chemical analyses on plants were done. The beginning of flowering was the date when 25% of the flowers in the different parts of the crown were in full bloom. The blooming intensity was noted from 0 (absent) to 5 (abundant), according to the research methodology of fruit tree breeding (Cociu, 1989). The trees and fruit characteristics were evaluated according to the Methodology for trying new varieties of fruit trees, shrubs and rootstock in order to approve the homologation and International Union for the Protection of New Varieties of Plants (UPOV) guidelines.
During 2010-2014 the fruit yield was recorded starting with the 6th year after planting, when the fruit production was considered stable. Yield was described numerically by visually estimating the yield weight (kilograms per tree) approximately one week before the harvest maturity.

RESULTS AND DISCUSSIONS

The studied biological material is represented by eight peach cultivars, three of them created in Romania and five varieties from abroad, but well adapted in our climatic conditions.
The beginning of blooming at peach varieties is started on the 23th of March (in the years with early spring) at 'Springold' and 'Springcrest', follow by the other studied varieties (at 2-3 days apart), table 2. In the years with late spring, the blooming started on the 7th of April and ended on the 19th of May ('Redhaven'). The blooming time period at each variety was about 12-15 days in every year. The end of blooming was grouped on the 9.04 to 18.04 in the years with early spring and on the 19.04 to 03.05 in the years with cold spring.
The blooming intensity was noted with 3, 4 and 5 (maximum) at 'Springold', 'Springcrest', 'Raluca', and 'Southland', that means a large number of flower buds were differed.
The fruit ripening was recorded between 18.06 (early varieties) and 5.09 (late peach varieties), the fresh consumption of the peach lasting two and a half month.

Table 2. Phenological stages of some peach genotypes (multiannual data/RSFG Constanta)

Genotype	Origin	Beginning of flowering	End of flowering	Flowering intensity	Ripening time
Springold	USA	25.03-7.04	11.04-19.04	5	18.06-30.06
Springcrest	USA	24.03-9.04	11.04-22.04	5	22.06-03.07
Cardinal	USA	27.03-10.04	18.04-29.04	3-4	2.07-12.07
Raluca	Romania	29.03-15.04	25.04-03.05	5	11.07-21.07
Redhaven	USA	31.03-19.04	13.04-30.04	4-5	17.07-28.07
Southland	USA	31.03-21.04	9.04-20.04	5	27.07-15.08
Superba de toamnă	Romania	1.04-15.04	10.04-22.04	4	5.09-15.09
Flacăra clon 1	Romania	31.03-17.04	7.04-16.04	3-4	27.08-6.09

Fruit of all those peach varieties have a nice appaearance, bright red colour, big size and a good balanced taste (table 3). The dry matter content was higher for 'Raluca' (12.8 %) and 'Flacăra clon 1' (11.3). The acidity was 0.37 mg/100 g flesh fruit ('Raluca') and 0.78 ('Flacăra clon 1').

During 2010-2014, the average fruit weight was 75 g ('Springold') and 220 g ('Flacăra clon 1'), table 3; average yield for 'Flacăra clon 1' is 14.7 t/ha while for the 'Raluca' cultivar is 24.66 t/ha (table 4). The fruit destination is for fresh consumption and processing.

Table 3. Quality test of fruit (multiannual date/RSFG Constanta)

Genotype	Fruit appearance	Fruit flesh	Fruit mean weight (g)	Dry matter (%)	Acidity* (mg%)
Springold	Spherical, yellow with 30% red	Yellow, ferm, good quality	75	9.0	0.67
Springcrest	Spherical, yellow with 60% red	Yellow, juicy	97	7.7	0.66
Cardinal	Spherical, yellow with 60% red	Yellow, juicy, good taste	160	9.3	0.58
Raluca	Spherical, yellow with 80% red	Yellow, very juicy, good flavor	195	12.8	0.37
Redhaven	Spherical, yellow with 90% red	Yellow, juicy, very good taste	180	9.4	0.71
Southland	Ovoidal, yellow, 55% orange	Orange flesh, ferm, very good taste	250	9.9	0.61
Superba de toamnă	Spheric-ovoidal, white-pink,70% red	White, juicy, good flavor	150	10.0	0.56
Flacăra clon 1	Ovoidal, yellow, 60% red	Yellow, juicy, good taste	220	11.3	0.78

*Acidity: mg malic acid/100 g flesh fruit

Table 4. Average yield of some genotype of peach (2010-2014) RSFG Constanța

Genotype	Yield (t/ha)**						Fruit destination
	2010	2011	2012	2013	2014	Average (t/ha)	
Springold	19.5	23.0	25.2	21.7	23.3	22.54	Fresh consumption
Springcrest	21.7	24.9	25.7	23.0	25.1	24.08	Fresh consumption
Cardinal	14.3	15.0	14.8	15.3	14.5	14.78	Fresh consumption and proccesing
Raluca	21.8	23.0	25.7	26.0	26.8	24.66	Fresh consumption and proccesing
Redhaven	19.4	22.0	26.0	25.3	22.7	23.08	Fresh consumption and proccesing
Southland	19.7	21.7	25.0	27.7	26.0	24.02	Fresh consumption and proccesing
Superba de toamnă	13.3	15.0	15.3	16.1	13.8	14.7	Fresh consumption and proccesing
Flacăra clon 1	13.0	14.7	16.0	19.4	15.0	15.62	Fresh consumption and proccesing

**The orchard density: 833 trees/ha

CONCLUSIONS

The studied peach varieties could be successfully extended in areas were the peach finds favorable conditions, enriching the present assortment due to their qualities such as: superior fruit taste and flavor, annual high and constant productivity, precocity of fruiting.
Blooming at the intervals on the same tree had the advantage that the spring frosts damaged only partially the flower buds, even when flowering was early (24.03-1.04).
The earliest varieties are 'Springold', 'Springcrest', 'Cardinal' and 'Raluca' (middle of June-beginning of July), and the latest is Flacăra clon 1 (September).
During the study (2010-2014) the highest average yield proved to be recorded at 'Raluca' (24.66 t/ha) and 'Southland' (24.02 t/ha), followed by and 'Springcrest' (24.08 t/ha) and 'Redhaven' (23.08) t/ha.

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THE ADAPTATION CAPACITY OF THE HYBRID VT 66.30.52 IN DOBROGEA CLIMATE CONDITIONS

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Abstract

The expansion of apricot consumption depends on the marketing, the quality of the fruit upon harvesting and on their cost. Market trends that have an impact upon apricot consumption are: globalization, the need for year round supplies of produce, the high cost labour, the diversification of the safety issues of pesticide use and bacterial contamination of fresh fruit. These pressures have renewed the interest in production systems in order to extend the harvest season, to reduce chemical inputs and to ensure a consistent fruit quality. Therefore, our efforts were focused on developing new varieties with high quality of fruit, higher levels of production, a greater diversity of fruit types to market and the adaptation to climate changes which have recently begun to occur. In this paper we studied the characteristics of the VT 66.30.52 hybrid, having as witness the variety Marculesti 22/4. The experimental determination was conducted the at Research Station for Fruit Growing Constanța between 2012 and 2015. The hybrid VT 66.30.52 proved to be superior to the witness in terms of weight of the fruit, their firmness, the dry matter and the colour of the skin.

Key words: *Prunus armeniaca, assortment, variety, promotion.*

INTRODUCTION

Throughout the entire world the research carried out concerning the apricot trees have among its main objectives the relationship between the climatic conditions and the cultivation of apricot trees. In our country, this relationship has been studied by many authors, among which we should mention the results obtained by Constantin N. ş.a (1955), Burloi Niculina (1957), Bordeianu T. et all. (1961), Cojean Natalia (1961), Mănescu Creola et al. (1975), Topor Elena (1987, 2002), Stancu T. et al. (1989), Roman Ana Maria (1998), Topor Elena (2009).

The results obtained by all these studies corresponded to a certain period of time and to a certain assortment of varieties, thus creating the link between the biology of the apricot tree and the climatic conditions in the area where it is cultivated (Topor E. 2009).

The purpose of this paper is to study the apricot tree hybrid VT 66.30.52 as concerns its phenological phases, its growth and productivity as well as the quality of the fruit, in order to improve the assortment of apricot trees in the climatic conditions of Dobrogea.

MATERIALS AND METHODS

The studies were carried out between 2012 and 2015 at the Research Station for Fruit Growing Constanta, within the Breeding Apricot Tree Laboratory. The biological material consisted of 2 apricot tree hybrids, part of a national collection planted in spring of 2009. Each hybrid is represented by 3 trees, planted at a distance of 4/4, with the shape of the head being a free flat palm in the direction of the row. The applied culture technology is that specific to apricot trees, containing fructification cuts, phytosanitary treatments, soil works, irrigation, harvesting, conditioning and capitalisation of the fruit. In order to construe the data, observation were made concerning the triggering and the evolution of the vegetative and fructification stages, as well as the quantity and quality of the fruit production. The determination of physical and organoleptic characteristics was performed according to the regular methodology for the study of varieties, as follows: the weight of the fruit was determined by weighing all fruit within a sample (25 fruit) and the average weight was calculated in g/fruit; the colour of

the fruit and of the pulp was established through direct visualizing, with the aid of colour codes (plastic tags with specific colours); the fruit's content of dry substance was determined by means of a digital refractometer, in Brix degrees.
The main chemical components were determined within the Chemistry Laboratory of ICDP Piteşti, as follows: the total quantity of sugar through the Fehling volumetric method; the total acidity through the titrimetric method, using phenolphthalein as indicator. The results were statistically processed by means of variance.

RESULTS AND DISCUSSIONS

In order for the blossoming phenophase to begin in the pedoclimatic conditions of SCDP Constanta, the VT 66.30.52 hybrid required 205.6 – 271.1ºC and a period of 5 to 10 days, enough for the pollination and the fertilization.
For the 'VT 66.30.52', the blossoming took place in 2013 on the 21th of March, six days earlier that the witness variety Marculesti 22/4, and in 2015 on the 19th of March (table 1).

Table 1. Main fructification phenophases (2013-2015)

Variety	Year	Beginning of blossoming		Ending of blossoming		Duration of the bloss. (days)	Ripening of the fruit		Duration of ripen. (days)
		Date	tºC	Date	tºC		Date	tºC	
VT 66.30.52	2013	21.03	142.2	2.04	218.8	13	23.06	1479.7	93
	2014	25.03	191.3	4.04	271.1	11	20.06	1526.8	88
	2015	19.03	139.8	30.03	205.6	12	15.06	1413.1	87
Marculesti 22/4 mt.	2013	27.03	159.5	5.04	242.9	10	1.07	1700.3	107
	2014	28.03	191.3	4.04	271.1	9	23.06	1693.4	87
	2015	25.03	191.3	4.04	271.1	11	1.07	1501.7	97

The date when the fruit become ripen is a biological characteristic for the 'VT 66.30.52'; this stage took place in 2014 and 2015 on the 15th of June, eight and nine days respectively earlier than the witness variety, proving that it belongs in the category containing varieties with extra-early ripening of the fruit (until the 30th of June).

The vigour of the trees' growth, represented by the growth in thickness of the trunk and the total growth of the annual sprouts between 2013 and 2015 (vegetative years V - VII) reveal a lower vigour for the 'VT 66.30.52', as compared to the witness variety Marculesti 22/4, this hybrid being considered as having a medium vigour (table 2).

Table 2. Surface of the trunk section and the total growth of annual sprouts
Years V-VII since cultivation (2013-2015)

Variety	Surface of the trunk section cm²				Growth rate 2013 - 2015			Average growth rate	Average number of sprouts/tree			Total growth of annual sprouts linear meters		
	2012	2013	2014	2015	2013	2014	2015		2013	2014	2015	2013	2014	2015
VT 66.30.52	37.3	49.1	54.9	59.9	11.8	5.8	5.0	7.5	70	40	79	18.3	17.3	21.9
Marculesti 22/4	34.2	62.5	71.8	98.1	28.2	9.3	26.3	21.2	58	60	93	21.2	31.7	34.8

Considering the tree as a whole and judging by the surface of the trunk section, the rate of the growth in thickness and the sum of the annual vegetative growth, it is revealed that the VT 66.30.52 has the tendency of having a moderate habitat, the fructification occurring mostly on branches that are 1 year old – mixed branches and May bouquets.

Analysing the average fruit production over the three years, we can state that the VT 66.30.52 fits in the category of productive varieties, its production ranging from 11 to 15 t/ha. Thus, it proves that it is worth being taken into account due to this quality (table 3).

Table 3. Fruit production over the period 2013-2015.

Variety	Year`	Average prod. 2013-2015		Diff. comp. to the witness +-	Signif.	Production dex kg/cm trunk sect.
		Kg/tree	t/ha			
VT 66.30.52	2013	23.7	14.8	+8.9	xxx	0.48
	2014	16.0	10.4	+4.1	xxx	0.29
	2015	22.4	11.2			0.37
	Average	20.7	12.13			
Marculesti 22/4	2013	9.4	5.9	-	-	0.15
	2014	10.0	6.3	-	-	0.13
	2015	15.0	7.5	-	-	0.15
	Average	11.4	6.5			

DL. 5% - 0.45; DL.1% - 0.61; DL. 0.1% - 0.81

From the determinations that were carried out, the conclusion was the fruit's loss of weight was a consequence of the draught in 2013. This hybrid managed to accumulate an average quantity of 13.7g/100g S.P. total sugar and 15.9% S.U.T., (table 4) which is a predominant characteristic of chosen genitors.

Table 4. Main physical and chemical characteristics

Variety	Year	Average weight of the fruit g.	% core	S.U.T. %	Total sugar g/100gS.P.
VT 66.30.52	2013	56	6.0	14.5	10.8
	2014	22.6	8.3	16.0	14.5
	2015	54.5	5.6	17.3	15.8
	Average	44.3	6.6	15.9	13.7
Marculasti 22/4	2013	60	6.0	14.5	10.3
	2014	34.6	5.4	14.2	12.5
	2015	50.2	9.3	14.5	12.9
	Average	48.2	6.9	14.4	11.9

The fruit which ripens early has a small to medium size, it is symmetrical and round (table 5). The main colour of the skin is orange with carmine on the sunny side. The pulp is light orange, with a smooth texture and a soft consistency and it does not adhere to the core.

Table 5. Shape and size of the fruit

Variety	D	d	H	Shape index (mm)
VT 66.30.52	48	40	55	1.1
Marculesti 22/4	41	37	53	1.2

According to the value of the attack degree of the frequency, the 'VT 66.30.52' hybrid fits into the resistance group 1=easily attacked in the conditions of the performance of phytosanitary treatments. In order to establish the resistance to disease of the 'VT 66.30.52', the relative resistance index was calculated, R=0.8, which proves that its value is higher than 0.7, this being a resistant variety (table 6).

Table 6. Resistance to *Stigmina carpophila* (2013-2015)

Variety	Frequency of attack %				Intensity of attack %			Resistance group			Degree of attack		
	2013	2014	2015	Average	2013	2014	2015	2013	2014	2015	2013	2014	2015
VT 66.30.52	4.8	4.8	1.5	3.7	+	+	+	1	1	1	0.1	0.4	0.01
Mt.	6.5	3.2	1.3	3.6	+	+	+	1	1	1	0.3	0.1	0.01

CONCLUSIONS

The 'VT 66.30.52' hybrid can be considered a variety with early ripening and it can improve the structure of the current assortment which still lacks in early varieties (in the area). In the district of Constanta, with the aid of the 'VT 66.30.52', the apricot season between the 15th and the 23rd of June become more diverse as concerns the consumption of fresh fruit, this variety being considered a perspective one.

This hybrid offers for the first time in this area the possibility of extending the consumption of early fresh fruit, given the fact that the fruit become ripen until the 20th of June (beginning with the second decade of June) compared to the witness variety. Thus, the hybrid is superior in terms of elements such as: the ripening precocity, the productivity, the commercial aspect, the resistance to diseases, organoleptic traits.

The guarantee for this variety's value is its adaptability to local conditions of climate and soil, expressed through its increased resistance to extreme temperatures in the area, to diseases and pests, which recommends its extension in cultivation.

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SOIL CHARACTERISTICS INFLUENCE THE FATTY ACID PROFILE OF OLIVE OILS

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Abstract

Olive tree is a typical Mediterranean plant grown in Marmara, Aegean, Mediterranean and South East Anatolian regions of Turkey and important oil sources for Mediterranean countries, fulfilling 90 % of the world olive oil production. Turkey is one of the important producer and stakeholders of olive oils after Spain, Italy, Greece and Tunisia. Cultivated olive cultivars in Turkey represent high genetic diversity, which may result in a standardization problem in terms of olive production and their fatty acid composition because the constituents of the fruit and the composition of its oil mainly depend on several factors such as climate, maturity, index variety, etc. Hence, the present study was designed to investigate the optimal soil characteristics for the favourable oil quality. In the study, the olive oils from South-Eastern region in Turkey according to their fatty acid profiles using gas chromatography of their fatty acid methyl esters were characterized and compared using chemo-metrics techniques. In this context, fatty acid profiles characterization was determined on ten olive samples collected from ten different locations. For the statistical evaluation, principal component analysis, variance analysis and correlation analysis were used. Accordingly, physical properties of soils influence the chemical composition and subsequently the quality of olive oils.

Key words: *Soil, olive oil, fatty acid, chemo metrics*

INTRODUCTION

Traditionally, plants have been extensively used for medicinal, nutritional, flavouring, cosmetically and industrial purposes. Of those plants, *Olea europaea* L. (olive) belonging to the Oleaceae family is one of the most important crops especially in Mediterranean countries on which they cover around 8 million hectares on the worldwide (Guinda et al., 2004) and its fruit and oil have a major agricultural importance in Turkey. Besides its fruits as table olive, its fatty oil is characterized with distinguished fatty acid composition, of which sanitary importance has been proven by a number of studies (Leon et al. 2004; Matson and Grundy, 1985). The important property of olive oil, the odour, as well as flavours association with oil quality have been found to be correlated with fatty acid composition (Maestro and Borja, 1990; Leon et al., 2004). Moreover, the oil obtained from olive fruits have essential key roles of reactive oxygen species (ROS) which are associated with pathology of some diseases including cancer, diabetes, cardiovascular, age related, and neurological disorders has been well documented (Chacraborty et al., 2009; Ishii, 2007; Burhans and Weinberger, 2007; Polidori et al., 2007; Halliwell and Guteridge, 1999; Soholm, 1998).

The chemical and physical properties of the soil influence plant growth, development and subsequently main primary and secondary metabolite production, secretion and accumulation. It is worth to note that the produced metabolites transport among the organs of the plants is also significantly affected by soil properties.

Uptake of an element from soil to plant depends on not only on the structure of the element, but also on different physicochemical factors of soil. Herein, transfer factor presents important information with respect to the certain amount of element transport from soil to the plant. Physicochemical parameters such as pH, $CaCO_3$ content, conductivity, organic matter content and soil texture are important factors affecting the transport of elements from the soil to plant species and consequently influence the plant growth, development and subsequently biochemical and physiological aspects of plant (Adriano, 2001: Lindsay and

Norvell, 1978; Kabata-Pendias and Mukherjee, 2007).

Application of chemo-metric approach in characterization of experimental samples has been extensively applied to quantitative evaluation of discrimination of variable results. In the current study, olive oil samples collected from different ten locations were compared for their fatty acid profile using analysis of variance (ANOVA) followed by the multiple comparison test of Duncan using SPSS. Furthermore, some characteristics of the sampling soils including pH, ($CaCO_3$), total salt, P (P_2O_5), K (K_2O) and organic matter were also determined and subsequently correlated with the fatty acid components by Pearson correlation matrix in Excel. Due to the existence of different experimental factors, chemo- metric techniques including Principal Component Analysis (PCA) were applied for analytical evaluation of fatty acid components between locations.

MATERIALS AND METHODS

Experimental Material:

The olive fruits were sampled from the Kilis Yaglik cv. (approximately the same aged trees) from Kilis district of south-eastern part of Turkey. Fruits were also harvested in the same ripening period (mid-December 2015) from the same position on the sampled trees.

Analysis of soil characteristics:

Organic matter by a modification of the Walkley and Black, calcium carbonate ($CaCO_3$) contents by Scheibler Calcimeter, total dissolved salts by Saturation Extract Method, soil phosphorus content by Olsen method, soil potassium content by flame photometer were determined for each samplings locations (Ure, 1990; Kaçar, 1995; Falciani et. al., 2000; Kaçar and İnal, 2008; Marin et al., 2008) The measurements were done in three replicates.

Oil extraction and fatty acid composition analysis:

The oils were extracted from olive fruits (each 10 g sample) with n-hexane for four hours using a Soxhlet Extraction Apparatus (Thermal). Then the solvent was evaporated under reduced pressure and temperature using a Rotary Evaporator (Heidolph). 0.5 g of olive oil was added 10 ml n-heptanes into a screw-capped tube for esterification. The fatty acid analyses were conducted according to the official method COI/T.20/Doc.no.24 2001. 0.1 g of olive oil was taken into screw-capped tube. 2 ml n-heptanes were added to it and shaken. After 0.2 ml methanolic potassium hydroxide was added for esterification, tubes were vigorously shaken for 30 sec. after the vials were closed. The supernatant of the solution was taken followed after one hour of incubation at room temperature. Then, the supernatant was put in 2 ml vials for injection. Gas chromatography with flame ionization detector (GC-FID) analyses of fatty acids methyl esters was carried out on a Shimadzu gas chromatography (GC-2010 series) equipped with an Supelco SP 2380 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 µm film thickness). Helium was used as carrier gas, at a flow rate of 3 mL/min. The injection and detector temperature were 140 ºC and 240 ºC, respectively. The oven temperature was held isothermal at 140 ºC for 5 min, then raised to 240 ºC

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 fatty acid composition is a quality indicator parameter of olive oils and hence the component profile of fatty acids should be monitored. For all the samples, 13 fatty acids were identified and quantified but the major fatty acid components including arachidic acid, behenic acid, linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid and stearic acid were compared using variance analysis and correlated with soil characteristics (Table 1-2). Accordingly, oleic and palmitic acid were the major fatty acids and ranged between 68.77–73.32% and 12.74–14.64%, respectively. No statistical differences were found in sampling locations for oleic acid and palmitic acid different for each location ($p< 0.05$).

Table 1. Fatty acid profile of the samples olive fruits and soil characteristics of the sampling locations

	(C20:0)	(C22:0)	(C18:2)	(C18:3)	(C18:1)	(C16:0)	(C16:1)	(C18:0)	pH	$CaCO_3$ (%)	Total salt (%)	P (P_2O5, %)	K (K20, (%)	Organic matter (%)
1	0.57 a	0.15 bc	8.94 b	0.86	69.71	14.02 ab	0.85 bcd	3.65 cd	7.85 bcd	19.10 e	0.017 bc	2.21 g	87.23 d	2.75 d
2	0.53 c	0.14 d	8.64 c	0.81	70.27	13.98 ab	0.92 abc	3.54 d	7.83 bcd	28.43 b	0.033 ab	2.29 g	75 f	3.50 b
3	0.50 c	0.13 d	8.60 c	0.89	69.67	14.81 a	1.08 a	3.20 e	7.87 bc	46.175 a	0.011 a	0.46 h	52.42 h	2.30 f
4	0.60 a	0.17 a	6.41 f	0.78	73.32	12.74 b	0.64 ef	3.88 ab	7.67 d	11.44 h	0.033 ab	3.30 e	92.5 b	0.99 h
5	0.59 a	0.16 bc	7.29 e	0.81	72.24	13.06 ab	0.69 def	3.86 ab	7.76 cd	13.35 g	0.034 ab	2.79 f	102 a	3.06 c
6	0.58 a	0.15 ab	9.30 a	0.90	68.77	14.64 ab	0.80 cde	3.63 cd	7.86 bcd	24.98 c	0.031 ab	5.51 c	63.15 g	2.56 e
7	0.60 a	0.16 ab	8.70 c	0.86	69.39	14.40 ab	0.84 cd	3.73 bc	7.86 bcd	23.11 d	0.037 a	4.52 d	21.5 j	2.57 e
8	0.60 a	0.17 a	7.43 e	0.94	71.19	13.54 ab	0.61 f	4.11 a	8.10 a	4.725 i	0.035 a	5.82 b	90.42 c	1.51 g
9	0.60 a	0.16 ab	7.41 e	0.81	72.31	12.78 b	0.66 ef	3.91 ab	7.99 ab	18.66 f	0.025 bc	6.88 a	78.32 e	4.00 a
10	0.43 c	0.11 e	7.88 d	0.78	72.06	13.40 ab	1.02 ab	3.18 e	7.76 cd	25.145 c	0.027 ab	2.195 g	43.5 i	2.16 f

C20: 0; Arachidic acid, **C22: 0;** Behenic acid, **C18:2;** Linoleic acid, **C18:3;** linolenic acid, **C18:1;** Oleic acid, **C16:0;** Palmitic acid, **C16:1;** Palmitoleic acid, **C18:0;** Stearic acid

Table 2**:** Correlation matrix (Pearson (n)) for the fatty acid components and soil properties

Variables	C20:0	C22:0	C18:2	C18:3	C18:1	C16:0	C16:1	C18:0	pH	$CaCO_3$ (%)	Total salt (%)	P (P_2O5, %)	K (K_20, (%)	Organic matter (%)
C20:0	**1**	**0.972**	-0.229	0.265	0.041	-0.226	**-0.838**	**0.898**	0.274	-0.602	0.437	**0.635**	0.421	0.023
C22:0	**0.972**	**1**	-0.405	0.227	0.196	-0.346	**-0.896**	**0.944**	0.258	**-0.699**	0.495	0.598	0.499	-0.139
C18:2	-0.229	-0.405	**1**	0.455	**-0.952**	**0.889**	0.606	-0.483	0.144	0.594	-0.325	-0.173	-0.511	0.356
C18:3	0.265	0.227	0.455	**1**	**-0.655**	0.623	-0.042	0.181	**0.700**	0.046	-0.115	0.242	-0.082	-0.187
C18:1	0.041	0.196	**-0.952**	**-0.655**	**1**	**-0.950**	-0.477	0.322	-0.251	-0.523	0.246	0.097	0.487	-0.196
C16:0	-0.226	-0.346	**0.889**	0.623	**-0.950**	**1**	**0.640**	-0.506	0.136	**0.682**	-0.334	-0.294	-0.576	0.034
C16:1	**-0.838**	**-0.896**	0.606	-0.042	-0.477	**0.640**	**1**	**-0.967**	-0.257	**0.883**	-0.576	**-0.721**	**-0.635**	0.121
C18:0	**0.898**	**0.944**	-0.483	0.181	0.322	-0.506	**-0.967**	**1**	0.384	**-0.850**	0.581	**0.713**	0.576	-0.065
pH	0.274	0.258	0.144	**0.700**	-0.251	0.136	-0.257	0.384	**1**	-0.165	-0.039	0.579	0.025	0.196
$CaCO_3$ (%)	-0.602	**-0.699**	0.594	0.046	-0.523	**0.682**	**0.883**	**-0.850**	-0.165	**1**	**-0.647**	-0.573	-0.581	0.260
Total salt (%)	0.437	0.495	-0.325	-0.115	0.246	-0.334	-0.576	0.581	-0.039	**-0.647**	**1**	0.495	0.084	-0.149
P (P_2O5, %)	**0.635**	0.598	-0.173	0.242	0.097	-0.294	**-0.721**	**0.713**	0.579	-0.573	0.495	**1**	0.096	0.148
K (K_20, (%)	0.421	0.499	-0.511	-0.082	0.487	-0.576	**-0.635**	0.576	0.025	-0.581	0.084	0.096	**1**	-0.030
Organic matter(%)	0.023	-0.139	0.356	-0.187	-0.196	0.034	0.121	-0.065	0.196	0.260	-0.149	0.148	-0.030	**1**

Values in bold are different from 0 with a significance level α= 0,05

C20: 0; Arachidic acid, **C22: 0;** Behenic acid, **C18:2;** Linoleic acid, **C18:3;** linolenic acid, **C18:1;** Oleic acid, **C16:0;** Palmitic acid, **C16:1;** Palmitoleic acid, **C18:0;** Stearic acid

The average linolenic acid level of olive oil samples ranged between 0.78-0.94% in south-eastern region of Turkey, below the maximum value fixed by the IOOC (1.0%) (International Olive Oil Council, 2003); but within the ranges proposed by the Turkish Codex (0.9%). However, it is worthy to note that no statistical differences were determined under different growing conditions.

Pearson correlation coefficients among fatty acid profiles are presented in Table 2. The maximum positive correlations were found

between C22:0 and C20:0 (r=.972), C18:0 and C22:0 (r=.944), C18:0 and C20:0 (r=.898), C16:0 and C18:2 (r=0.889) whereas strong and negative ones were observed between C18:1 and C18:2 (r=−.952), C18:1 and C16:0 (r=−.819). The major component, oleic acid (C18:1) was negatively correlated with pH (r=-.251), $CaCO_3$ (r=-.523) and organic matter (r=-.0196) but positively moderate associated with K content (r=.487).

On the other hand, salt content also positively-but weak-correlated with oleic acid content (r=.246). Of those major components, palmitic acid (C16:0) composition significantly varied with $CaCO_3$ content (r=.682) but negatively affected with salt content (r=.-334), P content (r=-.294) and K content (r=-.576). Linoleic acid (C18:2) displayed similar reaction with palmitic acid against $CaCO_3$ content (r=.594) and positive moderate relation with organic matter content (r=.356) but negative correlation with salt content (r=-.325), P content (r=-.173), K content (r=-.511) were found with linoleic acid.

Data of the fatty acids compositions corresponding to all olive oil samples were submitted to Principal Component Analysis (PCA) to transform a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components (PC). Only eigenvalues of greater than 1.0 are considered significant descriptors of data variance, according to Kaiser's rule (Kammoun and Zarrouk, 2012). Eigen analysis of the correlation matrix loadings of the significant principal components were summarized in Table 3.

Table 3. Eigen analysis of the correlation matrix loadings of the significant principal components

	PC1	PC2	PC3	PC4
Eigenvalue	6.701	3.294	1.247	1.073
Variability (%)	47.867	23.531	8.909	7.662
Cumulative (%)	47.867	71.398	80.308	87.970

The first four components (PC1, PC2, PC3, and PC4) had eigenvalues of 6.701, 3.294, 1.247 and 1.073, and accounted for 47.867 %, 23.531 %, 8,909 % and 7,662 % of the variance in the data, respectively.

Table 4. Correlations between variables and factors

	PC1	PC2	PC3	PC4
Arachidic	-0.771	0.463	0.007	0.043
Behenic	-0.858	0.348	-0.118	-0.005
Linoleic	0.707	0.595	0.120	0.138
Linolenic	0.068	0.873	-0.327	-0.283
Oleic	-0.591	-0.756	0.064	-0.111
Palmitic	0.733	0.613	-0.226	0.073
Palmitoleic	0.985	-0.130	-0.010	0.019
Stearic	-0.947	0.297	0.014	-0.017
pH	-0.201	0.710	0.229	-0.318
$CaCO_3$	0.910	0.024	0.116	-0.062
Total Salt	-0.612	0.039	-0.131	0.685
P	-0.657	0.473	0.283	0.241
K	-0.641	-0.186	0.047	-0.565
Organic matter	0.153	0.157	0.944	0.045

The first PC accounted for more 47.867 % of total explained variance. Linoleic acid. palmitic acid. $CaCO_3$ content were the most important factors in PC1 whereas linolenic acid. pH were the most important factor in PC2 (Table 3).

The ten sampling locations are successfully discriminated by their fatty acid compositions and physicochemical factors of soil. Oleic acid, palmitic acid and linoleic acid-major fatty acid components- were discriminated with K content, $CaCO_3$ content, organic matter content, respectively (Figure 1-3).

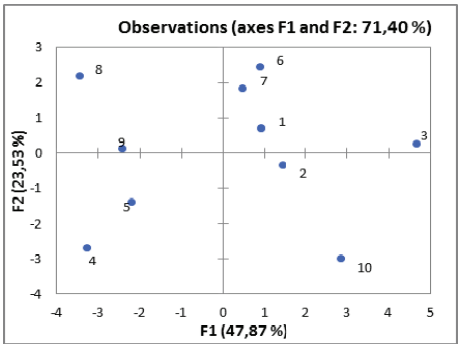

Figure 1. Observations (axes F1 and F2: 71.40 %)

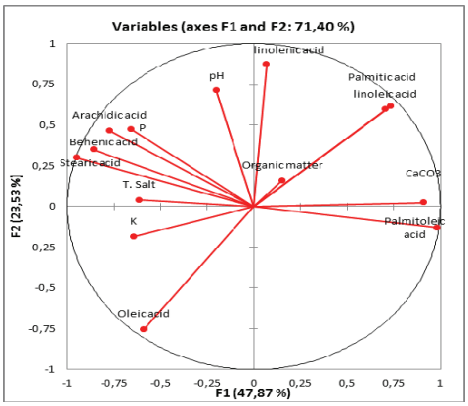

Figure 2. Variables (axes F1 and F2: 71.40 %)

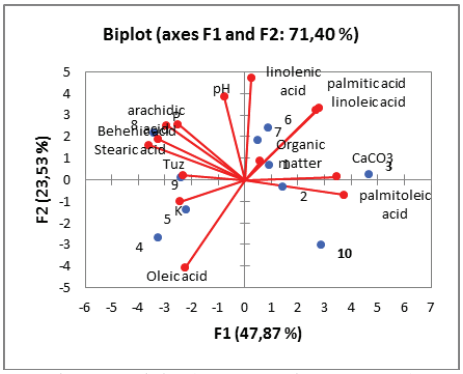

Figure 3. Biplot (axes F1 and F2: 71.40 %)

CONCLUSIONS

Of major fatty acids, oleic acid content was not affected in relation to the sampling locations. The possible effects of physicochemical characteristics of the sampling soils on the fatty acid profiles of olive oil were investigated by correlation test and then principal component analysis was performed to reduce the dimension of the experimental samples.

Oleic acid, palmitic acid and linoleic acid were more pronounced under K. $CaCO_3$ and organic matter content rich soils, respectively.

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A BRIEF OVERVIEW OF HAND AND CHEMICAL THINNING OF APPLE FRUIT

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Abstract

A brief overview of the fruit thinning effect on apple fruit quality is presented in the current paper. Handy and chemical fruit thinning has been previously studied, existing many published reports in literature that examine various aspects of this technological procedure. The state of the art in the field of apple thinning reveal that this operation can improve fruit size, increase return bloom and reduce alternate bearing habit of apple trees.

Key words*: apple, quality, thinning, fruit, blossom.*

INTRODUCTION

Apple thinning can be done in different ways: handy, chemicaly or mechanicaly.

Apple is characterized by heavy bloom and heavy set of fruits throughout the growing season with several negative consequence associated with small, poorly coloured, low quality fruits. Furthermore, flower bud formation for the following year is significantly reduced, resulting in low cropping and inferior quality fruit that has a reduced postharvest storage life. Thinning of the fruitlets is the removal of a portion of the crop before it matures on the tree to increase the marketability of the remaining fruit and to break the biennial bearing tendency of the tree (Greene, 2002).

Fruit thinning can improve fruit size, increase return bloom and reduce alternate bearing habit of apple trees.

Chemical and hand contribution on maintain the physiological balance between growth and fruiting and increasing the quantity and quality of fruit (Vămăşescu and Bălan, 2014).

Frequently, apple trees bloom abundantly and set too many fruits to optimize fruit size and return bloom. Therefore, most producers attempt to increase fruit weight by reducing the number of fruits on a tree (Treder, 2008).

Chemical thinners include different chemicals, but plant growth regulators and some insecticides are used for thinning in most cases. Result from the recently published literature point to advantages of combining certain growth regulators and insecticides for thinning. Combination of carbaryl and 1-naphtaleneacetic acid has given good results in some years but caused excessive thinning in others (Rogers, 1977).

Chemical thinning is important measure for the profitable agricultural production of fruits. Chemical thinning provides a good yield potential for the following vegetation. Thinning the apple crop during the post bloom period is absolutely essential to ensure large fruit size, superior fruit quality, and reliable annual cropping (Peşteanu, 2015).

Most of the thinning literature is focused on the effect of thinners on yield or other production aspects, while the fruit quality becomes a secondary issue (Jemrić et al., 2005).

Jemrić et al. (2003) cited on Link (2000) have reviewed the Germany experience with chemical thinning accumulated over three decades and concluded that there are two groups of quality components. First group includes size, colour, skin performance, firmness, sugar and acid content. The second group includes calcium and potassium levels which are important for storability and

occurrence of physiological disorders. Thinning intensity differently affects these two groups, therefore it is important to select an optimal thinning strategy by growing and local market conditions.
Hand thinning of the apple trees can be very accurate, but it is extremely expensive and requires skilled labor inputs (Costa et. al, 2001).

MATERIALS AND METHODS

A literature search strategy was used, starting on the older to the recent scientific papers on the hand and chemical thinning of apple fruit.

RESULTS AND DISCUSSIONS

Modern fruit thinning studies focus on the relationships between the thinning agent or combination of agents, thinning parameters such as the rate and timing of the application, and the effects on crop load (a measure of fruiting density typically expressed as number of fruit per trunk or branch cross-section area, or per tree canopy volume), fruit size (expressed as weight or diameter), fruit quality, and return bloom (the crop for the following year) (Davis, 2004).
Hand thinning of fruits is a very important process in fruit culture because it is a method of obtaining the optimum quantity of fruits, which have high physical and chemical qualities (Iordanescu et al., 2009).
Hand thinning is a common and high cost practice, not only due to the labor involved, but also because seedling and viguros clonal rootstock result in large trees (Reyes et al., 2008).
Iwanami et al. (2015) examined the efficiency of hand thinning on some apple cultivars. They reported that time required for hand thinning were very similar among clusters with four, five, or six flowers/fruitlets, which was twice as long as that required for clusters with two or three flowers/fruilets. They also concluded that time required for hand thinning cluster of axillary buds became significantly longer from bloom to 7 days after bloom and then decreased gradually from 7 to 25 days after bloom.
Chemical fruit thinning methods were tested by different researchers in many countries. Generally, plant growth regulators are used such as NAA, NAD, BA and ethephon for fruit tinning (Peşteanu, 2015).
In the literature there is information on the effect of different plant regulators in the chemical thinning of apple.
Naphthaleneacetic acid (NAA) is an auxin type thinner and was the first hormone type thinner used commercially (Stopar et al., 2007). The most effective time to apply NAA as a chemical thinner is when fruit diameter is 10-12 mm.
Synthetic cytokinin 6-benzyladenine (BA) has been found to be a good thinning agent. BA it is most effective if applied when fruit diameter averages about 10 mm and has a positive influence on return bloom, reduced the crop load and increased the fruit size (Greene, 2002; Robinson et al., 1998).
Naphthaleneacetamide (NAD) has long been in use, it acts as a synthetic auxin. NAD is considered to be a weak thinning agent who does not give satisfactory results if used alone (Stopar, 2006).
NAD induces early mild thinning, which starts the differentiation process among the flower cluster (Peşteanu, 2015).
Ethephon has long been used as a thinning agent. The main advantages of ethephon are that it greatly improves return bloom and that it can be applied over a longer period (Stopar, 2006).
The thinning response to chemical agents can be highly variable among cultivars and is strongly influenced by environmental conditions.
Weather, timing, choice of chemicals and concentration affect chemical thinning (Autio et al., 2005).
Šebek (2014) tested several thinning procedures comparing applications of NAA and BA in different concentration on three cultivars of apples. All treatments of NAA and NAA + BA had a positive effect on thinning of fruits, in terms of fruit weight and number of fruits per unit cross-sectional area of the trunk.
The highest reduction in ‘Golden Delicious’ fruit set was found on the NAA – sprayed trees, when evaluated at 45 days after full bloom (Reyes et al., 2008).
Peşteanu (2015) tested NAD (Geramid-New) and ethephon agents on ‘Idared’ apple variety.

The effect of the treatments with Geramid-New in dose 1.5 l/ha and Cerone 480 SL in dose of 0.3 l/ha had a positive effect on the number of inflorescences ant the placement of fruits in the trees crown. Also, these agents had an essential influence on quality of production.

NAD did not reduce fruit set. NAD also had no effect on fruit growth, and did not cause an increase in the proportion of pygmy fruit. NAD applied alone can not be recommended for thinning 'Fuji' apple trees (Stopar, 2006).

Wertheim (2000) mentions that NAD cannot be used on 'Delicious' trees because it induces the formation of a high number of pygmy fruit which stay on the tree until harvest time.

Radivojević et al. (2011) reported several experiments thinning two apple cultivars 'Gala' and 'Granny Smith', by hand and NAA and carbaryl. Average fruit size was consistently increased, especially in cv. 'Granny Smith'. A high return bloom was recorded in cv. 'Granny Smith' than cv. 'Gala'.

Šebek (2015) cited on Marini (2002) states that it is very difficult to adequately apply the procces of thinning in spur varieties from the Red Delicious cultivar, and that the use of NAA obtained satisfactory result in terms of fruit size and share of small fruits.

Milić et al. (2011) reported that the number of fruits per native branchet, fruit weight, fruit diameter and height yields, the best results in the chemical thinning of apple cultivar 'Golden Delicios' were obtained using BA in the concentration of 200 mg/L.

Turk and Stopar in 2010, studied the effect of 6-benzyladenine on apple thinning of cultivars 'Golden Delicious' and 'Idared'. The authors reported that BA sprayed at 10 mm did not support the findings contained in many reports which indicated that the best application time coincides with about 10 mm stage of fruit development (Greene, 2002). They concluded that BA can be active as a thinner in a wider period of phonological stages, from the end of bloom up to 20 mm of fruit diameter.

Single application of thinning agents ethephon 200 ppm at ballon stage and NAA 10 ppm or BA 100 ppm at 10 mm fruitlet diameter did not cause thinning response of 'Golden Delicious' (Stopar et al., 2007).

Recently, Peşteanu (2015) tested combination 6-BA and NAA. Therefore, it was established that the combined application of BA + NAA influenced on the nymber of inflorescences formed in the tree crown. Simultaneously the weight of single fruits increased from 16.8 to 28.2%, registering more favorable values where it was tested the combination of BA 100 ppm + NAA 10 ppm.

Significantly increased fruit weight resulted in the combined use of BA + NAA (Šebek, 2014).

Šebek (2015) reported the effect of single or combined use of products based on NAA and BA in thinning of the cultivars 'McIntosh', 'Jonathan' and 'Prima'. The author reported a significant reduction in the yield of 'Prima' apple fruits had the NAA 17.82 ppm treatment. Significantly higher yield compared to the control was determined by combined application of BA 100 ppm + NAA 4.29 ppm. Cultivar 'Prima' show a positive relation between the crop load and yield efficiency. The application of all treatments (individual application of NAA and combination of NAA + BA) in thinning cultivar 'McIntosh' has led to an increase in fruit weight. The application of NAA and NAA + BA combination of all treatments in thinning of fruits of 'Jonathan' cultivar has led to a statistically significant increase in fruit weight and statistically significant decrease in yield and total number of fruits per unit cross-sectional area of the trunk in relation to the control.

Yildirim-Akinci (2014) noticed that the combination of BA+GA_{4+7} is highly effective on fruit size when applied before cell division completed. It also increased fruit diameter, fruit lengh and fruit shape. The application of 100 ppm BA + GA_{4+7} was significantly increased fruit weight by 37% and improved the fruit shape.

Greene et al. (2006) applied a new BA thinning product, respectively MaxCel. This contains no gibberellins, contains more BA, the label was changed to apply more active ingredient, and the formulation was changed to improve foliar penetration and increase formulation stability. MaxCel significantly increased fruit weight at harvest.

Thinning showed no effect on fruit shape of apple cultivars 'Empire', 'Jon-A-Red', 'Braeburn" (Ouma and Matta, 2003).

Meland (2009) mentioned that it was more difficult to adjust the crop load at first bloom

than at the 20-mm fruitlet stage due to higher levels of fruit drop at first bloom. This was confirmed, where thinning at first bloom, rather than at the 20 mm fruitlet stage, improved the mean fruit weight and fruit quality, when comparing similar crop levels. Thinning at first bloom gave an annual crop of high quality.

The author Basak (2006) reported that on 'Gala' trees need to be intensively thinned. Because they blossom over a long period, the fruitlets are at different stages of development at thinning time. Thinning is most effective when too or more preparations are used in sequence. Thus, fruit quality was particularly good when BA was mixed with carbaryl or applied after NAA. In case of mixture of BA and carbaryl, yield and fruit size were better, but color was worse. When BA was used with NAA, fruit color was the same but refractive index was lower.

Peşteanu (2015) reports that in case of using BA 100 ppm + NAA 10 ppm application it was registered a decrease of fruit production, but and increase of their quality. Increasing the dose of BA 140 ppm + NAA 14 ppm does not permit fruit development in comparison with the previous variant. This is explained by the fact of pygmy fruit type appearance, diminishing the fruit number per a tree, average weight and diameter decreases, finally influencing the fruit production quantity and quality.

NAA has shown a strong thinning effect in spur-type 'Red Delicious' trees, but it may also induce an excessive development of pygmy fruits (Marini, 1996).

Milić et al. (2012) reported several experiments thinning three apple cultivars 'Braeburn' and 'Camspur' with NAA and BA. Thinning with NAA and BA has a potential risk of oversized fruits in 'Braeburn' and abnormally small (pygmy) fruit occurrence in 'Camspur'. The average fruit weight was increased, while effects of thinning on fruit parameters in 'Braeburn' were not consistent.

When ethephon was sprayed at a dose of 200 ppm did not reduce fruit set. When used alone, ethephon had no significant effect on mean fruit weight and fruit size distribution (Stopar, 2006).

Stopar et al. (2007) reported that spraying of ethephon at 200 ppm at the balloon stage slightly reduced the fruit number per tree at harvest time.

Al-Absi (2009) related that the ethephon at 500 ppm in absence or presence of BA at 200 ppm significantly increased the number of fruits per cm^2 trunk cross sectional area. A positive correlation existed between intensity of fruitlet thinning and fruitlet retention. These results are in agreement with that of Stopar and Lokar (2003), who reported that ethephon, BA and their combination significantly increased flower bud retention of 'Summerred' apples.

When ethephon treatment was followed up by BA treatment after blossoming, the thinning rate did not increase. Results were best when ethephon treatment was followed up by NAA treatment (Basak, 2006).

Marini (2004) applied combination of ethephon and Accel for thinning 'Delicious' apple trees. BA applied to 'Delicious' and 'Golden Delicious' at 11 to 12 mm fruit diameter reduced fruit set, and fruit weight and length/diameter ratio increased with concentration. The direct effect of Accel and ethephon on fruit weight appears to be inconsistent. Accel did not improve return bloom, even when trees were adequately thinned.

Iordănescu et al. (2009) cited on Cepoiu (1978) which reports that when hand thinning is done sometime in June, after the physiological fall of apples when they have 3-4 cm diameter, it has a good influence upon fruits physical and chemical qualities, but a very small impact upon buds differentiation.

Stopar et al. (2007) has noted that hand thinning performed at the end of June drop resulted in a reduced final number of fruit per tree and an increased mean fruit weight, but they were not significant. Hand thinning should be done more rigorously to reach the commercial fruit size about 150 g per fruit.

CONCLUSIONS

Fruit thinning is intended to address both horticultural and economic concerns as the grower simultaneously seeks to protect the tree from damage due to excess cropping, ensure adequate return bloom, and increase the number of larger, more valuable, fruit both in the current year and subsequent years (Davis, 2004).

The few studies summarized in this review illustrate that the effects of fruit thinning differ widely and successful thinning, resulting in increase fruit size, increased yield and improved certain parameters of the apple quality.

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EFFECTS OF CLIMATE CHANGE ON OLIVE CULTIVATION AND TABLE OLIVE AND OLIVE OIL QUALITY

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Abstract

Climate change is undoubtedly the most imminent environmental issue the world is facing today. Climate changes could heavily affect olive oil producing areas, especially in the Mediterranean basin. Olive trees are tougher than vines and can thrive on many different terrains and under various climate conditions. Researchers reported that a key area of Spanish olive oil production in Catalonia may become unviable within 20 years due to these increasing temperatures and water shortages. Extreme temperatures pose risks for olive production. The Mediterranean basin region, where more than 90% of the world's olive oil is produced is expected to be exposed to higher temperatures in the future due to global climate changes, which caused unfavorable growing conditions and showed negative effects on oil production and quality. According to result of studies there is an urgent need for approaches to estimate the consequence of climate change and its effects on olive quantity and quality of olive oil and table olive. This research was aimed to present result of studies which focused on effects of climate change on olive growing and production and also quality of table olive and olive oil products.

Key words: *global warming, olive industry, table olive, olive oil.*

INTRODUCTION

Climate change is undoubtedly the most imminent environmental issue the world is facing today. The rise in climate temperature will have certain major effects on ecosystems, wildlife, food chains and eventually human life (Appels et al., 2011). According to result of studies, in less than forty years, three-quarters of the wine producing areas on earth will not be suitable for vine farming due to the effects of climate change. Soon enough those vineyards will move to other territories that will have the conditions to grow the grapes, like northern Europe, northwestern America and areas of central China (Hannah et al., 2013; Vasilopoulos, 2013). Similarly, the climate changes could heavily affect olive oil producing areas, especially in the Mediterranean basin (Vasilopoulos, 2013). Climate change alters both average and extreme temperatures and precipitation patterns which in turn influence crop yields, pest and weed ranges and introduction and the length of the growing season (Anon., 2008). Olive trees are tougher than vines and can thrive on many different terrains and under various climate conditions. They give olive oil with little effort and care throughout the year, often without much watering. This is why countries like India, Libya and Australia are planting more olive trees; it is relatively easy to grow them and they can yield a profit (Vasilopoulos, 2013). According to result of studies climate chage have caused effects on olive cultivation and alteration on quality of table olive and olive oil (Ponti et al., 2014; Dag et al., 2014; Tupper 2012). This review aimed to present these effects and alterations on olive cultivation and olive product quality.

EFFECTS OF GLOBAL WARMING ON OLIVE CULTIVATION

Climate change threatens agro-ecosystems of olive which is an ancient drought-tolerant crop of considerable ecological and socioeconomic importance in the Mediterranean Basin (Ponti et al., 2014). Researchers reported that a key area of Spanish olive oil production in Catalonia may become unviable within 20 years due to these increasing temperatures and water shortages. Spain is thought to be highly susceptible to climate change. Studies have shown that the flowering period of olive trees is highly dependent on the yearly spring

temperatures, which are rising steadily over time (Tupper, 2012).

The most immediate issue for the olive cultivation is rainfall which is highly affected by climate change. 2013 has been reported as the driest year on record since dating back over 150 years for California (Moran, 2014). Less rainfall means low olive oil production, with few options for farmers when water prices remain high (Moran, 2014; Tupper, 2012). This pertains to the bulk of American olive oil production, considering that 90 percent of olives grown domestically come from California (Moran, 2014). If Spain is to continue its supremacy as an olive oil producing nation, new and innovative irrigation alternatives will have to be created to combat the constantly changing climate. This is no easy task however, as increasing irrigation can have negative effects on water supplies for the area, leading to desert like areas and water shortages for other purposes, as has previously been seen in Greece, Italy and Portugal when irrigation demands increased (Tupper, 2012).

When the weather becoming warmer, olive groves on high hills or slopes will probably suffer less, but groves located on low altitude areas or plains could become totally unproductive. There are already signs of the oncoming change, with this year's harvest in Spain crippled by the drought and the phenomenal weather variations (Vasilopoulos, 2013). Water scarcity affects every continent and countries such as Greece and Italy have already suffered the devastating effects of drought, with olives dying at high temperatures and from lack of water. In addition to the direct effects of a changing climate on the olive population, variations in weather can also cause changes in other environmental factors such as insects and disease. These may then influence the olive tree population, as an indirect effect of changing climates (Tupper, 2012).

Extreme temperatures, pose risks for olive production. In 1998, severe cold temperatures caused significant losses for olives in California. Olive trees can normally handle brief cold snaps, but sub- freezing temperatures that last longer than a few hours will damage new and small branches and may prevent fruit production (Moran, 2014).

Olive phenology has been reported as a good indicator of future climatic change (Osborne et al., 2000). This could be explained by the fact that photoperiod would also affect the start of flowering in the late spring flower species. Moreover, one of the most expected consequences of climate change will be the increase in minimum temperatures, especially in winter and early spring (Ahmad, 2001).

The variability in chilling hours, which garner less attention than frost, are equally important to overall olive vitality. Accumulated winter chill hours are declining in the growing regions of California, which affects a range of crops from olives to almonds. A substantial amount of chilling hours (32-45^{o}F) are necessary for olive flower bud development, which facilitate the plant's movement out of its vegetative state so fruit can be produced (Moran, 2014).

Inability to determine reliably the direction and magnitude of change in natural and agro-ecosystems due to climate change poses considerable challenge to their management. This level of climate warming will have varying impact on olive yield and fly infestation levels across the Mediterranean Basin, and result in economic winners and losers (Ponti et al., 2014).

Mild temperatures in summer are reported as a reason for increased olive fly infestations in the region without stretches of summer heat to reduce the fly population. Together, these effects create a complex web of changing climate and olive oil production, whose future will require further scientific research, careful monitoring (Moran, 2014; Marshall et al., 2011). Recycling to soil olive mill waste has the potential to improve soil fertility, thus reducing CO_2 emission associated to global warming (Altieri and Esposito, 2010).

Climate change will impact the interactions of olive and the obligate olive fruit fly (*Bactrocera oleae*) and alter the economics of olive culture across the Mediterranean Basin. The effects of climate change on the dynamics and interaction of olive and the fly using physiologically were estimated based on demographic models in a geographic information system context as driven by daily climate change scenario weather (Ponti et al 2014).

Climate does not only affect olive trees directly, but changing temperatures also influences insect diversity and frequency for a given area. Rising carbon dioxide levels will

exacerbate most insect and pest problems. This is particularly relevant to the olive fly, olive's most notorious and costly pest, but studies show that this effect may actually operate in a counter intuitive way (Moran 2014).

Decreased level of production may become common place if continued scarcity of water and increased temperatures start to effect groves in Spain. While high temperatures are optimal for growth and development of olives, heavy rain is also necessary to complete the ripening process (Tupper 2012). Irrigation of olives with saline water will inevitably increase in the future in the Mediterranean due to negative effects of population growth and climate change on the availability and quality of existing fresh water supplies. As a consequence, the risk land salinisation will exacerbate threading the agricultural production particularly in countries with a semi-arid or arid climate (Chartzoulakis 2005).

Spanish olive oil production has doubled in the last ten years, but ongoing drought and climate change may mean a setback for the global leader in olive oil production. Spain may fall to the same fate as fellow olive oil producing power houses Greece and Italy due to the effects of climate change. Italy has seen a drop of 50 % in production since 2001 and Greece has also seen its annual production levels decline by half with climate change thought to be an important factor (Tupper 2012).

EFFECTS OF GLOBAL WARMING ON TABLE OLIVE AND OLIVE OIL PRODUCTION AND QUALITY

Climate warming will affect olive yield and oil quality across the Mediterranean Basin, resulting in economic winners and losers at the local and regional scales. At the local scale, profitability of small olive farms in many marginal areas of Europe and elsewhere in the Mediterranean Basin will decrease, leading to increased abandonment (Ponti et al., 2014).

Emerging players of the olive industry like China and India with vast lands for cultivating olive trees could challenge European producers. Because of rapid changing weather, reduction of olive oil production is on the way for traditional olive oil powerhouses such as Spain, Italy and Greece (Vasilopoulos, 2013).

Decline of olive oil production in Italy and Greece has had a temporarily positive effect on Spain, which is now producing twice the joint production of Greece and Italy. Current harvest in Spain will be a poor one, with a 40 % drop in production due to drought, leading to a huge leap in market prices for olive oil (Tupper, 2012).

More than 90% of the world's olive oil is produced in the Mediterranean basin where is expected to be exposed to higher temperatures in the future due to global climate changes which caused unfavorable growing conditions and showed negative effects on oil production and quality (Dag et al., 2014). Early harvest (relatively low ripening index) is reported as one of the major findings to prevent from those climate changes (Dag et al., 2014; Vasilopoulos, 2013,).

Determination of the optimal fruit ripening stage for the production of olive oil represents a critical choice based on the best combination of oil quantity and quality (Dag et al., 2014). As olive ripening proceeds, fruit characters such as weight, pulp to stone ratio, color, oil content, enzymatic activities and profiles of various phytochemicals, including fatty acids and total polar phenol content, are constantly changing. These changes in fluence fruit firmness, olive oil chemical composition and sensory qualities (Beltrán et al., 2004). Climate has a major in fluence on the ripening process and hence on oil accumulation and its chemical composition (Aparicio and Luna, 2002).

As well as the quantity of fruit, the qualitative components of olive oil produced can be influenced by the environmental conditions of the growing year (Lombardo et al., 2008). This relates to the absolute variations in fatty acids and the relationships between these individual components such as the oleic acid/linoleic fatty-acid ratio, and the ratio between oleic acid and the sum of palmitic and linoleic acids (D'Imperio et al., 2007). Getting a high quality olive oil requires several different factors which are healtly trees, appropriate climate and proper farming. Also the ground morphology and the moisture levels of the area play an important role in shaping the oil characteristics. European olive oils in fifty years from now could be very different in terms of their qualities and organoleptic characteristics (Vasilopoulos, 2013).

One of the most important aspects is the use of irrigation while monitoring for potential water stress caused by water deficit in the summer months. Thus, greater attention needs to be paid on the part of the olive growers for evaluation of the vegetative–productive state of the trees and their reproductive cycle, and of the hydro-pedological conditions of the terrain that vary with the seasonal meteorological trends (Orlandi et al., 2012).

A water deficit during initial development of the fruit (in June in the northern hemisphere) can result in a decrease in the size of the cells of the mesocarp that cannot be recovered, except, at least in part, if the plants are regularly irrigated in the following stages (Servili et al., 2004). Water deficit affects fruit maturation, which occurs earlier and more rapidly, and can result in more intense pre-harvest fruit fall (Inglese et al., 1996). However, a number of studies have shown that the water state of the plant has marginal, if any, effects on free acidity and peroxide value of the olive oil produced (Servili et al., 2007). A direct relationship connects the water stress to the levels of linoleic and linolenic acids, where higher stress corresponds to high levels of these fatty acids. This therefore provides further support for the concept of qualitative irrigation (Servili et al., 2004).

The phase of maturation of the olive fruit influences not only its acid composition, but also the composition of its minor constituents, and particularly its phenolic and volatile compounds. Thus, factors that affect the evolution of maturation of the drupe can also affect the qualitative characteristics of the resulting olive oil (Fiorino and Nizzi Griffi, 1991).

Variability in acid composition has been correlated to the temperature sum of the period from fruit setting to fruit maturation. The high temperatures during this phase that arise in hot seasons and environments can result in decreased oleic acid content, which is accompanied by increased palmitic and/or linoleic acids (Lombardo et al., 2008). A very high temperature sum also tends to reduce total polyphenol content (Ripa et al., 2008). Similarly, in cooler areas, a positive correlation has been shown between the temperature sum from August to October and the total polyphenol content of olive oil (Tura et al., 2008).

The water state of the plant also has marked effects on concentrations of volatile compounds in the oil. Thus, oil from plants grown without irrigation, as opposed to those with, can be more bitter and biting to the taste (Servili et al., 2007). Plants grown under conditions of water stress therefore tend to produce oils that are more full bodied and strong in their taste, with strong bitter and biting notes, but that are relatively less aromatic (Orlandi et al., 2012).

CONCLUSIONS

According to result of these mentioned studies there is a urgent need for approaches to estimate the consequence of climate change and its effects on olive quantity and quality of olive oil and table olive. Water uses for irrigation should be good planned for olive orchard to reach maximum olive yield and olive oil or table olive quality with minimum water consumption. Global warming and rising carbon dioxide levels will increase the most of the insect and pest populations and change their life cycle. So that olive orchard should be kept under constant observation to determine the time and use the type and amount of pesticide and insecticide uses. According to climatic changes, harvest time of olives should be redefined which should be provide a balance between for high yield and final product quality. New breeding studies should be focused on the behavior of olive genotypes with respect to climate change. Water stres and diseases resistance of olive trees and olive oil quaity or table olive quality under increased temperatures and water stres should be use as a dominant advanced selection criteria for new olive cultivar candidates.

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A NEW APPROACH OF SWEET CHERRY *(PRUNUS AVIUM* L.) POLLINATION: CORIANDER *(CORIANDRUM SATIVUM* L.) ESSENTIAL OIL

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Abstract

The objective of this study was to examine the effect of coriander essential oil applied as an alternative method to increase fruit set of sweet cherry which is an important fruit for the Isparta region. In the trial, coriander essential oil was applied to the branches in blooming period and comparisons were made with branches on which essential oil was not applied (control). When average values are considered, it has been determined that the application increased fruit set from 27.86 to 53.28 in comparison with the control group.

Key words*: 0900 Ziraat, pollination, linalool, aspect, fertilization, bee*

INTRODUCTION

The main factors determining fertilization and yield of the sweet cherry include temperature and rainfall during blooming period, the period of overlapping blooming of the main and pollinizer cultivars and their self and cross incompatibility status. Other environmental factors such as bees are referred to as pollen vectors (Sutyemez and Eti, 1999; Choi and Andersen 2001; Pırlak, 2002). 0900 Ziraat is an export cultivar grown in Turkey which has dark colour, sweet taste and is crack resistant. Fertilization is essential for fruit set and yield in sweet cherry growing (Tosun and Koyuncu, 2007; Beyhan and Karakas, 2009). Successful fertilization of sweet cherries depends on the transfer of compatible pollen by honeybees, as most commercial cultivars are self-incompatible (Janıck and Moore, 1996; Thomson, 2004). Flowers are usually sufficient to obtain a product in cherries 25-50% of the flowers must become fruits. 0900 Ziraat sweet cherry cultivar has some problems in terms of efficiency. The biggest problem that causes decrease in yielding can be caused by self-sterility and late bloom, pollination and outcrossing (Bekefi, 2004). Sweet cherry pollen must come from another – and compatible - cultivar; therefore, a high degree of bee activity on the tree and between trees is required (Tonitti et al., 1991). Entomophily is a form of plant pollination whereby pollen is distributed by insects, particularly bees, Lepidoptera (e.g. butterflies and moths), flies and beetles. Honey bees will pollinate many plant species that are not native to their natural habitat but are often inefficient pollinators of such plants. Pollinators of sweet cherry are honey bees, bumblebees, solitary bees and flies (Bosch and Kept, 2002). Hussein and Abdel-Al (1982) also reported that honey bee consisted more than 67% of the total bees are visiting coriander and others. Diederichsen (1996) attributed that coriander is also a good melliferous plant; one hectare of coriander allows honey bees to collect about 500 kg of honey.

Coriander (*Coriandrum sativum*) is a valuable weed and spice plant from the Umbelliferae family (Baydar, 2009). The essential oil ratio in its fruits varies between 0.03-2.60% (Kaya et al., 2000). Coriander oil is among the 15 most produced essential oils. The previous researches indicate that linalool (1,6-octadien-3-ol, C10H18O, Mr=154.25, d20= 0.862 and boiling point 196– 198°C) is the main component in coriander essential oil, and it has potential usage as antisposmadic immunostimulatory antinociceptive (Peana et al., 2003; Telci et al., 2006). α-pinene, γ-terpinene, geranyl acetate, p-cymene and hexadeconoic acid in the coriander essential oil are also other

important components (Anitescu et al., 1997; Baydar, 2009). In our study, the effect of using the indirect bee activity of essential coriander oil has been examined as an alternative method to increase fruit set in sweet cherry.

In this study, coriander essential oil which is used as an activator to increase bee activity has been applied to cherry branches during blooming period and the number of fruits on the coriander applied branches along with that on the branches with no coriander application have been compared.

MATERIALS AND METHODS

Plant material

In this study, *Coriandrum sativum* essential oil has been applied to Gisela 5 rootstocks of the 0900 agricultural cherry type 10 years old which belong to an individual at the Atabey district of the city of Isparta in order to increase fruit set ratio. Starks Gold species at a ratio of 1/8 is used as a pollinator in the garden where the application was done.

Statistical analysis

The study was planned as a randomized block design with 3 repetitions. Three trees were randomly selected during each repetition and coriander essential oil was applied in 4 directions (North, South, East and West) to the fruit buds on the branches of each tree selected at the same level. Whereas the buds that were left as control were isolated during the application. Fruit set ratio in the study was calculated by counting the number of fruits on the essential oil applied branch along with the number of fruits on the control branch.

Data analyses

Data were subjected to analysis of variance for the essential oil application. All data were analyzed by computer software (Standard ANOVA analysis). The means were compared by using the LSD test described by Steel and Torrie 1980. Mean percentage and standard deviation of essential oil values of the collected samples were calculated by MS Excel program.

Isolation of essential oil

The essential oils were extracted by hydrodistillation for 3 h using Clevenger type apparatus using 10 g of the air-dried aerial parts of the plant samples. The volatile oils were stored in dark glass bottles at 4^{o}C until analysis (British Pharmacopoeia 1980).

GS-MS analyses of essential oil

Essential oil constituents were analyzed by (%) gas chromatography method, and GC-MS analysis was carried out by utilizing Shimadzu GC/MS-QP 5050 A in Suleyman Demirel University Experimental and Observational Student Practice and Research Center. CP Wax 52 CB (50 m x 0.25 mm i.d., film thickness 1.2 μm) capillary column and Helium as a carrier gas were used. The temperature program reached from 60^{o}C to 22^{0}C with 2^{0}C increases in temperature in a minute, and was applied by maintaining 22^{0}C for 20 minutes. Temperature of the injector was of 24^{0}C. Mass spectra were used at 70 eV. After the compounds were ionized in gas chromatography column and separated, mass spectrum of each of them were obtained. Evaluation procedures were conducted using "Wiley, Nist and Tutor" libraries.

RESULTS AND DISCUSSIONS

The effect of coriander essential oil application to increase fruit set in cherry can be seen in Table 1.

Accordingly, it is observed that the application has increased the number of fruits and that this increase is statistically significant.

When the average values are examined, it is determined that the application has doubled the number of fruits.

Table1. Effect of coriander essential oil on cherry fruit set

	Number of Fruits				
	North	**South**	**East**	**West**	**Average**
Control	26.22	27.11	29.22	28.89	27.86 a
Application	48.06	58.99	51.11	54.44	53.28 b
Average	37.44	43.00	40.17	41.67	40.57
$LSD_{app\ (\%1)}$: $LSD_{asp:\ ns}$	4.244				

Even though it was not determined to be statistically significant, it has been observed when the number of fruits in the directions is evaluated that the highest number is 43 on average for the south direction which is an expected result since blooming is expected early and fruit set is expected to be high on

trees that face south (Janick and Moore, 1996). This is followed respectively by west, east and south directions. When directions and application are evaluated together, it is observed that the highest number of fruits is found on essential oil applied on trees and that the increase was almost double in comparison with the control.

It is thought that this increase is due to linalool which is the main component in coriander essential oil (Table 2).

Table 2. Essential oil composition of *Coriandrum sativum*.

Element	Rt	Area (%)
2-dodecenal	59.9	1.80
Geraniol	58.5	2.91
Geranyl acetate	53.3	14.39
Linalool	40.0	80.9

Coriander plant is named in some resources as the honey plant and its attractive effect can be thought of as the reason for this. Raguso and Pichersky (1999) stated that the monoterpene alcohol, linalool, is present in the ßoral fragrance of diverse plant families and is attractive to a broad spectrum of pollinators, herbivores and parasitoids.

Priority in such applications is not only to increase fruit set; the effect on the quality parameters of the fruit should also be examined. Even though there are some studies on the effect of essential oils on seed germination, the number of studies on pollen germination and tube growth is scarce. The effect of coriander oil on pollen germination and pollen tube growth will be examined in future studies and we believe that this will put forth a new perspective for the solution of fertilization problems in cherry.

CONCLUSIONS

Coriander essential oil application increase fruit set in cherries express in the number of fruits is examined.

It has been determined that the application has doubled the number of fruits and linalool which is the main component of the coriander essential oil has been presumed as the reason for this.

In the light of these results, it is thought to carry out the application using different doses of coriander essential oil and that the increase in the number of fruits is due Linalool which is the primary component of the coriander essential oil; carrying out the application with all the other components can be thought of as well.

In addition, carrying out the application of different essential oils on different trees instead of only coriander essential oil on cherry trees will enrich the further studies.

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EFFECT OF DIFFERENT DOSES OF POTASSIUM ON THE YIELD AND FRUIT QUALITY OF 'ALBION' STRAWBERRY CULTIVAR

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Abstract

This study was carried out to determine effect of the potassium fertilizer rate on yield and fruit quality of day-neutral strawberry cultivar 'Albion' during 2013-2015 years. Frigo seedlings of Albion cultivar were planted in June 2013. In the experiment, 0-5-10-15 kg/da K_2O fertilizer was applied and identified as K-0, K-5, K-10 and K-15. Fruit weight, fruit length, fruit width, yield per plant, total soluble solid (TSS), titratable acidity (TA), pH, total phenolic and anthocyanin were determined. Applications exhibited a range of 10.95-27.94g for fruit weight, 24.95-37.91mm for fruit width, 34.44-52.18mm fruit length, 3.87-4.26 for pH, 7.00-8.10% for total soluble solids and 0.97-1.20% for titratable acid. Total phenolic content was observed in 650.28-900.02mg (Gallic acid equivalents (GAE)/100g) and anthocyanin content was observed 155.06-204.18mg/100g. We can recommend the K-10 and K-15 applications according to obtained data in our research for producers.

Key words: *Strawberry, albion, K_2O, phenolic, anthocyanin*

INTRODUCTION

Although commercial strawberry (*Fragaria x ananassa* Duch.) cultivation started towards the end of 1970 in Turkey, it is currently one of the biggest strawberry producers in the world with 372.498 tons production annually (FAO, 2015). Strawberry cultivation is generally carried out in open field or walk-in plastic tunnels to provide earliness in Turkey. There is a big demand for greenhouse strawberry production, during the recent years to extend the harvest period. The cultivation of strawberries in greenhouses in Turkey was also increasingly expanded in the Mediterranean Region during the last few decades. (Ercişli et al., 2005). The quality characteristics of strawberry fruit is concerned with: sugar rate, acidity, durability, brittleness, verjuice, aroma, allure and nutritive value (Kader, 1991; Salame-Donoso et. al., 2010).

Albion is a day neutral strawberry cultivar. Day neutral strawberry varieties show better performance in areas with highland climate. Because the day-neutral strawberry varieties are not affected by the length of day and consistently produces fruit. Therefore, they are recommended for areas with highland climate.

A study published in 2001 showed that strawberries actually contain in three basic flowering structures: short-day, long-day, and day-neutral. These refer to the day-length sensitivity of the plant and the type of photoperiod that cause flower formation. Day-neutral cultivars produce flowers regardless of the photoperiod (Hokanson and Maas., 2001).

The objective of this study was to determine the effect of different doses of potassium on the yield and fruit quality in 'Albion' strawberry variety.

MATERIALS AND METHODS

This study was carried out in the Corum region during 2013-2015. Albion strawberry seedlings were planted on black mulch for the commercial plantation, in June 2013. The soil properties of experimental area are presented in Table 1. Potassium applications were made as 0-5-10-15 kg on decares (K-0, K-5, K-10 and K-15). Pomological and chemical characteristics that is fruit weight, fruit length, fruit width, yield per plant, total soluble solid content (TSS), titrable acidity (TA) and pH, total phenolic (mg/100g) and anthocyanin (mg/100g) were investigated.

Table 1. Physical and chemical characteristics of the soil before cropping of strawberry.

Soil Properties	Analysis Results
Sand (%)	67
Silt (%)	19
Clay (%)	14
Salinity (mmhos/cm)	140.80
pH (1:2,5)	7.95
Calx (%)	34.23
Organic Matter (%)	2.28
Azote (ppm)	1141.96
Phosphor (ppm)	8.97
Potassium (ppm)	128.40
Calcium (ppm)	4078.60
Magnesium (ppm)	60.94
Iron (ppm)	2.74
Cupper (ppm)	0.45
Manganese (ppm)	4.75
Zinc (ppm)	0.47

The fruit characteristics of the strawberries were determined and cluster samples were randomly selected in 40 units of fruits. The weight and yield of per plant were determined using a 0.01g-sensitive weighing. The measurements of both the length and width (diameter) of fruits were made using a 0.01 mm-sensitive digital compass.

Total soluble solids contents (TSS): Samples of the examined cultivars were pooled to obtain a composite sample and analyzed for SSC using a digital refractometer. (Atago Model PR-1, Tokyo).

pH measurements were performed using a Hanna HI 98103 pH meter at 20 °C.

Titrable acidity (TA) was determined with potentiometrically using 0.1M NaOH to the end point of pH 8.1 and expressed as grams of citric acid per litre. (AOAC, 1984).

Determination of total phenolic content: Total phenolics content of strawberry were determined by using the Folin-Ciocalteu phenol reagent method. (Singleton and Rossi, 1965). Absorbance was measured on a spectrophotometer (MRX Dynex Technologies, USA) at 765 nm. The total phenolic contents were expressed as mg of Gallic acid equivalents (GAE)/l of extract.

Anthocyanins: Total anthocyanin content was measured with the pH differential absorbance method, as described by Cheng and Breen (1991). Briefly, absorbance of the extract was measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid–potassium chloride, 0.2 M) and 4.5 (acetate acid–sodium acetate, 1 M). Anthocyanin contents were calculated using a molar extinction coefficient of 29,600 (cyanidin-3-glucoside).

$A = (A\ \lambda 510 - A\ \lambda 700)\ pH\ 1.0 - (A\ \lambda 510 - A\ \lambda 700)\ pH\ 4.5$

Results were expressed as mg cyanidin-3-glucoside equivalents $100g^{-1}$ fw.

RESULTS AND DISCUSSION

In this research, fruit weight, fruit length, fruit width, yield per plant, total soluble solid content (TSS), titratable acidity (TA) and pH were determined in 2014 to 2015 growing seasons. Some pomological and chemical properties of Albion cultivar are presented in Table 2. The Fruit weight was found to vary from 10.95 to 27.94g according to the applications. K-10 application was the highest at 27.94g. K-15 application was followed by 26.71 g. Both of these applications were included in the same group. There was not significantly different between K-10 and K-15 applications. But there were observed significant differences between this group and other applications (K-5 and K-0) ($P<0.05$). The highest fruit length was obtained from K-15 application (52.18 mm). Other applications were followed by 40.48 mm, 38.11 mm and 34.44 mm (K-0, K-10 and K-5 respectively). Both of these three applications were included in the same group and there were significant differences ($P<0.05$) between this group and K-15 application (Table 2). Applications K-10 and K-15 were obtained (37.91 and 34.05 mm respectively) higher value of fruit width from others. There were not significant differences between these applications. The lowest value of fruit width was obtained from K-5 application (21.83 mm), but difference was not statistically significant between K-5 and K-0 application (Table 2).

The weight of albion fruits were reported by Hughes et. al. (2010) as 15.7 to 16.4 g. Our results were much higher than those values. These are considered to be caused by potassium treatments. The same researchers (Huges et. al., 2010) and Ballington et al., (2008) were declare the yield value as 2.1 to 2.4 kg/m and 309-775 g/plant respectively. Our findings were lower than those reported in the literature.

In our research, the highest value in terms of yield per plant was obtained from K-15 appli-

cation (285.20g) taken from the lowest yield K-0 applications (146.09g). However, differences between treatments were not statistically significant (Table 2). The highest SSC value was obtained from K-5 application (8.10%). The others applications followed by 7.90, 7.60 and 7.0 (K-0, K-10 and K-15 application respectively). Differences between K-15 (lowest SSC value) and other treatments were statistically significant (Table 2) ($P<0.05$).

SSC values were reported by Ruan et al., (2013) and Ornelas-Paz et al., (2013) as 8.35% and 6.6 to 9.0% respectively. Along with partially coincide, the values obtained in this research were lower than reported values in the literature generally. The reason for this the fruits used in this study were very large and they contain large amounts of water.

The highest value in terms of titratable acid was obtained from K-5 application (1.20%). This treatment was followed by the K-15 and K-0 applications. The differences between these three treatments were not statistically significant. The differences between K-10 (having the lowest value) applications and other treatments were statistically significant ($P<0.05$). Titratable acidity values were reported by Akhato and Recameles, (2013) and Ornelas-Paz et al., (2013) as 0.50 to 0.84% and 0.70 to 1.2% respectively in strawberries. Our findings were upper than these values.

When our findings were observed with respect to pH, the highest value was determined in K-15 treatment. This was followed by application of K-5 and K-10 respectively.

Differences between other applications and K-0 treatment (the last rank) were statistically significant ($P<0.05$). The determined pH values were reported by Akhatou and Recamales, (2013) and Ornelas-Paz, et al., (2013) as 3.51 to 3.82 and 3.39-3.80 respectively. The data determined in our study were higher than those reported in the literature.

Table 2. Effect of application on fruit weight, fruit length, fruit width, yield per plant, soluble solid content (TSS), titratable acidity (TA) and pH.

	Fruit Weight (g)	Fruit Length (mm)	Fruit Width (mm)	Yield Per Plant (g)	TSS (%)	Titratable Acidity (%)	pH
K-0	10,95±0,64* c	40,48±2,25* b	24,95±0,91* b	146,09±7,32	7,90±0,10* a	1,08±0,02** ab	3,87±0,13** b
K-5	17,26±1,79 b	34,44±0,57 b	21,83±0,34 b	181,20±11,90	8,10±0,15 a	1,20±0,07 a	4,06±0,04 ab
K-10	27,94±1,58 a	38,11±2,29 b	37,91±2,51 a	215,70±54,10	7,60±0,12 a	0,97±0,04 b	4,02±0,04 ab
K-15	26,71±1,05 a	52,18±0,65 a	34,05±0,21 a	285,20±46,00	7,00±0,06 b	1,16±0,01 ab	4,26±0,03 a

In this research, total amount of phenolic were determined as 650.28-900.02 mg/100g in Albion fruits (Table 3 and Figure 1).

The differences between applications were statistically insignificant in terms of total phenolic and anthocyanin amount (Table 3, Figure 1 and Figure 2) ($P<0.05$).

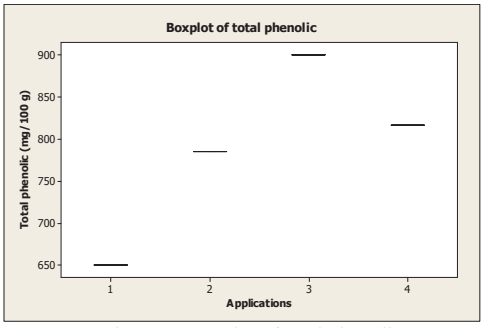

Figure 1. Boxplot of total phenolic

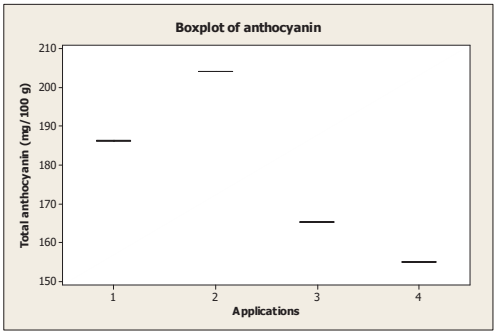

Figure 2. Boxplot of total anthocyanin

The highest phenolic contents were obtained from K-5 application. Application K-15 took place at the last in ranking.

The total amount of phenolic and anthocyanin were reported by Diamante. et al. (2012) as

1576-2466 mg gallic acid /kg FW and 385-470 mg Pel-3-GL/kg FW respectively.
The total amount of phenolic was reported by Capocasa et al., (2008) as 1.8-3.2mg GAE/g in strawberry. Medina, 2011 is reported the total phenolic content as 282 mg GAE/100g in organically grown strawberries.

Table 3. Total phenolic and anthocyanin contents

Application	Total Phenolic (mg/100 g)		Anthocyanin (mg/100 g)	
	Mean	Ave Rank	Mean	Ave Rank
K-0	650.28±0.01 d	2.0	186.27±0.01 b	8.0
K-5	785.37±0.01 c	5.0	204.18±0.00 a	11.0
K-10	900.02±0.01 a	11.0	165.34±0.01 c	5.0
K-15	816.87±0.01 b	8.0	155.06±0.00 d	2.0

The total amount of phenolic and anthocyanin were reported by Ornelas-Sun 2013 as 195.6-325.0 mg GAE/100g and 0.9-56.4 mg/100 g respectively.
Our findings were higher than the value reported in the literature in terms of both the total amount of phenolic and anthocyanins.

CONCLUSIONS

We can recommend the K-10 and K-15 applications according to the obtained data in our research for producers. Because, these applications showed higher values than control applications and K-5 application in terms of both yield and some quality characteristics and the total phenolic.

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THE EFFECT OF ROOTING MEDIUM TEMPERATURE AND MOISTURE ON ROOTING OF BLACK MULBERRY HARDWOOD CUTTING

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Abstract

In this study, the effect of different temperature and humidity content of rooting medium on rooting percentage of black mulberry hard wood cutting was investigated. For this purpose, different temperature and humidity contents were applied. In the result of the study, the effect of interaction of temperature x humidity and temperature on rooting ratio was not significant. On the other hand, humidity content caused significant changes on rooting percentage. The highest rooting ratio (63,11%) was observed in cutting planted in medium with 40% humidity. The lowest rooting percentages were obtained from two other media contained higher humidity compared to medium with 40% humidity. Both temperature and humidity treatment caused significant changes in root length. Among humidity treatment, 80% humidity produced the longest roots. The longest roots were obtained from 22°C of basal heating treatment. In term of the number of root per cutting, significant difference was not found among humidity treatments while temperature treatment caused significant changes. The highest root number per cutting was observed in cutting planted at 22°C.

Key words*: mulberry, Morus nigra, hardwood cuttings.*

INTRODUCTION

Mulberries (*Morus* spp.) belonging to the *Moraceae* family are widely distributed in many parts of the world, mainly in the northwest of South America and some parts of Africa (Datta, 2002) and their fruits are used for fresh consumption and syrup production (Davis, 1972). Their cultivation in Anatolia has been known since ancient times (Özbek, 1977). Hence, Turkey has a significant genetic potential for mulberries but the number and production of mulberry fruit decreased in the last decades due to the shift away from farming, increase of cutting and lack of sufficient maintenance. The anthocyanin found in berries and red fruits have been recently reported to possess preventive and curative properties against mouth, larynx, oesophagus, stomach and colon cancer (Prior, 2003; Zarfa et al., 2007). An excess accumulation of cyanidin-3-glucoside and cyanidin-3-rutinozit are of anthocyanin found in mulberries (Chen et al., 2005) and the contents are higher than other red fruits and some berries (Özgen et al., 2009). Expanding in application area and growing interest in the nutritional value of mulberry are increasing day by day and subsequently this interest leads to an increase in demand for mulberry seedlings and resulting higher costs. Also, the demands are not fully met due to some practical difficulties experienced in the black mulberry seedlings. Therefore, in the study, the effect of different temperature and humidity content of rooting medium on rooting percentage of black mulberry hard wood cutting was investigated. For this purpose, three temperature and humidity contents were applied. This study was performed under greenhouse conditions.

MATERIALS AND METHODS

Hardwood cuttings taken from an old branch of the rest period were prepared as 15 cm and then firstly soaked 0.3% fungicide for precaution against fungal infection. Then cuttings were submerged in indole butyric acid (IBA, 5000 ppm prepared in ethanol) for 5 seconds about 1 cm below the basal part of the cuttings. After holding the cuttings for 1-2 minutes in order to evaporate the alcohol, cuttings were planted in

rooting medium. The experimental design was composed of nine rooting chamber, of which temperature and humidity were separately controlled (Figure 1).

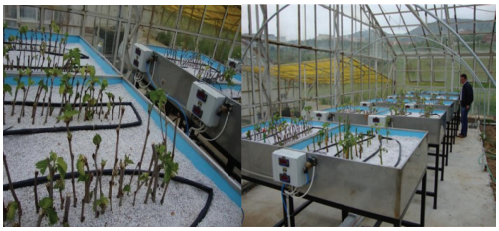

Figure1. Rooting chambers under control conditions

Temperature and humidity level for the study were determined as follows. The data, collected from Ministry of Food, Agriculture and Livestock, have been statistically processed and interpreted, building the trend line and setting up the forecast based on simulation models for the period 2012-2015. Subsequently, the rooting media were applied three different temperature degrees and humidity levels. Root zone humidity value was based on the field capacity. The desired temperature and humidity values of the table based on real-time measurements were performed with a computer-controlled system (Figure 2).

Figure 2. Computer-controlled system for temperature and humidity

The study was performed according to the complete randomized experimental design with three replicates and each replicate corresponded to 20 cuttings. The cuttings were kept in rooting media for 90 days. At the end of this period, the following measurements were made.

Callus formation: Callus was detected from cuttings and the results are expressed as a percentage of total cuttings.

Rooting rate: Adventitious root formation was detected from cuttings and the results are expressed as a percentage of total cuttings.

Root length and diameter: Adventitious root length and diameter were measured with a calliper.

Root number: Root number was expressed as the total number of adventitious formed per number of cuttings forming root.

Statistical Analysis: SPSS 17.00 statistical programme was used to determine statistical significance levels. The independent one-way ANOVA followed by Duncan multiple range test and the differences between individual averages were considered as statistically important at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Callus formation rates were collectively represented in Table 1. Whereas the highest callus formation rate (86.1 %) was obtained under 40 % field capacity and 18°C, the lowest rate was determined under 60 % field capacity and 26°C.

Concerning callus formation, no statistical significant difference was found among temperature and moisture content interaction but the effects of temperature and moisture content were significant ($p \leq 0.05$). Compared the means of temperature applications but not considering the moisture content, the highest callus formation was (71.72 %) under 22°C and the average results were statistically different and significant for each temperature application.

Table 1. Callus formation under different growth conditions

Temp.(°C)	Humidity (%)			Mean
	40	60	80	
18	86.10	60.53	55.13	67.26 AB
22	70.66	67.60	76.90	71.72 A
26	79.43	47.80	51.80	59.68 B
Mean	78.73 A	58.64 B	61.28 B	

Mean in the same column by the same letter are not significantly different to the test of Duncan ($p \leq 0.05$).

For the comparison of moisture content without evaluation of temperature, the highest value (78.73 %) was ascertained under 40 % moisture content but no statistical differences were found for 60 % (58.64 %) and 80 % (61.28 %) moisture contents (Table 1).

The highest root formation rate (69.06 %) was obtained under 40 % field capacity and 18°C, the lowest rate was determined under 80 % field capacity and 18°C. With respect to the root formation, no statistical significant difference was found among temperature and moisture content interaction but comparison of moisture content without evaluation of temperature, the highest value (63.11 %) was ascertained under 40 % moisture content but no statistical differences were found for 60 % (43.47 %) and 80 % (49.57 %) moisture contents (Table 2).

Table 2. Root formation under different growth conditions

Temp. (°C)	Humidity (%)			Mean
	40	60	80	
18	69.06	38,60	36.30	47.99
22	64.16	48,46	66,96	59.87
26	56.10	43,33	45.43	48.29
Mean	63.11 A	43.47 B	49.57 B	

Mean in the same column by the same letter are not significantly different to the test of Duncan (p≤0. 05).

Concerning with root length, no significant difference was found among temperature and moisture content interaction but the effects of temperature and moisture content were significant (p≤0.05).

Compared the means of temperature applications but not considering the moisture content, the highest root length (43.21 cm) was under 22°C but the average results were not statistically different at 18°C and 26°C.

Also, the highest root length value (42.64 cm) was obtained from 80 % but moisture content (40 % and 60 %) did not elicit statistically significant differences on root length (Table 3).

Table 3: Root length (cm) under different growth conditions

Temp.(°C)	Humidity (%)			Mean
	40	60	80	
18	29.47	30.07	26.71	28.75 B
22	33.90	37.72	58.02	43.21 A
26	28.23	28.47	43.21	33.30 B
Mean	30.53 B	32.08 B	42.64 A	

Mean in the same column by the same letter are not significantly different to the test of Duncan (p≤0. 05).

Regardless moisture content, average results for temperature were significant for root diameter but no difference between 18 °C and 22 °C. In agreement with root length, the widest diameter was determined under 80 % moisture content (Table 4).

Table 4: Root diameter (mm) under different growth conditions

Temp. (°C)	Humidity (%)			Mean
	40	60	80	
18	1.76	2.12	2.11	2.00 A
22	1.85	2.13	2.26	2.08 A
26	1.30	0.95	1.60	1.29 B
Mean	1.65 B	1.73B	1.99 A	

Mean in the same column by the same letter are not significantly different to the test of Duncan (p≤0. 05).

Table 5: Root number per cutting

Temp. (°C)	Humidity (%)			Mean
	40	60	80	
18	4.99	4.40	4.80	4.72 B
22	6.37	5.57	7.70	6.54 A
26	4.87	7.27	4.33	5.49 AB
Mean	5.41	5.74	5.61	

Mean in the same column by the same letter are not significantly different to the test of Duncan (p≤0. 05).

Average results related with temperature were significant for root number per cutting for each applications but soil moisture content did not elicit any significant changes.

In cutting propagation, no direct relationship with the rooting and callus formation, which occurs as a response to injury but increase the survival time of cuttings by preventing the decay in rooting medium has been reported (Kaşka and Yılmaz, 1990; Koyuncu et al., 2003a). However, in the present study, there was a parallel variation between callus formation and rooting percentage. These results are agreement with the report by Yıldız and Koyuncu (2000) but disagree with the studies (Sezgin 2009, Koyuncu et al., 2003a). In addition to delaying the decay of cuttings through formation of a protective layer, callus tissue was reported to help the water intake in some cases (Hartman and Kester, 1974). Herein, the highest callus formation percentage was found less than 22°C but no difference was determined between 18°C and 22°C. An increase up to 26°C slowed down callus formation. In general, keeping temperature in rooting media around 24°C promote cell

division and callus formation (Ağaoğlu et al., 1995) but it is worthy to note that those temperature values might vary according to the different plant species.

Moisture content in rooting media effected callus formation and 40 % of field capacity was the most favourable for callus formation. Up to our best knowledge, there is no study on the relation between moisture content and callus formation but in general, moisture content at a level is required in order to prevent ventilation (Hartman and Kester, 1974). Basal heating of rooting media can enhance root formation percentage (Alexandrow 1988); Yıldız and Koyuncu (2000) recorded an increase from 60 % to 89 %.

Even the highest average of root length was obtained from the rooting media with highest moisture content; there was decay in many cutting samples. This condition is probably caused by obstruction of the ventilation due to the high moisture content. Indeed, a good aeration of the rooting medium, in addition to good moisture retention, is stated to be the best (Hartmann and Kester, 1974).

CONCLUSIONS

In the study, the best root width was obtained under 18°C and 22°C but it decreased by 26°C. An increase in temperature of the rooting media decreased the width of root. Those coincided with the studies by Yıldız et al. (2009) and Sezgin (2009). In this study, moisture content in rooting media did not cause any change in number of root formation. The present results concerned with root formation are in good agreement with the previous studies (Koyuncu et al., 2003b, Yıldız et al., 2009; Sezgin, 2009, Erdoğan and Aygün, 2006).

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CHANGES IN THE PHYTOCHEMICAL COMPONENTS IN WINE GRAPE VARIETIES DURING THE RIPENING PERIOD

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Abstract

The aim of this study was to determine phytochemical components of Tannat, Cabernet Sauvignon, Malbec, Merlot and Shiraz wine grape varieties during the ripening period. As amounts of total phenolic compounds in different parts of the grape varieties, the highest total phenolic values for berry peel were found to be 300.58 µg GAE/mg in Cabernet Sauvignon, 974.23 µg GAE/mg in Malbec for pulp, 447.01 µg GAE/mg in Merlot for seed. The total flavonoid content in peel, pulp and seeds of varieties were found to be varied between 46.95 µg QUE/mg and 148.01 µg QUE/mg. In conclusion, total bioactive compounds of the grape differed significantly based on variety and grape part. Since higher bioactive compounds were found in pulps for all grape varieties, grapes should be consumed as a whole grape. This study also showed that these grapes are a potential source of natural bioactive compounds. It can be concluded that selected grape varieties and their parts can be considered a good source of phenolic and antioxidants.

Key words: *Grape, Diyarbakir, phenolic, flavonoid, cluster, berry, seed*

INTRODUCTION

Grapes are considered as a significant source of antioxidants in fruit species in the world (Pirinccioglu et al., 2012). Due to this property, it is a kind of fruit with the importance increasing day by day (Macheix et al., 1990). Among the types of grapes, especially red grapes, grape juice and major phenolic compounds found in red wine are called as flavonoids anthocyanins and flavonols (Rice-Evans et al., 1996; Singleton 1982; Palomino et al., 2000). It is reported that (Morris and Cawthon 1982; Bravdo et al., 1985; Matthews and Anderson 1988; Iland, 1989; Nadal and Arola 1995; De La Hera Orts et al., 2005) these substances that are important in terms of human health and found in grape vary according to the varieties of the grapes (Landrault et al., 2001), climate and soil conditions of the place where it grows (Spayd et al., 2002 ; Mateus et al., 2001), the maturity levels (Cangi et al., 2011), cultural practices (Babalik et al., 2009) and post-harvest transactions (Revilla et al., 2001).

Among the climate features of the vineyard areas, the place and vector issues especially the temperature, humidity and insolation are encountered as the important factors affecting the synthesizing the of phenolic compounds and antioxidant substances and the other phytochemicals. Considering these factors having very important impact on the ripeness and all compounds of ripeness, it is seen that some studies have been carried out to identify the phytochemical features of grape varieties grown in different ecologies in our country recently. (Deryaoglu and Canbas 2003; Karadeniz et al., 2005; Aras, 2006; Orak, 2007; Kelebek, 2009; Ozden and Vardin 2009; Uluocak, 2010; Bayir, 2011; Toprak, 2011; Kaplama, 2012).

Increased competition in the wine sector has led to the increasing demand for quality wine grapes and thus the emergence of the concept of quality in the grapes for wine to the forefront. In growing quality grapes, as well as the effects of the ecological factors in growing grapes especially the climate factors, the cultural applications performed in the viticulture have very significant effects on the the phytochemicals properties. By identifying the effects of the range of cultural applications like pruning (Pehlivan and Uzun, 2015), cultivation (Babalik et al., 2009) and irrigation (Bravdo et al., 1985; Matthews and Anderson

1988; Nadal and Arola, 1995) performed to receive production with the highest efficiency and quality on the phytochemical properties such as phenolic compounds, tannin and antioxidants and performing due to the information obtained will be useful.

Except for the effects of cultural applications performed in the vineyards on the physical changes in the bunch and berry features of the grape varieties and the properties such as brix, pH and total acidity in unfermented grape-juice (Tangolar et al., 2002; 2005 and 2010), it is very important to identify its effects on the amount of phenolic substance that is one of the most important quality indicators, and phyto-chemical properties including anthocyanin and antioxidant capacities. In the literature, it is seen that the studies to identify the effects of the cultural applications such as pruning, irriga-tion and fertilization in the varieties grown in the ecological conditions of our country are not too much (Pehlivan and Uzun, 2015).

This study was conducted to determine phyto-chemical components of Tannat, Cabernet Sauvignon, Malbec, Merlot and Shiraz wine grape varieties during the ripening period in the ecological conditions of Diyarbakır/Turkey.

MATERIALS AND METHODS

The research was carried out in the Dicle University Faculty of Agriculture Department of Horticulture in 2011 and 2012 years. In the research, Cabernet Sauvignon, Merlot, Shiraz, Malbec and Tannat red wine grape varieties are used as materials.

Grape varieties are grown as grafted into 110 R rootstocks. Vines are 7 years old. Planting distances rows are 1 m and intra-row is 2.5 m. Mid wire cordon training system was applied to the vines from 60 cm height. While making yield pruning to the vines, it was loaded as 18 buds/vine stock. Vineyard area has clay loam soil type. The applications such as irrigation, fertilizers and disease and pest control are done regularly in the vineyard.

Within the scope of the research, the chemical change occurred in Total Soluble Solids (TSS), total acidity and pH values during veraison and maturity periods of grape varieties has been determined (Ozdemir et al., 2006; Tangolar et al. 2010).

Besides, in order to determine the phyto-chemical change in peel, pulp and seeds of the grape samples, total phenolic compound amount and total flavonoid amount were detected (Chandler, 1983; Slinkard et al., 1997; Baydar et al., 2007).

RESULTS AND DISCUSSIONS

As a result of the study, total soluble solids (TSS) values of grape varieties showed significant differences according to the types and years. When TSS values of the grape values during veraison period of 2011 were examined (Table 1), it was determined that the highest value was in the Merlot variety (14.00%) and the lowest value was in Malbec varieties (11.33%). The difference between TSS values of the varieties was not found to be significant in 2012. TSS values were determined between 12.00% and 13.66%. When TSS values of the maturity times of the grape variety between the years of 2011 and 2012 were examined, the highest value was determined in Merlot grape varieties (25.33%) and the lowest value was determined to be in the Malbec grape variety (20.66%) in 2011 among the varieties included in the same letter group.

In all varieties, TSS amount has rapidly increased since the period of veraison and the yields reached to the period of harvest time after 3-6 week maturation process according to the varieties. In both years, TSS amount that was low in the period of veraison was determined to reach 20-25% of values that is desired during the harvest time.

In the trial made in Adana by Tangolar et al., (2005) in the years of 2002-2003, the TSS amounts in Chardonnay, Cabernet Sauvignon, Narince and Okuzgozu were determined to be 24.1-23.2-20.7 and 19.9% respectively in the second year. The researchers reported that there may be significant changes in the rate of TSS according to the varieties and the years. Indeed, as a result of this study, while the difference between TSS values in the veraison time of the grape varieties was important in the year of 2011, it was insignificant in 2012.

Ozdemir and Tangolar (2005) examined phenological stages (EST), temperature total values and some quality features in some table

grape varieties in Diyarbakır and Adana conditions, for two years. TSS was determined to be 12.6% and 12.7% respectively for Diyarbakır and Adana provinces in 1997 and 12.5% and 12.5% in 1998.

In both provinces, it has been reported that EST values were at the level that will not create problems for viticulture, the physiological activity in the vicinity of Diyarbakır started earlier but fruit ripening occurred in Adana earlier. Cluster, berry and grape-juice characteristics vary according to the varieties but the values among the provinces are close to each other.

As a result of a study carried out in the region of Kazova during two years, it has been reported that TSS increased in Bogazkere, Cabernet Sauvignon, Chardonnay, Emir, Merlot, Narince, Okuzgozu and Riesling varieties from the period of veraison until the harvest and the varieties except for Bogazkere and Okuzgozu reached to the sufficient level according to the desired TSS criteria in the harvest period (Sen, 2008).

As a result of the study carried out by Cangi et al., (2011) on wine grape varieties grown in Kazova (Tokat) region (Gewurtztraminer, Pinot Noir, Narince and Shiraz) TSS was determined to change 20.2% (Narince) and 22.3% (Shiraz) in the harvest period.

In maturation period, the findings related to the amount of TSS indicated that the differences emerged according to the years and varieties were related to the general characteristics of climate conditions and varieties according to the years and varieties in different years. In addition, in the cultural applications such as summer pruning performed in the vineyards in that year, the varieties can be seen to have significant impacts on TSS accumulation.

Table 1. Total soluble solids (%) during the veraison at harvest stages 2011 and 2012 year for varieties evaluated

	Veraison			Harvest		
Varieties	2011	2012	Average	2011	2012	Average
CabernetSauvignon	12.33bc	12.00	12.16c	23.33a	23.33ab	23.33b
Tannat	13.00ab	12.33	12.66bc	24.00a	24.00a	24.00b
Merlot	14.00a	13.66	13.83a	25.33a	25.33a	25.33a
Malbec	11.33c	12.66	12.00c	20.66b	21.33b	21.00c
Shiraz	13.33ab	13.33	13.33ab	24.66a	23.33ab	24.00b
LSD %5	1.01	N.S.	0.7	1.98	2.09	1.26

The difference between the means with different letters in same column was significant (P<0.05)

When the values obtained as a result of total acidity analysis of grape samples received in the process of veraison and maturity process of the varieties were examined, the acidity values were determined to be from 14.58 g/l (Merlot) to 19.02 g/l (Tannat) in the veraison process in the trial years and they ranged from 5.86 g/l (Cabernet Sauvignon) to 8.25 g/l (Tannat) in the maturity process (Table 2).

Table 2.Total acidity (g/L) during the blooming at veraison stages 2011 and 2012 year for varieties evaluated

	Veraison			Harvest		
Varieties	2011	2012	Average	2011	2012	Average
CabernetSauvignon	15.83b	15.74bc	15.83b	5.86c	5.89b	5.86c
Tannat	19.02a	18.84a	19.02a	7.92a	8.25a	7.92a
Merlot	14.58c	14.57d	14.58c	6.23bc	6.37b	6.23bc
Malbec	15.41bc	14.79cd	15.41bc	6.40b	6.43b	6.40b
Shiraz	16.18b	16.28b	16.18b	6.30bc	6.28b	6.30bc
LSD %5	1.17	1.08	1.17	0.43	0.6	0.4

The difference between the means with different letters in same column was significant (P<0.05)

Looking to the acidity values in the maturity values in 2011 and 2012, it was determined to be an increase generally and the highest acidity in 2011 and 2012 was seen in Tannat grape variety (respectively 7.92 and 8.25 g/l), the lowest value was seen to be in Cabernet Sauvignon grape variety (respectively; 5.86 and 5.89 g/l). In all varieties from the period of veraison, a great amount of decrease was observed in the total acidity amount with the

maturation in all varieties and the total acidity value has varied according to the varieties and years in the period of harvest. In the research, the lowest total acidity was determined to be from 5.86 to 5.89 g/l and the highest values were determined between 7.92 and 8.25 g/l (Table 2).

They reported in their study about the development of the grapes from the veraison stage to the extreme maturity, the berry weight increased from the date of first sample taking date and the amount of TSS increased from the process of veraison and the total acidity amount increased up to the process of veraison; it started to decrease after this process and the ratio of decline decreased up to the maturity; the amount of tartaric acid has continuously decreased since the beginning of the maturity and the amount of it remained almost fixed up to the end of the maturity (Agaoglu, 2002; Sen 2008).

In a study carried out with Bogazkere, Cabernet Sauvignon, Chardonnay, Emir, Merlot, Narince and Okuzgozu, Riesling varieties in Kazova region for two years, it is reported that the total acidity decreased rapidly from the period of veraison, the highest total acidity was detected in Bogazkere and Okuzgozu varieties and the lowest one was detected in the variety of Emir in the harvest period (Sen, 2008).

When pH values of grape varieties determined during veraison and maturity periods were examined (Table 3), the highest pH values were determined in Merlot variety (respectively; 2.87 and 2.86) and the lowest values were determined in Malbec variety (respectively; 2.44 and 2.45) in veraison periods in the years of 2011 and 2012.

Table 3. pH during the blooming at veraison stages 2011 and 2012 year for varieties evaluated

	Veraison			Harvest		
Varieties	2011	2012	Average	2011	2012	Average
CabernetSauvignon	2.84a	2.84a	2.84b	3.90bc	3.91b	3.90b
Tannat	2.53b	2.51b	2.52c	3.84c	3.82c	3.83b
Merlot	2.87a	2.86a	2.86a	4.01ab	4.03a	4.02a
Malbec	2.44c	2.45c	2.45d	4.05ab	4.06a	4.05a
Shiraz	2.44c	2.46c	2.45d	4.07a	4.09a	4.08a
LSD %5	0.03	0.02	0.02	0.13	0.06	0.06

The difference between the means with different letters in same column was significant (P<0.05)

As approaching to the maturity time of the grape varieties, it was determined that pH values increased. During the maturity process, pH values among the varieties were determined to vary from 3.82 to 4.09.

As a result of being examined of pH value in the berries during the maturation process of Gewurtztraminer, Pinot Noir, Narince and Shiraz that are among the wine grape varieties grown in Kazova (Tokat) region, pH was determined to vary from 3.27 (Pinot Noir) to 4.20 (Shiraz). When the values obtained from our study are examined, the pH values among the varieties at the time of maturity are seen to vary from 3.82 to 4.09.

Winkler et al., (1974) have reported that pH significantly increased until the grapes matured; with this change in pH, the unsuitable tastes in flavor and eating quality are covered and changed. It is reported that the pH during the grape increased in parallel with the increase in TSS during maturation and used as a decisive criteria in determining optimum harvest time. As the maturity criteria of grapes especially grown in warmer areas, pH can be used as maturity criteria (Fanizza, 1982). Indeed, Amerine et al., (1972) reported the lowest acceptable acid amount must be 0.65 g/100 ml, also pH in red table wine should be lower than 3.4.

When the maturity index values of grape varieties grown in Diyarbakir province in 2011 and 2012 are analyzed (Table 4), it is seen that the highest value belongs to Merlot grape variety (40.63) and the lowest value (30.37) belongs to Tannat grape variety in 2011.

In 2012, similar to the previous year, the highest value was found to be in the Merlot grape variety (39.92) and the lowest value was in the Tannat grape variety (29.18). The maturity index values of grape varieties are determined to vary from 29.77 to 40.27 on average.

Table 4. Maturity index (Total soluble solids (TSS) / Acidity)
during the veraison at harvest stages 2011 and 2012 year for varieties evaluated

Varieties	2011	2012	Average
CabernetSauvignon	39.81a	39.60a	39.71a
Tannat	30.37b	29.18c	29.77b
Merlot	40.63a	39.92a	40.27a
Malbec	32.34b	33.23bc	32.79a
Shiraz	39.15a	37.15ab	38.15a
LSD %5	4.41	5.62	3.08

The difference between the means with different letters in same column was significant (P<0.05)

In 2007, TSS was found to be 21.7 (%) during maturation period in Shiraz grape variety grown in Kazova; Total Acidity to be (g/l) 6.45 and maturity index to be 33.64, while in 2008 TSS was found to be 22.3 (%), Total Acidity to be (g/l) 7.05 and maturity index to be 31.63 (Uluocak, 2010). In our study, the average maturity index value in Shiraz variety was found to be slightly higher amount as 38.15.

As a result of the studies performed in Kazova region, the maturity index values of Bogazkere, Cabernet Sauvignon, Chardonnay, Emir, Merlot, Narince, Okuzgozu and Riesling varieties during harvest periods vary from 18.18 (Bogazkere) to 33.90 (Emir) according to the varieties (Sen, 2008).

When the total phenolic compound amounts in pulp, peel and seeds of grape varieties grown in Diyarbakir province are examined, it has been determined that the differences among the varieties are statistically significant (Table 5).

Table 5.Total phenolic content (µg GAE/mg) in grape berry skin, flesh and seed

Varieties	Peel	Pulp	Seed	Total
CabernetSauvignon	300.58a	107.03c	68.33c	475.94
Tannat	167.06b	697.86b	445.76a	1310.68
Merlot	92.50c	657.46b	447.01a	1196.97
Malbec	108.16c	974.23a	390.23b	1472.62
Shiraz	89.05c	667.13b	411.16ab	1167.34
LSD %5	34.931	129.89	43.96	

The difference between the means with different letters in same column was significant (P<0.05)

When the amount of phenolic compounds found in many different parts of the berries of grape varieties are examined, in the highest values in the peel are found in Cabernet Sauvignon variety, in pulp in Malbec variety and in seed in Tannat variety. When the phenolic compound amounts found in the different parts of the berries of grape varieties are examined, the highest values in the peel are found in Cabernet Sauvignon variety, in the pulp, they are found to be in Malbec variety and in the seed they are found in Tannat variety. The maximum amount of phenolic compounds found in Cabernet Sauvignon variety (300.58 µg GAE/mg), and the least amount is found in the Shiraz grape variety (89.05 µg GAE/mg). Considering the amount of phenolic compounds in the pulp, being different from the peel and the seed, the highest value was found to be in the Malbec grape varieties (974.23 µg GAE/mg) and the least was found to be in the Cabernet Sauvignon variety. When the total amounts of phenolic compounds in the seed are compared, it has been determined that the highest value is found in the Merlot grape variety and the lowest value is found in Cabernet Sauvignon grape variety. The total phenolic compound identified in the peel, pulp and seeds of the varieties has been identified to vary from 475.94 to 1472.62 µg GAE/mg (Table 5).

It has been identified in different research that total phenolic amounts vary according to the variety and year and decreased during maturation period (Doshi et al., 2006; Navarro et al., 2008; Jin et al., 2009). Saidani Tounsia et al., (2009) examined the total phenolic amount in the methanol extract of three types of red grape seeds and found the equivalent of respectively 427.00 mg/100g, 218.00 mg/100g and 112.81 mg/100g of gallic acid for dry weights of Muscat, Shiraz and Carignan varieties. A similar study was carried out by Hogan et al., (2009) and it was examined the

total phenolic content of the three types of Virginia black wine grapes in various regions of northern France by made and Cabernet in Virginia black wine grapes in northern France as Cabernet Franc 1, Cabernet Franc 2 and Cabernet Franc 3 and, as a result, they were identified to be equivalent of respectively 1.82 ± 0.07mg/g, 1.47 ± 0.05, mg/g 0.63 ± 0.02 mg /g of gallic acid.

As a result of their study, Ozden and Vardin (2009) have found that the total phenolic compound concentration of some grape varieties grown in Sanliurfa conditions such as Merlot, Chardonnay, Cabernet Sauvignon and Shiraz *(V. vinifera* L.) grape varieties vary from 1805 mg/kg to 3170 mg/kg in terms of total antioxidant activity and certain phytochemical properties. While the highest concentration of phenolic compounds was being found in Chardonnay variety, the lowest concentration was found in Shiraz variety.

In their study, Gokturk Baydar et al., (2011) have identified grape seeds and peel extracts belonging to Cabernet Sauvignon, Kalecik Karasi and Narince grape varieties, antioxidant properties of wine and the content of phenolic compounds. Total phenolic content was determined to vary from 522.49 to 546.50 mg GAE g^{-1} in seed extracts and from 22.73 to 43.75 mg GAE g^{-1} in peel extracts and from 217.06 to 1336.21 mg L^{-1} in wine. The radical scavenging effects of the samples and reducing capacities have varied depending on grape varieties, the parts of the grape and wine type.

Kanner et al., (1994) analyzed total phenolic compound amounts by harvesting the grapes in optimal harvest ripeness in their study conducted with seven different table (Miabell Concord, Flame Seedless, Emperor, Thomson Seedless, Red Globe and Red Malaga) and seven different wine (Calzin Petite Shiraz, Merlot, Cabernet Sauvignon, Cabernet Franc, Sauvignon Blanc and Chardonnay) grapes. They reported that phenolic compounds in wine grapes vary from 230 to 1236 mg/l and Calzin and Petit Shiraz grape varieties have the highest phenolic content.

It has been identified in different researches that total phenolic types vary according to the year and variety and they decrease during the maturity process (Doshi et al., 2006; Navarro et al., 2008; Jin et al., 2009). Saidani Tounsia et al., (2009) examined the total phenolic amount in methanol extract of three types of red grape seeds and they found equivalent to respectively 427.00 mg/100g, 218.00 mg/100g and 112.81 mg/100g of gallic acid for Muscat, Shiraz and Carignan varieties in their dry weights. A similar study was carried out by Hogan et al., (2009) and it was examined the total phenolic content of the three types of Virginia black wine grapes in various regions of northern France by made and Cabernet in Virginia black wine grapes in northern France as Cabernet Franc 1, Cabernet Franc 2 and Cabernet Franc 3 and, as a result, they were identified to be equivalent of respectively 1.82 ± 0.07mg/g, 1.47 ± 0.05, mg/g 0.63 ± 0.02 mg /g of gallic acid. As a result of the analysis made in grape varieties examined in Diyarbakir conditions, the total amount of flavonoids in the pulp, peel and seed of the berry were found to vary greatly among varieties (Table 6).

Table 6.Total flavonoid content (µg QUE/mg) in grape berry skin, flesh and seed

Varieties	Peel	Pulp	Seed	Total
CabernetSauvignon	5.18e	60.99b	9.72ab	75.89
Tannat	13.28b	53.62c	7.61bc	74.51
Merlot	5.85d	29.46d	11.64a	46.95
Malbec	10.17c	58.37bc	7.28bc	75.82
Shiraz	19.65a	122.25a	6.11c	148.01
LSD %5	0.65	5.34	3.06	

The difference between the means with different letters in same column was significant (P<0.05)

When the total amount of flavonoid compounds is examined, the total flavonoid amount found in the peel, pulp and seed was detected to vary from 46.95 to 148.01 µg QUE/mg. It has been detected that the flavonoid content in the peel is found mostly in Shiraz grape variety (19.65 µg QUE/mg) and the lowest one is found in Cabernet Sauvignon (5.18 mg QUE/mg) grape variety. Considering the flavonoid substance content in the pulp, it has been detected that the

highest value is found again in Shiraz grape variety (122.25 µg QUE/mg) and the lowest one is found in the Merlot grape variety (29.46 µg QUE / mg).
When flavonoids substance amounts found in the seed were analyzed, the highest value was found to be in Merlot grape varieties (11.64 µg QUE / mg), and the lowest value was found to be in Shiraz grape varieties (6.11 µg QUE/mg) (Table 6).

CONCLUSIONS

In the chemical analysis performed with one-week intervals from the period of veraison to the maturity, it has been determined that while the grapes are ripening, there is an decrease in tartaric acid and an increase in TSS and pH and these parameters vary in accordance with the years and varieties.
As a result of the examination of the grape-juice features of the varieties used in the research, it has been seen that the amount of TSS rapidly increased in all varieties since the period of veraison and it reached to the harvest period of the grapes after 3 to 6-week maturation period according to the varieties. In both hears, the amount of TSS that was low in the period of veraison reached to the 20-25% of desired values during the harvest period.
When the values obtained as a result of tartaric acid analysis of the grape samples received in the periods of veraison and maturity are examined, the acidity value in the period of veraison in trial years was determined to vary from 14.58 g/l (Merlot) to 18.84 g/l (Tannat) and in the period of maturity, it was determined to vary from 5.86 g/l (Cabernet Sauvignon) to 7.92 g/l (Tannat).
When the pH values of grape varieties determined during the periods of veraison and maturity are examined; the highest pH values during veraison periods in 2011 and 2012 were determined to be in Merlot varieties (respectively; 2.87 and 2.86), the lowest values in the Malbec variety (respectively, 2.44 and 2.45). As approaching to the maturation time of the grape varieties, an increase was determined in the pH values.
According to the results of maturity index that is another feature examined in the research, in both trial years it has been determined that the highest value belongs to Merlot grapes variety while the maturity index value varied between 29.77 and 40.27 on average.
As a result of the research, the highest values in terms of the amounts of phenolic compounds in the research have been determined to be in the peel in Cabernet Sauvignon variety (300.58 µg GAE/mg), in the pulp they are found to be in Malbec variety (974.23 µg GAE/mg) and in the seed they are found in Merlot variety (447.01 µg GAE/mg). When the flavonoid amounts are compared, it has been determined that the total flavonoid amount varied from 6.95 µg QUE/mg to 148.01 µg QUE/mg. the flavonoid content found in the peel is found mostly in the Shiraz (19.65 µg QUE/mg) grape variety and the lowest value was found in Cabernet Sauvignon (5.18 µg QUE/mg) grape variety.
Considering the flavonoid substance content in the pulp, the highest value was found again in Shiraz (122.25 µg QUE/mg) grape variety like in the peel and the lowest value was found in Merlot (29.46 µg QUE/mg) grape variety. When the flavonoid amounts in the seed are compared, it has been determined that the highest value belongs to Merlot grape variety (11.64 µg QUE/mg) and the lowest value belongs to Shiraz grape variety (6.11 µg QUE/mg).
Among plant-derived foods, fruits and vegetables are natural sources that are rich in phenolic substances. Today, it is clear that the increase in escaping from the artificial substances will increase the significance of the natural phenolic substances. Besides the use opportunities in the fields of food, ladder and pharmacology, it is seen that understanding the mechanism of action of phenolic substances with significant effects on human health and it is important to investigate the paths to be able to use technologically.

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DETERMINATION OF POMOLOGICAL AND BIOCHEMICAL COMPOSITIONS ON BERRIES IN DIFFERENT PARTS OF CLUSTERS IN SOME TABLE GRAPE VARIETIES

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Abstract

This research work has been conducted in the trail areas of the 'Research and Application Vineyard of Table Grape Varieties' situated in 'COMU Dardanos Campus' during the years 2013 and 2014 in order to determine pomological and biochemical compositions on berries in different parts of clusters of some table grape cultivars namely, 'Cardinal', 'Yalova Cekirdeksizi' and 'Yalova Incisi'. In the research, the samplings have been done from the berries randomly on top of clusters (TC), middle outer side of clusters (MOC), middle inner side of clusters (MIC) and tip of cluster (TIC) of grape varieties (initial clusters). The heaviest amount of berries has been obtained from the MOC of the 'Cardinal', 'Yalova Cekirdeksizi' and 'Yalova Incisi' cultivars. The ripest berries (SSC TA^{-1}) had been taken in TC in 'Cardinal', TC, MOC and MIC in 'Yalova Cekirdeksizi' and TC, MOC and MIC in 'Yalova Incisi' cultivars. In particular, berries of TIC were found more small–light, and a little bit unmatured than berries of other parts of clusters in 'Cardinal' and 'Yalova İncisi' grape cultivars. Pomological and biochemical compositions of berries on different parts of cluster may vary considerably, and also it has been changed at different grape cultivars. For this purpose, the regular monitoring of the maturity level of table and wine grape cultivars should be done by following the precautionary measures. It has also been determined that the samplings should have to be done in equal number from at least 3 different parts such as top, middle and tip sides of clusters. Nevertheless, the tip portions, having the latest blooming on flower clusters to be cut in certain proportions just after the berry formation, have been projected that they will provide an increase in that of the volume and maturity in those berries remaining on clusters.

Key words*: Vitis vinifera* L.*, table grape, position of grape on cluster, pomological and biochemical composition of grape berries, grape quality.*

INTRODUCTION

According to FAO statistical data; there were 67,067,128 tons fresh grape produced in 6,969,373 ha of vineyards in 2012 in the world. In the same year, the total fresh grape production was recorded as 4,275,659 tons in 462,296 ha grapevine area in Turkey (FAO, 2014). Clusters, uniform in size and colour, have bigger berries is an important factor for increasing the market values of table grapes. Therefore, many researches have been carried out related to the training system and development of new hybrid grape types and varieties aimed to improve the grape quality.

Every berry fertilized on flower cluster, has occurred from pericarp that has juicy pulp and skin (Agaoglu, 1999). Size of berries may vary by variety, growing power of vine stock, water uptake, berry set, berry count and maturity (Celik, 2011). Balance between direct sun exposure on leaf area and size of berries is significantly affected the yield and quality of product (Reynolds et al., 1994). Stoev (1974) reported that leaves found in same direction have been supported by roots in same direction. Similarly, nutrition of bunch on shoot was affected by shoot that have bunches. Todorov (1970) explained that there is a positive correlation between sizes of cluster and shoot having the cluster. The same author also reported that the clusters and berries on strong and productive shoots are more heavy clusters and berries than that of others. However, there are many factors that control the development and composition of grape berries. Therefore, there are significant differences on pomological and biochemical composition between clusters

on a vine stock or first and second clusters on same summer shoot or berries of same clusters (Smart et al., 1985; Yılmaz and Dardeniz, 2009). Yılmaz and Dardeniz (2009) mentioned that the best developing clusters in terms of fruit width, length and weight were first clusters on summer shoots. Moreover, maturity indexes of first clusters have been found higher than second clusters in case of 'Amasya' and 'Cardinal' grape cultivars. Besides, clusters located on summer shoots from growing on second bud showed a better development in terms of cluster width, length and weight than those that located on summer shoots growing on first bud. Though there were not any significant differences found on maturity index by the pruning of 2 buds.

Nowadays, different application methods are being used for the improvement of grape quality. For example, Ilgin (1997) reported that the thinning of cluster at the level of 25% in 'Yalova Cekirdeksizi' grape cultivar decreased the yield of grafted vineyards but had not effect on ungrafted vineyards. Although the grape yields decreased 50% of thinning of 75% of flower clusters in ungrafted vineyard while the grape quality has been increased by 25% level of thinning found more of flower clusters in ungrafted vineyard. Dardeniz and Kismali (2002) determined the effect of cluster thinning, a week before blooming at 0%, 30% and 60% levels, on the yield and quality of grapes, and also on the vegetative growth of 'Amasya' and 'Cardinal' cultivars. But in this study, authors have not recommended 30% levels of cluster thinning of 'Amasya' cultivar, but 60% level of cluster thinning has been recommended only in southern latitudes. In case of 'Cardinal' variety, 30% level of cluster thinning at was enough for grape quality.

Florescence starts from top to bottom on the flower clusters. After berry set, berries on the bottom of the cluster impair the quality and image of whole of the cluster because of smaller and late maturing berries. Therefore bunch thinning is recommended for these berries on the bottom of cluster in 2 or 3 weeks after berry set at 25% or 33% level. Thus either enlargement for cluster or increase in berry size was provided. Also it is possible to harvest at 3–7 days early to clusters that have good coloured, allured and uniform size. When the berries were 5–7 mm, the clusters were tipped at 1/3rd, 1/6th and 1/12th of the cluster length on 'Cardinal' and Uslu table grape cultivars by Dardeniz (2014). In Uslu, cluster length (cm), cluster width (cm), cluster compactness (1–9), number of berries/cluster (n), berry weight (g) and titratable acidity (TA) (%) parameters were affected by the applications. In 'Cardinal', cluster length (cm), cluster compactness (1–9), number of berries/cluster (n), berry weight (g), total soluble solid (TSS) (%), titratable acidity (TA) (%) and maturity index parameters were affected by the applications. Yield was not affected by cluster tipping in both grape cultivars. It was concluded that the cluster tipping applied to the Uslu in a proportion of one–third and to the 'Cardinal' in a proportion of one–sixth of the cluster length would be positively sufficient in terms of increasing the grape quality.

Dardeniz et al. (2012a) compared the growth and productivity of primary and secondary summer shoots, which primary summer shoots were cut at the base following 10–15 cm of growth and secondary buds were forced to sprout giving rise to new summer shoots, of two different table grape varieties, 'Yalova İncisi' ('Honusu' x 'Siyah Gemre') and 'Yalova Cekirdeksizi' ('Beyrut Hurması' x 'Perlette'). In both years (2010 and 2011), vinestock that has secondary shoots showed significant decreases in the levels of fresh grape yield, with especially small grape bunches obtained. In terms of maturity of the grapes, significant differences were not observed among the applications due to low sprout growth on secondary shoots. The results of this study show that some grape products still may be harvested even if primary buds from summer sprout are damaged in cases of late spring frost.

Another research has also been carried out by Dardeniz et al. (2012b) evaluating the changes in chlorophyll content on 8 different table grape varieties leaves in 3 different (5th node, 10^{th} node and 15^{th} node) branch nodes, at 4 periods (15^{th} of June, 1^{st} of August, 15^{th} of September and 1^{st} of November) by SPAD digital chlorophyll meter. Although chlorophyll contents were low on the leaves of 10^{th} and 15^{th} nodes, chlorophyll contents were observed to be equal during the 2^{nd} measurement period in

leaves at the 5th and 10th nodes, and also during the 3rd measurement period in leaves at the 10th and 15th nodes. Additionally during the last study period, a reduction of chlorophyll content was observed in leaves at the 5th and 10th nodes. 3–5 leaves per lateral shoot, the terminal 3 leaves at the tip of the shoot contained low chlorophyll content during the 1st study period; however a gradual increment in chlorophyll content was observed in subsequent periods. High total chlorophyll content was observed in lateral shoot leaves throughout all periods of study when compared to leaves at the tip of the shoot. Turker and Dardeniz (2014) aimed to determine the effects of 3 different levels of axillary shoot removal applications (High Level Axillary Shoot Removal (HLASR); Normal Level Axillary Shoot Removal (NLASR); None Axillary Shoot Removal (NASR) on the yield and quality characteristics of the 6 different varieties of *Vitis vinifera* L. As far as the HLASR application is concerned, it reduced the yield of table grape by causing a decrease in the potential of vine stock in all grape varieties especially in the second year of research. Consequently, this application in which all of the axillary shoots are taken from the bottom of vine stock is not recommended for any variety of table grapes. In the case of well–organized spraying program, NASR method of application will contribute to high yield, good quality and early grape production in all table grape varieties.

A research that carried out on 'Horoz Karasi' and 'Gok uzum' grape varieties, was to determine the effects of 1 3^{-1} cluster reduction (CR), 1 3^{-1} CR + herbagreen (HG) and 1 3^{-1} CR + humic acid (HA) applications on grape yield and quality of cultivars were examined. It was suggested that 1 3^{-1} CR + HA application increased grape yield, berry weight, berry red and blue colour intensity values of Horoz Karasi grape variety and 1 3^{-1} CR application increased grape yield and maturity index values of Gok üzüm grape variety (Akin, 2011).

Akin et al. (2012) reported that the combined leaf fertilizer (TARIS–ZF) significantly increased quality parameters such as berry length, berry weight, maturity index, juice yield and drying index of grapevine cv. 'Gok uzum'. Increasing crop load values (16, 21, and 26 buds/vine) increased fresh grape yield and juice yield; however, maturity index and drying index decreased in comparison to the control. As a result of this study, it was suggested that produce a high yield and to increase quality parameters 16 buds/vine pruning, and fertilization by TARİŞ–ZF may be applied on grapevine cultivation especially on 'Gok uzum' cultivar.

A research that in order to determine the effects of 9 different winter and summer pruning practices in 'Yalova İncisi' grape cultivar is suggested that the thinning practices such as EP + CT (early pruning + cluster thinning) and EP + CT + BT (early pruning + cluster thinning + bunch thinning) have been found recommenddable for acquiring early and high–quality crop yields in those regions of our country where early spring frosts are not considered as dominant. By applying pruning on normal date with high level axillary shoot removal practice; the increases both in the average yield and quality, and ripeness of grapes were quite satisfactory resulting to the increase in leaf size and vine stock potential. On the other hand, overall ripening of grapes resulting by the increase found in leaf size and vine stock potential after the application of GB+SUB practice (Sezen and Dardeniz, 2015).

However, it is known that different treatments affected quality of table grapes, for determinate to effect of these treatment it is required to sample the right way on every cluster. Because it is seen that some grape cultivars' berries on different parts of clusters have significantly pomological and biochemical differences. Therefore, this research in order to determine pomological and biochemical compositions on berries in different parts of clusters in some table grape cultivars such as 'Cardinal', 'Yalova Cekirdeksizi' and 'Yalova İncisi'.

MATERIALS AND METHODS

This research has been conducted in the trail area of the 'Research Vineyard of Table Grape Varieties' situated in 'COMU Dardanos Campus' in the years 2013 and 2014 in 'Yalova İncisi' which has white colour berries with seeds on short cluster and very early cultivar, 'Cardinal' which has red colour berries with seeds on middle–long cluster and early cultivar and 'Yalova Cekirdeksizi' which has white colour seedless berries on middle–short cluster

and mid early cultivar. Plant materials, used in this research, have been grafted onto 41B American grape rootstock ('Yalova Incisi' cultivar) and 5BB American grape rootstock ('Cardinal' and 'Yalova Cekirdeksizi' cultivars) in 11 years old. Vines were trained to unilateral cordon system. The spacing in between rows and within rows was 3.0 m and 1.5 m; respectively. The soil was loamy clay, slightly alkaline, medium calcareous and unsalted. Summer shoots that reached sufficient lengths were bended between first and second wire and tipped above 20–30 cm on second wire. 2nd offshoot tipping treatments did 1 month later after 1st treatments. Tipping of offshoots was cut above 1st or 2nd leaves on bottom of the offshoots by secateurs.

All grape cultivars were thinned first and second leaves of summer shoots, lateral, offshoot, water sprouts, and secondary and tertiary shoots just before full blooming in both 2 years (2013–2014). While harvest time was coming, first clusters of grape cultivars packed with plastic bags and labelled and brought to Pomology Laboratory in Canakkale Onsekiz Mart University, Faculty of Agriculture, and Department of Horticulture.

In this experiment grape berries were sampling randomly on Top of Clusters (TC), Middle Outside of Clusters (MOC), Middle Inside of Clusters (MIC) and Tip of Clusters (TIC); and berry width (mm), berry length (mm), berry weight (g of 1 berry), Chroma value of berry, Hue value of berry, soluble solid content (SSC) (Brix%), Titratable acidity (TA) (mg tartaric acid 100 g^{-1}), maturity index (SSC TA^{-1}) and total phenolic compounds (TPC) (mg GAE 100 g^{-1}) were investigated with these berries.

This research, was settled in randomized plot factorial design with 3 replications and each replication was had 100 berries which took 1st clusters on 4 vine stock. LSD multiple comparison test was used determining the differences among treatments. All of the data analyses were done with SAS system for Windows (ver. 9) statistical package program.

RESULTS AND DISCUSSIONS

Statistical data, in order to determine the pomological and biochemical compositions found into the berries in different parts of clusters of some table grape cultivars, are given in Table 1–6.

In the light of the results of this research, the values regarding to the highest berry width and length have been determined on the TC (20.98 mm and 21.68 mm, respectively) and MOC (21.61 mm and 21.61 mm, respectively) in 'Cardinal' cultivar, when MOC (6.46 g) and TC (6.01 g) had the heaviest level of berries. However; the shortest, narrowest and the lightest berries have been found at the TIC and MIC, respectively. The lowest values of chroma were measured into the MOC (8.67) and TC (9.57). Berries on the TC were found more red tones and good coloured as compared to the other parts of clusters due to their highest values of Hue, shown in Table 1.

The highest SSC have been recorded in those berries which were found on the TC (14.00%) while the lowest SSC were calculated on the berries located at the TIC (12.62%) and MIC (13.16%). The lowest TA was determined in TIC (0.817%) for 'Cardinal' grape cultivar. The highest pH values have been found on the TC (3.47) and MOC (3.42). The ripest berries (SSC TA^{-1}) have been obtained from the TC in case of 'Cardinal' cultivar. Berries of TIC (2029 mg GAE 100 ml^{-1}) and MOC (2056 mg GAE 100 ml^{-1}) had significantly lower TPC in comparison to the other parts of clusters. TC (2112 mg GAE 100 ml^{-1}) having the highest content of phenolic compound, shown in Table 2.

Table 1. Some pomological compositions on berries of different parts of clusters in 'Cardinal' cultivar*

Parts of clusters	Berry width (mm)	Berry length (mm)	Berry weight (g of 1 berry)	Chroma value	Hue value
	2013–2014	2013–2014	2013–2014	2013–2014	2013–2014
TC	20.98 a	21.68 a	6.01 ab	8.67 c	49.32 b
MOC	20.88 a	21.61 a	6.46 a	9.57 bc	62.30 a
MIC	19.97 b	20.91 b	5.60 b	10.15 b	57.82 a
TIC	19.99 b	20.27 c	5.46 b	11.18 a	59.95 a
LSD	0.5855	0.3418	0.6523	0.9553	5.6721

*: Means of 2 years data. TC: Top of clusters, MOC: Middle outer side of clusters, MIC: Middle inner side of clusters, TIC: Tip of clusters.

Table 2. Some biochemical compositions on berries of different parts of clusters in 'Cardinal' cultivar*

Parts of clusters	SSC (%)	TA (%)	pH	Maturity index (SSC TA^{-1})	TPC (mg GAE 100 ml^{-1})
	2013–2014	2013–2014	2013–2014	2013–2014	2013–2014
TC	14.00 a	0.663 b	3.47 a	22.24 a	2112 a
MOC	13.21 ab	0.678 b	3.42 a	20.43 b	2056 b
MIC	13.16 b	0.671 b	3.39 ab	20.88 ab	2068 ab
TIC	12.62 b	0.817 a	3.30 b	16.84 c	2029 b
LSD	0.7965	0.0429	0.0972	1.5628	58.9

*: Means of 2 years data. TC: Top of clusters, MOC: Middle outer side of clusters, MIC: Middle inner side of clusters, TIC: Tip of clusters.

Although, the highest values of the width of berry have been calculated from the MOC (16.03 mm), MIC (15.14 mm) having the narrowest berries. The largest berries in their sizes have been observed into the MOC (19.21 mm) and TC (18.79 mm). While the berries that located onto the MOC (3.34 g) bearing the heaviest number of berries. On the other hand, the berries found on the MIC (2.77 g) possessing the lowest values of weight. There were no any significant differences found in the berries located on different parts of clusters in accordance to the values of Chroma and Hue, given in Table 3.

Table 3. Some pomological compositions on berries of different parts of clusters in 'Yalova Cekirdeksizi' cultivar*

Parts of clusters	Berry width (mm)	Berry length (mm)	Berry weight (g of 1 berry)	Chroma value	Hue value
	2013–2014	2013–2014	2013–2014	2013–2014	2013–2014
TC	15.74 ab	18.79 a	3.06 ab	13.85	106.59
MOC	16.03 a	19.21 a	3.34 a	13.95	106.61
MIC	15.14 b	17.63 b	2.77 b	14.24	106.79
TIC	15.68 ab	17.60 b	3.04 ab	14.72	106.40
LSD	0.6022	1.1285	0.4548	NS[1]	NS

*: Means of 2 years data. TC: Top of clusters, MOC: Middle outer side of clusters, MIC: Middle inner side of clusters, TIC: Tip of clusters.
[1]NS= Non–significant.

Differences between SSC and TA berries, found on different parts of clusters, have not been significantly different when the lowest pH values, maturity index values and TPC values have been determined into the berries located at the TIC which were 3.12, 13.52 and 1890 mg GAE 100 ml^{-1}, respectively.

The highest pH values were measured in berries that found on the TC and MOC. Statistical analyses showed that the berries while taking place on the TC, MOC and MIC had the highest maturity (15.32, 15.03 and 14.80, respectively) while the berries found on the MIC (1963 mg GAE 100 ml^{-1}) and TC (mg GAE 100 ml^{-1}) having the highest numbers of the total phenolic compounds. Monagas and Bartolomé (2005), reported that the maturation and sun exposure factors affected synthesis of phenolic compounds especially flavones are found in the skins of berries for the berries against sun burn (Table 4.).

Table 4. Some biochemical compositions on berries of different parts of clusters in 'Yalova Cekirdeksizi' cultivar*

Parts of clusters	SSC (%)	TA (%)	pH	Maturity index (SSC TA^{-1})	TPC (mg GAE 100 ml^{-1})
	2013–2014	2013–2014	2013–2014	2013–2014	2013–2014
TC	14.53	0.951	3.20 a	15.32 a	1959 a
MOC	14.18	0.943	3.20 a	15.03 a	1907 ab
MIC	14.55	0.984	3.18 ab	14.80 a	1963 a
TIC	13.89	1.029	3.12 b	13.52 b	1890 b
LSD	NS[1]	NS	0.0716	0.9129	45.41

*: Means of 2 years data. TC: Top of clusters, MOC: Middle outer side of clusters, MIC: Middle inner side of clusters, TIC: Tip of clusters.
[1]NS=Non–significant.

According to the results of this research work, the highest berry width have been determined on MOC (19.29 mm) and the lowest berry width have been calculated on MIC (18.00 mm) in 'Yalova Incisi' cultivar.
Berries on MOC and TC (24.41 mm and 23.94 mm, respectively) had the longest berries. When MOC (5.79 g) had the heaviest berries, berries on TIC (4.67 mm) and MIC (4.68 mm) had the lightest grapes.
The lowest chroma values were measured on MIC (13.04), MOC (13.58) and TC (13.65). Berries on TC had more yellow tones and good coloured than the other parts of clusters due to the highest values of Hue (Table 5).

Table 5. Some pomological compositions on berries of different parts of clusters in 'Yalova Incisi' cultivar*

Parts of clusters	Berry width (mm)	Berry length (mm)	Berry weight (g of 1 berry)	Chroma value	Hue value
	2013–2014	2013–2014	2013–2014	2013–2014	2013–2014
TC	19.01 ab	23.94 a	5.54 b	13.65 b	106.74 c
MOC	19.29 a	24.41 a	5.79 a	13.58 b	108.16 b
MIC	18.00 c	23.07 b	4.68 c	13.04 b	109.58 a
TIC	18.54 bc	23.01 b	4.67 c	15.46 a	107.46 bc
LSD	0.5548	0.8148	0.1656	0.8094	1.3988

*: Means of 2 years data. TC: Top of clusters, MOC: Middle outer side of clusters, MIC: Middle inner side of clusters, TIC: Tip of clusters.

The highest SSC have been recorded in berries on TC (13.47%) while the lowest SSC were calculated on the berries of TIC (12.35%).
The lowest TA was determined in MIC (0.472%) for 'Yalova Incisi' grape cultivar. The highest pH values were found on MOC (3.84) and TC (3.81). The ripest berries (SSC TA^{-1}) have been obtained from MIC (28.24), TC (27.59) and MOC (26.95) in 'Yalova Incisi' cultivar.
Berries of TIC (1900 mg GAE $100ml^{-1}$) had significantly lower TPC in comparison to other parts of clusters. MIC (1924 mg GAE $100ml^{-1}$) was highest content of phenolic compound (Table 6).

Table 6. Some biochemical compositions on berries of different parts of clusters in 'Yalova Incisi' cultivar*

Parts of Clusters	SSC (%)	TA (%)	pH	Maturity Index (SSC TA^{-1})	TPC (mg GAE 100 ml^{-1})
	2013–2014	2013–2014	2013–2014	2013–2014	2013–2014
TC	13.47 a	0.488 ab	3.81 a	27.59 a	1911 ab
MOC	12.99 b	0.483 ab	3.84 a	26.95 a	1904 ab
MIC	13.29 ab	0.472 b	3.73 b	28.24 a	1924 a
TIC	12.35 c	0.528 a	3.67 b	23.55 b	1900 b
LSD	0.3795	0.0460	0.0534	2.4693	21.101

*: Means of 2 years data. TC: Top of clusters, MOC: Middle outer side of clusters, MIC: Middle inner side of clusters, TIC: Tip of clusters.

CONCLUSIONS

According to research results, the largest berries were determined on TC and MOC in 'Cardinal' cultivar and on MOC in 'Yalova Cekirdeksizi' and 'Yalova Incisi' grape cultivars. The largest berries were taken from TC and MOC in case of all cultivars, though grape berries of MOC had the heaviest berries in all cultivars.
The ripest berries (SSC TA^{-1}) have been obtained from the TC in 'Cardinal'; TC, MOC and MIC in 'Yalova Cekirdeksizi' and the TC, MOC and MIC in 'Yalova Incisi' cultivars. Particularly, the berries of TIC have been found tinier, lighter and lesser mature as compared to the berries of other parts of the clusters in case of 'Cardinal' and 'Yalova Incisi' grape cultivars.
Pomological and biochemical compositions of berries on different parts of clusters may vary considerably and also they have changed in different grape cultivars. Therefore, the tip reduction for monitoring maturity on table and

wine grape cultivars should be treated with caution and it was determined that samplings have to be done equally at least 3 different parts (top, middle and tip) of clusters. Nevertheless, the tip portions, having the latest blooming on flower clusters to be cut in certain proportions just after the berry formation, have been projected that they will provide an increase in that of the volume and maturity in those berries remaining on clusters.

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KNOWLEDGE OF QUALITY PERFORMANCE OF SOME TABLE GRAPE VARIETIES GROWN AND OBTAINED IN THE EXPERIMENTAL FIELD FROM U.A.S.V.M. BUCHAREST

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Abstract

In our country, the first varieties of grape-vines with known genetic origin, have been created since the sixth decade of the last century, and the outstanding achievements obtained in improving varieties were presented and published over the years, through many treatises. In general, the main objectives of the unitary genetic improvement of grapevine program were sought in obtaining varieties (table grapes, wine, seedless), that would be characterized by a higher production potential than the genitors and that would show greater resistance to pests and diseases specific to grapevines. Unfortunately, these new obtained varieties mostly are known neither nationally nor externally, only a few managed to get in and pass through this transition period, the border of the area where they were created. Although some of these are very valuable, both in terms of productivity and quality, in these circumstances, they will be doomed to anonymity. In this paper, we will refer to the five varieties of table grapes produced in our university - Muscat Timpuriu de Bucuresti, Augusta, Chasselas de Băneasa, Triumf and Select varieties classified in three different eras of maturation (early, middle, tardive). The productive and qualitative performances achieved by these varieties, expressed through carpometric values and organoleptic perspectives (yield, gluco-acidometric index, shape, color of skin, firmnes of flesh, particular flavor), can become attractive for vineyard in the south of Romania and can effectively contribute to fill the conveyor varietal of grape varieties for table grapes. Therefore, the promotion of these local creations, through various means, would be a win for both wine growers (producers) and consumers due to very high production potential, but also because of the particular organoleptic qualities that they posses.

Key words*: capacity, grape table, performance, quality, varieties*

INTRODUCTION

In our country, the first varieties of grape-vines with known genetic origin, they have been created since the sixth decade of the last century, and outstanding achievements obtained in improving varieties were presented and published over the years, through many speciality papers, over the years (Constantinescu et al., 1959, 1960, 1962,1965, 1966; Constantinescu, 1975; Constantinescu and Negreanu, 1960; Dvornic, 1960, 1974; Gorodea et al., 1976; Gorodea, 1983; Neagu et al., 1968; Negreanu and Lepădatu, 1971; Oprea and Gorodea, 1980; Oprea et al., 1983, 1986; Ioniţă et al.,1981; Lepădatu, 1979; Toma and Ispas, 2008). The main objectives of the unit program of genetic improvement of grapevines, coordinated by the Research Institute for Winegrowing and Winemaking Valea Calugareasca, sought and is still soughting in particular at creating varieties with higher potential of productivity and quality, with installment periods of maturation at table grapesvarieties, with increased resistance to diseases and pests, but also with higher resistance to weather and extreme phenomena (Stroe et al., 2013). Therefore, in our country in the period 1970-2000 were approved 22 varieties of table grapes and three seedless, and after 2000, six more varieties of table grapes. Given these goals, mentioned above, creating a new varieties of grapevines and improving the main varieties of table grapes from the range which makes up the conveyor varietal of our country were the main concerns of the research under taken in within our institution – U.A.S.V.M. Bucharest beginning with the year 1957. Among the achievements obtained in our institution, we mention the creation and approval of two early varieties of table grapes: Muscat Timpuriu de Bucureşti and Augusta,

two varieties of table grapes with middle maturity - Chasselas de Baneasa and Triumf and a variety with late maturity- variety Select (Table 1). The main data about these varieties can be found in Vitis International Variety Catalogue (www.vivc.de).

This study aimed to follow the elements that define the quality of the five new varieties in the south area of Romania in order to popularise them.

Varieties come basically from the same wine area where they imposed, but in Romania, except the first two, were rarely investigated and even much less cultivated.

The first two and last two varieties are distinguished by a slight degree of similarity between them, having in common some genetic lineage (Variety Queen Vineyards as paternal variety, in the first case and the variety Afuz ali in the second case).

This study was addressed on the need to know the quantitative and qualitative performance of these varieties in order to promote them at least at national level.

Table 1. Genetic origin of studied varieties

Prime name	Muscat Timpuriu de Bucuresti	Augusta	Chasselas de Baneasa	Triumf	Select
Variety number *V*IVC	8256	14781	2480	12655	11471
Country of origin of the variety	Romania	Romania	Romania	Romania	Romania
Species	*Vitis vinifera* L.	*Vitis vinifera* L.	*Vitis vinifera* L.	*Vitis vinifera* L.	*Vitis vinifera* L.
Pedigree as given by breeder/bibliography	Coarna albă x Queen vineyards	Italia x Queen vineyards	Chasselas dorė	Lignan x Afuz li	Bicane x Afuz ali
Pedigree confirmed by markers	-	Italia x Queen vineyards	-	-	-
Prime name of pedigree parent 1	Coarna albă	Italia	Chasselas blanc	White Luglienga	Bicane
Prime name of pedigree parent 2	Queen vineyards	Queen vineyards	-	-	Afuz ali
Year of crossing	1970	1984	1978	1970	1970
Last update	15.01.2016	22.12.2015	22.12.2015	22.12.2015	22.12.2015

MATERIALS AND METHODS

They were studied five varieties of table grapes, obtained in U.A.S.V.M. Bucharest-Muscat Timpuriu de Bucuresti, Augusta, Chasselas de Băneasa, Triumf and Select. The varieties were conducted on semi-stem type of pruning Guyot on semi-stem with a load of 42 buds/vine. Were watched mainly the elements of fertility and productivity, currently used in studying varieties of grape-vines, in special on those who have shown interest in the appreciation of carpometric and organoleptic elements covered by this study: average weight of a grape, weight of 100 berries, production/vine (kg/vine), sugars (g/l), total acidity (g/l tartaric acid), gluco-acidometric index, shape, color of skin, firmnes of flesh, particular flavor. Grape harvesting was performed at full maturity of each variety. This study was approached from two perspective: knowledge of these varieties to promote and popularize them at least at national level. For this, were used data sheets of each individually varienty, from speciality literature and for updating the variety data were pursued the viticultural year 2014-2015 being known that the variety must provide a high intrinsec quality given by the constant productions obtained year after year, regardless of the direction of production (for table, for wine, a raw material for distilled products etc.).

Figure 1. Variety Muscat Timpuriu de Bucuresti

Variety Muscat Timpuriu de Bucuresti - Short presentation. Vigorous variety with a short growing period between 155-165 days, which is well suited to the lead half stem form. Presents poor tolerance to cold (-16°C -...-18°C), medium drought tolerance, manifesting a high sensitivity to mildew and powdery mildew. The variety is characterized by abundant fruit fulness evenon secondary shoots.

Figure 2. Variety Augusta

Variety Augusta - Short presentation. Variety of medium vigor which obtain good results in 20 buds/m^2, divided on long elements. Presents middle frost tolerance (-18^0 C ...- 20^0 C), powdery mildew and gray mold of grapes and manifest greater sensitivity to mildew.

Figure 3. Variety Chasselas de Băneasa

Variety Chasselas de Băneasa - Short presentation. The variety is characterized by a medium growth vigor of the variety from which it was obtained. But it is more sensitive to cold than Chasselas doré and shows good disease resistance.

The best results were obtained at a load of 12 to 16 buds/m^2 spread over long strings with 12-14 buds, (Ţârdea and Rotaru, 2003).

Figure 4. Variety Triumf

Variety Triumf - Short presentation. The variety is very vigorous and the author (Dvornic, 1974; Indreas and Visan, 2000; Stroe, 2012), recommends Guyot type of pruning on semi-stem with a load of 14 to 15 buds/m^2.Presents good tolerance to cold (-18°C ... -20°C), drought and oidium, especially susceptible to powdery mildew and gray rot of grapes. It is not attacked by moths.
It yields the same as Muscat Timpuriu de Bucuresti on the secondary shoots and most often, they can be used to recover the production of grapes climate accidents.

Figure 5. Variety Select

Variety Select - Short presentation. Select varieties are distinguished by great vigor, with strong growth and a middle period growth. It manifests a good resistance to frost and oidium and tolerance to powdery mildew and gray mold. Select doesn't makes small berry and doesn't makes small grains. In plantations, the author recommends between 18-20 buds/m^2.

RESULTS AND DISCUSSIONS

The analysis of climatic elements for the wine year 2014-2015 and the values of the four synthetic indexes (Table 2) shows that when the thermal resources are high, the water resources are low and the most fluctuating indicator is the bioclimatic one, whose spectrum is within the 9.9 - 12.76. The observations made show that the area in which the didactic-experimental field of U.A.S.V.M. Bucharest is found is favorable for growing varieties of grape-vines studied (registered in the south of Romania), and the elements of microclimate positively put their mark on the behavior of the studied varieties.

Table 2 Evolution of climatic elements (2001-2015)

	Specification	Average	Year
		2001-2011	2014-2015
Indices agroclimatics	The hydrothermic coefficient CH)	0.75	0.73
	The real heliotermic index (IHr)	1.3	1.23
	The viticultural bioclimatic index (Ibcv)	9.9	12.76
	Index of the oenoclimatic aptitude (IAOe).	5231	5153
	Huglin index	2392	2548.9

In awarding the same charges of 42 buds/vine in the viticultural year 2014-2015 it observes, that there are differencies is their behavior, given by the fertility of varieties and the obtained values keeps the varieties in standard limits, and in some cases, such as the variety Triumf the values recorded are even higher (Table 3). Their qualitative appreciation is based not only by analyzing the elements of fertility (absolute fertility coefficient, absolute productivity index), which in general, are constant, but also in the accumulated sugar levels, amid a total acidities quite balanced. The data show a highlight from this stand point varieties Muscat Timpuriu de Bucuresti (171 g/l), Chasselas de Băneasa (178 g/l), but neither the other varieties are found in imbalance, the minimum being recorded by variety Select (145 g/l). Regarding the appreciation of organoleptic and carpometric elements which makes the subject of this study is observed and maintained a constant distinct, surpassing in terms the viticultural year analyzed average values found in the speciality literature (Dvornic, 1974; Gorodea et al., 1976; Oprea et al., 1983, 1986). The varieties of table grapes can be harvested before full maturity, practically at the maturity of consumption based on gluco-acidometric index. Normally this index is between 2.5 ÷ 4.5. At the tested varieties ranged from 2.2 - 3.06 (Table 4), the highest values recorded Muscat Timpuriu de Bucuresti and Chasselas de Băneasa (respectively 3.05, 3.06).

Although the variety Select, usually reach full maturity later belonging to maturing eras V-VI, in terms of the viticultural year 2015 it reached the optimal harvest in advance (mid-September). In the context of the results presented above, it can be appreciated that the viticultural area in which they were created and are current cultivated these varieties, leaves its mark on their quality potential, at the precocity maturing, as evidenced by productions obtained that are constant from quantity and quality point of view, year after year, no matter the variety and age of maturation.

Table 3. The synthesis of the main fertility elements of varieties study

Varieties	% fertile shoots	Absolute fertility coefficient	Relative fertility coefficient	Absolute productivity index (g/shoot)	Relative productivity index (g/shoot)
Muscat Timpuriu de Bucuresti	41 51.9*	1.5 1.0*	0.8 0.6*	483 380*	258 228*
Augusta	61 63	1,7 1.07	1.1 0.63	552 466	356 275
Chasselas de Baneasa	75 65	1,6 1.4	1.1 0.8	411 327	282 187.2
Triumf	40 53	1,1 1.2	0,8 0.9	484 547.2	352 410.4
Select	50 40	1.4 1.1	0,6 0.4	256 451	602 164

*years 2014-2015

Table 4. Physical and chemical characteristics of the grapes belonging to the studied varieties

Varieties	Average weight of a grape (g)	Weight of 100 berries (g)	Production (kg /vine)	Sugar (g/kg)	Total acidity (g tartaric acid/l	Gluco-acidome-tric index	Berry			
							Shape	Color of skin	Firmnes of flesh	Particular flavor
Muscat Timpuriu de Bucuresti	322 380*	383 402*	3.0 2.6*	190 171*	5.9 5.6*	3.22 3.05*	ovoid	green yellow	very firm	muscat
Augusta	325 436	440 502	3.74 3.04	149 155	5,3 5,9	2.8 2.62	ovoid	green yellow	slightly firm	none
Chasselas de Baneasa	257 234	448 398	3.7 3.2	151 178	6,0 5.81	2.51 3.06	globose	green yellow	soft	none
Triumf	440 456	408 430	3.5 3.7	150 152	6.8 6.73	2.2 2.25	ovoid	green yellow	very firm	none
Select	430 410	470 492	3.1 2.6	135 145	6.42 6.57	2.10 2.2	ovoid	green yellow	slightly firm	none

*years 2014-2015

CONCLUSIONS

Muscat Timpuriu de Bucuresti is maturing immediately after variety Muscat Perla of Csaba and far exceeds the size of berries and flavored taste. In addition, grapes precocity, discreet flavor, pleasant taste and attractive appearance make this new variety to have a good potential for the viticulture from our country. It can be grown allover the country, especially in the southern regions, where ensure the early supply of the market.

Variety Augusta is also a sort of early, maturing in the second decade of August, with 5-6 days after Cardinal variety has large berries, but shows a gradual ripening of the grapes.

Variety Chasselas de Băneasa presents larger berries than those of the variety Chasselas doré, but has its lower organoleptic qualities. It impose with large enough production and can contribute to diversification of varietal conveyor of varieties with middle ripening maturity.

Triumph variety is distinguished by the attractive appearance of the grapes very pleasant taste, refreshing and good resistance to transport.

Select variety has a quite compact grape, very showy and doesn't makes small berry and doesn't makes small grains, it retains their organoleptic qualities even after 2-3 weeks if left on the vine, but accumulate modest sugars.

Quantity and quality performance of the varieties analyzed, can become attractive for the decision taking them in culture, at least for the viticulture in the south of Romania.

Therefore, the promotion of these local creations, through various means, would be a win for both wine growers (producers) and consumers due to very high production potential, but also because of the particular organoleptic qualities they hold.

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STUDY OF THE INFLUENCE OF AGING IN DIFFERENT BARRELS ON SHIRAZ WINES

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Abstract

The Syrah/Shiraz grape variety is rather newly introduced in Romania and the producers are still working on establishing optimum winemaking technologies for it. Aging in oak barrels is an important technological step for this high tannic wine, therefore, the selection of the appropriate barrel to improve the structure, colour and flavour of this wine is of great importance. This study aims to compare the influences on the colour and sensory parameters induced on Shiraz wine by aging it for 1 year in barrels made of oak with various origins (French, Romanian, Russian and Hungarian oak) and various toasting levels. Wine kept in stainless steel tank was also used as control. It was observed that the French oak tends to differ compared to Russian and Hungarian oak as regards the colour differences induced in the wines, while the influence of Romanian oak is rather similar to that of French oak. Concerning the sensory quality of the wine aged in barrels, preliminary results point out that barrels from the provider "Transilvania bois" (Romanian and Russian oak) offer constant sensory quality, but some French oak barrels may ensure outstanding sensory quality. Regarding the toasting degree, for Shiraz wines the medium plus toasting offered the best results for structure and aromatic profile after 1 year of aging. These findings need to be confirmed with more precise aromatic profile analyses.

Key words*: oak barrel, Shiraz, flavor, maturation, wine.*

INTRODUCTION

Aging wine in oak barrels is a well known technique used to improve the quality of red wine by reducing the tannin astringency, increase the intensity of colour through tannin-anthocyians condensation and to improve the structure and taste under the influence of the substances extracted from the wood. The earliest literature on the use of oak containers for wine can be traced back to the Roman Empire (Zhang *et al*., 2015).

The topic is important for many wineries, therefore plenty of studies have been performed in order to determine the various effects that wood contact and aging in oak barrels induce in the wines. However, selecting the oak type and toasting degree for the oak to match the structure and complexity of a certain varietal wine is a difficult task, and not many studies are found on this subject.

The present study focuses on several oak barrel types destined for the aging of red wine from the Shiraz grape variety grown in Dealu Mare region of Romania.

The barrels selected for comparison are produced from wood of various origins (French, Russian, Hungarian and Romanian), with various levels of toasting (heavy, medium plus and medium).

The oak wood used in winemaking is mainly from two sources: American oak (Quercus alba - the white oak) and French oak (Quercus robur – the pedunculate oak or sometimes the less common Quercus petraea - sessile oak). American oak induces intense vanilla and coconut flavours, while the French oak induces a more subtle favour. The Eastern European Oak is actually Quercus robur too, as the French oak, but the flavour it imparts is considered in-between American and French Oak, due to a higher content of volatile phenols and phenolic aldehydes than the French oak, even though they belong to the same species (De Simon *et al*., 2003).

Studies dating back as far as 1974 (Singleton, 1974, Singleton 1995) showed that the

chemical composition of oak with various types is quantitatively different, but more recent studies are also available (Alanon *et al.*, 2011). Beside the botanical species, the geographic origin is also important regarding the proportions of various compounds found in the wood (Prida and Puech, 2006; Guchu *et al.*, 2006).

The aging of wines in oak wood barrels leads to the extraction of numerous compounds that have an impact on wine colour, astringency and bitterness, either directly or indirectly. The ellagitannins extracted from wood and the presence of oxygen have a major impact on taste and colour through the process of co-pigmentation of anthocyanins and tannins during red wine aging (Asen *et al*, 1972; Mateus *et al.*, 2001, 2002).

New types of compounds, such as oaklins, have been reported to contribute to changes in the colour and astringency of wines during aging (Sousa *et al.,* 2005).

The grape variety of the wine kept in barrels is also important, as the wine composition too influences the way in which the wine is aging. In this study Shiraz was selected as the grape variety, a rather rare variety for Romania, but for which the interest has been growing steadily during past 10 years.

The wines of Shiraz have a good structure, being quite tannic and requiring aging.

For these reasons, the aim of this work was to study the influences of oak barrels on the Shiraz wine sensory and colour characteristics, in order to determine the most appropriate type of wood in accordance to its origin.

MATERIALS AND METHODS

Raw material

To carry out the study regarding the influence of barrels on the aging of red wines, different types of barrels and Shiraz wines were used.

The Shiraz wine was produced in 2013, from grapes harvested in Dealu Mare Vineyard. The grapes were harvested in plastic boxes of 10 kg each, on October 3, 2013, when they reached the sugar concentration of 260 g/l, which corresponded too with the phenolic maturation phase and a moderate level of total acidity (5.48 g/l tartaric acid). Grapes harvested in this way allowed for both quality and high yield in the resulted wine. Shiraz is a grape variety that does not benefit from over-maturation, not only because it does not accumulate more sugar as in the case of other varieties, but also loses aroma and acidity.

Winemaking process

The harvested grapes were brought directly to the wine cellar and crushed within maximum four hours after harvesting. For antioxidant protection a dose of 40 mg/l of sulfur dioxide (SO_2) was applied during the crushing process.

The winemaking was based on classic red wine maceration fermentation process.

To accomplish the maceration-fermentation phase, 8000 kg of the crushed grapes were introduced in a 10,000 l stainless steel tank. To extract more color from the skins, 1 g/100 kg of enzyme Lallzyme EX-V was added during maceration. The fermentation was started by inoculation of 20 g/hl 15 RP selected yeast from Lallemand. A dose of 20 g/hl OptiRed (yeast membranes) was also added (from Lallemand products catalog).

Maceration-fermentation for this red wine took place at a temperature of 25-29°C, for 15 days. During all this period the homogenisation of the crushed berries was performed 3-4 times a day, by punching down the cap formed at the upper part of the tank.

After the alcoholic fermentation the wine was separated from the solid parts. Malolactic fermentation (MLF) began at the end of alcoholic fermentation, when the wine was still warm (18-20°C) and yeast population was decreasing. To facilitate MLF the wine was slightly aerated and inoculated with 0.5 g/hL malolactic bacteria. The MLF was slow and accomplished in 20-40 days. In the end the wine was again slightly aerated. After the completion of MLF the wine was separated from the lees by racking and then sent to maturation in oak barrels of 225 l volume.

The wine cellar where winemaking was performed is built on the principle of gravity, which prevents excessive pumping and oxidation, allowing for the production of wines with increased quality.

Types of barrels

For the wine maturation different types of barrels were used, obtained from various manufacturers. The oak used for barrels was of different origins and processed to have different

toasting levels. Furthermore, the barrels were either new or in their second or third year of use. For this experiment, the types of barrels available were as follows: barrels of Russian and Romanian oak from the Romanian producer Transilvania Bois; barrels of French oak from the French producers – Radoux, Boutes and Francois Freres; barrels of Hungarian oak from a Hungarian producer - Trust Hungary.
Toasting levels varied from medium, medium plus to heavy toast.

Analyses

The main grape and wine parameters were determined in accordance to the usual methods: the sugar content by refractometry, total acidity by automatic titration by using a Mettler Toledo titrator, alcohol concentration by ebuliometry and sulphur dioxide by Ripper method.
The evolution of wines from different barrels was monitored every 3 months over a period of 1 year, by tasting with an in-house panel of wine experts. Organoleptic analyses of the wines were done by examining and recording the visual, smell and taste traits on a special evaluation sheet. An OIV score sheet for the evaluation in international contests was used to score the wine on a scale of 100 points (OIV, 2008).
The determination of total polyphenol was performed with an UV-VIS – spectrophotometer, by diluting the wine 100 times and measuring the absorbance at 280 nm in cuvettes of 1 mm path length. The value of the absorbance measured at 280 nm was reported after multiplying it with 100.
The determination of color intensity, hue and chromatic characteristics of wine was also performed with an UV-VIS – spectrophotometer. The absorbance of wine was measured at three wavelengths, 620, 520, 420 nm, representative for the description of wine colour. Young red wines have a maximum absorbance at 520 nm, while for aged wines color absorbance shifts, their maximum getting closer to 420 nm.
The reference method for reporting the color parameters was a spectrophotometric method by which tristimulus values and chromaticity coordinates X, Y, Z, are determined according to the standards of International Commission on Illumination (CIE, 2015).

RESULTS AND DISCUSSIONS

The wines of Shiraz were introduced in barrels and aged for 1 year and then their parameters determined. As control, the Shiraz wine aged in stainless steel tank was used.
In Table 1 the main wine parameters are included. It can be seen that the barrel, irrespective of type of oak or year of production (and usage) does not influence much the main wine parameters. The only noticeable exception is the volatile acidity, which is clearly higher in all barrels (around 1 g/l acetic acid) as compared to the volatile acidity determined from wine kept in the stainless steel tank (0.75 g/l acetic acid).

Table 1. Main physico-chemical parameters of Shiraz wines aged in barrels and in stainless steel tank

Barrel type	Free SO_2 mg/l	Total SO_2 mg/l	pH	Alcohol % v./v.	Total acidity g/l tartaric acid	Volatile acidity g/l acetic acid
Control (Stainless steel tank)	53	54	3.68	14.8	5.05	**0.75**
Radoux R TGS H TH – French oak	48	93	3.51	15.5	5.78	0.99
Radoux R TGS M TH – French oak	32	43	3.52	15.4	5.63	0.97
Radoux R TGS Rev TH – French oak	39	74	3.53	15.4	5.93	0.98
Radoux Evol R TGS M+ TH – French oak	31	81	3.52	15.3	5.63	1.08
Francois Freres 2011 TG M TH – French oak	27	65	3.53	15.5	5.66	0.95
Boutes Tradition M – French oak	49	71	3.53	15.3	5.73	1.00
Transilvania bois - Romanian oak	39	55	3.52	15.4	5.76	0.98
Transilvania bois 2013 M – Russian oak	23	52	3.53	15.4	5.78	0.98
Transilvania bois M+ 2013 – Russian oak	30	43	3.52	15.3	5.78	0.99
Transilvania bois M 2011 – Russian oak	33	74	3.53	15.3	5.70	0.92
Transilvania bois M+ 2011 – Russian oak	39	77	3.53	15.3	5.70	0.92
Trust M+ – Hungarian oak	26	61	3.53	15.3	5.76	0.97

The organoleptic analyses revealed that the barrels induced significant differences in the aspect, smell and taste of the wines, and also that these characteristics evolved during the maturation.

In Table 2 the sensory evolution of wines during their maturation is presented, while Table 3 presents the sensory descriptions and scores obtained by the wines evaluated after 1 year of aging in barrels.

Table 2. Average scores awarded to Shiraz wines matured in barrels, evaluated at various time intervals during 4 wine tasting sessions

Barrel type and year of usage	Score March 2014	Score July 2014	Score October 2014	Score March 2015
FrFreres M - II	79.0	81.8	76.8	78.0
Trans B Rus M - I	82.2 Silver	80.0	84.2 Silver	78.0
Radoux H - I	78.5	81.0	82.2 Silver	82.0 Silver
Trust Hung M+ - III	76.6	79.4	76.2	83.0 Silver
Trans B Rus M - II	76.6	79.6	78.6	83.0 Silver
Trans B Rus M+ - I	85.0 Gold	80.2	81.4	83.0 Silver
Trans B Rom M -III	76.6	81.4	76.6	84.0 Silver
Radoux Rev M - I	81.8	82.4 Silver	77.4	84.0 Silver
Radoux Evol M+ - I	82.8 Silver	78.6	76	85.0 Gold
Radoux M - I	77.6	82.8 Silver	81.8	86.0 Gold
Trans B Rus M+ - I	76.6	77.8	77.6	86.0 Gold
Boutes Trad M - I	76.4	73.2	78.0	86.0 Gold

As it can be seen in Table 2, most of the wines aged in new barrels (first year of usage) were highly appreciated by the winetasters after 1 year of maturation. Among the them, those aged in Transilvania Bois – Russian oak, Radoux (both classic and evolution style of toasting) – French oak and Boutes – French oak received top marks, irrespective of the toasting level.

The scores for all the wines ranged from 78-86, which, in accordance to the OIV rules for wine contests signify: good wines (78-81.9 points), silver medal wines (82-84.9), gold medal wines (85-91.9), and great gold medal wines (92-100). Generally, keeping the wines in contact with oak for 1 year improved the perceived quality, but in some cases the quality was not much improved, remaining around 78-82 during all the evaluated period. The Romanian oak only improved the wine up to a silver medal level, but this should be interpreted with caution as the barrel employed was in its third year of usage. It can be noticed that the greatest improvement is generally achieved in new barrels, followed by barrels used in their second and third year.

Aside of the effect on wines' aroma, the barrels are expected to influence the polyphenolic composition of wines (Table 4).

The total polyphenol index of the wine stored in barrels for a year was, on average 37.95 ± 3.46, with only one wine exceeding the range 35.5-38.0 and reaching TPI = 48.57.

This higher polyphenol wine was kept in a French oak new barrel from Radoux, obtained with a special toasting technique called "Evolution" (Radoux, 2009), in which the maximum toasting temperature was lowered compared to the classical toasting process, while the length of the toasting operation extended through a "re-cooking" phase.

Colour has also undergone some changes during barrel aging. The samples were analyzed after one year of storage in tank or in barrel and the CIE*Lab* colour parameters determined are included in Table 4.

Table 3. Wine sensory description and average scores awarded to Shiraz wines matured in barrels after 1 year of aging

Type barrel	Score	Description
FrFreres M - II	78	The medium toast induces less structure and complexity in the taste, a volatile acidity covering the wine fragrance
Trans B Rus M - I	78	Intensely colored, pigments remaining on the tasting glass surfaces, due to a perceptible volatile acidity, which, through acetaldehyde favors copolymerisation
Radoux H - I	82	Intensely colored, with a dominant aroma of vanilla, but still with harsh tannins which dry the mouth; some sweetness is also perceivable
Trust Hung M+ - III	83	Fruity aroma of cherry and sour cherry, quince and fig jam, with a slight hint of volatile acidity, still aggressive tannins
Trans B Rus M - II	83	Powerful, astringent, with aggressive tannins in taste, but with a well balanced acidity
Trans B Rus M+ - I	83	Floral aroma and vanilla notes, still rough, but long, with a burning aftertaste due to alcohol and tannins
Trans B Rom M -III	84	Fine olfactory quality, well integrated medium toast oak and long taste
Radoux Rev M - I	84	More classic style wine, giving the feeling of a shorter term contact with wood, with notes of vanilla and spices, complex in taste, yet harsh, but ready for the market
Radoux Evol M+ - I	85	Long, lingering, peppery aftertaste with persistent, complex flavour of vanilla, tobacco, toast, coffee and caramel
Radoux M - I	86	Fruity aroma of cherries and bitter- sweet cherries, vanilla and truffles notes, long, complex, drinkable, but still a bit harsh in aftertaste
Trans B Rus M+ - I	86	Very well balanced wine, round, lingering, complex, elegant, with notes of over-ripen fruits and spices
Boutes Trad M - I	86	Elegant wine, intense and round in the same time, well balanced acidity and bitterness, well integrated oak flavor

Table 4. Total polyphenolic index and CIE*Lab* parameters of Shiraz wines aged in barrels and in stainless steel tank

Barrel type	TPI	a	b	c	h	L	ΔE*
Control (Stainless steel tank)	46.14	49.38	6.11	49.76	0.12	52.86	0
Radoux R TGS H TH – French oak	36.51	49.56	15.78	52.01	0.31	46.07	11.82
Radoux R TGS M TH – French oak	37.86	54.22	11.87	55.50	0.22	41.39	13.72
Radoux R TGS Rev TH – French oak	37.91	51.35	13.12	53.00	0.25	44.40	11.16
Radoux Evol R TGS M+ TH – French oak	48.57	52.57	13.89	54.37	0.26	40.78	14.72
Francois Freres 2011 TG M TH – French oak	36.91	55.56	13.22	57.11	0.23	40.66	15.41
Boutes Tradition M – French oak	37.86	48.78	11.83	50.20	0.24	50.68	6.15
Transilvania bois - Romanian oak	35.54	52.67	11.06	53.82	0.21	47.23	8.19
Transilvania bois 2013 M – Russian oak	36.28	51.72	14.13	53.62	0.27	34.87	19.83
Transilvania bois M+ 2013 – Russian oak	37.28	54.63	12.11	55.96	0.22	39.58	15.49
Transilvania bois M 2011 – Russian oak	37.76	54.85	13.85	56.57	0.25	42.07	14.36
Transilvania bois M+ 2011 – Russian oak	37.43	52.95	10.86	54.05	0.21	46.92	8.41
Trust M+ – Hungarian oak	35.49	54.55	15.03	56.58	0.27	40.83	15.85

* ΔE is the colour difference calculated against the colour of wine in tank

All the wines have an intensely red colour, with some differences in the colour tone. Some samples aged in barrels, in accordance to parameter *a*, have their colour shifted on thee green-red axis toward more red tones (*a*=50-55), as compared to the wine stored in stainless steel tank (*a*=49.4). Not all the wines kept in barrels evolved toward a higher value of parameter *a*, showing that the type of barrel and the amount of oxygen which enters through it is important, although no clear tendency regarding the origin of oak or type of toasting was identified. As regards the parameter *b*, the position on the blue-yellow axis, this one was clearly influenced by the barrel. The values of parameter *b* are more or less double (*b*=10.9-15.8) for the wines in barrels than the value of the wine kept in tank (*b*=6.1), clearly showing a shift toward yellow components, which means that they lost their blue-violet tones and acquired more yellow-brown tones, typical for wine oxidative maturation. This tendency is easier to observe when we place the samples in the *ab* colour space (Fig. 1).

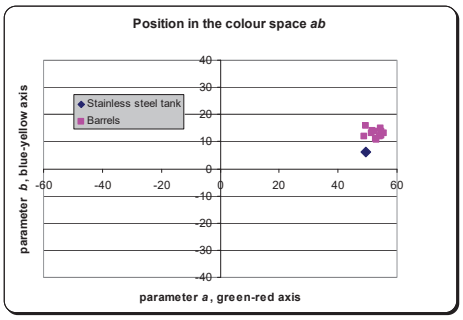

Figure 1. The position of wine the tank (blue) and in barrels (magenta) in the *ab* colour space

As expected, the evolution of wine in barrels is faster than in tank, the hue increasing in all the wines kept in barrels, irrespective of the oak origin and type of toasting (Fig. 2).

However, although for the separate colour parameters changes related to a certain type of oak could no be clearly demonstrated, the overall colour difference, ΔE, which takes into account the variations of a, b and L parameters ($\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{(1/2)}$) seems to show that some types of oak induce more colour changes than others (Fig. 3).

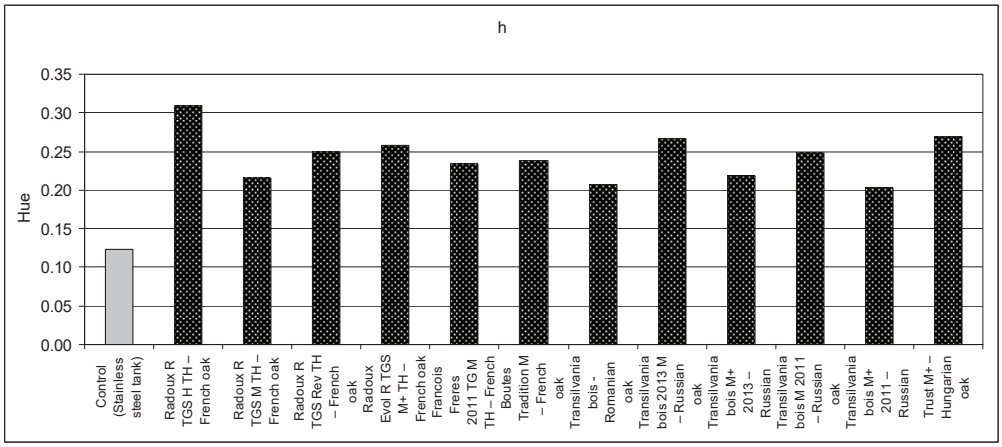

Figure 2. The wine hue in the tank (left sample) and in barrels

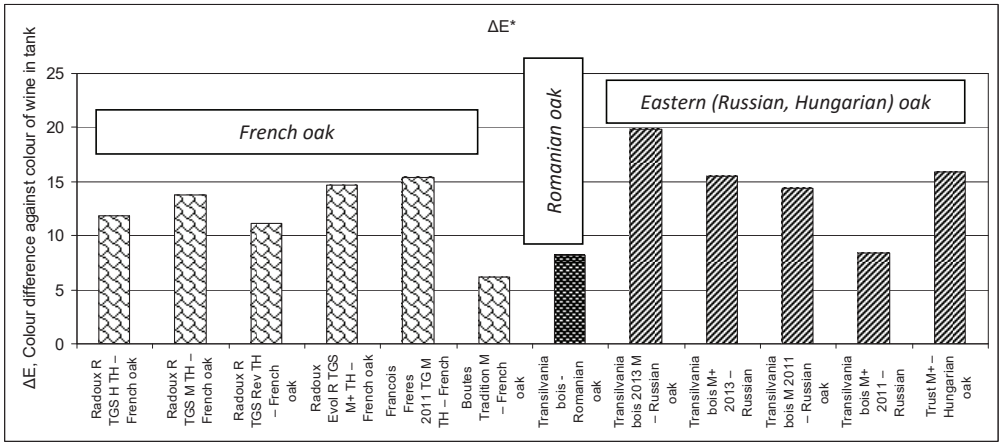

Figure 3. The difference in color ΔE wines depending on the type of barrel compared with the control sample kept in tank (groups of barrels made of French oak, Romanian Oak and Russian or Hungarian oak were outlined with different patterns)

It was observed that Eastern oak (Russian and Hungarian oak) tends to induce more colour differences in the wines (Fig 3 – the right group, average ΔE=14.8±1.8) as compared to French oak (Fig 3 – the left group, average ΔE=12.2±1.4). In this respect, the Romanian oak (Fig. 3, the middle sample, ΔE=8.19) tends to behave more like the French oak, although, by applying statistical analysis for the three groups, due to the small number of barrels analyzed and the inherent barrel-to-barrel variation (Towey and Waterhouse, 1996), no statistically significant difference was found at the 0.05 level of certainty. Moreover, it must be reminded that the barrel made of Romanian oak is in its 3rd year of usage and that the colour is not only influenced by the porosity and oxygen allowed to dissolve in wine, but also by the tannins transferred by the barrel to wine and their condensation with the existing polyphenols of wine. However, also in the third year of usage are the barrel Boutes Tradition-French oak (ΔE=6.68) and barrel Trust M+ – Hungarian oak (ΔE=15.85), showing that the Romanian oak behaves rather like the French oak than the others, suggesting that the Eastern oak is allowing more oxygen in the wine than the French oak.

CONCLUSIONS

In order to determine the evolution of Shiraz wine in barrels and select the most appropriate barrels for the wine style the simplest analyses to perform are colour determination by CIE*Lab* method and sensory analysis.

Some colour differences were induced by aging in barrels, and based on these changes we observed that the French oak effects tend to differ compared to Russian and Hungarian oak, while the Romanian oak places itself closer to the French oak in this respect.

Transilvanian bois barrels (Romanian and Russian oak) offer constant sensory quality (silver and gold medal scores), but some French oak barrels led to outstanding sensory qualities in wines (gold medal scores).

Among the tested barrels, the Radoux Evolution barrel, produced by a special toasting technique, stood out by producing the wine with highest total polyphenol index, one of the highest colour differences compared to the control wine and the gold medal score obtained in sensory evaluation.

Based on the studied parameters, no clear correlations were found regarding the colour of the final wine and the toasting degree and the barrel year of usage. Apparently, the medium plus toasting degree is more suitable for the structure and aromatic profile of Shiraz wine, thus they were described as being more complex and round in the same time, with the classical notes of coffee and spices. However, more precise analyses are needed to draw accurate conclusions regarding the changes in aromatic profile induced by certain types of barrels or toasting levels.

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EVALUATION OF PARENTAL FORMS AND HYBRID POPULATIONS DESCENDING FROM TOMATOES, FOLLOWING HEAT RESISTANCE AND PRODUCTIVITY

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Abstract

Climate change has a negative impact on agriculture. In the last decade, drought and high temperatures have become more frequent with strong negative effects on crop productivity. Selection based on resistance to extreme temperatures now becomes the actual objective because in some years, the air temperature reaches 35-45ºC during the flowering stage of the day, which considerably reduces fruits setting and the yield per hectare. The aim of our research was to test the level of heat resistance of genotypes and hybrid populations descending from tomatoes to select the forms with high resistance level. As a result of research we found that the highest resistance to heat was manifested by genotypes of F_3 generation Mihaela x Irisca (93.9%) and F_3 Maestro x Irisca (82.9%) and the lowest - by the variety Maestro (31. 2%). The genotypes with increased resistance can be used subsequently in research for genetic breeding for resistance to high temperatures.Testing of the selected material based on the characters complex, including heat resistance, demonstrated the possibility of creating new forms of tomato combining productivity with high air temperatures resistance.

Key words: *tomatoes, intra-specific hybridization, breeding, resistance, heat*

INTRODUCTION

Tomatoes are one of the most common vegetable crops in Moldova and worldwide because of high nutritional value of its fruits, both, for fresh consumption and many types of processed products.

In Moldova, permanently, climate change has had a negative impact on agriculture. We can mention that in the past decade drought and high temperatures have become more frequent, with strong negative effects on crop productivity. Selection based on resistance to extreme temperatures now becomes the actual objective because in some years the air temperature reaches 35-45ºC during the day at the blossoming stage, which considerably reduces fruits setting and the yield per hectare. Therefore, creating tomato genotypes with resistance to extreme environmental factors and high productivity and quality indicators presents a serious improvement (Mihnea et al., 2010; Mihnea, 2011; Mihnea et al., 2005; Moldovanu et al., 2000. Sato et al., 2000; Saltanovici et al., 2003; Saltanovici et al., 2012). According to the author V.L Erşova (1979) the air temperature is of 30-33ºC for most tomato varieties, the pollen losses its fertility, fecundity is compromised, the flowers fall, growth either stops or interrupts and the intensity of photosynthesis decreases. The harmful impact of high temperatures is intensified under deficit of soil water. It has been established that the optimum temperature of pollen germination is from 22 to 26ºC and fruit development - from 20 to 24ºC.

Positive effects of breeding can be achieved by using a sufficient number of genotypes specially selected for certain agro-environmental areas and, at the same time, taking into account the considerable variability of the plants cultivation conditions. Contemporary breeding demonstrates the need to create lines, varieties and hybrids with high environmental resistance. The importance of the adaptive breeding to create varieties combining resistance and stressogenic factors with high productivity factors has been recognized by many researchers (Pivovarov et al, 1990; Kilchevsky, 1997; Zhuchenko, 2005).

The aim of our research was to test the level of heat resistance of genotypes and hybrid

populations for selecting forms descending from tomatoes with the high level of heat resistance.

MATERIALS AND METHODS

The experiments were conducted in the year 2011 under field conditions in the experimental plot of the IGFPP. As the initial material for the planned research were used genotypes selected from F_3 hybrid generations using a character complex of 4 combinations and backcrossing hybrids obtained on the base of intra-specific hybridization of Maesrto x Irisca, Maestro x Dwarf Moneymaker, Maestro x Dwarf Moneymaker, Mihaela x Irisca. Field experiments were conducted in triplicate in randomized blocks of seedlings cultivation without irrigation. The sowing took place in greenhouses in the first decade of April according to the scheme 7 x 10 cm and field planting - in the scheme of 70 x 30 cm. Field planting was performed in the second decade of May, and harvesting was done gradually (4-6).
Caring for and tomato growing were performed in accordance with agro- technical norms adopted in Moldova. High temperature resistance of genotypes was evaluated according to methodological recommendations (Ivakin, 1979) based on plant growth capacity after their exposure to high temperatures (43ºC) for 6 hours. The data obtained were statistically processed using the software STATISTICS 7.
Graphical representation, tabular and textual, was performed through the Microsoft Office and Microsoft Excel software.

RESULTS AND DISCUSSIONS

By testing the response of 4 parental forms and 43 phenotypes of tomatoes selected from four hybrid combinations and backcrossing hybrids obtained on the base of intra-specific hybridization of Maesrto x Irisca, Maestro x Dwarf Moneymaker, Maestro x Dwarf Moneymaker, Mihaela x Irisca hybrids under the impact of high temperatures (43º C) it was established that genotypes/populations selected for two years under field conditions manifested different responses to the heat. The data obtained on the response of tomato hybrid populations at elevated temperatures demonstrated that in some hybrid combinations F_3, F_2BC there were recorded values of high resistance of hybrids obtained in comparison with the genitors, while in some value combinations there were less average parents. The result of evaluating genotypes / populations on heat resistance is shown in Figure 1. Analysis of these forms under heat resistance showed that variability from 31.2 to 93.9% was within the limits. As the data show, a high resistance was demonstrated by genotypes 4, 6, 7, 8 selected from F_2 hybrid combination Maesrto x Irisca, genotypes 11, 13 from the backcross combination F_1BC (Maesrto x Irisca) x Maestro, genotype 15 from combination F_1BC (Maestro x Irisca) x Irisca. According to the degree of resistance to temperatures indicated by genotypes selected from combinations in which as the paternal form was used the variety Dwarf Moneymaker, there were found two genotypes of 21 (5 - F_3 Maestro x Dwarf Moneymaker and 11 -F_1 BC (Mihaela x Dwarf Moneymaker) x Dwarf Moneymaker]) that demonstrated resistance of 77.9% and 60.3%. In terms of the heat response of genotypes selected from the combination Mihaela x Irisca, it was seen that that genotypes 5, 6, 9 (Fig. 1C) demonstrated the resistance of 63.1; 93.9 and 61.4%, respectively. It should be mentioned that most simple and backcrossed hybrid populations showed high values of the resistance level of plants. However, hybrid populations created with participation of Irisca variety had the highest indices of the examined character.
Concerning another examined quantitative indicators - the seedling length that can be considered both, as the genotype peculiarity and the index of resistance to high temperature and it is evident that it differs much from genotypes included in the study (Fig. 2).
Plants length under optimum conditions was within the limits of 70.0 ... 114.2 mm while under stressful conditions - 24.2 ... 66.6 mm in case of the combination Maesrto x Irisca whose parents showed pronounced differences of the analyzed index (24.2 and 41.0 mm); it was found that most offspring had much higher values than the best parents, with the exception of genotypes 10, 16 in which the plant length was 33.9 and 33.2 mm. The offspring from the hybrid combination Mihaela x Irişca (Figure 2

C) showed a higher level of plats growth and was within the limits from 46.6 to 66.6 mm. In genotypes selected from the combination Maestro x Dwarf Money Maker (Figure 1) the plants length under stress was greater than in the parental forms while in combination of Mihaela x Dwarf Moneymaker, only one descendent ceded the best parent.

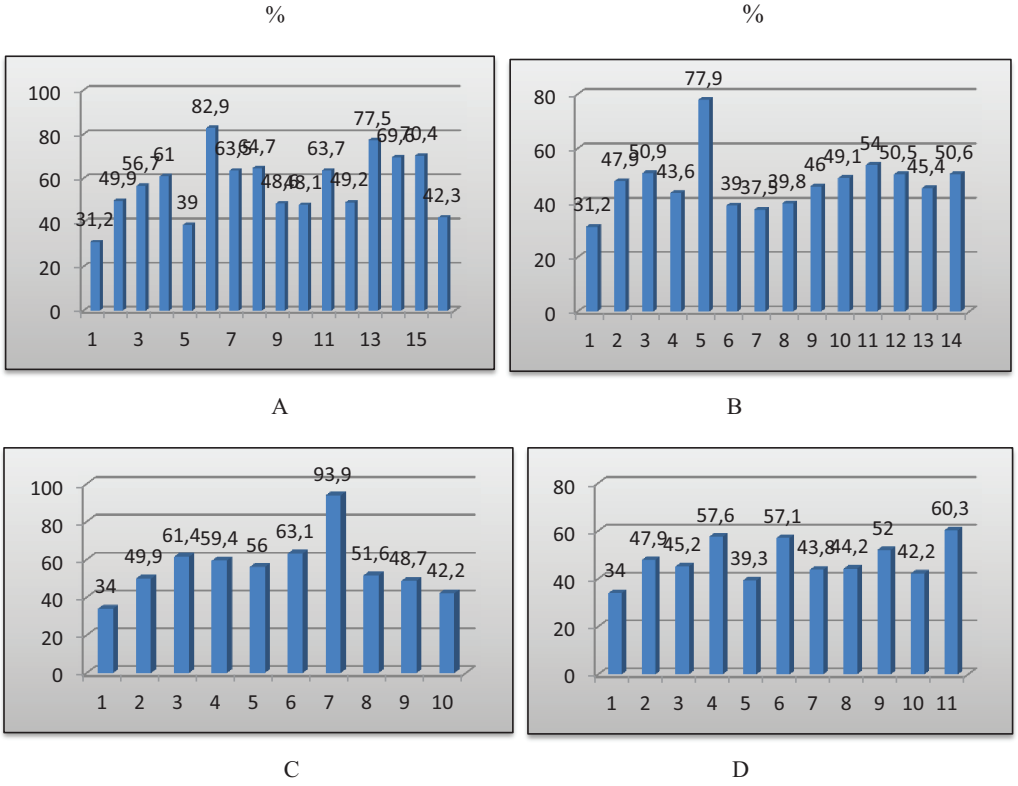


Figure 1. Evaluation of tomato genotypes selected from intra-specific combinations on heat resistance in comparison with parental forms

A. 1 – Maestro, 2 – Irişca, 3-9 – F_3 (Maesrto x Irişca), 10-13 – F_2 BC (Maesrto x Irişca) x Maestro, 14-16 – F_2 BC (Maesrto x Irişca) x Irişca.

B. 1 – Maesrto, 2 - Dwarf Moneymaker, 3-10 – F_3 (Maestro x Dwarf Moneymaker), 11-12 – F_2 BC (Maestro x Dwarf Moneymaker) x Maestro, 13-14 – F_2 BC (Maestro x Dwarf Moneymaker) x Dwarf Moneymaker.

C. 1 - Mihaela, 2 – Irişca, 3-7 – F_3 (Mihaela x Irişca), 8-9 - F_2 BC (Mihaela x Irişca) x Mihaela, 10 – F_2 BC Mihaela x Irişca) x Irişca.

D. 1 – Mihaela, 2 - Dwarf Moneymaker, 3-8 – F_3 (Mihaela x Dwarf Moneymaker), 9-10 – F_2 BC Mihaela x Dwarf Moneymaker) x Mihaela, 11 – F_2 BC (Mihaela x Dwarf Moneymaker) x Dwarf Moneymaker.

Under the influence of temperature of 43^0 C the repression of plant growth occurred in all analyzed forms, whose values were presented in genotypes of the combination MaestRo x Irisca - 31.8; -46.4; -42.5; - 27%; -38.9; -44.0; -41.2; -49.2; -34.9; - 43.9; -29.5; - 53.3 -54.6; -56.0%, while in the combination Mihaela x Irisca -44.; -25 4; -37.9; - 22.8; -31.5; -24.5; -43.9; -57.1% compared with the check. In the combination of Maestro x Dwarf Moneymaker the repression of plant growth was within the limits of 36.9 ... 65.5, while in Mihaela x Dwarf Moneymaker - 42.3 ... 57.9%. Testing of the selected material on the base of the parent sporophyte heat resistance revealed the forms possessing a lower depression of the plants under the thermal stress and high resistance to heat. They were included in the process of further improvement.

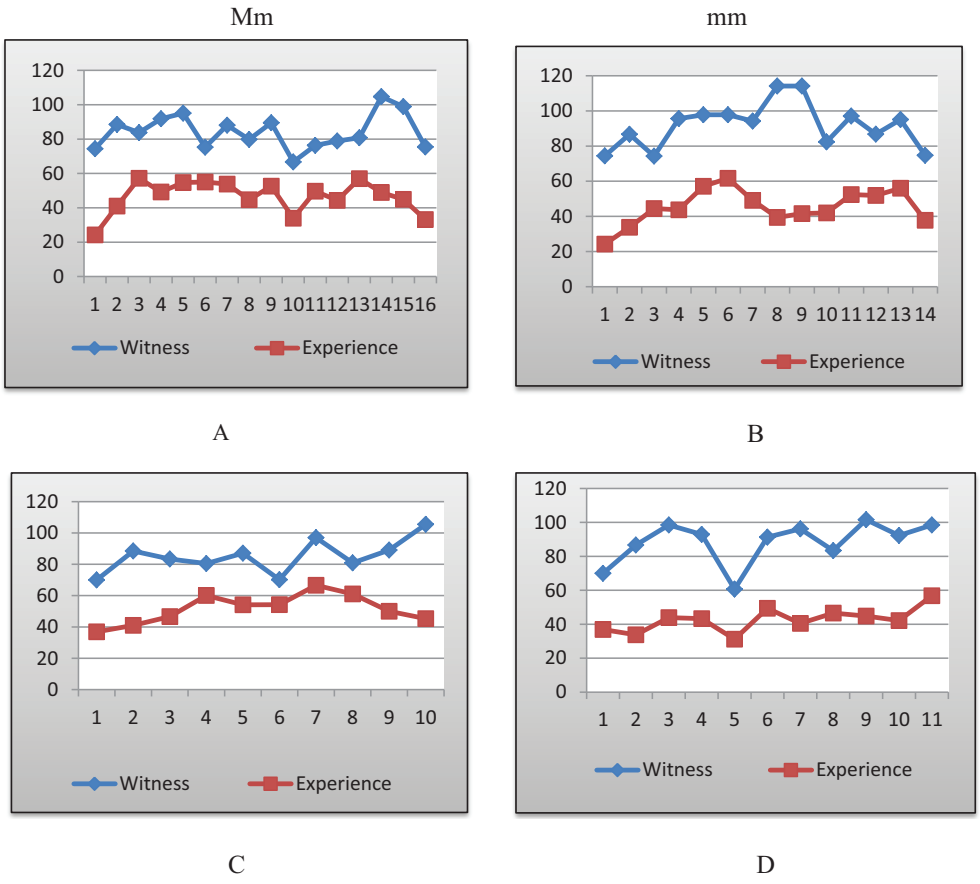


Figure 2. Tomato plant length under optimal conditions (25^0 C) and stress (43^0 C)

A. 1 – Maestro, 2 – Irişca, 3-9 – F_3 (Maesrto x Irişca), 10-13 – F_2 BC (Maesrto x Irişca) x Maestro, 14-16 – F_2 BC (Maesrto x Irişca) x Irişca.

B. 1 – Maesrto, 2 - Dwarf Moneymaker, 3-10 – F_3 (Maestro x Dwarf Moneymaker), 11-12 – F_2 BC (Maestro x Dwarf Moneymaker) x Maestro, 13-14 – F_2 BC (Maestro x Dwarf Moneymaker) x Dwarf Moneymaker.

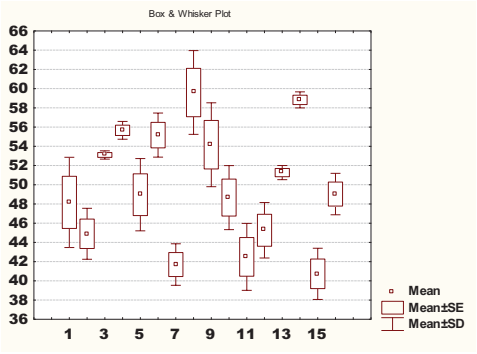
C. 1 - Mihaela, 2 – Irişca, 3-7 – F_3 (Mihaela x Irişca), 8-9 - F_2 BC (Mihaela x Irişca) x Mihaela, 10 – F_2 BC Mihaela x Irişca) x Irişca.

D. 1 – Mihaela, 2 - Dwarf Moneymaker, 3-8 – F_3 (Mihaela x Dwarf Moneymaker), 9-10 – F_2 BC Mihaela x Dwarf Moneymaker) x Mihaela, 11 – F_2 BC (Mihaela x Dwarf Moneymaker) x Dwarf Moneymaker.

Evaluation promising forms under field conditions in respect of productivity revealed a rather high variability of both the total harvest (Figure 3 A) and the share of market fruit (Figure 3 B).The total harvest of the initial forms ranged from 44.9 t / ha (variety Irişca) to 55.2 (variety Dwarf Moneymaker), the rate of market fruit was 87.0 ... 97.0%. The total harvest in hybrid combination F_3 (Maestro x Irishka) and F2 BC (Maestro x Irişca) x Maestro was 53.0 and 55.7 t / ha, much higher than of the parent with high values, variety Maestro (48.2 t / ha). The hybrid combinations F_2 BC (Maestro x Irişca) x Irişca the harvest was at the level of the best parent, and the hybrid combinations where for crosses were used varieties Maestro and Dwarf Moneymaker, lines showed a lower harvest with the exception of the combination F_2BC (Maestro x Dwarf Moneymaker) x Maestro. In combinations derived from crosses of varieties Mihaela and Irisca, Mihaela and Dwarf Moneymaker the total harvest was lower than the best parent except combination F3 (Maeda

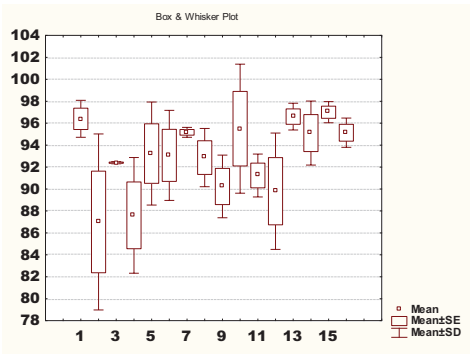
x Dwarf Moneymaker) for which the total harvest was much higher than of the parent with high values. After evaluating the market rate of fruit it was found that hybrid combinations showed a variability of 87.8 ... 97.0%. A low market rate of fruit was recorded for the hybrid combinations F_2BC (Mihaela x Irişca) x Mihaela (89,8%).

t/ha



A

%



B

Figure 3. Productivity of tomato varieties and lines obtained as a result of intra –specific hybridization, year 2011

1 – Maestro, 2 – Irişca, 3 - Dwarf Moneymaker, 4 – Mihaela, 5 – F_3 (Maestro x Irişca); 6 – F_2 BC (Maestro x Irişca) x Maestro, 7 – F_2 BC (Maestro x Irişca) x Irişca, 8 – F_3 (Maestro x Dwarf Moneymaker), 9 – F_2 BC (Maestro x Dwarf Moneymaker) x Maestro, 10 – F_2 BC (Maestro x Dwarf Moneymaker) x Dwarf Moneymaker, 11 – F_3 (Mihaela x Irişca), 12 – F_2BC (Mihaela x Irişca) x Михаела, 13 – F_2 BC (Mihaela x Irişca) x Irişca, 14 – F_3 (Mihaela x Dwarf Moneymaker), 15 – F_2 BC (Mihaela x Dwarf Moneymaker) x Mihaela, 16 – F_2 BC (Mihaela x Dwarf Moneymaker) x Dwarf Moneymaker

CONCLUSIONS

The highest heat resistance was found in the genotypes of the F_3 generation (Mihaela x Irisca (93.9%) and F_3 Maestro x Irisca (82.9%), and the lowest in the variety Maestro (31.2%).

The genotypes tested showed differential response to heat and can be used later in the research of genetic resistance improvement to high temperatures.

Testing of the selected material based on a character complex, including heat resistance, demonstrated the possibility of creating new tomato forms that combines productivity with resistance to high air temperatures.

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BACKGROUND OF VEGETABLE MULTI-CRITERIA ANALYSIS IN MUNICIPALITY OF KALOYANOVO, BULGARIA

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Abstract

Horticulture is one of the importance parts of Bulgarian agricultural sectors. Crop growing specification requires deep and good knowledge about agricultural development and practices. Advanced research and carefully gathered and analysed information is the first step of the successful result. This publication presents the structure and more necessary data for complex analyzing of environmental factors and their influence on agriculture structure in Municipality of Kaloyanovo, Plovdiv region, Bulgaria. The study area is 347 sq.km and average elevation - 198 m. An overview would show the present state of physiography, climate, crop cultivation, landscape, elevation, labor force, marketing, farmers practices, areas under cultivation, land use, soil type, water resources, road systems, erosion level, the most spread vegetable marketing, demography and socio-economy opportunities. Methodology is based on thematic maps and analysis, creating by GIS application. It aims to identify current status of the most appropriate plants development, land management and changes in land use form. Spatial data and thematic maps present increase and decrease of agricultural availability in the study area. Results obtained will be relate to multi-criteria land suitability evaluation, accordingly to vegetable crop characteristics. This model of land evaluation helps farmers and vegetable growers to realise potentiality of their land parcel and required management procedures.

Key words*: multi-criteria analysis, GIS, environmental factors, vegetable crops.*

INTRODUCTION

Agriculture is permanent developing structure, connected to the sensible land use and land management. From the beginning of the civilization man has used the land resources to satisfy his needs. The land resources regeneration is very slow while the population growth is very fast, leading to a nunbalance. On a global scale, agriculture has the proven potential to increase food suppliesfaster than the growth of the population (Davidson, 1992).
Sustainable vegetable farming system is associated with good practices related to people cantered development, sustainable live lihood, agro-ecological practices, sustainable forestry system, community based natural resources management, participatory policy development, indigenous farming system, fair labour condition, good agricultural practises, equitable access to water and others (Baniya, 2008). Sustainability is the ability of an agricultural system to meet evolving human needs without destroying and, if possible, by improving the natural resource base on which it depends (USAID, 1988). In order to determine the most desirable direction for future development, the suitability for various land uses should be carefully studied with the aim of directing growth to the most appropriate sites.
Necessary of sustainable agricultural development is mentioned by different authors (Stoeva, 2013; Christova et al, 2013; Toskov, 2013; Nikolova, 2013). According to them, it is important to create developing strategies about vegetable production and management. Natural resources have to be ruled and used carefully for good sustainable future yields. Careful planning of the use of land resources is based on land evaluation, which is the process of assessing the suitability of land for alternative land uses (Fresco et al, 1994). Information on land resources is a key to their careful and effective evaluation.
The main purpose of this article is to present the important and necessary environmental and

human developing factors for formulating sustainable vegetable developing. Establishing appropriate suitability factors is the construction of suitability analysis. Agricultural evaluation is concerned with the assessment of various land actions and used for specified purposes. It involves an analysis of some basic surveys of geographic situation, soils, land using, demography state, saved areas by lows, road systems, water resources, etc. All data is response with plant requirements. This is mean to present the importance of preliminary environmental assessment of some area and its positive and negative sides for sustainable vegetable developing.

Study area

In Bulgaria it is not so spread and usual, farmers to make a preliminary study of the future cultivated area. This study presents the essence of necessary factors for making good vegetable production. The study area is Municipality of Kaloyanovo. Situated in the north part of the Upper Thracian plain, covering area of 347 sq. km., the municipality is part of the region of Plovdiv and consists of the municipal center Kaloyanovo, and 14 settlements as well - Begovo, Glavatar, Gorna Mahala, Dolna Mahala, Duvanlii, Dalgo Pole, Zhitnitsa, Ivan Vazovo, Otets Paisievo, Pesnopoy, Razhevo, Razhevo Konare, Suhozem and Chernozemen. Up to the present moment the population of the municipality amounts to 12800 people. Municipality of Kaloyanovo has an important transport-geographic position. The main thoroughfares, which connect the north and south part of Bulgaria, respectively the countries from North-East Europe and Scandinavian countries with Turkey and the Near East pass through its territory. The railway road Plovdiv-Karlovo passes through six of the settlements and the railway road Plovdiv-Hisarya passes through other three settlements. Kaloyanovo is situated 24 km. from the regional center Plovdiv and 16 km. from "Trakia" highway, which connects Western Europe with Near East.

The selected municipality is good representative for analysing agricultural practices and making important points of preliminary knowledge about environmental structure. The extensive research of natural factors gives opportunities to increase positive sides of vegetable production. So it is essential to have profound analysis and necessary information before doing agricultural actions in some area.

MATERIALS AND METHODS

The base information about land management for making successful vegetable developing presents the present situation of land use and land management. The necessary data contents coordinated geographical borderlines of villages in the Municipality of Kaloyanovo, road systems, water resources, land using data, public or social property, population, classify-cation of cultivated terrains. All information is gathered from Municipality of Kaloyanovo and statistic data from different researches. Some of them are:

- Cadastre maps- The digital model formats are ZEM, CAD. Information source: the Geodesy, Cartography and Cadastre Agency.
- Statistic data about population, land use, land category, road and water systems– source http://www.kaloianovo.org/.
- Soil characteristics- Information source: The Soil Resources Agency and the Institute of Soil Science “Nikola Pushkarov”.

All action related to spatial data as collecting new information, organize in groups, creating connection between them, logical links, correct and sufficient presentation and sharing can be realized by Geographical Information Systems, named GIS (Stefanova et al., 2014). Methodology is based on using GIS platforms and application for analysing and presenting the results. All transformation actions of collected data are made by ArcGIS software and appropriate spatial data filters. The results are thematic maps of collected information.

RESULTS AND DISCUSSIONS

Multi-criteria analysis is complex information from different branches. It includes geographic information, coordinated location of the studied area, climate, soil type, elevation, population, demographic data, statistic information, agro-ecological settings, land use and management, etc. All necessary information is collected and arranged carefully by different experts from various scientific branches. Information

transformation from paper to spatial data and introduced into computer managed software is obligatory action for easily dealing with a huge amount of heterogeneous data. The core of this study is presenting the necessity of background agricultural analysis and making well-arranged future actions for more profits. Brief description of the study area in general is presented by thematic maps about some of the important agricultural points. Attributes of the study area has marked effects on the tradition and culture and in turn to the cultivation practices. The information would show the basic facts to be considered for the data analysis and interpretation of the results. Environmental factors and technics progress urge forward making consideration of the study area information and put it on prime importance. It includes information from the socioeconomic, demography, land use and vivid dimensions. All data is collected from different sources, geographic transformed and coordinated and introduced into GIS software. The spatial data can be easily manipulated, manual added, clearly presented and used for future predictions. GIS application allows additions in every time and enriching the past and present data with new information. So GIS tools allow making multi-criteria analysis and combined various data from different branches.

The studied area is situated in South Bulgaria and it is a part of Plovdiv region. This is the most developed agricultural region for vegetable production (Arnaudova et al., 2014). Today, Municipality of Kaloyanovo faces a number of various environmental and ecological innovations and yield increased challenges. The development and implementation of an environmental action plan for the valleys are associated with the strategy position and closely connection with another neighbour agricultural areas. The picture above presents the Municipality of Kaloyanovo position into Bulgaria map (Figure 1).

Figure 1: Bulgaria map and Municipality of Kaloyanovo (original)

In the municipality are situated 14 villages with one center city, named Kaloyanovo. The bigger village is named Razhevo Konare with area of 46.01sq.km and the smallest one is Glavatarwith area of6.23 sq.km. Next map (Figure 2) presents the position of 14 villages in Municipality Kaloyanovo. The relief is plane to hilly and average elevation is 250 m. The climate is transcontinental, characterized with an open winter and a hot summer. These factors are essential and favorable for vegetable developing.

Next maps (Figures 3 and 4) present information about land use and soil distribution in the studied area. Dominant soil types are sandy-clay and loamy, presented in the next map (Figure 3). These soil characteristics are useful for cultivation of various vegetable plants- tomatoes, papers, cabbages, carrots, etc.

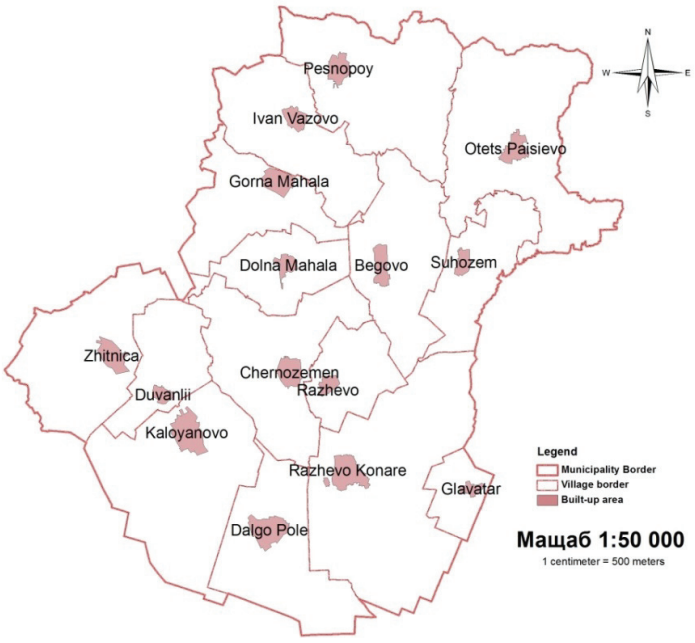

Figure 2: Map of the studied area- Municipality of Kaloyanovo (original)

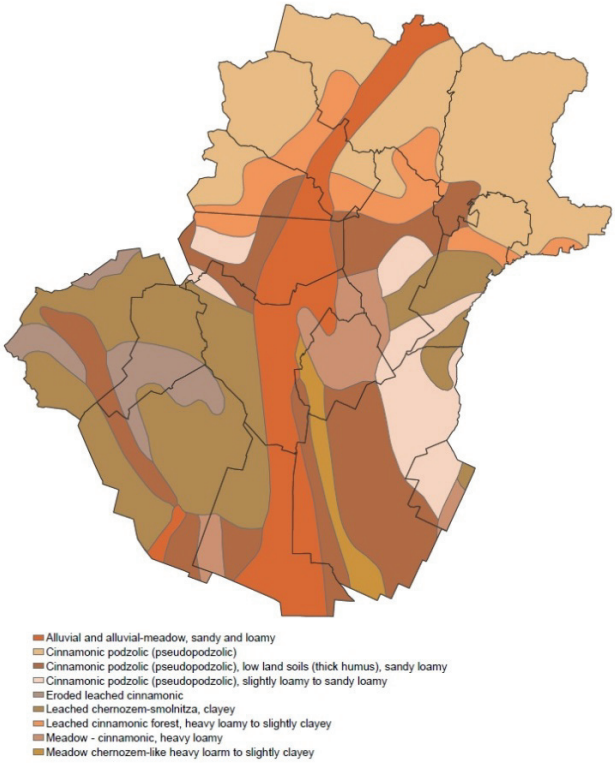

Figure 3: Map of Soil type in the studied area- Municipality of Kaloyanovo (original)

Good environmental factors advantage good agricultural actions. Above 50% of the land area is cultivated. Only the latest north terrains are covert with forest. More over 75% of the cultivated land is covered by corn fields, vegetable sorts and fruit plants. Forest area is 14,5%, almost 3.99% of urban area, 4.16% water resources and another 2.35% for road systems, useful fossils, depots, scraps, etc. More agricultural land allows cultivating and developing of more different vegetable plants and opportunity of increasingly yields. All this information is presented by the next map (Figure 4).

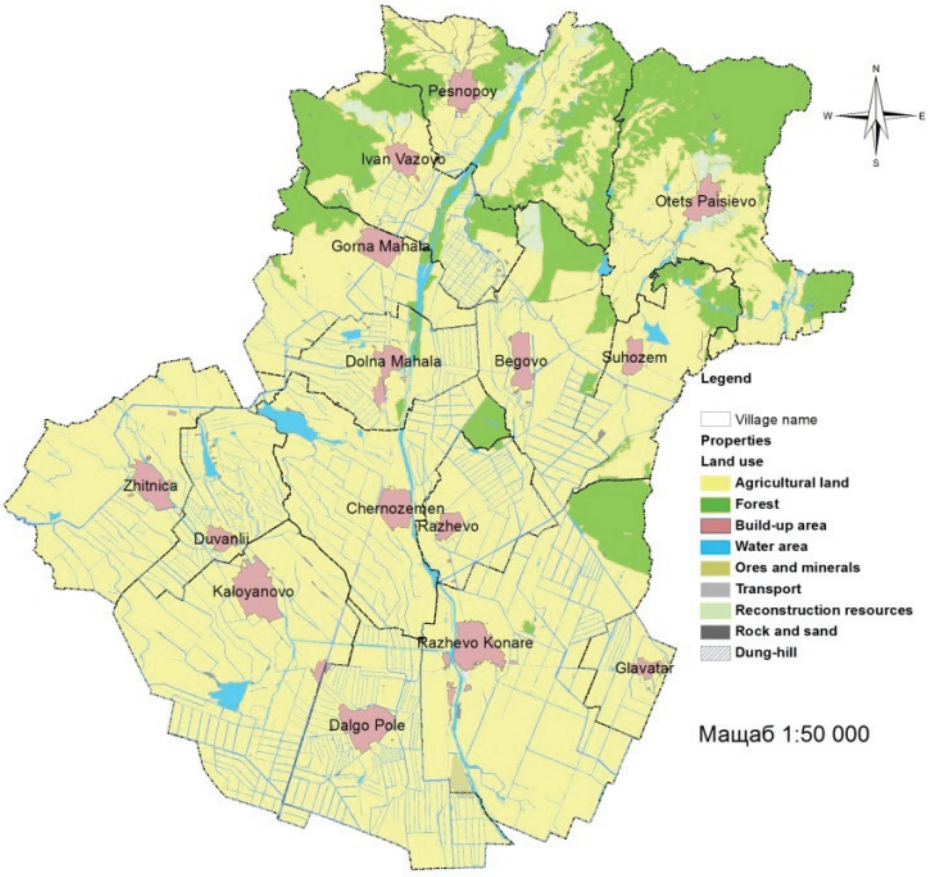

Figure 4: Land use map of Municipality Kaloyanovo (original)

According to the property of land in Municipality of Kaloyanovo (Figure 5) almost all cultivated area is private – 60.22%. State property is 16.8%, municipal area is 18.66% and built-up area 4.32%. So the most appropriate area for agricultural actions is private terrains. Private section is more developing and can be in funds by its own profits. So it is important for vegetable farming systems to be subsidized and to rely on its own production.

Categorization of cultivated land area (Figure 6) is made by 10 categories of comparison. This method of land classification is the most spread in Bulgaria and it is consistent with climate conditions, relief, soil characteristics, plant requirements, etc. The classification is based on number from 2 to 10. Land category is assessment of land characteristics. The lowest number shows the best land for cultivation. Dominant land category is between 2 and 4, so it is very good for agricultural actions. Categorization is presented on the next map and categories are shown by different colors.

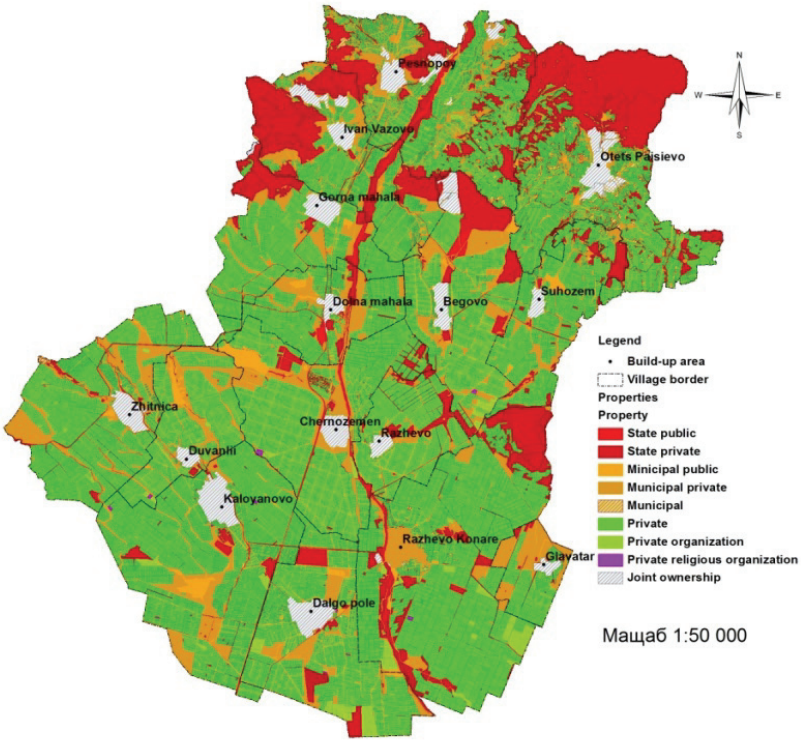

Figure 5: Property land map of the studied area (original)

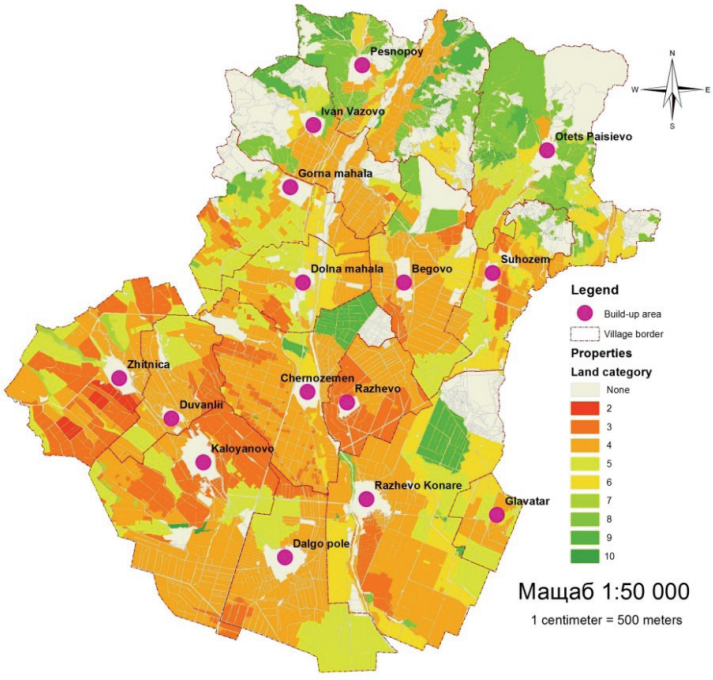

Figure 6: Map of Land category in Municipality Kaloyanovo (original)

Transport system (Figure 7) is amount to 152 km. It mainly contents roads from class 2 with length 24 km, class 3 with length 21 km and class 4 with length 62 km. During the studied area is situated railway with length 45 km. Municipality has important transported role of connecting North with South Bulgaria. Road information has influence on fast transportation of agricultural production to the market systems. Good structured roads help for connecting to the nearest factories and vegetable shops.

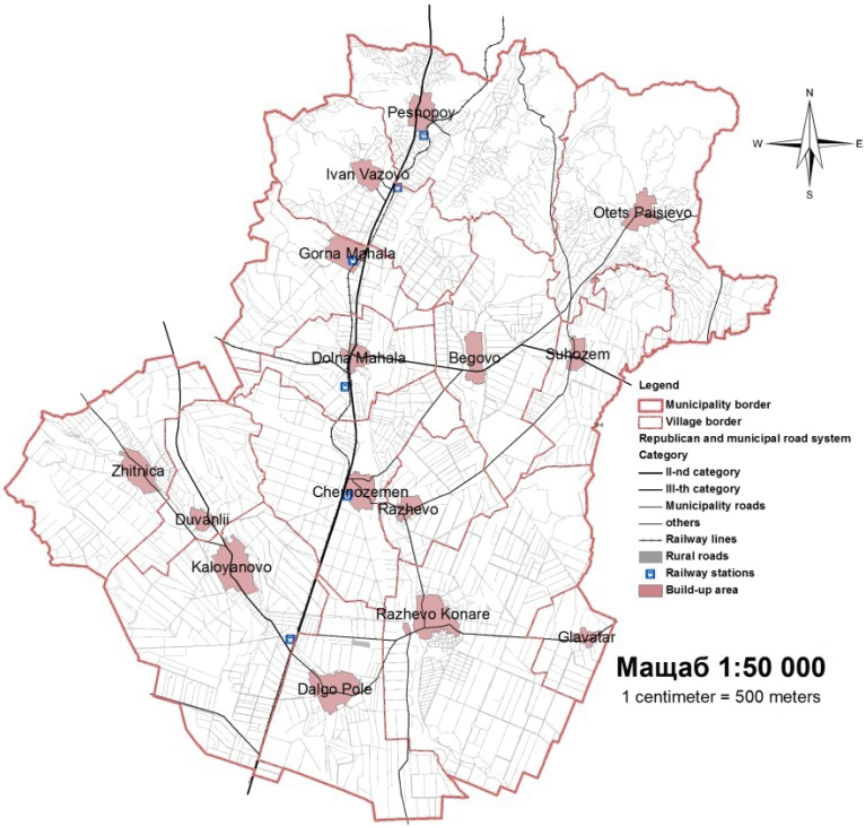

Figure 7: Map of road system (original)

Water resources (Figure 8) covered 14 500 ha area. Water system is presented by irrigation systems, rivers and canals. Length of water systems is 116 km. Next thematic map illustrates the situation of present irrigation systems and water resources in the Municipality of Kaloyanovo. Water resources are very substantial for vegetable growing. Analysed data face on good built and supported irrigation systems, which is permanently used and upgraded for agricultural practices.

Demography situation is presented on the next map (Figure 9). By the last census of the population in the studied area, organized during 2011 year, the population is 11 879 people. Dynamic is shown about the period 1934-2007 years. The most developed area is municipal center Kaloyanovo (Figure 10), so it is center city. In comparison with past years, population is decreased from small villages and move to the cities. The new technology tempts young people to the bigger cities, so the smaller villages become uninhabited areas. These results will have negative reflection on future agricultural development. It can be improved by implanting new facilities and more modern agricultural machines.

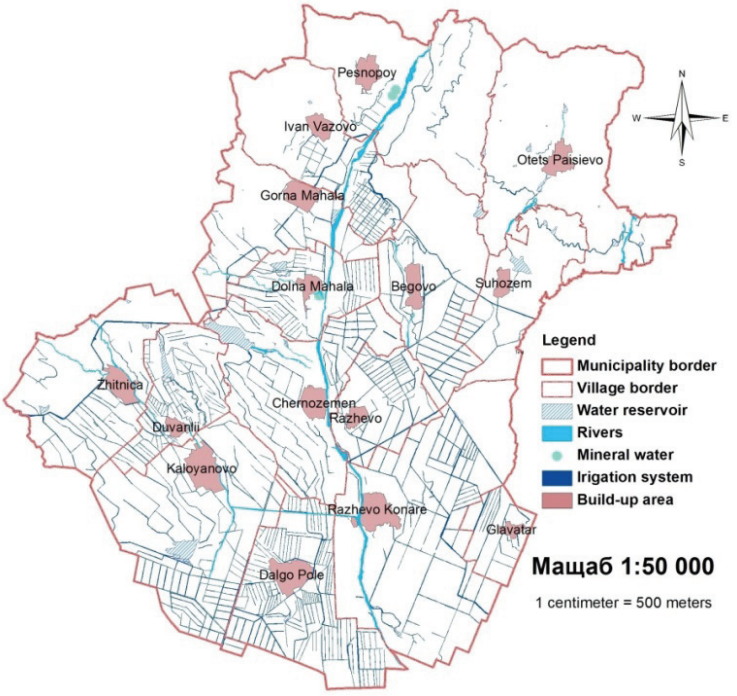

Figure 8: Map of water resources (original)

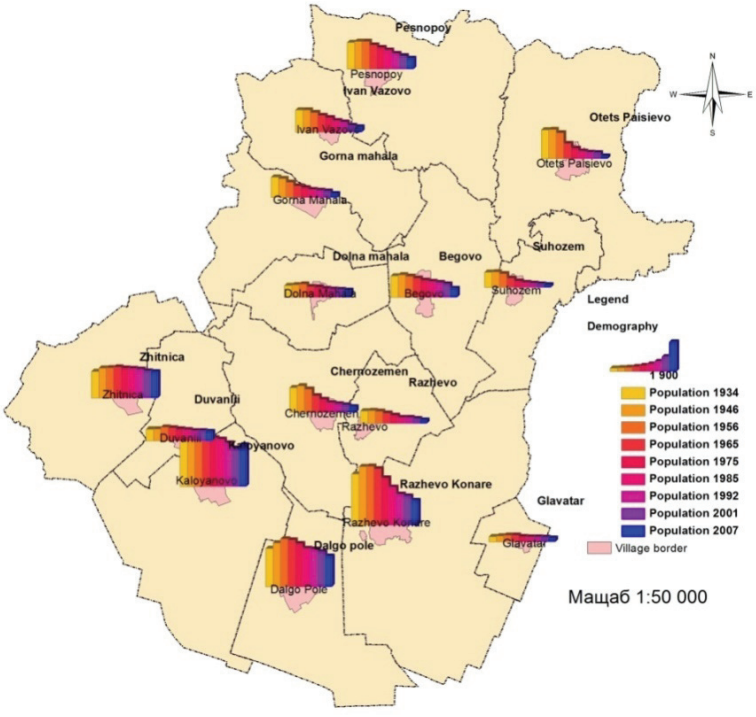

Figure 9: Demography map of studied area (original)

The main objective of the land evaluation is the prediction of the inherent capacity of a land unit to support a specific land use for a long period of time without deterioration, in order to minimize the socio-economic and environmental costs (De La Rosa 2000). Finding suitable land area for demanding agriculture crops is the need of present day farming system. So it is essential to know in advance if one area is good for agricultural practice or not. Background study is important part of farming management and it is the base level of expert knowledge.

All presented information is linked to the latest years. Spatial data transformation and implementation it in GIS show an easily way to analyze present information about nature and environmental resources. Agricultural development depends on environmental facilities. For good success and minimized losses, farmers have to do advanced research about environmental factors and nature resources. Results from the study have to be suitable for all plans requirements.

Figure 10: View from the studied area (http://www.kaloianovo.org)

CONCLUSIONS

Agriculture is a sector influenced by different parameters: nature resources, land use and management, water and road systems, population, plant requirements, etc.

All information is combined and presented by GIS tools. The data related to spatial information and boundary are calculated with the scale of 1:50000. Resulted thematic maps present the importance of environmental and human practices.

Municipality of Kaloyanovo is good area for agricultural development. All natural factors-soil distribution, water resources and land category will have positive effects on crops development. Good arranged road system and private land property increase product transportation to the vegetable markets and factories.

All nature factors have positive effects on distribution of land use - mainly cultivated areas. As a negative factor is a result about decreasingly population in the smaller villages. It may be change by using more modern techniques.

Cultivation is difficult decision making and based on a huge factors. So advanced multi-criteria analysis is the method of mixing necessary information. GIS being one of the powerful tools, efficacy of the evaluation process will be maintained (Baniya, 2008). Non spatial parameters can also be analysed in the spatial basis to help making decision process easier. It is very essential to understand land capacity to support appropriate plants cultivation. So farmers are advised to use research for vegetable cultivating, according to nature and environmental potentiality. Site specific classification in order of suitability is main interest of vegetable growers for further profitable vegetable crop development.

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MAINTAINING OF THE QUALITY AFTER HARVESTING OF THE WHITE CABBAGE, DEPENDING ON NITROGEN FERTILISER DOSES APPLIED TO THE CROP

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Abstract

The purpose of this paper is to establish the influence of nitrogen fertilization of the cabbage crop on its preserving capacity. The paper presents the results obtained in the period 2014-2015 on quality maintaining of the white cabbage after harvesting. There were studied two varieties of cabbage: Gredana - summer, Danish variety and Buzoiana, autumn, Romanian variety. The culture was fertilized with nitrogen in the following doses: 100; 200; 300 and 400 kg active substance N/ha. Cabbage was kept in cold conditions (temperature = 0-2ºC, relative humidity = 85-90%) for a period of 50 days - summer variety or 110 days - autumn variety. After storage determinations were performed on the weight losses (expressed by evaporate-transpiration), the losses by conditioning (resulted by removing yellowed leaves, of diseased ones and of the spine) and the main biochemical components. Researches show that the influence of nitrogen fertilization on maintaining of the quality of white cabbage during the preservation period is negative only at high doses (over 200 kg active substance N/ha). In case of dosages of 300 and 400 kg active substance N/ha losses were recorded of up to over 30% after 110 days of preservation of autumn cabbage. Therefore, in the case of cabbage destined to preservation it is not recommended the use of fertilizers in dosages greater than 200 kg active substance N/ha. The value of the main biochemical components is not strongly influenced by the dose of fertilizer applied to the crop. However one can notice an increase in the protein substances and a slight decrease of the values of ascorbic acid, total sugars and cellulose, from variant control (unfertilized) to variant with 400 kg active substance N/ha.

Key words*: storage capacity, losses, biochemical components*

INTRODUCTION

The therapeutic virtues of cabbage - this so common aliment - are known since antiquity. Its qualities are undeniable; therefore cabbage can be used successfully in the prevention and treatment of a very large number of diseases, being a real natural pharmacy. White cabbage is rich especially in pro-vitamin A, vitamins C and E, vitamin B1, vitamin B2, vitamin PP and in fibers, elements that provides cells health, giving it valuable therapeutic properties. Cabbage is richer in vitamin C than oranges, has few calories and a lot of substances with antioxidant effect, contains large amounts of magnesium, potassium, calcium, iron, copper, phosphorus, sulfur. These features and many others recommend the cabbage as a natural remedy against a large number of diseases (Popoescu et Zavoianu, 2011; Bogoescu, 2015). It is an alkalizing, nutritional, energetical, remineralizing and tonic aliment, and it is preferable to be eaten raw, in order to keep intact its properties. The research conducted in the last 30 years (Salunkhe et al.,1985.) confirmed that a regular consumption of cabbage has a beneficial effect in the prevention of the colon cancer in particular, of stomach cancer, but also of lungs, esophageal and rectal cancer (researches conducted at

University of Minnesota and J. Hopkins from USA, in Greece, Israel, Japan, Norway). Storage capacity of the cabbage depends on the quality of raw material for storage, which is influenced by culture conditions and variety (Ciofu, 2011, Ciuciuc et Toma, 2007, Stoleru et al., 2011). The variety imprints to the plants a certain chemical, histological and cytological structure (Jelea et Jelea, 2007, Salunkhe et Kadam, 1998, Stoleruet al., 2011), detectable through different methods of analysis (Hura, 2006). As far as produce destined for long term storage are concerned, as it is the case for cabbage, respecting the technological links which influence the forming and maintaining of the quality and the storage capacity is of great importance because choosing the adequate type of cabbage for the storage spaces is of the essential importance when aiming to maintain the quality during storage. In order to achieve this goal the crops destined for storage must be kept under observation beginning with the growing periods, meaning the moment the crop is started.

The purpose of this paper is to evaluate the preserving storage capacity of summer white cabbage of the 'Gredana' variety and of autumn cabbage, 'Buzoiana' variety, depending on the doses of nitrogen fertilization during culture period.

MATERIALS AND METHODS

The researches were conducted during period 2014-2015, using summer and autumn white cabbage, obtained in a vegetable farm located in an area of the Romanian seaside.

The trial was organized as a bi-factorial experience, with following experimental factors:

- A - variety
  - a1 - Gredana (summer variety)
  - a2 - Buzoiana (autumn variety)
- B - fertilization level
  - b1 – control (unfertilized)
  - b2 – 100 kg active substance N/ha
  - b3 – 200 kg active substance N/ha.
  - b4 – 300 kg active substance N/ha. (for summer variety, only)
  - b5 – 500 kg active substance N/ha. (for autumn variety, only)

The storage was effectuated in refrigeration conditions (temperature = 0-2ºC; air relative humidity = 85-90%) for a period of 50 days (summer cabbage) and 105 days (autumn cabbage), thereupon the following determinations were effectuated:

- weight losses, resulted by evaporate - transpiration;
- losses by conditioning, resulted by removing yellowed leaves, of diseased ones and of the spine.
- identification of pathogens that caused the rot of the cabbage;
- main biochemical components (soluble solids, soluble sugars, titratable acidity, ascorbic acid, protein substances, cellulose).

The methods for determining the biochemical components were the following:

- the refractometric method, using the ABBE refractometer in order to determine the content of soluble dry substance;
- the Bertrand titrimetric method, for the determination of the content of soluble carbohydrates;
- the titrimetric method, for the determination of the titratable acidity;
- the spectofotometric method, for the determination of the ascorbic acid
- the gravimetric method, for the determination of the cellulose;
- Kjeldahl method, for the determination of the proteins.

During storage the hydro-thermal factors in the storage room were verified on a daily basis in order to ensure the respecting of the optimal conditions for the maintaining of the quality. Also, appreciations were made concerning the cabbage' capacity to maintain their quality during storage, as well as the possible occurrence and development of various specific diseases.

RESULTS AND DISCUSSIONS

1. Level of losses

Data presented in Table 1, on the preservation for short time of the summer cabbage, show that between the three graduations of fertilization with nitrogen that were studied, after 50 days of cold storage, there were no pronounced differences of the losses. It finds that weight losses recorded values very close to the three variants: from 4.8% in the case of fertilization b1 variant (unfertilized) to 5.3% in the case variant b3, with the dose of

fertilization of 200 kg active substance N/ha.

Table 1. Loses recorded by summer white cabbage after 50 days of cold storage

Variant	Fertilization level ((kg a.s. N/ha.)	Losses (%)		
		quantitative losses	qualitative depreciation	total
b1	unfertilized	4.8	5.0	9.8
b2	N 100	5.2	5.1	10.3
b3	N 200	5.3	5.5	10.8

Because of during storage period have not reported disease attacks, the conditioning consisted only in the removing of 1-2 yellowed leaves, which made that the conditioning losses be small (between 5.0% in the case of variant b1 and 5.5% in the case of variant b3). Total losses recorded values between 9.8% at the control variant and 10.8% at b3 variant.

Based on these results it can be considered that the fertilization with nitrogen in amounts up to 200 kg don't influence negatively the preservation capacity of summer cabbage, 'Gredana' variety, if it is storage a period of 55 days.

Analyzing the data presented in Table 2, on the preservation of long time of autumn cabbage, variety 'Buzoiana', it finds that after 110 days of refrigerated storing, weight losses increase proportionally to the dose of nitrogen: from 6.0% in the case of control, up to 10.8% in the case of variant b5, fertilized with 400 kg active substance N/ha.

Qualitative depreciation also increased proportionally with the dose of nitrogen applied to the crop. They are between 17.6% in the case of the control variant and 20.7% in the case of variant b5, but the differences are not significant compared to control only at variant b5.

Unlike summer cabbage, kept a short period, were there was no attack disease, at keeping for a long period of the autumn cabbage, there appeared various diseases and the conditioning losses resulted largely by removing rotting leaves.

Among the pathogens that caused the rot of outer leaves can mention: *Botrytis cinerea, Sclerotinia sclerotiorum, Alternaria brassicae* and bacteria of the genus *Pseudomonas*. The attack was emphasized at the cabbage of b4 and b5 variants. In addition, at variant b5 were found brownings of the inner leaves oh the heads.

Table 2. Loses recorded by autumn white cabbage after 110 days of cold storage

Variant	Fertilization level (kg a. s. N/ha.)	Losses (%)		
		quantitative losses	qualitative depreciation	total
b1-control	-	6.0	17.6	23.6
b2	N 100	6.9	17.8	24.7
b3	N 200	7.8*	18.7	26.5
b4	N 300	8.3**	19.1	27.4*
b5	N 400	10.8***	20.7*	31.5***
		DL 5%=1.70	DL 5%=2.33	DL 5%= 3.69
		DL 1%= 2.48	DL 1%=3.39	DL 1%=5.36
		DL 0.1%=3.72	DL 0,1%=5.09	DL 0,1%=8.05

Total losses ranged from 23.6% at the control variant to 31.5% at variant b5, differences between these variant being very significant. In case of variant b4 differences were significant, compared to the control.

From the data presented up to now, it results that the effect of nitrogen fertilization on the storage capacity of the cabbage is negative only at high doses (over 200 kg a.s. N/ha). Until this dose there have not been reported the negative effect neither summer cabbage nor autumn cabbage, for which we can consider as limit to which we can apply nitrogen fertilizer without affecting the storage capacity of the cabbage is 200 kg a.s. N/ha.

2. Level of the biochemical components.

The analysis of the main biochemical components of the autumn cabbage (Table 3) reveals the fact that between the variants of nitrogen fertilization does not occur essential differences from this point of view, except protein substances that, from the value of 0.64% seen in variant b1, increase to 1.12% at variant b5.

It is observe a slight decrease in values of the ascorbic acid, total sugars and cellulose, from variant b1 to variant b5.

It also noted that during the storage of cabbage, the quantities of the biochemical components did not modify noticeable.

Table 3. The main biochemical components of autumn cabbage at the beginning and after the cold storage

Specification	M.U.	Moment of determ.	Variant				
			b1	b2	b3	b4	b5
Dry soluble substance	%	I*	6.4	6.3	6.0	6.0	6.0
		II**	6.5	6.4	6.1	6.1	6.1
Ascorbic acid	mg/ 100g	I	35.99	35.11	34.73	34.50	32.64
		II	35.15	34.80	33.45	32.30	30.40
Titratable acidity	%	I	0.15	0.15	0.15	0.15	0.14
		II	0.16	0.16	0.15	0.16	0.15
Total carbohydrates	%	I	3.68	3.58	3.68	3.42	2.94
		II	3.23	3.48	2.94	2.75	2.80
Proteins (Nx 6.25%)	%	I	0.64	0.71	0.76	0.80	1.00
		II	0.80	0.87	0.88	1.05	1.12
Cellulose	%	I	0.73	0.73	0.72	0.63	0.61
		II	1.17	0,96	0.85	0.78	0.88

* at harvest
** after 110 days of cold storage

This is explained by the fact that the cabbage was subjected to low temperature and so by the slowing of the metabolic process. Very small increases observed after storage in dry matter values, titratable acidity and cellulose, but content in ascorbic acid and total sugars decrease.

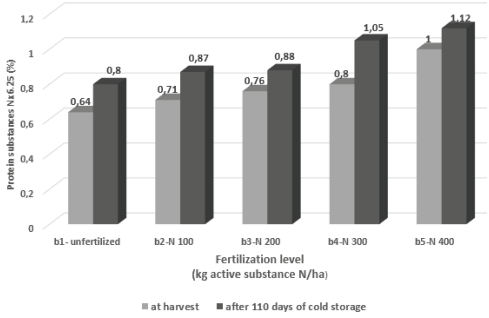

Figure 1. The proteins content of the autumn cabbage, fertilized with different nitrogen doses

CONCLUSIONS

Fertilization of the white summer and autumn cabbage with nitrogen fertilizer up to 200 kg active substances/ha not affect the preservation quality of the cabbage.

After 50 days of storage of the summer cabbage and after 110 days of storage of the autumn cabbage the total losses are insignificant compared to the control.

At higher doses of nitrogen fertilizers, 300 and 400 kg active substances N/ha, the capacity for storage of autumn cabbage was affected. After 110 days of storage total losses were over 30%.

Therefore, for the cabbage destined for preserving for long time is not recommended fertilizer doses greater than 200 kg N/ ha.

The value of the main biochemical components of the autumn cabbage is not strongly influenced by the dose of fertilizer applied to the crop.

In the case of different fertilization level, there are no essential differences in the values of the main biochemical components, beside the proteins. There was an increase in values at protein substances (0.64%-b1 variant, 1.00%-b5 variant) and a slight decrease in values of ascorbic acid, total sugars and cellulose, from control variant to variant b5 (400 kg active substances N/ha).

Because of cold storage, intensity of the metabolic processes decreased, for which, during storage, the biochemical component did not suffer major changes.

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EFFECT OF SOME "BIO-INSECTICIDES" USED AGAINST TWO SPOTTED SPIDER MITES (*TETRANYCHUS URTICAE* KOCH.) IN THE CUCUMBERS CROP UNDER PLASTIC TUNNEL CONDITIONS

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Abstract

Experiment was performed in the summer of 2015, in a cucumbers crop under high plastic tunnel conditions in order to determine the efficacy of some bio-insecticides in the control of two spotted spider mites (Tetranychus urticae) attack. Bio-products Oleorgan - 0.3% (saponified neem oil 40%), Konflic - 0.3% (Quassia amara 50%+saponified extract of different oils 50%), Canelys – 0.3% (cinnamon extract 70%), Kabon – 0.3% (potassium soap from vegetable oils 50%), Zicara – 0.15% (citrus peel extract and essential oils 70%), Lasser 240 SC – 0.05% (spinosad 24%) and Vertimec 1.8 EC – 0.08% (abamectine 1.8%) were applied single or mixed, repetitive or alternated in different variants. Were performed 6 applications. Intervals between treatments decreased from 7 to 3-4 days, depending of pest infestation level. Percent of attacked area/leaf and attack/plant established by visual estimation, and the harvest/plant were registered at the end of crop vegetation cycle. In addition, for a better appreciation of obtaining results were calculated the average attack and production increasing. Analyzing of the all parameters take into account showed that the most relevant of them proved to be the average attack (noticed as "general attack"), harvest/plant and harvest increasing. Yield data were statistical assured. Based on these parameters, best results (6.1%, 3.914 kg, and 224.8%, resp.) were obtained with a combination of spinosad 24% and abamectine 1.8% applied alternate. Appropriate values (6.4%, 3.076 kg, and 176.7%, resp.) were registered with product Oleorgan based on saponified neem oil 40%. Relatively good results (17.7%, 2.504 kg, and 143.8%, resp.) have been obtained by applying a mixture containing Oleorgan + Kabon + Canelys (0.3% each) alternately with another mixture: Konflic + Canelys + Zicara (0.3%; 0.3%; 0.15%). In the cases of spinosad 24% and untreated check, the three parameters take into account presented values of 75 and 92.5% resp., 2.248 and 1.741 kg, resp., and 130.8% (untreated check was reference). The study revealed that the saponified neem oil 40% applied alone had lower but appropriate performances to those of spinosad and abamectine alternately applied, proving a high acaricidal action, which promote it to be used in IPM and organic farming practices.

Key words*: bio-insecticides, plant extracts, plant oils, efficacy, cucumber, mites*

INTRODUCTION

The use of bio-insecticides based on plant extracts or metabolites of the various categories of bodies has experienced a revival in the past decade because of its positive impact on public and enviromental health. Plant species whose insecticidal action was known long time ago came to the attention of scientists, along with the start of investigations for finding new botanical source-species (Grdiša and Gršić, 2013). Despite their beneficial effect on the quality of the environment, bio-pesticides in general have several limiting characteristics (quick degradation in sunlight, air and moisture) that make them less agreed by large farmers communities, being recognized and used mainly in organic farming from developed countries. They have initiated extensive researches to identify their own botanical source-species and the formulation of their own bio-insecticides (Khater, 2012).

The present work aimed to establish the action spectrum and efficacy of some comercial bio-insecticides applied alone or mixed, repetitive or alternatively to control populations of two spotted spider mites (*Tetranychus urticae*) in a cucumber crop under high plastic tunnel.

MATERIALS AND METHODS

Experiment was performed in a cucumber crop (cultivar „Mirabelle F1") under high plastic tunnel, natural infested with two spotted spider

mites (*Tetranychus urticae* Koch.), during summer season, 2015 in Vidra-Ilfov, Romania. Tested bio-insecticides were: Oleorgan (saponified neem oil 40%), Konflic (*Quassia amara* 50% + saponified extract of different oils 50%), Canelys (*cinnamon* extract 70%), Kabon (potassium soap from vegetable oils 50%), Zicara (*citrus* peel extract and essential oils 70%), Lasser 240 SC (*spinosad* 24%) and Vertimec 1.8 EC (*abamectine* 1.8%).

Each of treatment variant was organized in three replicates (20 plants/replication), liniar arranged. The treatment variants were:

1. Mixed bio-insecticides (Oleorgan 0.3% + Kabon 0.3% + Canelys 0.3% mixed and alternately applied with Konflic 0.3% + Canelys 0.3% + Zicara 0.15% mixed);
2. Laser 240 SC 0.05% alone, alternately applied with Vertimec 1.8 EC, 0.08% alone;
3. Oleorgan 0.3% alone, repetitive applied;
4. Laser 240 SC 0.05% alone, repetitive applied;
5. Untreated check.

The bio-insecticides water solutions were applied by spraying of plant leaves, using a manual pump.

It were applied 6 treatments during the vegetation season, intervals between applications decreasing from 7 days (at the beginning of pest infestation) to 3-4 days (in the top of pest development).

A single visual observation was performed 4 days after last treament by this way being estimated the percent of attacked area/leaf (sample size: 4 leaves/5 plants) and the percent of attacked area/plant (5 plants/replicate). Yield was registered also, during experimental period and statistical analyzed.

RESULTS AND DISCUSSIONS

Obtained results had confirmed the scientific researches (Martinez, 2001; Hummel and Kleeberg, 2001) showing the great efficacy of saponified neem oil 40% (Oleorgan) in the control of two spotted spider mites populations (fig. 1).

In this case had emphasized a compensatory effect between low value of the attack/leaf (6.2 %) and that easy greater of the attack/plant (6.5 %) which proves that attacked areas on leaves were small but the number of infested leaves on plant was greater (fig. 2).

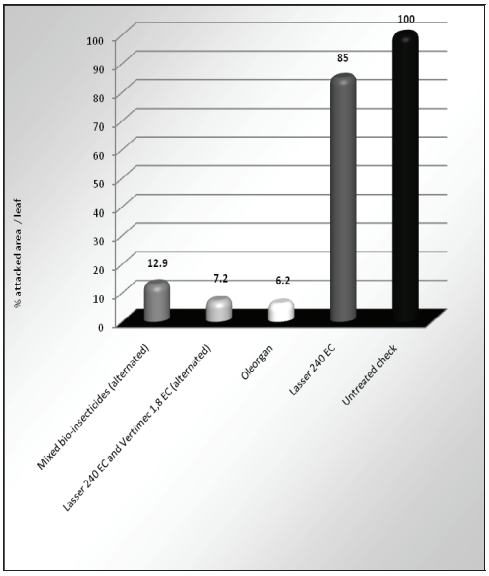

Figure 1. Effect of some "bio-insecticides" used in the control of mites on cucumbers crop under high plastic tunnel (attack/leaf)

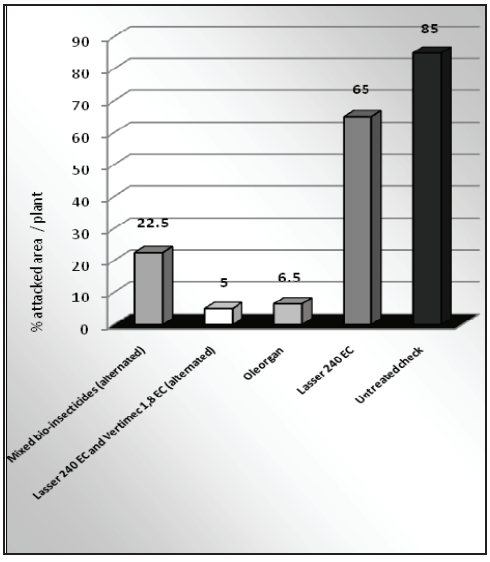

Figure 2. Effect of some "bio-insecticides" used in the control of mites on cucumbers crop under high plastic tunnel (attack/plant)

By alternating *spinosad* (Laser 240 SC) and *abamectine* (Vertimec 1.8 EC) applications the effect was opposite, the attacked areas on

leaves being greater while the number of infested leaves on plant was lower.
For a balanced appreciation of the obtained results it was calculated the average values of the two registered parameters noticed as "general attack" (fig. 3).

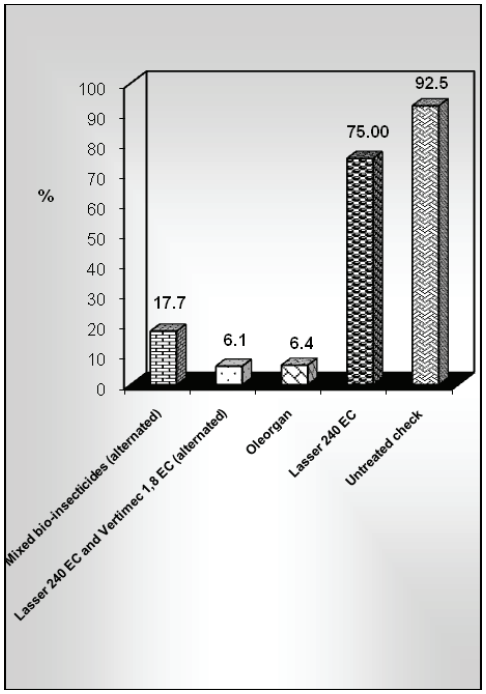

Figure 3. Effect of some "bio-insecticides" used in the control of mites on cucumbers crop under high plastic tunnel (general attack/plant)

They showed the similar effects of neem oil and alternatively treatments with *spinosad* and *abamectine* in the control of two spotted spider mites populations. Mixed bio-insecticides, alternatively applied gave good results also, by comparison with *spinosad* and untreated check. Despite of appropriate results obtained with neem oil and spinosad/abamectine alternate application, the differences between the yields of these two variants were bigger (fig. 4) suggesting a certain negative influence of oil compound on cucumbers plants. However, all the four treatment variants experimented gave better results than untreated check regarding harvest/plant. The biggest yield difference comparing to untreated check was obtained with *spinosad*/*abamectine* alternate applications followed by *neem* oil, mixed bio-insecticides alternatively applied and *spinosad* repetitive applications (fig. 5).

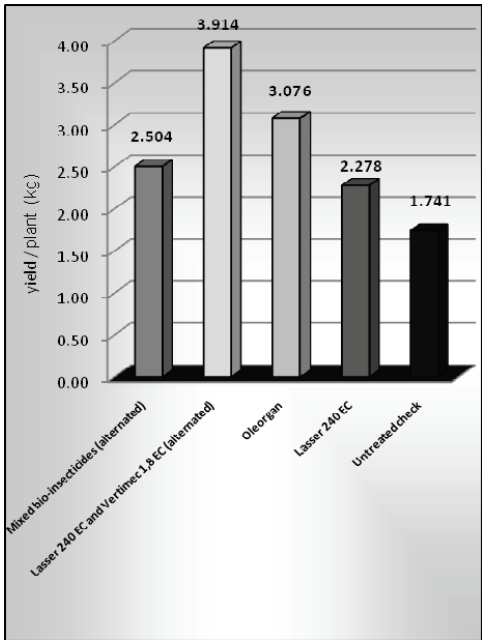

Figure 4. Harvest obtained in the cucumber crop treated with different „bio-insecticides"

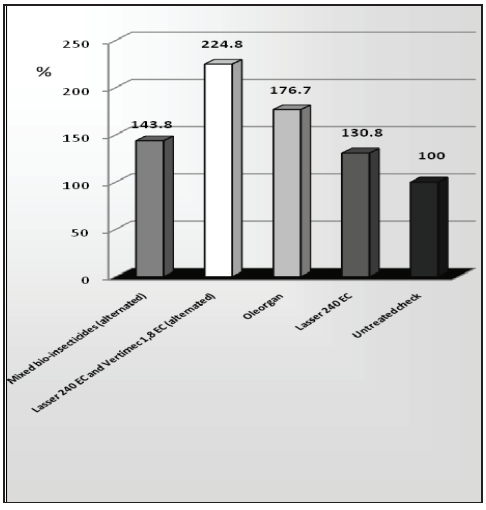

Figure 5. Harvest increasing at cucumbers crop treated with different „bio-insecticides"

Statistical interpretation of yield data were made by analysis of variance (table 1).

Table 1. Significance of yield differences between treated variants and untreated check

Variant	Yield (t/ha)	Yield (%)	Differences (t/ha)	Significance
1	31.73	143.8	+ 9.66	***
2	49.60	224.7	+ 27.53	***
3	39.00	176.7	+ 16.93	***
4	28.87	130.8	+ 6.80	***
5	22.07	100.0	-	-
LSD 5% LSD 1% LSD 0.1%	= 1.78 = 2.59 = 3.88			

Differences between the experimental variants can be observed in the photos 1, 2, 3, 4 and 5.

Photo 1. Effect of mixed bio-insecticides in the control of red spider mites on cucumber plant (V1)

Photo 2. Effect of *spinosad* and *abamectin* alternate applied in the control of red spider mites on cucumber plants (V2)

Photo 3. Effect of saponified *neem* oil in the control of red spider mites on cucumber (V3).

Photo 4. Effect of *spinosad* in the control of red spider mites on cucumber plants (V4).

Photo 5. Effect of red spider mites attack on untreated cucumber plants (V5).

CONCLUSIONS

The most relevant parameters in this study proved to be general attack, harvest/plant and production increasing compared to untreated check. Based on them, it can appreciates that *spinosad* and *abamectine* applied alternately gave the best results (6.1 %; 3.914 kg; 224.8 %, respectively), being followed of *neem* oil alone (6.4 %; 3.076 kg; 176.7 %, resp.), mixed bio-insecticides alternate (17.7 %; 2.504 kg; 143.8 %, resp.) and *spinosad* alone (75 %; 2.278 kg; 130.8 %, resp.). At untreated check were registered the most poor results (92.5 %; 1.741 kg).

On the other hand, the relatively great decreasing of yield registered at *neem* oil by comparison with *spinosad* and *abamectine* alternately applied, it could suggest a slowly phytotoxic reaction to the oil compound that reduce the ability of plant respiration, but this should be studied in depth.

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FOLIAR BIOACTIV TREATMENTS INFLUENCE ON EGGPLANTS SEEDLINGS

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Abstract

Eggplants (Solanum melongena) is known for its weak root system and for the particular sensitivity towards different stressors such as heat, water stress, nutritional stress. Therefore, is extremely important to obtain eggplants 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 eggplants seedling with Spraygard 1%, Razormin 0.1% and BAC Foliar Spray 0.3%. The treatments were performed in two distinct stages of development: at one, respectively three weeks after the seedlings transplantation. Analysis of the acquired data indicated that 0.1% Razormin treatment showed the best results in obtaining of eggplants seedlings with a strong roots systems and a good development. These results are supported by physiological and biochemical processes, which were intensely expressed at the plants in this experimental variant. Also good results in terms of quality seedlings were obtained when treated with BAC Foliar Spray 0.3% and in the case of simple application of adjuvant Spraygard 1%.

Key words*: eggplants, foliar treatments, photosynthesis, root, transpiration.*

INTRODUCTION

In recent years, it was important to reconsider treatments with bioactive substances used in horticultural practice in the context of integrated horticulture development concepts. This comes to align vegetable production in our country to the European Community directives regarding the decrease of environmental pollution through the horticultural technologies, knowing that the horticulture system involves highly intensive cultivation technology which frequently endanger their integrity and the food security.

Vegetables species like eggplant, grown in the temperate climate zone using transplants, are subjected to environmental stress which limits seedlings growth, crop productivity and quality (Sękara et al., 2012). A common consequence of the environmental stress is the increased production of toxic compounds, especially reactive oxygen species produced as result of oxidative metabolism in chloroplasts, mitochondria and peroxisomes (Kim et al., 2004). Previous researches reported that it is absolutely necessary to accomodate the seedlings with variable stress condition which they will cope in the cultivation place so that allow the achievement of "stress memory" (Jennings and Saltveit, 1994; Knight et al. 1996; Mangrich and Saltveit, 2000; Sękara et al., 2012).

In our country, similar researches have been made on pepper and tomato seedlings that have benefited from fertilization with Razormin and Cropmax, or of treatment with Spraygard (Chilom et al., 2000, Bălan et al., 2014; Dobrin et al., 2014).

The research reported in this study were performed on eggplant (*Solanum melongena*), a main specie of vegetables grown in the field, the summer-autumn crop established by seedlings. It were used different growth regulators (Razormin, Spraygard, BAC Foliar spray, Bio Roots) as foliar treatments on eggplant seedlings and the comparative results were studied.

MATERIALS AND METHODS

The experiments established in 2015, April - June, aimed to test the action of fertilizers Razormin and BAC Foliar Spray and of the

universal adjuvant Spraygard on growth and development of eggplants 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, avoidance pests and diseases attacks by strengthening the immune system of plants with implications for reduction of production cost.

The experiment was installed into an experimental greenhouse of the Hortinvest Research Centre – USAMV Bucharest.

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 ethoxylated alcohol 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 applying 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.

We established a monofactorial experiment with 4 variants, considering application of bioactives substances Sparygard 1%, Razormin 0.1% and BAC Foliar Spray 0.3% on egg-plants seedlings (Pana Corbului – an appreciate Romanian varieties) in two distinct stages: at one, respectively three weeks after the seedlings transplantation (7 May and 22 May).

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%. The experiment was installed using the block method in linear alignment with 4 repetitions. The total number of plants in the experiment was 240, each variant containing 60 plants, with 15 plants per repetition.

Sowing was made directly into alveolar pallets (alveolar ø = 5 cm) on April. Because heat and water were optimal provided, mass emergence of seedlings occurred after 12 days. The eggplants seedlings transplanting was done on April 26, in large plastic pots (400 ml) filled with professionally nutrient substrate KEKKILA BP 75% + 25% perlite. It has used this type of pots, with high volume, to counteract any imbalances that may arise in terms of installing a high or even excessive thermal and hydric conditions in the production are, specific at this time of year. During the growth period specific agrotechnics for seedling production was applied: daily ventilation, watering, weeding weeds. The temperature was kept at 22–24 ^{o}C to 28 ^{o}C at day and 18–20 ^{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 week and respectively five weeks after transplantation.

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 physio-logical 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;
- **determinations of the assimilatory pigments content** in the active leaves**:** c*hlorophyll* and *carotenoid pigments* were extracted in 80% acetone and determined spectrophotometrically (wavelenghts 663 nm, 647 nm and 480 nm) using the extinction coefficients and equations described by

Schopfer (1989). The results were expressed in mg/100 g fresh weight.

RESULTS AND DISCUSSIONS

The results of the analysis of the first stage (10 days after the first treatment) are shown in Table 1. Eggplant seedlings have reacted differently to the treatments applied. Plant height is quite different, from 12.4 cm (V1) to 13.6 cm from V4 - fertilized with BAC Foliar Spray, 0.3%. Also in this variant it was recorded the highest number of leaves 5, while at the untreated variant was of 4.2 leaves. The frequency is 0.34 leaves/cm PA to the V1 and 0.38 leaf/cm PA for V2. and V4.

Table 1. Growth and development of eggplant seedlings 10 days after the first fertilization

Variant	Plants height HPA (cm)	No. of leaves	Leaves frequency (nr/ cm HPA)
V_1	12.4	4.2	0.34
V_2	12.8	4.8	0.38
V_3	13.4	4.8	0.36
V_4	13.6	5.0	0.37

Context analysis at this time points out that the application of different treatments have a defining influence on plant growth and development ($R^2 \geq 0.9692$ for plant height and $R^2 \geq 0.8$ for the number of leaves formed) and allows placement variant treatment BAC Foliar Spray, 0.3% on top position, closely followed by treatment with Razormin 0.1% (figure 1).

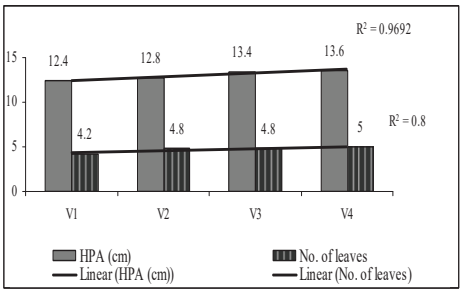

Figure 1. Influence of applied treatment on growth of eggplant seedlings ten days after treatments

In order to determine the overall effect of the treatments program applied on the eggplant seedlings were made observations and measurements also two weeks after application of the second treatment. The results obtained are shown in Tables 2 and 3, respective in Figures 2, 3 and 4.

Table 2. Growth of eggplant seedlings at two weeks after the second treatment

Variant	No. of leaves	Plants height HPA (cm)	Roots length HR (cm)	Plants total lenght HT(cm)	Leaves frequency (nr./cm HPA)
V_1	5.4	21.8	10.6	32.4	0.25
V_2	6.4	24.6	12.2	36.8	0.26
V_3	7.4	24.8	17.2	42.0	0.30
V_4	6.6	23.2	16.0	39.2	0.28

Applied treatment program determined differences regarding on the growth of eggplant seedlings. 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 the higher values (7.4 leaves formed, 24.8 cm plant height, 17.2 cm root length, total length 42 cm plant; 0.3 frequency leaves). In contrast, V1 untreated produced the smallest increase, all the analyzed indicators registering the lowest values (5.4 leaves formed, 21.8 cm plant height, 10.6 cm root length, total length of 32.4 cm plants, leaves frequency 0.25).

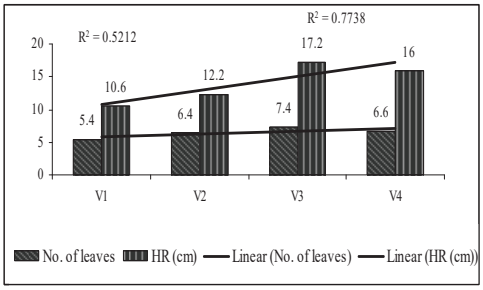

Figure 2. Influence of applied treatment on eggplant seedlings growth two weeks after the second treatment

As can be seen from Figure 2 and 3, schedule treatments with bioactive substances exert a greater influence on the growth of eggplant seedlings, respectively, a very significant influence on the growth of roots ($R^2 = 0.7738$) and a significant one on the number of leaves and on frequency leaves ($R^2 \geq 0.52$).

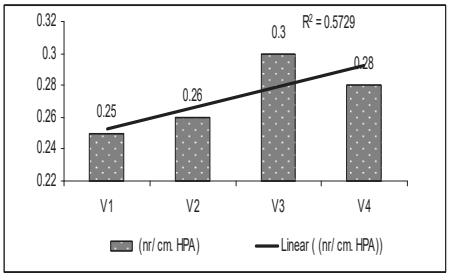

Figure 3. Influence of applied treatment on the leaves frequency two weeks after the second treatment

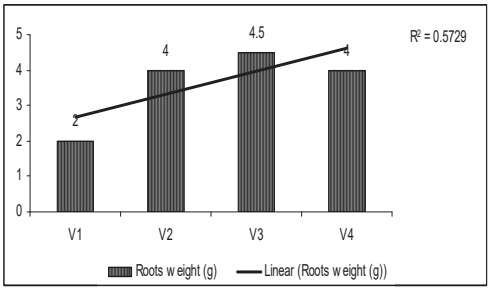

Figure 4. Influence of applied treatment on the roots weight two weeks after the second treatment

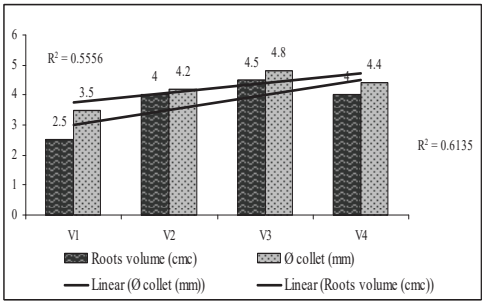

Figure 5. Influence of applied treatment on the roots volume and the collet diameter two weeks after the second treatment

Taken together the results obtained for eggplant seedlings morphometry, we estimate that the most balanced variant is V3 (fertilized with 0.1% Razormin) regarding on plants growth.
Developing of eggplant seedlings two weeks after the second treatments was quantified by various indicators of weight and volume and by diameter of collet. The obtained results (Table 3; Figure 4 and 5) regarding eggplant mass ratio highlights two situations:

1. at V3 variant all indicators recorded the highest values compare to the other variants (mass root 4.5 g; 10 g total mass, volume root 4.5 cm^3; 4.8 mm collet diameter), excluding the aerial part mass (5.5 g);

2. in contrast, V1 variant recorded the lowest values (mass root 2 g; 8 g total mass; volume root 2.5 cm^3; 3.5 mm collet diameter), excluding the aerial part mass (6 g).

Good results have also recorded the seedlings treated with BAC Foliar spray and Spraygard. A strong influence of the treatment on the development of the root system and of the collet diameter was noticed.

Table 3. Developing of eggplant seedlings at two weeks after the second treatments

Variant	Aerial part mass (g)	Roots weight (g)	Total weight (g)	Roots volume (cm^3)	Ø collet (mm)
V_1	6.0	2.0	8.0	2.5	3.5
V_2	5.5	4.0	9.5	4.0	4.2
V_3	5.5	4.5	10.0	4.5	4.8
V_4	5.5	4.0	9.5	4.0	4.4

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 (27.2–27.6 ^oC) and light intensity (Q) registered the value of 1280-1360 mmol/m^2/s.

Table 4. Physiology of the eggplant seedlings two weeks after the second treatment

Var.	A [µmol/ m^2/s]	E [µmol/ m^2/s]	A/E	GS [µmol/ m^2/s]	Leaf temp.T [^0C]	Q [µmoli/ m^2/s]
V_1	9.95	1.84	5.41	0.07	27.2	1280
V_2	6.35	0.84	7.56	0.045	27.6	1280
V_3	8.34	1.80	4.63	0.06	27.6	1360
V_4	9.08	1.57	5.78	0.06	27.2	1360

The results analysis revealed that V1 untreated variant recorded the highest values (Photosynthesis rate A = 9.95µmol/m^2/s; Transpiration rate E = 1.84 µmol/m^2/s; Stomatal conductance Gs = 0.07; A/E = 5.41) for all studied parameters. This intense physiological activity is not supported by plant growth and can be explained only by the theory that nutrition, regime of water and the temperature may be major factors of stress for seedlings of eggplant (Acatrinei, 2010; Sekara et al., 2012). The intensify of physiological processes without correlation with 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. It was also noticed V2 - Spraygard, which amid of low rates of transpiration and stomatal conductance, recorded very good photosynthetic yields.

Table 5. Assimilatory pigments of the seedlings leaves

Var.	Assimilatory pigments (mg/100 g)		
	Chlorophyll a	Chlorophyll b	Carotenoids
V_1	102.43	50.42	5.70
V_2	112.00	38.98	5.27
V_3	136.50	47.97	5.41
V_4	142.67	36.35	4.35

Biochemistry leaves are somewhat contradictory to physiology in the sense that an increased rate of photosynthesis is not necessarily correlated with an increased content of chlorophyll pigments. Thus, V1 untreated, recorded the lowest content of chlorophyll pigments (chlorophyll 102.43 mg/100 g, 50.42 mg/100 g chlorophyll b) but, due to an increase in photoprotection status, had the highest content in carotenoids pigments (5.7 mg/100 g). For other variants studied, the results were correlated with physiology and morphometry. It was again emphasized the V3, with the highest content of chlorophyll pigments and a very good photoprotective activity. V4 occupied second place, which was given mainly to synthesis of chlorophyll a (142.67 mg/100 g).

CONCLUSIONS

In horticultural tehnology were applied treatments with bioactive substances as a frequence practice for accelerating or inhibiting the growth of vegetable seedlings or plant or as a life support under different stressful conditions.
In the first stage of analysis the context results shows that treated variants have superior biometrics comparing to the control untreated variant. BAC Foliar Spray 0.3% highlights as variant in which seedlings have realised the most favorable growth.
At the second moment of analysis V3 - fertilized seedlings Razormin 0.1% it was noticed as the best option working variant. In this variant plants achieved the best and balanced growth, all indicators analyzed having higher values (7.4 leaves formed, 24.8 cm plant height, 17.2 cm root length, total length 42 cm plant; 0.3 frequency leaves). Treatments program applied of eggplant seedlings exerted at least a significant influence on the growth of eggplant seedlings, of roots, of the number of leaves and of frequency leaves.
Mass ratio analysis reveals that at V3 variant all indicators recorded the highest values compare to the other variants excluding the aerial part mass
Biochemical and physiological analysis also revealed V3 with the highest content of chlorophyll pigments and a very good photoprotective activity. V4 occupied second place, which was given mainly to synthesis of chlorophyll a.
In conclusion, the analysis of the acquired data allow to remark that treatment with Razormin 0.1% led to obtaining of eggplant seedlings with high quality and strong roots. Very good results in terms of quality seedlings were obtained when treatment with BAC Foliar Spray 0.3% was used and also with simple application of adjuvant Spraygard 1%.

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DYNAMICS OF PHYSIOLOGICAL PROCESSES IN TOMATOES DURING THE PHENOLOGICAL STAGES

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Abstract

Tomato (Solanum lycopersicum L.), known to belong to the Solanaceae family, is considered one of the most important vegetable in the world since the fruits are widely consumed either fresh or processed. The ripe fruits are a valuable source of vitamin C, carotenoids and minerals such as iron and phosphorous that is daily required for a healthy diet. Fruit growth and ripening are the result of multiple physiological and metabolical processes that occur during the plant development. A thorough knowledge of the physiological characteristics of tomato plants is necessary to improve the technology of cultivation under greenhouse conditions. Research regarding the intensity of physiological processes as photosynthesis, respiration, transpiration have been made on some tomato hybrids cultivated in protected spaces in different phenological stages of plant development: in the vegetative growth period, at the flowering time and at the fruiting time. The measurements were performed with electronic analyzer LCA-4. It has been noticed that dynamics of the physiological processes varies depending on the hibrid type and on the development phenophase.

Key words*: tomato, phenology, photosynthesis, respiration, transpiration*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), known to belong to the *Solanaceae* family, is considered one of the most important vegetable in the world since the fruits are widely consumed either fresh or processed. Beside the high nutritional value, the ripe tomato fruits are a valuable source of vitamin C, carotenoids and minerals such as iron and phosphorous that are daily required for a healthy diet (Nour et al., 2013).

Fruit growth and ripening are the result of multiple physiological and metabolical processes that occur during the plant development. Leaves are considered to be the main providers of carbon for fruit growth (Hetherington et al., 1998). Therefore, the major functions of the leaves was studied in order to relate the influence of different cultivation technologies or varying environmental conditions on fruit growth and development. A thorough knowledge of the physiological characteristics of tomato plants is necessary to improve the technology of cultivation. The physiological parameters depends on genetics, environmental factors (temperature, light, water and nutrient availability, air composition), agricultural techniques (Schwarz et al., 2002; Islam, 2011; Zhu et al., 2012).

Recently new tomato hybrids with improved nutritional content and potential health benefits are being developed. Consequently, it has become increasingly important to assess their physiological parameters in order to recommend the use of certain cultivation technologies. For this purpose study of the intensity of physiological processes as photosynthesis, respiration, transpiration was performed on some tomato hybrids cultivated in protected spaces (greenhouse). The determinations were made in different phenological stages of plant development (growth, flowering and fruiting phenophase) so that some peculiarities of selected tomato hybrids to be emphasized.

MATERIALS AND METHODS

Four tomato hybrids from collection of Faculty of Horticulture (USAMV Bucharest) were investigated: Principe Borghese, Maressa, Izmir and Ruxandra.

Principe Borghese hybrid is a cherry tomato with determined growth, small pear-shaped

fruits, ideal for consumption in fresh and preserved condition.
Maressa hybrid is characterised by undetermined growth, uniform round fruits of middle size (150-170 g), suitable for consumption in a fresh state.
Izmir and Ruxandra are tomato hybrids with undetermined growth, which produce big round fruits (180-200 g).
The selected tomato hybrids were cultivated in protected systems (greenhouse), that provided controlled conditions for plant growth, so that the determinations were made at 720-880 μmols/m^2/s light intensity and a temperature of 22-24°C.
Photosynthesis, respiration and transpiration rates were determined with LCA-4 analyzing portable system (ADC Bioscientif, UK) on the fifth leaf from the top of plant. The measurements were made on 10 tomato plants randomly chosed in the greenhouse and average of these 10 measurements was calculated. The obtained results were expressed in μmols $CO_2/m^2/s$ for photosynthesis and respiration rate and μmols $H_2O/m^2/s$ for transpiration rate.

RESULTS AND DISCUSSIONS

The performed research approached the variations of some physiological parameters of certain tomato hybrids during the development stage, in different phenological phases: vegetative growth, flowering and fruiting phases.

The photosynthesis process

Tomatoes are included in C_3 photosynthetic type. The intensity of photosynthesis determines growth and development of plants, so directly influences the yield quantity and quality (Burzo and Dobrescu, 2005).
The obtained results (Figure 1) pointed out that in *the vegetative growth stage* it can be noticed that the most reduced photosynthetic rate was determined at Principe Borghese hybrid (3.21 μmols $CO_2/m^2/s$), while Izmir hybrid registered a 1.5 times higher value (4.88 μmols $CO_2/m^2/s$).
Also Maressa and Ruxandra hybrids reached similar values, no significant differences were reported by comparison with Izmir hybrid.

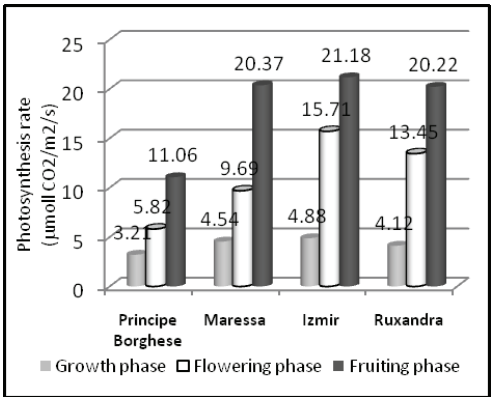

Figure 1. Dynamics of photosynthesis in the leaves of selected hybrids

The photosynthesis of tomato hybrids has been monitored also in *the flowering phenophase*, which debuted in May. In this period Izmir hybrid was noted with an increased value of photosynthesis rate (15.71 μmols $CO_2/m^2/s$), which was 1.17 times higher compared to Ruxandra hybrid and 2,7 times higher than the one registered by the Principe Borghese hybrid.
Comparing the data obtained in the two analyzed phenophases, it can be appreciated that in the flowering stage the process of photosynthesis increased in all selected hybrids, but with a different rhythm: it was 3.21 times more intense at Izmir hybrid, 2.44 times higher at Maressa and only 1.8 times higher at Principe Borghese. This dynamics of photosynthesis can be correlated with the achievement of the growth of leaves, which reached the characteristic dimensions. These results are in according with Ludwig and Withers (1984), which determined the highest value of photosynthesis intensity when the tomato leaves reached 30-50% of their maximum area.
Also in *the fruiting phase* were made determinations of the photosynthetic rate, which reached higher values compared to flowering phase in all the selected hybrids because the formation and growth of the fruits stimulate the photosynthesis process. Thus, the photosynthesis increased by 2.1 times at Maressa hybrid, by 1.9 times at Principe Borghese hybrid and by 1.5 times at Ruxandra hybrid.
Izmir hybrid recorded the highest photosynthetic rate in this phenophase (21.18

μmols $CO_2/m^2/s$) compared to the other analyzed hybrids. However, in this hybrid the photosynthesis process was stimulated only 1.3 times by fructification, while the flowering determined a 3.3 times more intense rate of photosynthesis in comparison with the growth stage.

It is notable Izmir hybrid as having an elevated biological potential, given the high value of the photosynthetic intensity performed during monitored phenophases.

The transpiration process

Transpiration process consists in removal of the water excess by the plants, thus avoiding supersaturation of the cells with watter and overheating (thermoregulator role). Most importantly, transpiration generates the suction force of the leaf, which is involved in roots activity of water and minerals absorption. Thus it can be appreciated that transpiration and photosynthesis are related processes, as the suction force of leaf provides the raw materials needed to develop leaf photosynthesis (Burzo et al., 2004).

The obtained data (Figure 2) indicated that in *the growth phenophase* the transpiration rate varied between 2.33 μmols $H_2O/m^2/s$ in Principe Borghese hybrid and 3.31 μmols $H_2O/m^2/s$ in Izmir hybrid. Comparing the values determined for the selected hybrids, it was noticed that Izmir hybrid performed a transpiration rate by 1.42 times more intense than Principe Borghese, by 1.38 times higher than Maressa and by 1.4 times higher than Ruxandra hybrids.

In *the flowering phenophase* an increasing of transpiration rate can be observed compared to the growth phase in all the selected tomato hybrids: by 1.36 times at Ruxandra, by 1.11 times at Maressa, but insignificantly at Principe Borghese and Izmir. It appears that Principe Borghese hybrid registered similar values of transpiration rate both in growth and in flowering phenophase. In contrast, Ruxandra hybrid showed an increased rate of transpiration in the flowering phase, which is corelated with an increased rate of photosynthesis in the same phenophase.

The determinations performed in *the fruiting phenophase* indicated an increase of the transpiration rate in all the studied tomato hybrids as result of fruit formation and growth. The transpiration increase during this development stage may be corelated to an increased water demand provided as result of the roots absorbtion stimulated by an intense transpiration.

It is notable that Izmir and Ruxandra hybrids registered the highest transpiration rate in flowering and fruiting phases, so a positive correlation with photosynthesis rate was observed.

On the contrary, Principe Borghese hybrid registered the lowest rate of transpiration, an almost constant value (2.33-2.45 μmols $H_2O/m^2/s$) during the monitored phenophases. Also the photosynthesis process was the least intense throughout the research in this hybrid.

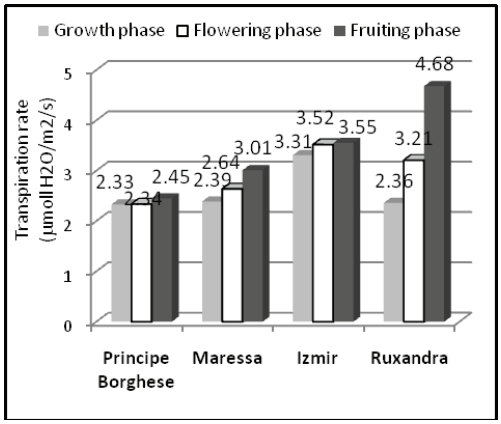

Figure 2. Dynamics of transpiration in the leaves of selected hybrids

The respiration process

The respiration is the only process that provides biochemical energy to achieve the plant growth and development. Tomatoes are climacteric plants, so the respiration process follows a characteristic dynamics: it achieves a maximum in the growth phase, then decreases, but reaches a second maximum (the climacteric maximum) during the maturity phase (Gherghi et al., 2001; Burzo et al., 2005).

Regarding the intensity of respiration process (Figure 3) in the selected tomato hybrids it was noticed high values (2.3-2.22 μmols $CO_2/m^2/s$) at Izmir and Maressa hybrids in *the growth phase*, which are positively correlated to photosynthetic process. The lowest value of respiration rate in this phenophase was registered by the Principe Borghese hybrid (1.49 μmols $CO_2/m^2/s$).

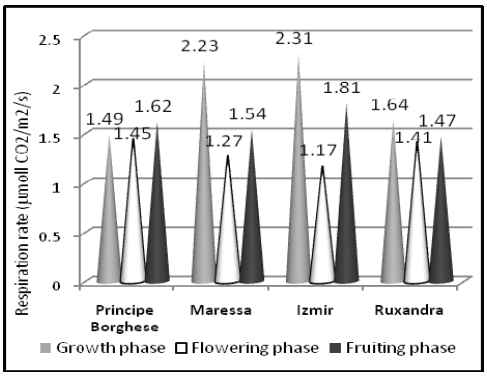

Figure 3. Dynamics of respiration in the leaves of selected hybrids

A decrease of respiration rate was noticed in *the flowering phase* in all the studied tomato hybrids, in according to speciality literature. The reduction rate of respiration was different: by 1.96 times lower in Izmir hybrid, 1.74 times at Maressa, 1.17 times at Ruxandra and insignificant decrease at Principe Borghese hybrid.

In *the fruiting phenophase* a second maximum of respiration process was determined in all the selected hybrids, the highest value (1.81 µmols $CO_2/m^2/s$) being registered at Izmir hybrid, which was characterized by an intense metabolism during the entire research period. A reduced respiration rate was measured at Maressa hybrid (1.54 µmols $CO_2/m^2/s$) and Ruxandra hybrid (1.47 µmols $CO_2/m^2/s$).

CONCLUSIONS

It has been noticed that dynamics of the physiological processes varies depending on the hibrid type and on the development phenophase.

The photosynthesis process follows an ascending evolution during the research period: both the flowering and the fruiting stage stimulate the photosynthesis, which registered increased values compared to the one determined in growth phenophase in all the studied hybrids.

The transpiration process is positively correlated to the photosynthesis, so it increased constantly, but in different rhythm during the development phenophases in all the studied hybrids.

The respiration process follows the dynamics characteristic for climacteric plants: it achieved a maximum in the growth phase, decreased in the flowering phase, but reached a second maximum (the climacteric maximum) in the fruiting phase.

Among studied hybrids, Izmir hybrid was noted with a high biological potential, given the increased values of physiological parameters during the monitored research period, which indicate an intense metabolism.

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NEW PROCESSING TOMATO VARIETIES OBTAINED AT V.R.D.S. BUZĂU

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Abstract

Researchers for improving tomatoes at V.R.D.S.Buzau have been since its beginning, 1957. Here were obtained for the first time in Romania, the tomato hybrid seed under Bulgarian license, the 10 x Bizon famous hybrid. Over time the new valuable, with well-defined genetic constitution varieties were created which currently occupy significant surfaces in culture. Among these are: Buzau 22, Buzau 47, Buzau 1600. Studies undertaken by V.R.D.S.Buzau about the Romanian varieties of tomatoes have spotlighted that old varieties of tomatoes were patented as mixed, with destination for fresh consumption and industrialization. At the same time it was found that these varieties do not always meet the appropriate parameters for both destinations, so after the year 1990 researches were undertaken by the Improvement Laboratory in an intensive system, with the aim of obtaining creations strictly specialized, according to destination. The research started with the establishment of a solid germplasm database, followed then by its knowledge and use in the improvement process. The unit currently has a total of over 1000 genotypes from this species in various stages of improvement. The main objectives for improvement of which were obtained new creations were: productivity, quality of fruit in accordance with the requirements of the processors, the dry substance content, low acidity, pigmentation, the content of lycopene, sugar-acidity ratio, the jointless gene (breaking the fruit without peduncle), and genetic resistance to the main pathogens, concentrated fruit maturation suitable for mechanized harvesting. The researches were completed for a total of 5 new varieties patented and registered in the Official Catalogue of the Crop Plants from Romania. Among these, the varieties Darsirius and Daria present ovoid, plump fruits, Kristinica and Florina 44 R have round fruits, and Florina 44 T has also round fruits that show an easy mucrone, transmitted by the beck gene. Regarding the earliness, the first place is occupied by the variety Kristinica, its fruits reaching the physiological maturity at 90 days after planting and the tardiest is the Darsirius variety, with fruits that reach the complete maturity at 130 days after planting.

Key words*: Darsirius, breeding, Florina , germplasm, Kristinica.*

INTRODUCTION

V.R.D.S. Buzău showed interest for tomatoes breeding since its establishment, in 1957. There were created the first Romanian varieties of tomatoes intended for both industry and fresh consumption. At the same time was obtained hybrid tomato seed for the first time, under the Bulgarian license, the famous hybrid 10 x Bizon, followed by Romanian Export II hybrid made from the crossing of line 24 x XIII.

Currently, the institution has a portfolio of 18 approved and patented varieties, registered in the Official Catalogue of Romanian Crop Plants.

It was found that most Romanian varieties so far have a mixt destination, both for fresh consumption and industrialization. As a result of the increasing demands imposed by growers, processors and consumers, starting with 1996, research undertaken intensively at V.R.D.S. Buzău in terms of tomatoes breeding had the aim of obtaining specialized strictly specialized varieties.

At the same time it was found that Romanian industry varieties offer is currently quite limited in comparison with market needs. In terms of per capita consumption tomatoes are the leading processed vegetables. (Gould et al., 2013). Tomatoes intended for processing must meet certain conditions, to have certain features that are implemented through the breeding process of improvement and genetic resource used.

For instance, certain phenological traits (early flowering and concentrated fruit set) were associated with a set of morphological traits (smaller canopies and low vegetative biomass),

along with gains in physiological traits (biomass N concentration and photosynthetic rates) in modern varieties (Felipe et al., 2014).
The breeding program aimed the fruit quality, especially in terms of their chemical composition. Processing of fresh tomato into paste had an overall positive effect on the contents in phenolic compounds, no effect on lycopene and a slight and high detrimental effect on β-carotene and ascorbic acid, respectively, (Chanforan et al., 2012).
This work presents recent varieties obtained for industry as results of research undertaken in 1996-2015 at V.R.D.S. Buzău

MATERIALS AND METHODS

The research started with the collection of germplasm, its evaluation and division by type (sp- self prunning, SP- half self prunnig and SP^{+}- indeterminate) and depending on the degree of genetic stability (stable, advanced and segregant) (fig.1.).

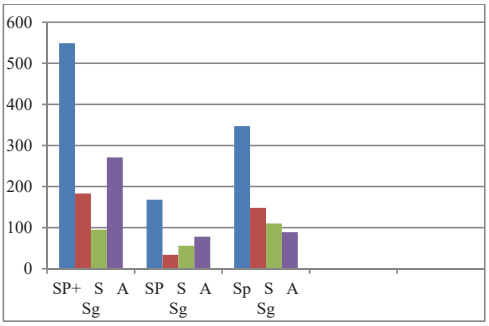

Figure 1. Tomatoes germplasm composition

SP+ (indeterminate)- 549 lines from which:
S (Stable) 183,
A (advanced) - 95
Sg (segregant) - 271
SP (half self-pruning lines) - 168 lines from which:
S (Stable) 34,
A (advanced) - 56
Sg (segregant) -78
Sp-(self-pruning lines)- 347 lines from which:
S (Stable) 148
A (advanced) - 110
Sg (segregant) -89
After germplasm evaluation, it was divided into two fields, the general collection field where were maintained the collected genotypes and work field composed from promoted genotypes in accordance with the breeding process objectives.
Priority had self-pruning genotypes genetically stabilised totalling 148.
Breeding applied methods were specific for tomatoes, especially individual repeated selection.
After the evaluation and tests carried out in the test fields over a long period of time, 5 genotypes showed stability and genetic superiority in terms of productivity and meeting specific industry characteristics and were approved under the following names: Daria (Măriuca), Florina R, Florina T, Darsirius and Kristinica.
As control variant in the experience has been used well-known variety Rio Grande.
The applied technology was specific to tomatoes specifying that the establishment of culture, for all the cultivars was done through direct sowing and planting stock. Sowing for seedling production has been carried out on the 10th of March and the planting was carried out on the 25th of April (Fig.2.).


Figure 2. Seedlings

For direct sowing variant, all cultivars were seeded on the 20th April.
Soil preparation was made in sSeptember through leveling, followed by fertilization. The work was followed by deep ploughing. In spring, the soil has been mobilized with disc harrows, followed by soil modeling. Maintenance work was specifically, irrigations (7-8) during the vegetation period, filling in gaps, manual and mechanical cultivation.
Planting has been carried out using the following scheme of crop establishment (fig.3.):

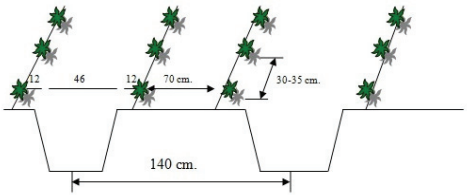

Figure 3. Crop establishment scheme on modeled soil

The same establishment scheme was used to direct sowing crop variant specifying that the norm of seed was 1 kg/ha, followed by sparing work, after the plants have reached the cross stage.

RESULTS AND DISCUSSIONS

The researches were completed with obtaining a rich and varied assortment of new Romanian varieties at this species. The new varieties have distinct genetic and phenotypic features that correspond to the proposed objectives for industrialization.

The main features of the newly created varieties are:

Florina R variety of tomato (fig. 4.) has determined growth intended for field crop. Special taste and aroma and also the dry matter percentage over 6.2% recommends it for industrialization. Immature fruit is green, with green shoulder (U gene) and at physiological maturity turns bright red. The fruit are firm, split and burning sun resistant and has a long shelf life after harvest (over 10 days). The fruit has commercial, attractive aspect with pleasant and balanced taste. The fruit can be jointed harvested because of the short stem, increasing the duration of storage after harvesting.

Plant vigour is average, presents a number of 60-80 leaves with leaflets of medium size.

In the inflorescence can be found 4-6 large, round fruit, medium weight of fruit is 220 g.

In cross section the fruit shows a pericarp thickness of 7-8 mm and 4 seminal lodges.

The fruit has a small number of seeds, between 60-80 that are well developed and visibly covered with yellow thin hairs.

Production potential = 50-60 t/ha.

The Florina T variety (fig. 5) is similar to the Florina R variety, having at the obtaining base the same origin but differentiates itself by the following characteristics: the fruit is round with easy mucron (Beck gene). At the same time the yield level is low but the shelf life is longer due to the higher firmness.

The Darsirius variety (fig. 6.) has ovular shape fruits with an average weight of over 80 g, immature fruits have uniform green colour (U.G. gene) and riped fruits are glossy dark red. The fruits are firm, with small abscission area without hard tissues into the fruit, jointless, easily to be harvest and it is split resistant, reddish-burgundy colour in ripe. Due to the concentrate ripening and jointless fruits, the variety is suitable also for mechanized harvesting.

Fig. 4. Florina R variety

Fig. 5. Florina T variety

It has a high content of dry matter of more than 5.5 percent. It can be grown in organic farming, being created in our country concerning the specific climate conditions. Plant has determined growth with a height ranging between 50-60 cm with 4-6 vigorous shoots.

Figure 6. Darsirius variety

Variety is genetically endowed with genes of resistance to specific tomatoes diseases and Nematodes (Mi gene) and has a long shelf life after harvesting for over 10 days.

The Kristinica variety (fig. 7.) has a determined growth, registering an average height of 60 cm, small vigor and shallow foliage.

Figure 7. Kristinica variety

Thanks to this feature it can enlarge the surface density and shows an average number of 6 fruit per truss. Before maturity, the fruit presents green shoulders (U gene).

The fruit is round shaped and has an average weight of 120 g. Fruits are firm and are red colored. The shelf life of the fruit both on the plant and after ripened, after harvest is over 30 days, without yield depreciating. Yield production: 50-60 t/ha, 2, 5 kg/plant. Yield can increase significantly if it interferes with additional technological links.

Beeing the first obtained and patented variety of V.R.D.S. Buzau for industry, it was tested in the six main vegetable romanian institutions in comparative crop with Rio Grande. The obtained results are presented in table no.1.

Table 1. testing of potential yield production (t/ha) of tomato variety Kristinica in comparative crop, in six of the main romanian vegetable institutions

Variety	Locality						Average		STAS of total**	Early yield of total***
	Tc.	Ov.	Cl.	Cf.	Tu,	Tg.	t/ha	%	%	%
Rio Grande (control variant)	52.2	22.9	44.2	68.3	9.8	37.5	39.2	100	77.2	5.4
Kristinica	41.8	29.2	50.3	82,0	20.4	40.3	44	112.2	86.9	21.2

*Locality: Tc.=Tecuci; Ov.= Ovidiu; DL 5%=8,8 t/ha
Cl.= Calarasi; Cf.= Calafat; Tu.= Turda DL 1%=13,8 t/ha
Tg= Targoviste DL 0,1%=23,5 t/ha
**Fruits with weight of over 33 g;
***Yield obtained until 31st of July- in the south; 10th of August- in the rest of area

Daria (Măriuca) variety (fig. 8) is determined growth, medium vigor, with an average height of 55 cm, a vigorous stem, from which 6-8 sprouts start. The leaves are medium sized, dark green, slightly embossed.

Figure.8. Daria (Măriuca) variety

The fruit is slightly ovular shape, immature fruit is uniform green (UG gene) and red riped. The fruits are firm, with a pericarp of 8-9 mm and a total of 3 seminal lodges where there are between 60-80 seeds. The shelf life of the fruit is good, for more than 30 days. Average production per plant is 2.5 kg but can increase significantly if it interferes with additional technological links.

In terms of yields obtained at V.R.D.S. Buzău in 2010-2015 period, is noted that compared to Rio Grande control variant, the 5 varieties for industrialization have recorded higher yields (table.no. 2.).

Daria (Măriuca) registered in 2014, 108.9 t/ha, being the highest yield compared to Rio Grande who registered just 75.2 t/ha in the same year, and highest yield recorded by this variety.

The smaller productions were obtained in 2011 (21.5 tonnes/hectare at Florina R) but with the difference that the climatic conditions of that year were not favourable for the tomatoes.

On average, all 5 varieties are superior compared to the control variant with a maximum value of 17.8 percent registered by Daria (Măriuca).

In order to obtain an early yield the concentrated ripening fruit was aimed and Florina T recorded the highest percentage of total early yield, of 9.6%, while Florina R had 6.9 percent.

Table 2. Yield obtained at V.R.D.S. Buzău in 2010-2015

Variety	Yield (t/ha)								STAS* of total yield	Early yield of total**
	Year						Average			
	2010	2011	2012	2013	2014	2015	t/ha	%	%	%
Rio Grande	34.8	25.6	24.5	62.9	75.2	73.2	49.4	100	93.6	8.3
Florina T	33.4	25.5	36.0	54.2	71.8	63.1	47.3	95.7	94.3	9.6
Florina R	33.5	21.5	32.4	81.0	93.5	53.9	52.6	106.5	93.1	6.9
Daria	31.2	22.7	51.6	71.8	108.9	63.1	58.2	117.8	93.0	8.1
Darsirius	31.8	22.3	37.2	74.5	76.4	75.6	53.0	107.3	93.3	7.2
Kristinica	30.9	25.2	36.7	68.0	71.3	77.5	51.6	104.5	93.4	7.9

* fruits with weight of over 33 g
**Yield obtained until 31st of July

DL	5%	10.3	20.9 %
DL	1%	14.0	28.3 %
DL	0,1%	18.7	37.8 %

The industry varieties were biochemical analysed and it was found that in the case of dry matter content, due to the pericarp density, Darsirius variety ranked the first place with 5.8%; dry soluble matter remains at the rate of 5% as in the case of the variety Kristinica (table no. 3). In the case of varieties of Florina T, R and r. Daria (Măriuca) an equal value of 4.5% was recorded. Also, Kristinica has registered a value of 0.43% acidity, at the opposite side being Darsirius with 0.35%. The highest content in sugar was recorded by Darsirius while Daria (Măriuca) has registered a rate of 2.44 percent.

Regarding the sugar : acidity ratio, Darsirius was first valued with 8.97%, followed by Florina R with 16%. The highest content in ascorbic acid was measured at Florina T followed by 11.97% at Darsirius with 9.58%. The highest content of lycopene 9.08% was recorded by Florina R.

Table 3. Biochemical analysis of tomatoes for industrialization

Parametre	Variety					Average values*
	Daria (Măriuca)	Kristinica	Florina T	Florina R	Darsirius	
d.m.c. %	5.18	5.26	5.35	5.30	5.8	5.98±0.83 (cv=14%)
s.m.c. %	4.5	5.00	4.5	4.50	5.00	4.75±0.35 (cv=7%)
Acidity (Citric acid), %	0.41	0.43	0.39	0.37	0.35	0.38±0.03 (cv=8%)
Sugar total,%	2.44	2.97	2.88	3.02	3.14	3.37±0.77 (cv=21%)
Ratio sugar: acidity	5.95	6.91	7.38	8.16	8.97	10.76±0.09 (cv=1%)
Ascorbic acid, mg/100 g^{-1}	8	7.02	11.97	8.30	9.58	9.67±0.65 (cv=7%)
Lycopene, mg/100 g^{-1}	6.5	5.00	8.18	9.08	6.00	8.00±1.5 (cv=19%)
Average weight,g± a.s.	93.5±22.1 (cv=24%)	108.9±13.5 (cv=12%)	139.9±29.1 (cv=21%)	167.9±26.0 (cv=15%)	86.9±13.3 (cv=15%)	92±10 (cv=11%)

* Viorica and Vipon varieties (RDIVFG Vidra)
d.m.c. – dry matter content
s.m.c. – soluble matter content

CONCLUSIONS

The researches were completed so far with the achievement of a germplasm resource at this species consisting of 1064 genotypes, both evaluated and computerized stored from which in the future will be able to obtain new

varieties. 5 new varieties of tomatoes for industrialization have been obtained and approved: Kristinica, Darsirius, Florina T and R and Daria (Măriuca), genotypes with distinct features that enrich the current industry tomatoes assortment.

All five varieties behaved positively to the both crop systems direct sowing and planting stock specifying that the plants were more vigorous in the direct sowing crop, but with 15-20 days later yield. Recorded yields and physical and chemical properties of fruits demonstrates that the objectives of the proposed research breeding program were reached.

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NEW GENOTYPES OF *LUFFA* SPP. OBTAINED AT V.R.D.S. BUZĂU

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Abstract

Luffa cylindrica belongs to the Cucurbitaceae family, being an annual, herbaceous plant, multiplied by seeds. It is a well-defined genre, alongside with the species L. acutangula, L echinata,, L. graveolens, with their origin along with L. cylindrica in the tropics. And the species l. operculata, l. quinquefida and l. astorii are originating in the neo-tropical zone. Of these, these two species originating in India, L. cylindrica and L. acutangula are most common. They have been acclimatized for starters in India and America, then expanded and grown on a large scale for their immature fruits used as vegetables. With the passage of time, the areas of usage have diversified greatly. In Romania, the most known species is Luffa cylindrica, plant introduced in our country after the 1960s, at V.R.D.S. Buzau by dr. ing. Marcela Iosifescu. Though it has been successfully acclimatized in our country, especially in the protected spaces, promoting this culture was quite slow. At present, the spaces occupied by this species are pretty small, insignificant. After 1996, researches regarding the Luffa species were taken over time by the Laboratory of Improvement, aiming the acclimatization of new species and obtaining new creations with distinct biological phenotypical expression. Along with Luffa cylindrica, a special attention was given to the acclimation of new species, of which Luffa acutangula was successfully acclimated. The research continued with the crossing of L. acutangula x L. cylindrica species. By this crossing was obtained an F1 hybrid with intermediate sized fruit and high density of the fibre. From the segregation of the F1 hybrid were obtained in 6 new families, lineage with distinct features and numerous intermediate forms have been removed in the process of improvement. Varieties of Luffa acutangula have very large fruit, in green have recorded an average of 119 cm, unlike Luffa cylindrica, which recorded an average of 56 cm. F1 hybrid has an average length of fruit length of 65 cm. New varieties derived from the crossing of species ranged from L1 to L5 with 91 cm and 48 cm, the smallest value. The researches were completed with the obtaining of new genotypes Luffa cylindrica and L. acutangula with distinct features.

Key words**:** *acclimation*, *hybrid, improvement, L. acutangula*, *L. cylindrica.*

INTRODUCTION

Luffa species have exhibited a special interest for V.R.D.S. Buzău, which is also the first institution in the country where *Luffa cylindrica* was introduced in breeding programs by Ph.D. Engineer Iosifescu Marcela, after 1960. First researches have been channeled towards testing products to combat certain pathogens and diseases in *Cucurbitaceae,* knowing that *Luffa* spp. shows a remarkable resistance within this family.

The *Cucurbitaceae* or vine crop family is a distinct family without any close relatives and includes many important vegetables such as cucumber, melon, watermelon, squash, and gourds.

Plants within *Cucurbitaceae* consist of 95 genera *(*Kousik et al., 2015)

Is a well-defined genre, alongside the species *L. acutangula*, *L. echinata*, *L. graveolens*, which have their origins along with *L. cylindrica* in the tropics. The species *L. operculata*, *L. quinquefida* and *L. astorii* are originating in the Neotropical. Of these, two native species, from India, *L. cylindrica* and *L. acutangula* are most common. They have been acclimatized for starters in India and America, then expanded and grown on a large scale for their immature fruits used as vegetables. In time, the areas of *Luffa* spp. usage have diversified greatly.

Although stages of acclimatization and improvement of the species accounted for a great success in our institution, it did not occupy until now significant areas in culture and no Romanian variety was patented. After 1996, researches focusing on *Luffa* species were resumed in an intensive system with the aim of obtaining new genotypes with precise directions for use and also the acclimation of new species, with an emphasis on *L. acutangula.*

Recent researches confirm that *Luffa* species are plants with multiple uses. The fruit contains triterpenoid saponins: lucyosides A, B, C, D, E, F, G, H, I, J, K, L, M, ginsenosides Re, Rg1, etc. The leaf contains triterpenoid saponins: lucyin A, lucyosides G, N, O, P, Q, R, 21β-hydroxyoleanoic acid, 3-O-β-D glucopyranosyl - maslinic acid ginsenosides Re, Rg1; flavonoids: apigenin, etc. The seed contains polypeptides: luffins P1, S, luffacylin etc. (Partap et al., 2012). As an entomophilies species, preferred by pollinating insects, it presents numerous genotypes.

Rich morphological variability occurs in cultivated species of *Luffa* in different growing regions. (Prakash et al., 2013).

At the present, the interest for the species of *Luffa* grew considerably in our country, motivating the initiation of further researches presented in this paper.

MATERIALS AND METHODS

The researches started with the achievement and the enrichment of a germplasm collection for this species. Three species were taken in study: *Luffa cylindrica*, *Luffa acutangula* and *Luffa operculata*. Within the species *Luffa cylindrica* we managed to achieve a large number of genotypes but have been selected for study 3 genotypes with distinct characteristics and stable in descent, two of them obtained at the V.R.D.S. Buzău; one of them has white seed, the other one has black seed and the third one is from Bulgaria. Within the species of *Luffa acutangula,* researches started with the species acclimation, because so far these varieties were not cultivated in Romania. The germplasm collection was established with a total of five distinct genotypes, but one who has demonstrated adaptability and stability to our soil and climatic conditions was the one originating from China, codenamed G2. Within the species *Luffa operculata,* until now, we haven't managed any genotype acclimation that presents adaptability and genetic stability.

The selection methods were the specific ones for cucurbits, and the stabilized families were subjected for hybridization followed by the segregation process. Special attention was paid to isolation areas due to its entomophilies degree. They were cultivated in different greenhouse compartments, to avoid contamination.

RESULTS AND DISCUSSIONS

Researches finalised with the achievement of a solid germplasm collection at this species.

From the *Luffa cylindrica* group, a new variety was achieved that presents distinct characteristics recommending it to be used as a vegetable sponge. The plant is vigorous, with a well developed root system that explores the deeper layers of soil.

In protected areas, the plant reaches the height of six up to eight meters. From the stem, eight-twelve main shoots with numerous secondary shoots develop, and have a capacity of dispersion of over six meters. The stem is vigorous, edged, slightly lignified at the base, with a medium diameter of 16 millimeters. (Fig. 1).

Figure 1. Stem

The plant has a rich foliar device, consisting of scattered leaves on shoots at a distance of 14-18 cm, with a leaf petiole ranging between 18-24 cm, and a diameter between 6-8 mm. Length of leaf varies between 20-30 cm and the width register values between 18-28 cm. (Fig.2.).

Figure 2. Plant and leaf detail

The leaf is composed of 5 lobes, the inferior ones being serrated. The shoots present tendrils to facilitate climbing on the trellising system (Fig.3).

Figure 3. Tendrils

The flowers are yellow, distinct on the plant; the male ones show a long petiole, which varies between 20-40 cm long, with an average diameter of 10 mm and the peduncle length of 1-1, 2 cm. The sepals are sharp and have an average length of 10 mm. The corolla diameter is of 8 cm, flowers are type-5, and number of flowers in florescence is 3-12. Female flowers are solitary and are distinguished by the presence of the miniature fruit at the base of the corolla. First make their appearance are male ones, that are far more numerous than females (Fig.4).

Figure 4. Male and female flower

The fruit is a green cylindrical ridged pepo, with the base slightly narrow than the apex. It has an inner dense network of cellulose fibre, a characteristic that gives it more quality in its use. The plant has a great capacity production, but in normal nutritional conditions it retains a number of 18-22 fruits per plant. (Fig. 5). The production can increase significantly if it interferes with special directing and topping of works for the unnecessary shoots and if is ensured an adequate level of nutrition per phenological phase.

Figure 5. Unripe fruit, dried fruit, sponge

Referring to the recent studied species, *L. acutangula*, the researches finalised with its acclimation, this being a first in our country, and with the achievement of a new variety with distinct characteristics. Regarding the biological specific features of this species, is similar with the *L. cylindrica,* bearing the mention that its fruit are likely to be consumed when fresh. Optimal size of fruit for consumption is of 20 - 40 cm. With this dimension, the fruit diameter at the base is of 6.5 cm, ant the apex diameter is of 7.2 cm. If are not harvested, the fruits lose their softness, spongy fibres appear inside and are unfit for human consumption. Such unharvested fruit can exceed 1 meter in length and as they mature, they turn from green to brown, lose significant weight and decrease greatly its size. (Fig. 6.)

Figure 6. Fruits at maturity consumption

Culture can be established through seedlings or direct sowing. In field conditions, the plant

behaves like a tardy plant, therefore it is recommended to be cultivated in the warmer areas of the country. Seedling production is carried out just like the rest of the cucurbits, being performed in alveolar palettes with 28 holes, in order to ensure an appropriate nutrition space. Sowing, for the production of seedlings is made in the first decade of February, and for the field in the first decade of March (Fig. 7).

Table.1. Main fruit characteristics

Variety name	Fruit length (cm)			Weight of the sponge (g)	Seed no./fruit	Seed weight/fruit (g)
	green	dried	sponge			
L.acutangula	119	98	93	41.7	534	64.1
L. cylindrica	56	47	44	26.3	312	37.4
Luffa CxA[1]	65	56	53	28.9	428	51.4
L1	91	82	77	57.8	444	53.3
L2	73	65	61	36.4	462	55.4
L3	63	52	58	22.2	512	61.5
L4	49	40	37	33.8	335	40.2
L5	48	36	32	5.5	279	33.5
L6	59	50	46	38.8	320	38.4

[1]*Luffa C x A– Luffa cylindrica* X *Luffa acutangula*

Figure 7. Seedlings

Planting in protected areas is carried out around April 1, while plantings in the field are made after 1 May. Seedling age should be 55-60 days. Where the establishment of culture is made by direct sowing, sowing in unheated protected areas is carried out after March 20 while in the field after April 20. (Fig.8).

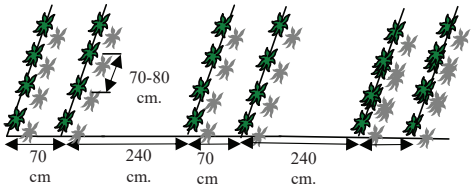

Figure 8. Crop on unshaped terrain establishment plan for protected areas

The species supports several technological variants. It can be established in equidistant rows or strips. It was found that the best results were obtained when the culture was established in strips - 70 cm between rows and 240 cm between bands and between plants on the row 70-80 cm depending on the vigor of cultivar.

After the stabilization and the achievement of the two varieties of *L. cylindrica* and *L. acutangula,* the researches continued with the hybrid combination execution between the selected genotypes, aiming to obtain valuable hybrid creations and the enrichment of thr autochthonous genetic heritage.

Although the research conducted worldwide indicate that the crossing between the species *L. cylindrica* and *L. acutangula* presents sterility in descendents, the hybrid combination achieved at V.R.D.S. Buzău had demonstrated compatibility and manifested a visible phenomenon of heterosis in F1, obtaining a hybrid with intermediate morphological features between the two species, a high yield production and a combined use destination. Young fruits can be used in nutrition and the mature ones are used as a vegetable sponge (Fig. 9).

L. cylindrica ♀ x *L. acutangula* ♂

↓

Hibrid F1

Figure 9. The hybridization plan

The research continued with the segregation of the hybrid made from the crossing of the two different species of *Luffa*. In F2 resulted 6 new genotypes with distinct features and numerous intermediate forms that have been removed in the process of breeding.

After intensive breeding works, these new genotypes were carefully selected and genetically stabilized in order to promote them in subsequent crops.

In what concerns the characteristics of the fruit in the green stage and as a sponger, the measurements showed significant differences between the studied genotypes (table 2).
Analyzing the fruit surface, G4 emphasized with the appearance of the protruding ribs on a smooth surface, unlike most genotypes that have exhibited a slightly rough and ribbed surface (Fig. 10).
Regarding the receptacle, the biggest diameter but also the greatest length was recorded by *L. acutangula,* having respectively 3,2 and 4,8 cm. Also the sponge color differs, having shades ranging from white, greenish white, yellowish and slightly brown.
In terms of sponge density, the genotypes with a small and medium density and by default a rare network are for fresh consumption, while those with high density cellulose fiber, and are intended for use as a vegetable sponge.

Figure 10. Types of surfaces and fibers of the fruit

A dominant character was the black colour of the seeds which was transmitted in lineage for most of the genotypes, an exception being made only by *L. cylindrica* that has white seeds (Fig. 11).

Figure 11. Black and white seeds

Observations were made regarding the fruit diameter in three stages: unripe, dried and as a sponge; the significant differences between the three stages were mainly due to dehydration of the fruit. (fig. 12).

Figure 12. Dehydrated fruits and cellulose fiber sponge

Table 2. Fruit characteristics

Variety name	Exterior color of the fruit	Fruit surface		Floral receptacle		Sponge colour	Sponge density	Seed colour
				Diameter (cm)	Length (cm)			
Luffa acutangula	Green	ribbed	Rough	3.2	4.8	Yellowish	Low	Black
Luffa cylindrica	Green	Slightly ribbed	Smooth	2.1	3.7	White	High	White
Luffa CxA	Green	Slightly ribbed	Slightly rough	2.7	4.1	Slightly brown	High	Black
G1	Green	Slightly ribbed	Rough	1.1	3.1	Slightly brown	Medium	Black
G 2	Green	Ribbed	Slightly rough	1.1	2.2	Greenish-white	High	Black
G 3	Light green	Ribbed	Smooth	1.4	4.1	Yellowish	High	Black
G 4	Light green	Heavily ribbed	Smooth	1.8	3.5	White	Low	Black
G 5	Light green	Ribbed	Smooth	0.8	2.8	White	Low	Black
G 6	Light green	Slightly ribbed	Slightly rough	2.5	4.2	Greenish-white	High	Black

Thereby, the upper part of the unripe fruit, the point of grip of the fruit from the stalk, has registered the largest diameter in the case of G2 with 6.6 cm reaching the stage of sponge to 5.1 cm. The greatest difference between the unripe fruit base diameter and the base diameter of sponge was recorded by G1 and G3, with a decreasing diameter of 1.7 cm from one stage to another.

In terms of the middle part of the fruit, the highest diameter value was registered at G2 with 9.9 cm for the unripe fruit and at the opposite boundary is G1 with 5.9 cm as a sponge. Regarding the apex diameter, it was found that G2 registered a maximal value of 11.5 cm as an unripe fruit and a minimal value of 6.7 cm as a dried pepo (Table no. 3).

Table 3. Unripe, dried and sponge fruit diameter

Variety name	Base (cm)			Middle (cm)			Apex (cm)		
	Unripe	Dried	Sponge	Unripe	Dried	Sponge	Unripe	Dried	Sponge
Luffa acutangula	6.1	5.5	4.9	8.9	8.4	7.8	10.8	10.2	8.7
Luffa cylindrica	5.5	4.6	4.0	8.3	7.8	7.1	8.9	8.3	7.6
Luffa CxA	6.3	5.6	4.7	9.5	8.9	8.4	10.9	10.1	9.3
G 1	5.6	4.7	3.9	7.1	6.6	5.9	9.4	8.6	7.9
G 2	6.6	5.8	5.1	9.9	9.6	8.8	11.5	10.8	10.1
G 3	5.6	4.5	3.9	8.9	8.2	7.7	9.7	8.9	8.3
G 4	5.3	4.5	4.0	9.6	9.0	8.4	10.4	9.5	8.9
G 5	3.7	2.8	2.2	7.8	7.0	6.5	7.5	6.7	6.8
G 6	6.3	5.4	4.8	9.0	8.5	7.8	11.1	10.4	9.8

CONCLUSIONS

At the present, researches finalised with the achievement of a solid germplasm collection both for *L. cylindrica* and *L. acutangula*.

For *L. acutangula*, we managed its acclimation, breeding and drafting of the crop technology.

Two new varieties were achieved with distinct destination use, *L. cylindrica* as a vegetable sponge and *L. acutangula* for fresh consumption.

A new hybrid was obtained from *L. cylindrica* X *L. acutangula* crossing, a hybrid that manifests the heterosis phenomenon with a high yield production, strength, genetic resistance at the main pathogens and a mixed use destination. The unripe fruits can be used in nutrition just like zucchini, and when they mature can be used as vegetable sponges because of the high density fibre, volume and their large water retention capacity.

After the achieved hybrid segregated, six new distinct genotypes were obtained, three of which can be used for producing vegetable sponges and three are for fresh consumption.

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THE BEHAVIOR OF SWEET POTATO *(IPOMOEA BATATAS)* IN TERMS PSAMOSOILS IN SOUTHERN ROMANIA

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Abstract

Variability of climate, especially lack of rain and low fertility psamosoils determines that surfaces quite stretched in most crop yields to be much reduced. In this context, the choice assortment of plants and varieties with high adaptability to the harsh conditions of climate and soil is a necessity for obtaining high yields, stable and reliable, that provide increased energy needs of the population food. Research conducted during 2013-2015 at the Research - Development Center for Agricultural Plants on Sands, Dabuleni, highlights a favorable microclimate for plant growth and development of sweet potato (Ipomoea batatas). The values recorded physiological indices sweet potato grown in climatic conditions in the sandy soils of southern Oltenia showed that it easily adapts to the conditions of excess heat here is a plant heat-loving and light. Productions made from sweet potato varieties Pumpkin (KSP1) and Chestnut (KSC 1), sandy soil conditions studied ranged 17428kg / ha and 35467 kg / ha depending on the crop and cultivated variety. We showed correlations between tuber production and the amount of degrees of temperature recorded in air (r = 0.904; r = 0.992). Also between rainfall and production carried out by two varieties of sweet potato is negative correlations (r = -0.642; r = - 0.848). These correlations highlight the specificity of the plant to dry climate. Nutritional quality presented values differentiated to the two varieties studied, according to the year of culture.

Key words*: sweet potato, tolerant, drought, physiology, quality*

INTRODUCTION

Sweet potato (*Ipomoea batatas*) belongs to the family *Convolvulaceae* and is native to Central America and the north-west of South America. Globally, is among the food crops the most important in the world after wheat, rice, corn, potato Irish and barley, being adaptable to climate tropical and subtropical zones, drought-tolerant and grows in conditions of fertility and soil pH decreased (Kareem I., 2013). It is a plant well adapted to tropical and subtropical climates, but can grow successfully in a wide range of climatic conditions in the cold season average to not more than 5 months. Research by James A. Duke 1983 plant underlines the sensitivity at low temperatures, frost tolerating plant (www.ncsweetpotatoes.com). It grows best at an average temperature of 24°C, with abundant sunshine and warm nights. Annual rainfall of 750-1000 mm are considered most appropriate, with a minimum of 500 mm in the growing season. Culture is sensitive to drought tuber initiation stage, 50-60 days after planting and is not tolerant to water stagnation, it can cause tuber rots and reduce root growth due to poor aeration. Abundant in nutrients and fiber (of which 40% soluble fibre that helps lower the blood sugar and cholesterol), sweet potato is the ideal food for diabetics, children and pregnant women (Betty J. Burri, 2011 Mihaela Cioloca et al., 2013). Through its qualities, a sweet potato variety with yellow and orange pulp is a valuable source of vitamin A and vitamin B6. Also, sweet potato provides a significant amount of vitamin C and vitamin D, essential in the formation of bones and teeth, for good digestion, wound healing and immune system. Sweet potato also contains iron, which helps metabolize protein, and magnesium, a mineral stress. Concerns for sweet potato cultivation in Romania took place in USAMV Bucharest (Ciofu R., 2005, Musat C., 2010) and were resulting in the creation of two varieties (Crux V., 1991, 1997). The variety of sweet potato varieties grown in Romania is quite limited and they are cultivated more experimental. Orange and yellow varieties have

a high content of beta-carotene, the precursor of vitamin A (Kareem I., 2013). Therefore encourages the cultivation of these species in places like Africa where vitamin deficiency is causing severe health problems (Wariboko C. et al., 2014, Ladokum OA, et al., 2007). All species of sweet potatoes are rich in antioxidants. Sweet potato, although it has a sweetish taste, the presence of complex carbohydrates, which help regulate carbohydrate and reduced insulin resistance, is beneficial for people with diabetes. Based on these considerations nutrients, Center for Science in the Public Interest of America awarded the highest score compared to other vegetables. They also have a low glycemic index, which means that hunger will appear later. Research - Development Center for Agricultural Plants on Sands, Dabuleni began in 2013 a collaboration with the Institute for Agricultural Technology and Science Kyungpook National University, based in Daegu, represented by Prof. dr. Jong - and Dr. Sang KIM Gi Cho Eun-, South Korea, under "Technical and Scientific Cooperation Memorandum between Kyungpook National University ASAS Bucharest and the Institute for Agricultural Science and Technology. Within this collaboration was initiated research on the behavior of two varieties of sweet potato (Ipomoea batatas): Pumpkin (KSP1) and Chestnut (KSC1) in terms of climate and soil from RDCAPS Dabuleni, Dolj County.

MATERIALS AND METHODS

Research at the culture of sweet potato (Ipomoea batatas), were conducted in 2013-2015 at RDCAPS Dabuleni on sandy soil with low natural fertility (0.42 to 0.82% humus) and pH (H_2O) = 5.9 to 6.9 in the Bilateral Cooperation Protocol between Kyungpook National University (KNU) in South Korea and the Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu sisesti" Bucharest. It studied the behavior of two varieties Korean: Pumpkin (KSP1) and Chestnut (KSC1) in terms of climate and soil in the sandy soils of southern Oltenia. Seedling product to the protected system type solar greenhouses as follows:
-between 20 to 25 March were planted sweet potatoes from seed in the greenhouse (Figure 1)

Figure 1. Planting sweet potato tubers in greenhouse

-between March and April are maintained in the greenhouse through irrigation and ventilation
After cutting the shoots, fertilize the solar N50 and watered with water necessary to obtain another generation of shoots (Figure 2)

Figure 2. Cutting the shoots in solar

-from May 5 to 10 they were cut shoots produced in solar and were planted in the field billon covered with polyethylene mulch. (Figure 3).

Figure 3. Planting the sweet potao shoots in field

In field was fertilized with $N_{80}P_{80}K_{80}$, to prepare the ground and in the vegetation was fertilized with N_{70}.Sweet potato tubers grown in two varieties were received from the Republic of Korea in accordance with the cooperation agreement. During the growing season, in root tubers stage was determined the photosynthesis and transpiration rate of plant to

leaf level with + Portable Photosynthesis System LCpro device in three times of the day. At harvest the production of tubers has been determined and the quality of production as follows: total dry substance and water (%) - gravimetric method; simple soluble carbohydrates (%) - Soxleth Reagent method; the starch (%) - colorimetric method; C vitamin (mg/100 g f.s.) - iodometric method.

RESULTS AND DISCUSSIONS

Climatic conditions during the period 2013-2015 reveals an increase in atmospheric drought, revealing the sweet potato vegetation period mean monthly temperature higher by approx. 0.15 to 1.45℃, compared to the annual average (Table 1). Although the average amount of rainfall recorded during the study period was 372.3 mm, with 102.92 mm above the annual average, they were unevenly distributed and water to meet the needs of the work was drip irrigation necessary to ensure the ceiling of 80% of active moisture range. In terms of ensuring the thermal requirements for growing sweet potato, 2015 it was the most favorable, followed by 2013 and then 2014. The average air temperature during the growing season (May to September) was between 20.48 -21.78 ℃ and rainfall were within 269.2 to 516.9 mm. 2014 was unfavorable for sweet potato and due to heavy rainfall that occurred in September, which coincided with the maturation period tubers, preventing dry substance accumulation and weight gain formed tubers per plant. Also, the average temperature in 2014 was lower by 1.02- 1.30 ℃, the values recorded in the other two years, which led to unevenness maturing tubers and finally to production results low, sweet potato is a heat-loving plant. To mature, sweet potato varieties needed about 3133.44 to 3332.34 ℃, built up during the growing season.

Table 1. Climate characterization of sweet potato vegetation period (2013-2015 Dabuleni)

Month	Climatic element	2013 Year	2014 Year	2015 Year	Multiannual average
May	Air temperature average ^{0}C	20	16.6	19.2	16.8
	Rainfall average (mm)	61	117.4	52.4	61.6
	Relative humidity %	71	76.5	73	
June	Air temperature average ^{0}C	22.1	20.7	20.5	21.6
	Rainfall average (mm)	105.2	92	134.2	68.5
	Relative humidity %	76.8	76.9	73.8	
July	Air temperature average ^{0}C	23.5	23.1	24.9	23.1
	Rainfall average (mm)	36.2	125.6	11.0	54.2
	Relative humidity %	73.7	77.9	62.9	
August	Air temperature average ^{0}C	24.1	23.7	24.3	22.4
	Rainfall average (mm)	30.8	16	48.4	37.7
	Relative humidity %	68	72	68.2	
September	Air temperature average ^{0}C	17.8	18.3	20.1	17.75
	Rainfall average (mm)	36	165.9	84.8	47.38
	Relative humidity %	72.5	82,3	77.3	
Air temperature average during the growing sweet potato 01.05– 30.09 (^{0}C)		21.5	20.48	21.78	20.33
Rainfall average during the growing sweet potato 01.05– 30.09 (mm)		269.2	516.9	330.8	269.38
Relative humidity average during the growing sweet potato 01.05– 30.09 (%)		72.4	77.12	71,04	-
$\sum$ ^{0}C in air during the growing sweet potato 01.05– 30.09		3292.6	3133.44	3332.34	3110.49
Rainfall (mm) during the growing sweet potato 01.05– 30.09		269.2	516.9	330.8	269.38
Production of sweet potato tubers (kg/ha)	Sweet potato variety / Year	2013	2014	2015	Average
	Pumpkin (KSP1)	23864	17428	33300	24864
	Chestnut (KSC1)	30176	18857	35467	28166

Results on physiological reactions to sweet potato depending on climatic factors:

During the growing season, in root tubers stage was determined the photosynthesis and transpiration rate of plant to leaf level with + Portable Photosynthesis System LCpro device in three times of the day (Figure 4). Figure 5 shows the diurnal variation of photosynthesis (micromoles CO_2 / m^2 / s) - determined the two varieties of sweet potatoes planted Pumpkin (KSP1) and Chesnut (KSC1) depending on the climatic conditions in the crop year 2014 -2015.

In 2014 the variety Pumpkin (KSP1) when determining the 15 o'clock when the daytime temperature reaches the highest values (38 -40 ℃), photosynthesis rate recorded 12,555 micromoles CO_2 / m^2 / s and the variety Chestnut (KSC1) at the same time of day photosynthesis rate was 18,975 micromoles CO_2 / m^2 / s. 2015 variety pumpkin (KSP1) when determining the 15 o'clock rate of photosynthesis recorded 21 715 micromoles CO_2 / m^2 / s and the variety Chestnut (KSC1) at the same time of day rate of photosynthesis was 27 175 micromoles CO_2 / m^2 / s. Every year the production was positively correlated with the amount of diurnal variation of photosynthesis.

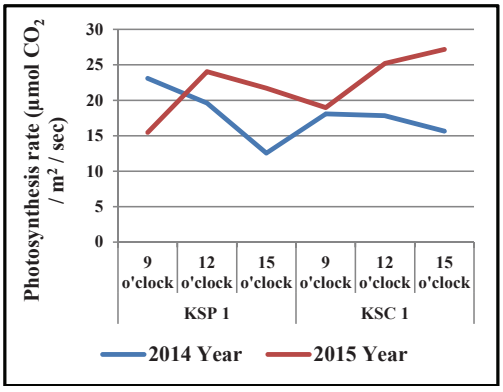

Figure 4. Diurnal variation of photosynthesis sweet potato plant, depending on the variety and the crop year

Figure no. 6 shows the diurnal variation of photosynthesis in the two sweet potato varieties in the 3 times of day in 2014 and 2015. In 2014 when the amount of degrees the temperature was lower than the annual average and exceeded the annual average rainfall amount, variety Pumpkin (KSP1) recorded higher values of photosynthesis diurnal variation in measurements performed at 9:12 and at 15, the variety Chestnut (KSC1) achieved the highest value of diurnal variation of photosynthesis. Climatic conditions during the vegetation period of 2015 influenced the different speed and pacing of physiological processes to sweet potato grown in the sands. In July, air temperature varied between 26.7 to 41.5 ℃ determinations when air humidity fell below 25% and photosynthetic active radiation varies between 1200 to 1700 mol / m2 / s. In 2015 when the amount of degrees the temperature was higher than the annual average, and the amount of rainfall was lower than the annual average, the variety Chestnut (KSC1) recorded higher values of diurnal variation of photosynthesis in tests carried out in all 3 points of the day. This indicates that the variety Chestnut (KSC1) has the capacity to behave better in drought conditions than the variety Pumpkin (KSP1).

Diurnal variation of the sweet potato leaf transpiration depending on the variety and the crop

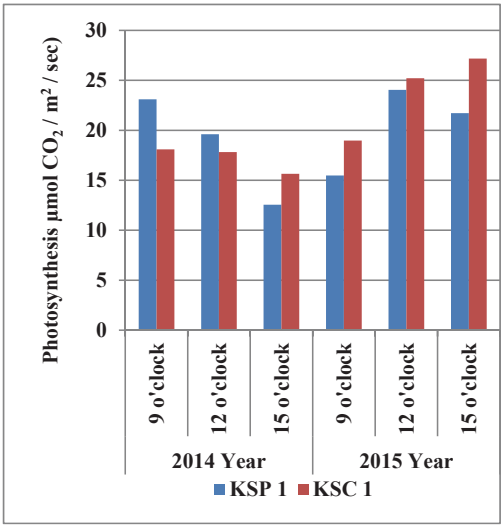

Figure 5. Diurnal variation of photosynthesis sweet potato plant, depending on the crop year and variety

Figure no. 7 presents diurnal variation of leaf transpiration (mmol H_2O / m^2 / s) - determined the two sweet potato varieties planted, Pumpkin (KSP1) and Chesnut (KSC1) depending on the climatic conditions

in culture in 2014 -2015. In 2014 the variety Pumpkin (KSP1), diurnal variation of leaf transpiration (mmol H_2O / m^2 / s) when determining from 9 was 3.32 mmol H_2O / m^2 / s at 12 was 3.44 mmol H_2O / m^2 / s and at 15, when daytime temperatures reach the highest values, diurnal variation of leaf transpiration recorded 2.3 mmol H_2O / m2 / s. Variety Chestnut (KSC1) diurnal variation of transpiration leaf (mmol H_2O / m^2 / s) when determining from 9 was 2.795 mmol H2O / m2 / s at 12 was 2.975 mmol H_2O / m^2 / s and at 15 pm, when daytime temperatures reach the highest values, diurnal variation of leaf transpiration of 4.46 mmol H_2O / m^2 / s.

In 2015 Year, Pumpkin variety (KSP1), diurnal variation of leaf transpiration (mmol H_2O / m^2 / s) when determining from 9 was 1.97 mmol H_2O / m^2 / s at 12 was 7.01 mmol H_2O / m^2 / s, and at 15, when daytime temperatures reach the highest values, diurnal variation of transpiration foliar registered 9.705 mmol H_2O / m^2 / s the variety Chestnut (KSC1) diurnal variation of transpiration leaf (mmol H_2O / m^2 / s) when determining from 9 was 2.58 mmol H_2O / m^2 / s at 12 was 7.11 mmol H_2O / m^2 / s and at 15, when daytime temperatures reach the highest values, diurnal variation of leaf transpiration recorded 9.93 (mmol H_2O / m^2 / s).

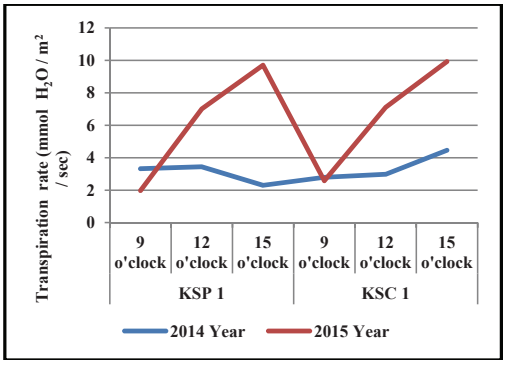

Figure 6. Diurnal variation of transpiration sweet potato plant, depending on the variety and the crop year

In 2014 when the amount of degrees the temperature was lower than the annual average and the amount of rainfall exceeded the annual average, the variety Pumpkin (KSP1) recorded higher values of diurnal variation of leaf transpiration tests carried out at 9:12 and at 15, variety Chestnut (KSC1) achieved the highest value of diurnal variation of leaf tanspiration. In 2015 when the amount of degrees the temperature was higher than the annual average and the amount of rainfall was lower than the annual average, the variety Chestnut (KSC1) recorded higher values of diurnal variation of leaf transpiration tests carried out in all 3 points of the day. This indicates that the variety Chestnut (KSC1) has the capacity to behave better in drought conditions than the variety Pumpkin (KSP1). Climatic factors have intensified leaf transpiration which recorded high values ranging from 1.97 to 9.7 mmol H_2O / m^2 / s variety Pumpkin (KSP1) and values between 2.58 to 9.93 mmol H_2O / m^2 / s variety Chestnut (KSC1). Both varieties to leaf transpiration maximum intensity registered between 12 to 15 hours and action when stress factors (drought atmospheric and pedological drought) was maximum.

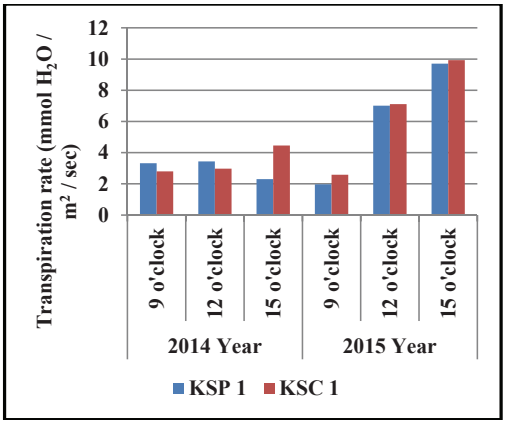

Figure 7. Diurnal variation of transpiration sweet potato plant, depending on the crop year and variety

The climatic conditions of the three years of study have influenced both the production of sweet potato and their nutritional quality (Table 2). The best production results were obtained in terms of 2015, the variety Chestnut (KSC 1) 35467kg / ha). The lowest production was obtained in the climatic conditions of 2014 (high rainfall and temperature below the annual average) (17428kg / ha variety Pumpkin (KSP1) and 18857kg / ha variety Chestnut (KSC 1).

Nutritional quality presented values differentiated the two varieties studied climate conditions of the three years.

The content of total solids of higher values under the years 2014 and 2015 (38.50% variety Pumpkin and 39.93% for the variety Chestnut), when rainfall was higher than 2013.

If the soil temperature is high dry substance can be lost through excessive breathing. In a warm season but soil moisture, dry substance will remain high due to the reduction in the intensity of process breathing.

The amount of soluble carbohydrates and vitamin C to higher values also in terms of 2014 (10.12% carbohydrate variety Pumpkin and 16.72 mg / 100g fresh substance, vitamin C variety Chestnut).

The starch content presented similar values in the years 2014 and 2015.

Figure 8. Determinations of plant physiology sweet potato

Table 2. Results on production of sweet potato tubers and their biochemical composition, depending on the variety and the crop year

Variety	Year	Tuber production (Kg/ha)	Total dry matter (%)	Water (%)	Soluble carbohydrates (%)	Starch (%)	C vitamin (mg / 100g fresh substance)
Pumpkin (KSP 1)	2013	23864	37.44	62.56	8.38	-	10.56
	2014	17428	38.50	61.50	10.12	12.70	11.44
	2015	33300	38.41	61.59	8.33	12.55	7.99
Chestnut (KSC 1)	2013	30176	35.44	64.56	7.97	-	13.20
	2014	18857	36.55	66.95	10.04	12.60	16.72
	2015	35467	38.93	61.07	8.09	12.69	10.56

The results obtained in the production of sweet potato tubers during 2013-2015, highlights a positive correlation with the amount of degrees of temperature recorded during the growing season (May-September) (Figure 9). This function connexion is more closely to KSC1, which is delayed compared to the variety Pumpkin 5-7 days, production is greatly influenced by climatic conditions during baking.

Production recorded in both varieties was negatively correlated with the amount of rainfall recorded during the growing of the plant (Figure 10). The climatic conditions in 2014, particularly abundant rainfall, were unfavorable to plant productivity, due to the growth of lush foliage at the expense of root tubers process;

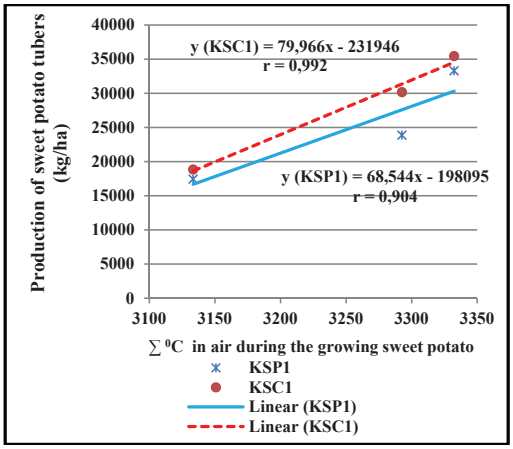

Figure 9. Relationship between production of tubers and the amount of degrees of temperature recorded during the growing season of sweet potato

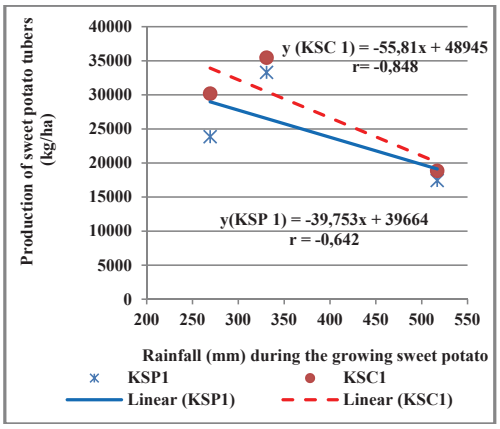

Figure 10. The relationship between production of tubers and rainfall recorded during the growing sweet potato

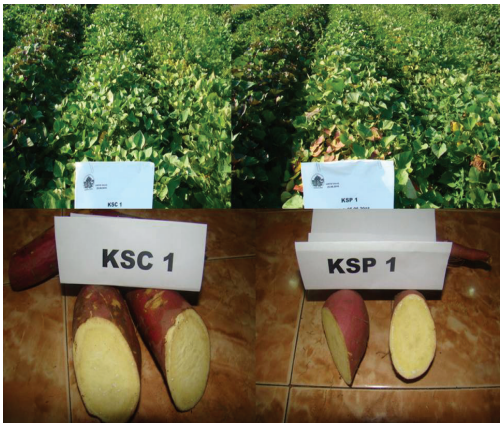

Figure 11. Sweet potato in the vegetation phase of and phase of tubers

CONCLUSIONS

Research conducted during 2013-2015 at the Research - Development Center for Agricultural Plants on Sands, Dabuleni, highlights a favorable microclimate for plant growth and development of sweet potato (*Ipomoea batatas*).

The values recorded physiological indices sweet potato grown in climatic conditions in the sandy soils of southern Oltenia showed that it easily adapts to the conditions of excess heat here is a plant heat-loving and light. Noting that the rate of photosynthesis in this species remains high throughout the day compared to other species (watermelons, peppers, eggplant), where the rate of photosynthesis decreases sharply from 12 to 15 hours under direct stress factors.

Production made sweet potato varieties Pumpkin (KSP 1) and Chestnut (KSC 1), studied under sandy soils ranged 17428kg / ha and 35467 kg / ha depending on the crop and cultivated variety.

Production of sweet potato tubers obtained from the period 2013-2015, is positively correlated with the amount of degrees of temperature and negatively with rainfall recorded during the growing season (May-September).

Nutritional quality presented values differentiated to the two varieties studied, according to the year of culture.

Given the level of production that was achieved in sweet potato grown in the period 2013 - 2015 is necessary to continue research on introduction in culture in the sandy soils of Romania sweet potato (Ipomoea batatas).

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RESEARCH ON THE PREPARATION A PROTOCOL OF THE DIRECT ORGANOGENESIS THROUGH *"IN VITRO"* CULTURE TECHNIQUES TO *HELIANTHUS TUBEROSUS* L. (JERUSALEM ARTICHOKE)

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Abstract

In the context of the current social and economic effects of the global crisis, it manifests itself with greater intensity and in one of the most important sectors of the Romanian economy - agriculture. Modern technique has transformed farming from a craft mastered in practice daily in a complex science that allows routing of the factors of vegetation and the transition to industrial agriculture. In this paper there will be presented arguments that underline the use of Jerusalem Artichoke (Sun root). Based on general principles of the techniques of "in vitro" culture, whose applicability was verified in the case of most species, our research aims to establish the optimal conditions for developing crops with pronounced morphogenetic capacity. The planting material at tuberous roots of artichoke was studied with greater efficiency by biotechnological methods. The working protocol was developed as a result of research undertaken on apexes "in vitro" cultivation taken from sprouts obtained by forcing the tubers in cold weather to grow shoots in the presence of additional fitohormones (BAP, K, NAA). These tubers were used to fulfill micropropagation requirements through an efficient growth "in vitro" that can succesfully be adapted to improved micropropagation for the varieties of Jerusalem artichoke.

Key words: *organogenesis, „in vitro" culture protocol, Jerusalem artichoke.*

INTRODUCTION

In Romania, Jerusalem artichokes is known under different names: kale, Jerusalem artichoke, carrots earth in Oltenia (SV part of Romania part), apple earth, in areas Brasov and Fagaras, potato crow in northern Transylvania. In Romania it was introduced over 200 years ago, but it was never expanded in culture. It is grown on small areas close to farms or livestock farms being used in pig feed as a steal. Jerusalem artichokes used in feeding these animals contribute to weight gain and changing the meat-fat percentage in favor of meat. Artichoke Romanian market is not developed to the extent properties of this plant. Due to its large strains, which in some varieties exceed 3 meters in height, it can be successfully used to create protection curtains for sensitive crops against cold winds or to prevent moisture evaporation from the soil. Strains can also be used to produce pellets for heating (***MADR, Romania, 2014). In home country (Mexico), Jerusalem artichokes used to be the staple food of the ancient Indians, and in Europe it was introduced at the beginning of the sixteenth century. It was first used in France during the Second World War, as the fermentation substrate for the production of alcohol as fuel.

Helianthus tuberosus perennial herbaceous plant belonging to the family *Asteraceae* and is cultivated as a vegetable for its tubers (Baillarge, 1942, Seiler 1993, Ciofu, 2004). *Astaraceae* family *(Compositae*) is one of the largest family dicotyledonous (approximately 20,000 species, of which 320 with high economic importance including power plants, industrial utilization of medicinal, decorative and honey plants. Type 5 clustering in widened or raised inflorescence (calatidiu) is a main pattern. Dried fruits are achenes (Axinte et al., 2006).

Jerusalem artichoke tubers contain an amount of up to 20% dry matter, which is found in abundance in a polymer of fructose called

inulin. May contain iron, calcium, magnesium, manganese, potassium, sodium, silicon, zinc, proteins, pectins, amino acids, vitamins B1, B2 and C. The stem and the leaves in the composition tryptophan, leucine, and beta-carotene (provitamin A).

Coming into being Jerusalem artichoke as a source for inulin (Gibson et al., 1995, Tassoni et al., 2010), a fructose polymer that may provide dietary health benefits for obesity, diabetes, and several other health issues (Gallaher and Schneeman, 1996). Inulin is a polysaccharide unique natural, with 95% fructose. Apart from artichoke inulin in the chicory roots is, dandelion, and other plants, but in smaller quantities (Monti et al., 2005, Serafini, 2010).

The possible use of the crop for biofuels is drawing tremendous recent interest (Hergert, 1991, Kays and Nottingham, 2008a, Diederichsen, 2010). With its ready cultivation and minimal pest and disease problems (Cassells and Walsh, 1995), Jerusalem artichoke is an underutilized resource that possesses the potential to meet major health and energy challenges (Kays and Nottingham, 2008a, 2008b, 2008c).

In vitro propagated techniques were used for propagating plants *Helianthus tuberosus*, explants of leaves was used in experiments to induce somatic embryogenesis (El Mostafa, 2006, Alla et al., 2014) and also in other research initiated on the formation of tubers of Jerusalem artichoke, using fragments of stems (Wissmann and Tripathi, 1977, Gamburg et al., 1999) in order to increase efficiency in obtaining biological material for planting. Taha et al., (2006) applied *"in vitro"* propagated techniques on Jerusalem artichoke (*Helianthus tuberosus*) for enhancement of inulin production. Preservation methods also are evalueted to facilitate the back-up of field collections of clonally propagated *H.tuberosus* (Volk, 2006, Diederichsen A., 2010, Alla et al., 2014).

The purpose of this study was the development of protocols for obtaining morphogenetic *Helianthus tuberosus* cultures and to assess the efficiency of multiplication obtained by using somatic explants taken from the tubers.

MATERIALS AND METHODS

Plant material

Tuberous root of the Jerusalem artichoke, which are the vegetative parts, spare and organs used for propagation of the plant presents adventitious buds that can be induced in a controlled manner as to produce sprouts. This is a major pattern to obtain the planting material in areas with cold seasons.

The tuberous roots used in this study to produce sprouts in order to initiate "*in vitro*" cultures, were not characterized in terms to belonging to a particular variety, it was chosen one depending on the periderm and pulp color phenotype harvested from local population plants grown in Cernatesti village near Buzau town, (located in the E region of Romania country). The Jerusalem artichoke tubers roots used to obtain shoot in aseptical conditions had purple and white periderm color, but in both cases the pulp was white (Figure 1).

Figure 1. Aspect of tuberous roots artichoke crops (local population from Cernăteşti village) with purple and white periderm color, used as a source of biological material for the initiation of *"in vitro"* cultures.

The roots tuberous had medium size, were placed on rockwool (Grodan type) moistened and kept in laboratory conditions at about 25°C and moderate atmospheric humidity for 3 weeks long. This time was necessary so that the level adventitious buds would be in the state of physiological rest to begin the shoot elongation. Therefore, depending on the size of the tuberous root after thermal pretreatment there were formed between 10-30 adventitious buds/1 piece.

Aseptic protocol for culture initiation

Tuberous roots were first cleaned by immersion for 8 hours in a dilute solution prepared from concentrated dishwashing detergent with tap water rinse followed by stirring in a stream supported by tap water for 20 minutes. Each root was cut crossectionally and then in quarters (Figure 2a). In order to sterilize the area under aseptic conditions fragments of Jerusalem artichoke as inocula were surface sterilized first of all with ethyl alcohol solution of 70% concentration (1 minut imersion) and after one rinsing in sterile distilled water (5 minutes) we continues the sterilization with another product represented by Na hypochlorite solution (under the form of the commercial product, Hey) 10% (w/v) concentration for 20 minutes. The explants were triple-washed in sterile water (3 times for 10 minutes each), dried on filter paper and after that was made the inoculation of the explant. In a first stage was carried out in culture vessels type Petri dishes of 10 cm diameter insulated with Parafilm tape (Figure 2b) and later, with the development of cultures, they were transferred (at the time of passages) in vessels with higher capacity (100 ml Erlenmeyer vessels).

Various combinations of induction media were used, all based on Murashige and Skoog (MS) medium, supplemented with 30.0 g/l sucrose, 7.5 g/l Difco Bacto Agar (Sigma) and different concentrations of phytohormones (0.01 mg/l) of naphthaleneacetic acid and 2 mg/l for cytokinins: kinetin (K) (6 furfuryl-aminopurine) and BAP (6-benzylaminopurine) in comparison with Control variant without added hormones in composition of MS (1962) basal medium (Table1).

a) b)

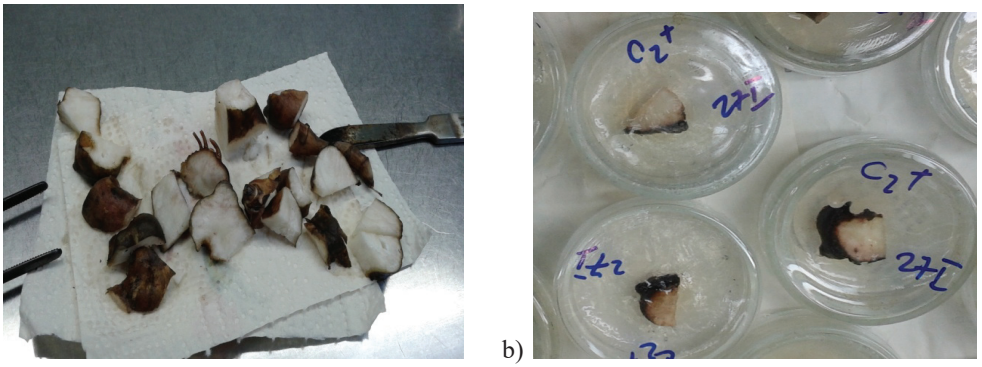

Figure 2**.** Appearance artichoke tuberous roots fragments used as a source of biological material before (a) and after inoculation (b) inoculare

Table 1. Experimental variants used in recipes to initiate artichoke organogenesis

Variants	The basal culture medium and hormonal balance	Other component
Control	MS (1962)	7.5 g./l agar+ 30 gr/l sucrose
C2	MS (1962) +2.0 mg/lBAP+0.01 mg./l NAA	7.5 g./l agar+ 30 gr/l sucrose
C 3	MS (1962) +2.0 mg/l K+0.01 mg./l NAA	7.5 g./l agar+ 30 g./l sucrose

Legend: MS – *Murashige&Schoog(1962);* BAP - 6- benzylamino purine*;*
NAA – *1-Naphthaleneacetic acid;* K- chinetine

All the explants were cultivated under illumination (16 h light/8 h dark) with light intensity of 110 µmol/m^{-2}.s^{-1} (ROMLUX fluorescent lamps,LFA 40W daylight-230V).
Explants transfers on fresh media with the same hormonal balance or medium other variants was performed observing aseptic conditions under the same conditions with sub-culturing period of 21 days.

RESULTS AND DISCUSSIONS

Observations made in the multiplication phase.
Inoculation of explants consisted of inoculating the culture medium variants selected for experimentation (Table 1), distributed into the culture dish, operations being performed in a sterile laminar flow hood (Activa HF Miniflo 120 model) by biotechnological methods developed on following standard operating plant requirements for explant cultures "*in vitro*" (Cachiţă-Cosma, 2007), with some adaptations to the specific conditions of the Laboratory of Plant biotechnology at the Faculty of Biotechnology (UASVM from Bucharest) described in the previous chapter Material and methods.
Subcultivation cultures initiated by us were made at intervals of 21 days on the variant culture medium used to originate (Variant C2), as it has proven effective in both stimulating the development of shoots multiples and in terms of their elongation. On the occasion of each subcultivations shoots elongated at longer dimensions than 3-4 cm were detached and used to manufacture new meristematic explants apexes and uninodal fragments (Figure 3). Also there were inoculated 3 series of explants on each variant / recipe used for fragments of the Jerusalem artichoke tubers. These three series are considered repetitions of used variants, the following results presented in the Table 2 represent the average of this assembly line. Inoculation of explants from all three series / variant culture medium was carried out simultaneously with Control date (in 3 repetition).
Inoculated plants reaction to the different hormonal combinations of cytokinins and auxins added to the culture medium Murashige Skoog (1962) was shown in the first three weeks of the initiation of culture through a hypertrophy of the explants for the Jerusalem artichoke tubers fragments. and poor axillary bud elongation in both variants C2 and C3 culture used. Slight differences were highlighted as compared to their control samples (Table 2).

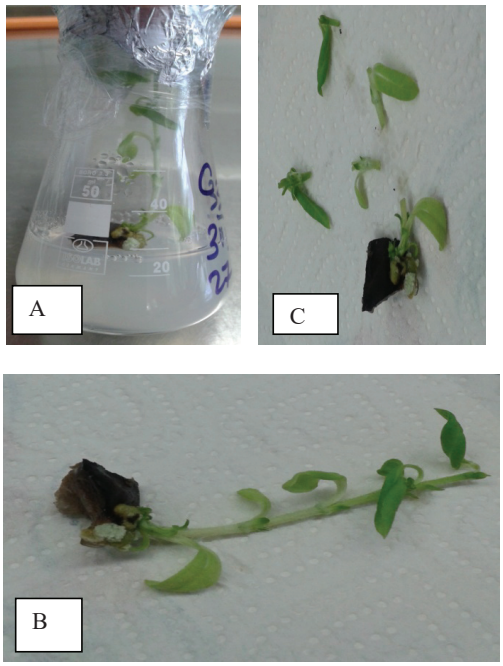

Figure 3. Induction development of shoots (A) to *H.tuberosus* by cultivating the tuberous roots sections on the recipe variant C3 (MS (1962) 2.0 mg/l K + 0.01 mg./l NAA) and multiplying elongated sprouts 3-4 cm long (B) by uninodal fragments explant (C)

Observations on the influence of the culture medium and hormonal balance composition.
Positive results in inducing caulogenesis (formation of adventitious shoots) to explants fragments tuber artichoke used as a source of biological material from plants grown in the area Cernatesti, Buzau county, did we obtain only on hormonal alternative C2 2.0 mg / lBAP + 0.01 mg / l NAA on the same basal medium Murashige & Skoog (1962) and added 7.5 gr./l agar Difco Bacto Agar (Sigma) + 30 gr/l sucrose after 55 days to initiation of the "*in vitro"* culture (Figure 4).

Table 2. The comparative effects of variants (C2 and C3) on *in vitro* morphogenesis leadership at Jerusalem artichoke explants (explants inoculated in 3 series of experimental compared to control)

The series of inoculation (Repeatition)	Recipe culture medium	Number of plants inoculated	Total number of cultures derived morphogenetic	%
Series I	**C2**	12	10	83.33
	C3	7	4	57.14
Series II	**C2**	10	10	100.00
	C3	10	8	80.00
Series III	**C2**	12	12	100.00
	C3	9	9	100.00
Control	MS (1962) for series I	5	0	0
	MS (1962) for series II	5	2	40.00
	MS (1962) for series II	5	1	20.00

Legend: MS – *Murashige&Schoog(1962);* C2=MS(1962) +30g/l sucrose +7.5 g/l agar +2.0 mg/lBAP+0.01 mg./l NAA; C3=MS(1962) +30 g/l sucrose +7.5 g/l agar+2.0 mg/l K+0.01 mg./l NAA; Control= MS (1962) without added hormones

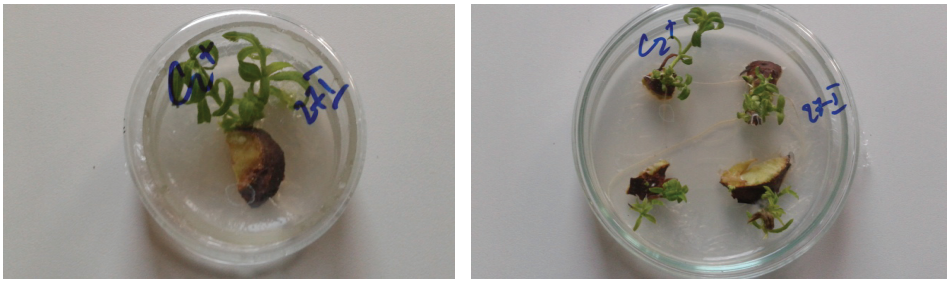

Figure 4. Morphogenetic cultures developed on the C2 recipe variant of culture medium (MS (1962) + 30g / l sucrose 7.5 g / l agar +2.0 mg/l BAP + 0.01/ mg./l NAA) after 55 days to initiation of the *in vitro* artichoke experiment

Our observations on the effect of plant hormones on artichoke explant evolution of grown "*in vitro*" variants C2 and C3 have concluded that both cytokinins used (BAP and K) in 2 mg/l concentrations stimulated elongation of axillary stems and development of morphogenetic culture with multiple adventitious shoots. After three months duration of making subcultivation on the initial inoculation environments the efficiency of developing new shoots doubled (5-7 adventitious shoots/initial inoculated explants) as you can see in Figure 5 and Table 3.

Figure 5. Morphogenetic cultures developed the recipe C3 variant of culture medium (MS (1962) + 30g / l sucrose 7.5 g / l agar 2.0 mg / NAA mg./l lBAP + 0.01) after 90 days of initiation of the artichoke experiment.

Table 3. The comparative effects of variants (C2 and C3) on "*in vitro*" morphogenesis at Jerusalem artichoke explants (explants inoculated in 3 series of experimental compared to control) after 90 days

The series of inoculation (Repeatition)	Recipe culture medium	Number of plants inoculated	Total number of cultures derived morphogenetic	%
Series I	C2	13	4	30.76
	C3	11	10	90.90
Series II	C2	28	16	57.14
	C3	23	16	69.56
Series III	C2	2	2	100.00
	C3	12	6	50.00
Control	MS (1962) for series I	5	5	100.00
	MS (1962) for series II	9	5	55.55
	MS (1962) for series III	15	5	33.33

Legend: MS – *Murashige&Schoog(1962);* C2=MS(1962) +30g/l sucrose +7.5 g/l agar +2.0 mg/lBAP+0.01 mg./l NAA; C3=MS(1962) +30 g/l sucrose +7.5 g/l agar+2.0 mg/l K+0.01 mg./l NAA; Control= MS (1962) without added hormones

CONCLUSIONS

In this work, we have established for the fist time protocol caulogenessis induction of *Helianthus tuberosus* L. (Jerusalem artichoke). The responsiveness of explants was optimal and was not recorded substantial losses due to contamination of biological material. The best morphological characteristics and growth behavior were observed for tuberous routs fragment culture with multiple adventitious shoots cultivated on MS(1962) basal media supplemented with low concentration of NAA (0.01 mg./l) and high concentration of K chinetine (2.0 mg/l) after a previous subcultivation on variant C2 (3-4 times). After this transfer on C3 recepies the yield was 10-12 multiplier adventitious shoots/explant which was inoculated and adventitious roots developed (Figure 6).

Figure 6. Explants with adventitious roots morphogenetic artichoke recipe developed on C3 [MS(1962) +30 g/l sucrose +7.5 g/l agar+2.0 mg/l K+0.01 mg./l NAA] environment.

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A COMPARATIVE ANALYSIS OF CURRENT BIOCLIMATIC LANDSCAPE DESIGN APPROACHES

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Abstract

In the contemporary landscape architecture, the development of bioclimatic design approaches in projects and studies appeared and evolved in the last 20-30 years, yet although some principles date back to the ancient gardens of the Middle East. Bioclimatic landscape design has been developed in order to improve the relationship between human habitats and the natural environment as well as to ameliorate the microclimate influence on human health and comfort. The approach is focused on analyzing and comparing five recent bioclimatic landscape analysis and design projects from several cities located in different climatic zones of the world, including hot arid areas (New Cairo, Egypt; Phoenix, Arizona, USA; Be'er Sheva, Israel), hot humid areas (Guangzhou, China) and temperate humid zones (Utrecht, the Netherlands). The research aims to identify, evaluate and compare methods of analysis, design and implementation used in each case study. The results of the study indicate the main factors involved in the development and implementation of projects in bioclimatic landscape architecture, presenting the impact of the proposed interventions on the bioclimatic variables. The conclusions set out the major principles and methods used in bioclimatic landscape design, underlying the benefits of woody vegetation, the most significant controlling factor of the microclimate in most geographical areas.

Key words*: Bioclimatic landscape planning and design, green infrastructure, urban microclimate, urban vegetation*

INTRODUCTION

Nowadays, bioclimatic planning and design are well known concepts in the field of architecture and urban planning which have been deeply researched in the last half century. In the case of landscape architecture, the modern bioclimatic approach have appeared in the 1980s (Robinette, 1983), but has been theorised, developed and applied mostly in the last 15-20 years (Brown and Gillespie, 1995; Attia and Duchhard, 2011). The main objective of bioclimatic landscape design is to ensure a more comfortable and safer microclimate for the human habitats, especially the urban ones. Thus, the green spaces should be planned in order to mitigate bioclimatic challenges such as urban heat island, wind and dust storms, air pollution, etc. In this context, the study reveals different recent approaches in the field of bioclimatic landscape design.

MATERIALS AND METHODS

The selected case studies are focused on the improvement of urban microclimate conditions through vegetation and water features. Within the research five projects from different climatic zones of the world are analysed through a comparative study (Tables 1, 2). The projects are located in: hot-arid areas – the case of New Cairo, Egypt (Attia and Duchhard, 2011), Phoenix, Arizona, USA (Declet-Barreto et al., 2013) and Be'er Sheva, Israel (Segal, 2015); hot-humid zones - Guangzhou, China (Chen et al., 2009) and temperate-humid areas – Utrecht, the Netherlands (Lensink, 2015). In most of the cases, the projects are approached at the neighbourhood scale, including residential areas and a university campus. The exception is the landscape planning and design strategy for Be'er Sheva, which refers also to the green spaces system at city scale (Figure 1). Regarding the implementation stage, the projects located in Be'er Sheva, Utrecht and Phoenix are approached only at conceptual level, while the bioclimatic landscape design proposal for the American University of Cairo has been already implemented (Figure 2). The proposed analysis criteria include the specific bioclimatic challenges for each type of climate, vision and objectives (Table 1), conceptual

principles, specific methods, results and findings (Table 2). The conclusions reflect the most significant findings in the case of each criterion as well as a general overview of the current approaches in bioclimatic landscape design and future perspectives.

Table 1. Comparative analysis of recent bioclimatic landscape design projects; challenges, visions and objectives

City	New Cairo, Egypt	Phoenix, Arizona, USA	Guangzhou, China	Utrecht, the Netherlands	Be'er-Sheva, Israel
Project title (author, year)	***Bioclimatic landscape design strategy for the American University of Cairo (AUC) Campus (Shaddy Attia, 2006)***	***Creating the park cool island in an inner-city neighbourhood: heat mitigation strategy for Phoenix (J. Declet-Barreto, A. J. Brazel, C. A. Martin, W. T. L. Chow, S. L. Harlan 2012)***	***Field measurements on microclimate in residential community in Guangzhou (Z. Chen, L. Zhao, Q. Meng, C. Wang, Y. Zhai, F. Wang, 2009)***	***Climate-Responsive Maarschalkerw-eerd (Rick Lensink, 2014)***	***Street garden – Trees and vegetation as generators of urbanism in the desert city (Lotem Segal, 2014)***
Type of project	Conceptual landscape design, Implemented	Landscape planning scenario	Analysis of an implemented landscape design project	Conceptual landscape planning and design project	Conceptual landscape planning and design project
Scale of project	University campus	Inner city neighbourhood	Residential community	Suburban area	Neighbourhood scale and city scale
Climate	Hot arid	Hot arid	Hot humid	Temperate humid	Hot arid
Bioclimatic challenges	- Solar radiation (Heat) control - Wind and dust storm control - Evaporation control	- Urban heat island - Heat wave - Wind and dust storm - Evaporation control	- Urban heat island - Wind speed - Air humidity	- Urbanization effects - Wind control - Air quality - Urban heat island in summer	- Poor quality of urban open spaces, lack of green spaces - Solar radiation (Heat) control - Wind and dust storm control
Vision and objectives	- Improvement of microclimate, thermal comfort and energy conservation through landscape elements (vegetation, water, structural elements)	- Assessment of the impact of vegetation in lowering temperature during extreme heat periods in an urban neighbourhood park	- Development of an analysis method to improve the microclimate of a residential community by changing the bioclimatic factors	- Planning a climate-responsive suburban neighbourhood by optimizing the thermal comfort - Minimizing the negative impact of urban development	- Creating an oasis for the desert city - "The Street Garden" concept proposes an alternative circulation channel connecting residential, commercial and public centres

RESULTS AND DISCUSSIONS

Bioclimatic challenges. In hot arid areas, the main bioclimatic challenges are the heat and solar radiation control, protection against wind and dust storms and evaporation control. In hot humid climate, the challenges include wind speed control, urban heat island and air humidity control. In the case of the temperate humid climate, the main bioclimatic challenge during winter is wind control, while during summer the prevalent is the urban heat island phenomenon. Beside these bioclimatic issues, the air quality level is a common factor

independently to the type of climate. Thus, it can be observed that mitigating urban heat island effect and reducing the impact of wind speed and dust storms are the main goals of bioclimatic landscape design (Table 1).

Vision and objectives. All the analysed projects are concerned on improving the microclimate and human thermal comfort through landscape elements, especially woody vegetation and watersheds. Nevertheless, several projects include specific and complementary objectives such as energy conservation, in the case of New Cairo (Attia and Duchhard, 2011), minimizing the negative impact of urban development, in Utrecht (Lensink, 2015) or creating an alternative circulation network through green infrastructure, in Be'er Sheva (Figure 1) (Segal, 2015). The differences between the projects in terms of visions and objectives depend also on the analysis and planning approaches, on the main principles and the specific methods used in each project (Table 1).

Conceptual principles and measures. It should be mentioned that part of the projects are focused mostly on analysis (Guangzhou and Phoenix), while the others combine analysis and planning principles and methods (Table 2). In Guangzhou and Phoenix, two residential neighbourhoods are assessed in order to identify and quantify the impact of vegetation on the microclimate. The difference between the two approaches is that in the first case, the effect of vegetation and watersheds on the microclimatic conditions is analysed on site (an already developed residential area), while in the second case the impact of a bioclimatic landscape planning scenario is evaluated through simulation methods. The projects focused on planning involve distinct approaches, based on specific principles. Thus, the bioclimatic strategy for the university campus located in New Cairo proposes a bioclimatic zoning concept with specific vegetation and microclimatic impact (Attia and Duchhard, 2011). Besides this, the walled-garden concept (used to protect from desert storms and heat waves) and intensive/extensive landscaping (used as an adaptation to water scarcity) are proposed (Figure 2). Even Utrecht and Be'er Sheva are characterized by different bioclimatic challenges the planning solutions for both urban areas are concerned on creating green infrastructure networks (Segal, 2015; Lensink, 2015). The main measures consist of preserving, expanding and connecting the green areas in order to improve the thermal comfort using vegetation. In Utrecht the planning proposal refers only to Maarschalkerweerd suburban area, while in the case of Be'er Sheva the green infrastructure strategy is proposed at the whole city scale (Table 2).

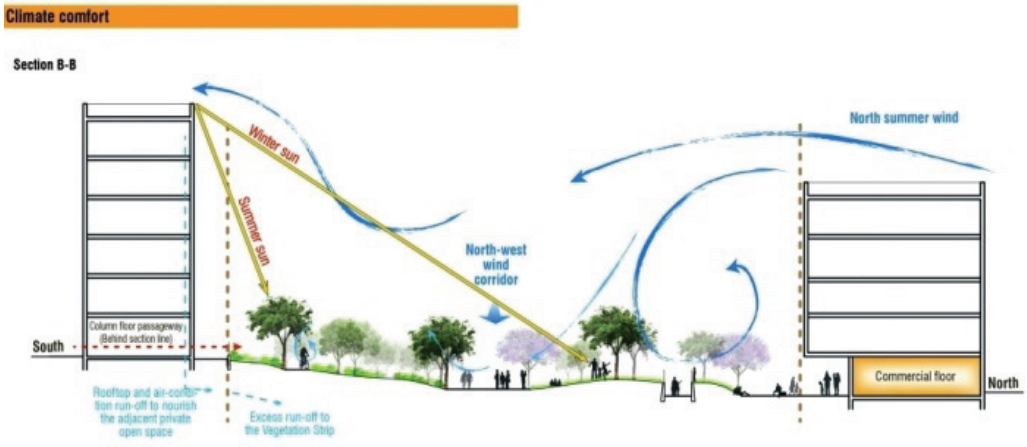

Figure 1 – An urban bioclimatic green corridor proposal for Be'er Sheva, Israel (Source: Segal, 2015)

Table 2. Comparative analysis of recent bioclimatic landscape design projects; principles, methods and findings

City	New Cairo, Egypt	Phoenix, Arizona, USA	Guangzhou, China	Utrecht, the Netherlands	Be'er-Sheva, Israel
Project title (year)	***Bioclimatic landscape design strategy for the American University of Cairo (AUC)* Campus *(2006)***	***Creating the park cool island in an inner-city neighbourhood: heat mitigation strategy for Phoenix (2012)***	***Field measurements on microclimate in residential community in Guangzhou (2009)***	***Climate-Responsive Maarschalkerw-eerd (2014)***	***Street garden – Trees and vegetation as generators of urbanism in the desert city (2014)***
Conceptual principles	Bioclimatic zones concept: 1. Shelterbelt, 2. Oasis, 3. Desert landscape, 4. Greenways, 5. Parking and roads, 6. Building / landscape interface, 7. Inner garden - Walled gardens (shelterbelt) - Extensive/intensive landscaping	- Comparing the current situation of a residential area with a landscape planning scenario in order to quantify the benefits of vegetation	- Researching the impacts of different surface materials with various thermal properties on the outdoor thermal environment, including large areas of water, tree shadows and pavement materials.	- Preserving, expanding and connecting the current green areas in order to prevent the urban sprawl and to ensure a thermal comfort for the local community - Principles: greenery, urban geometry, wind control, watersheds.	- Climate comfort (using vegetation) - A tool system for the Street Garden layout (determined by existing trees, watersheds, valuable open spaces) - Urban backbone Master plan (a green infrastructure network for the whole city)
Specific methods	- ENVI-MET simulations - ECOTECT shading analysis - PET index to calibrate and validate the simulation	- ENVI-MET simulations - Object-Based Image Analysis (OBIA)	- Field measurements in order to determine the microclimatic parameters (air temperature, black bulb globe temperature, humidity, wind speed): 9 spots placed in different conditions (trees, watersheds, pavements)	- A microclimate specific analysis shows the most suitable areas that can be developed as climate-responsive elements	- Behavioural, well-being and perceived quality analyses - Green infrastructure density analysis
Results and findings	- Vegetation plays the most effective role in surface and air temperature modification - The outdoor environment is moderated most efficiently by combining trees, ground cover vegetation and water surfaces	- Significant air and surface temperature reduction in the landscape plan scenario - A "Park cool island" effect, extended to the close non-vegetated areas - A net cooling of air in the planted areas, ranging from 0.9°C to 1.9°C during the warmest period of the day - Reduction of the surface temperature from 0.8°C to 8.4°C in planted areas	- Air temperature was 1.3°C lower near the lake and 25% less in high temperature (>35°C) compared to other areas - Spots placed in vegetation shaded spaces are 0.6-0.8°C lower and 20% less in high temperature - Humidity – the lake doesn't have a significant impact on the community - A safe wind environment	- Vegetation is presented as the main influencing factor: it provides shade, controls wind, cools the air temperature by water evaporation. - The green areas are preserved and made accessible for the inhabitants	- Trees and vegetation are the most significant factors in microclimate improvement, having also a positive social and psychological impact

Figure 2. The implemented landscape design project for the American University of Cairo, New Cairo, Egypt (Source: The American University of Cairo – Aviation Academy, 2013)

Specific methods. The specific methods used in bioclimatic planning projects include a wide range of analyses such as: ENVI-MET simulations; ECOTECT shading analysis; object-based image analysis, microclimatic field measurements; green infrastructure suitability analysis; behavioural, well-being and perceived quality analysis; density of green infrastructure.

ENVI-MET simulations were used both in analysis and design projects (Phoenix and New Cairo) in order to determine the impact of the vegetation on air temperature (Attia and Duchhard, 2011; Declet-Barreto et al., 2013).

The ECOTECT shading analysis was used within the bioclimatic landscape strategy for the American University of Cairo (Figure 2) to find out the percentage of the tree shaded area (Attia and Duchhard, 2011).

Microclimatic parameters have been measured on site in order to assess the impact of trees and water features in a residential community in Guangzhou (Chen et al., 2009).

The suitability analysis for the development of climate-responsive elements has been used within the planning strategy for Maarschalkerweerd suburban area (Utrecht) to identify the potential open spaces to be integrated into the green infrastructure network (Lensink, 2009).

Behavioural and green space quality analyses have been utilized in Be'er Sheva in order to assess the social and psychological impact of vegetation (Segal, 2015).

CONCLUSIONS

All the analysed projects illustrate the significant impact of vegetation (especially the trees) and water features in air temperature regulation and wind control. Beside this, a "cool island" effect, extended to the close non-vegetated environment, has been identified in the case of Phoenix landscape planning scenario (Declet-Barreto et al., 2013). The improvement of microclimate through vegetation has also had a positive psychological and social impact on the inhabitants (Segal, 2015).

Despite the fact that each project proposes a distinct approach, all the authors highlight the significant benefits of the woody vegetation on the microclimate conditions. It may be concluded that bioclimatic landscape planning projects are focused on the improvement of microclimate conditions through modelling vegetation and water features in order to minimize the negative impact of the built environment and to increase the thermal and psychological comfort.

Each project uses different analysis methods, which could be combined to develop more complex assessment tools in bioclimatic landscape architecture. Even if a there is a common aim in the field of bioclimatic landscape planning and design, the methodological approaches are still heterogeneous. Thus, the current comparative study reveals the necessity to develop further integrated analysis and design instruments in

order to optimize the bioclimatic impact of green infrastructure and its associated benefits.

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THE ENLARGEMENT OF *LEUCOJUM AESTIVUM* L. IN DIFFERENT SUBSTRATES UNDER GREENHOUSE CONDITION

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Abstract

Leucojum aestivum L. *(summer snowflakes) is one of the species permitted to export in a limited number from Turkey. 6-7 million summer snowflakes bulbs with more than 7.5 cm circumference are exported to the Netherlands, Denmark and USA. The bulbs obtained from propagation methods are only permitted to export. This study was conducted to enlarge the circumference of snowflakes bulb by using soilless culture techniques in different substrates such as sawdust, cocopeat and perlite+peat in a short time in a part of unheated glasshouse which belongs to Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Horticulture, 2014-2015. The study was established according to randomised plot design with 3 replications and each plot had 20 bulbs of Leucojum aestivum* L. *Some parameters like, bulb diameter, bulb height, bulb weight, stem diameter, stem and leaf weight, dry stem and leaf weight, leaf number, leaf length, leaf thickness, leaf width, root length, root weight, dry root weight and emerging ratio were determined. As a result of the study, substrates have a significant effect on bulb diameter, bulb height, bulb weight, stem and leaf weight, dry stem and leaf weight, leaf number, leaf length, leaf thickness, leaf width, root length, root weight, dry root weight and emerging ratio. However, there was no significant difference between substrate treatments for the stem diameter. The highest average value of bulb diameter (24.80 mm), bulb height (35.38 g) and bulb weight (10.29 g) were measured in perlite+peat. While perlite+peat gives the best result for leaf length (31.23 cm), the highest average value for root length (22.58 cm) were measured in cocopeat.*

Key words*: Leucojum aestivum, geophyte, soilless culture, cultivation, ornamental plants.*

INTRODUCTION

Turkey is quite rich in terms of bulbous plants. It has 1056 taxa geopyhtes and 424 of them are endemic. Endemism ratio is 40 (Özhatay, 2013). Some natural flower bulbs have been exported over a hundred years from Turkey. Turkey has income between 2.5-3 million dollar by exporting natural flower bulbs (Yazgan, 2005; Asil and Sarıhan, 2010).

There are four firms exporting natural flower bulbs in different region in Turkey. These firms have exported the bulbs of 15 natural flower species to the Netherlands, Denmark and USA. The bulbs of *Leucojum aestivum* L. (summer snowflake, loddon lily) is one of the species permitted to export with quota restriction. The bulbs which has more than 7.5 cm circumference and propagated with reproduction techniques are permitted to export (Anonymous, 2016).

Leucojum aestivum L. can be propagated from bulblet and seeds. Yet, propagation of seed takes five or more years from seed to develop plant capable of flower production. Snowflakes has dainty white flowers above attractive dark green foliage. It is a perennial plant growing from 30-60 cm and blooms in early spring, around late February or early March. Its linear leaves are about 3-6 mm wide (Figure 1). Summer snowflake is extremely tolerant of soil such as sand, loam and clay soils. It prefers soils with a more neutral or alkaline pH and tolerates both partial and full sun exposure (Davis, 1965-1984; Zencirkıran, 2002; Aksu et al., 2002).

The aim of this study was to enlarge the circumference of snowflakes bulb by using soilless culture techniques in different substrates in a short time and to use these bulbs in exporting.

Figure 1. Summer snowflakes

MATERIALS AND METHODS

This research was conducted in 2014-2015 growing period in a part of unheated greenhouse (Figure 2) which belongs to Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Horticulture with the dimensions; 10 m width, 50 m length, side 3 m height, 6 m roof height, and north-south directed with arched roof, Turkey.

Figure 2. Unheated glasshouse used in the research

Three different subrates such as cocopeat, sawdust and perlite+peat (1v:1v) were used in this research. Cocopeat block (5kg) was saturated with water (Figure 3) then by; it was mixed for airing and expanding (Figure 4). After this process, one block cocopeat was expanded to about 65 litres of moist coco peat. Sawdust was watered as far as drainage was out and then expanded. Perlite and peat were mixed on an equal basis (1:1 volumetrically). Styrofoam box as a pot were used for planting bulbs of *Leucojum aestivum* L.

Figure 3. Cocopeat block saturated with water

Figure 4. Ready for usage Cocopeat

Leucojum aestivum (summer snowflake, loddon lily) bulbs provided from a firm exporting flower bulbs (Figure 5) with 6 cm in circumference and 4.82 g in weight were used as a plant material (Figure 6) in the study.

Figure 5. The view from firm exporting flower bulbs

Figure 6. Bulbs of *Leucojum aestivum* L.

After the dry outer scales and roots of summer snowflakes were plucked, the bulbs of summer snowflakes were treated in 1% Captan and 0.5 % Mancozeb for 20 minutes against fungus reproduction. Then, they were left in the shade to remove the excess water and kept waiting until planting.

The styrofoam box were filled with cocopeat, sawdust and perlite+peat (1v:1v) as much as half of its depth.

Later the bulbs were planted in 7.5cm X 7.5cm spacing and depth of 7 cm and the styrofoam box were filled with those substrates completely on January, 5th 2014 (Figure 7,8).

Figure 7. Bulbs planted in styrofoam box

The bulbs were irrigated with only water till they formed their roots. Later on, they were irrigated with nutrient solution once a week. Nutient solution contains (ppm); N:193, P:64, K:242, Ca:182, Mg:37, S:55, Fe:4, Mn:1.23, Zn:0.22, Cu:3.92, Mo:0.02 (Resh, 1981). During growing period, the plants were irrigated with fresh water monthly. The study was designed as a randomized plot design with three replications and each plot (box) has 20 bulbs of *Leucojum aestivum* L. Data such as bulb diameter, bulb height, bulb weight, stem diameter, leaf number, leaf width, leaf length, leaf thickness and total bulb number obtained from the trial were analysed statistically by analysis of variance, multiple comparison test and correlation with SPSS 23 software. Separation of means was by the Duncan's multiple comparison test at p = 0.05.

Figure 8. The box filled with substrates fully

RESULTS AND DISCUSSIONS

In this research it was determined that different growing substrates have statistically ($p<0.05$) significant effect on bulb characteristics of snowflake (*L. aestivum* L.) (Table 1). In generally, diameter of bulb is an important indicator for commercial quality and flowering of bulbous plants (Kazaz and Özzambak, 2002). The highest value was obtained by perlite+peat (24.80 mm) for diameter of bulb. This was followed by cocopeat (23.29 mm) and sawdust (19.43 mm), respectively (Table 1).

Table 1. The effect of substrates on bulb characteristics

Substrates	Bulb Diameter (mm)	Bulb Height (mm)	Bulb Weight (g)
Sawdust	19.43 c	31.71 b	5.11 c
Cocopeat	23.29 b	33.33 b	8.01 b
Perlite+Peat	24.80 a	35.38 a	10.29 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$).

Also, some researchers stated that growing substrates have significant effects on bulb diameter in different bulbous plants, such as lilium, freesia and snowdrop (Yılmaz and Korkut, 1998; Akçal, 2014; Kahraman, 2015).

In addition to, the highest value of bulb height was measured in perlite+peat (35.38 mm). Consequently, the effect to the other two substrates on bulb height was similar. At the

same time, for the bulb weight the highest value obtained by perlite+peat (10.29 g), followed by cocopeat (8.01 g) and sawdust (5.11 g), respectively (Table 1).

Except the stem diameter, all of the leaf characteristics were significantly ($p<0.05$) effected by substrates (Table 2).

Table 2. The effect of substrates on stem and leaf characteristics

Substrates	Stem Diameter (mm)	Leaf Number (pieces)	Leaf Witdh (mm)	Leaf Thickness (mm)	Leaf Lenght (cm)
Sawdust	5.44 a	2.03 c	6.02 b	0.99 b	18.27c
Cocopeat	6.08 a	2.75 b	6.65 ab	1.46 a	26.61 b
Perlite +Peat	6.86 a	3.26 a	7.06 a	1.26 a	31.23 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$).

The maximum result for leaf number was observed with an average value of 3.26 pieces in perlite+peat, also minimum value was observed in sawdust (2.03 pieces). With a value of 7.06 mm and 6.65 mm, perlite+peat and cocopeat give the best result for leaf width. At the same time, the highest value was measured for leaf thickness, at cocopeat (1.46 mm) and (1.26 mm), perlite+peat respectively (Table 2). The means recorded for media conclude that maximum leaf length (31.23 cm) was observed in plants grown in perlite+peat, followed by those grown in cocopeat (26.61 cm), while minimum leaf length (18.27 cm) was recorded for the plants that were grown in sawdust. Similar results were observed for lamina length in a study on different freesia cultivars (Tahir et al., 2011), also these results confirmed the findings of Saygılı (2012), who observed various leaf lengths for lilium in different substrates.

The data regarding root, stem and leaf characteristics of snowflake are presented in Table 3. Planting media had a significant ($P<0.05$) effect on root, stem and leaf characteristics of snowflake. For the root length cocopeat (22.58 cm) gives the best result, while the highest values obtained from perlite+peat for root weight (2.03 g), dry root weight (0.29 g), stem and leaf weight (4.69 g), dry stem and leaf weight (0.62 g), respectively. However, perlite+peat and cocopeat showed same effects on root weight, also stem and leaf weight was affected by cocopeat and sawdust statistically at the same level.

Table 3. The effect of substrates on root, stem and leaf characteristics

Substrates	Root Length (cm)	Root Weigh (g)	Dry Root Weigth (g)	Stem and Leaf Weigth (g)	Dry Stem and Leaf Weigth (g)
Sawdust	10.68 c	0.92 b	0.12 c	1.36 b	0.19 c
Cocopeat	22.58 a	1.73 a	0.24 b	2.21 b	0.38 b
Perlite+Peat	19.17 b	2.03 a	0.29 a	4.69 a	0.62 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$).

According to Kakoei and Salehi (2013) similar results were observed in different pot mixtures for spathiphyllum. On the other hand, Merhaut and Newman (2005) stayed that, the use of coir and peat did not influence plant growth such as shoot dry weight in both of the lilium varieties.

At the research, there were 20 piece of bulb were used for each plot. As it was seen in Table 4, results indicate that, differences in various substrates markedly ($P<0.05$) affected total bulb number, bulb emerging ratio and also bulb loss ratio.

Table 4. The effect of substrates on bulb number and emerging

Substrates	Total Bulb Number (pieces)	Bulb Emerging Ratio %	Bulbs Loss Ratio %
Sawdust	15.67 b	88.33 b	11.67 a
Cocopeat	17.67 a	98.33 a	1.67 b
Perlite+ Peat	18.00 a	100.00 a	0.00 b

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$).

The highest value of total bulb number was obtained by perlite+peat (18.00 pieces), followed by cocopeat (17.67 pieces) and sawdust (15.67 pieces), respectively (Table 4). Also, maximum bulb emerging ratio was determined in perlite+peat medium with ratio of 100%, but minimum value was determined in sawdust medium with ratio of 88.33%. Hence, sawdust has taken the greatest value for the bulb loss ratio by 11.67%.

As it understood from the results, growth and development parameters were already better in perlite+peat medium, followed by cocopeat and sawdust (Figure 9 and Figure 10).

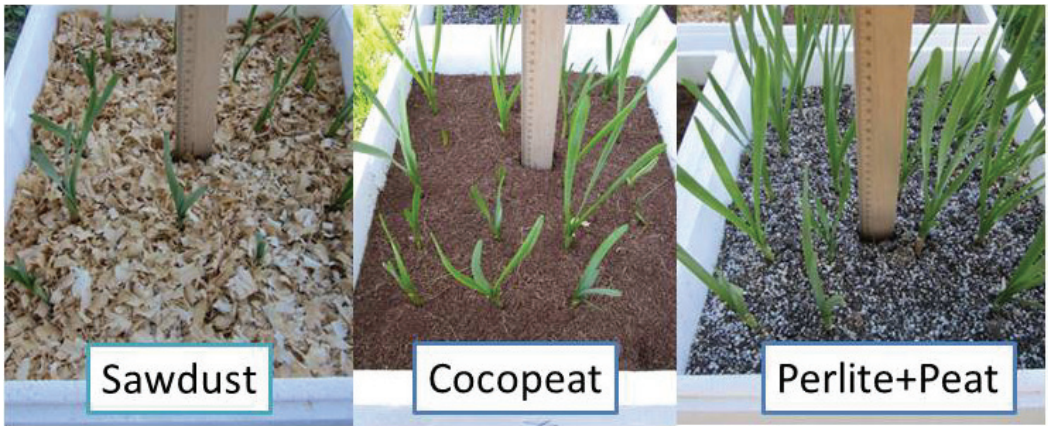

Figure 9. Plants of *Leucojum aestivum* L. growing on different substrates

Figure 10. Bulbs of *Leucojum aestivum* L. growing on different substrates

The relations between the plant characteristics of *L. aestivum* L. was seen in Table 5. According to this, it was conclude that there was a strong correlation between all the plant characteristics, except stem diameter and leaf thickness. In spite of this, strong correlations such as between bulb weight with bulb diameter (r=0.972) and bulb height (r=0.907), also between leaf length with bulb diameter (r=0.963), with bulb height (r=0.915) and bulb weight (r=0.986) indicates that there was a good relationship between underground parts with vegetative development.

Table 5. Correlation between the characteristics of *Leucojum aestivum* L.

	Bulb Diameter	Bulb Height	Bulb Weight	Stem Diameter	Stem and leaf Weigth	Leaf Number	Leaf Width	Leaf Thickness	Leaf Length	Root Length	Root Weigth	Dry Root Weigth	Dry Leaf Weigth	Total Bulb Number	Bulb Emerging Number
Bulb Diameter	1														
Bulb Height	**,856****	1													
Bulb Weight	**,972****	**,907****	1												
Stem Diameter	,464	,551	,551	1											
Stem and leaf Weigth	**,790***	**,829****	**,805****	,451	1										
Leaf Number	**,881****	**,787***	**,894****	,569	,733*	1									
Leaf Width	**,796***	**,798****	**,820****	**,752***	**,703***	**,945****	1								
Leaf Thickness	**,672***	,402	,588	,255	,202	,533	,463	1							
Leaf Length	**,963****	**,915****	**,986****	,601	**,760***	**,854****	**,817****	,631	1						
Root Length	**,825****	,587	**,735***	,290	,358	,651	,585	**,933****	**,781***	1					
Root Weigth	**,858****	**,836****	**,853****	,605	,614	**,690***	**,710***	,536	**,893****	**,741***	1				
Dry Root Weigth	**,987****	**,863****	**,979****	,524	**,740***	**,862****	**,788***	,662	**,978****	**,821****	**,913****	1			
Dry Leaf Weigth	**,915****	**,910****	**,979****	,590	**,820****	**,878****	**,812****	,523	**,955****	,630	**,782***	**,924****	1		
Total Bulb Number	**,865****	,572	**,797***	,557	**,675***	**,807****	**,749***	,634	**,777***	**,719***	**,727***	**,848****	**,728***	1	
Bulb Emerging Number	**,865****	,572	**,797***	,557	**,675***	**,807****	**,749***	,634	**,777***	**,719***	**,727***	**,848****	**,728***	**1,000****	1

*. Correlation is significant at the 0.05 level

**. Correlation is significant at the 0.01 level

CONCLUSIONS

As a conclusion it was seen that, plant characteristics of summer snowflake (*Leucojum aestivum* L.) were already affected by different substrates. This is an important factor for enlargement bulbous plants with soilless culture methods.

Fast development may obtained by different substrates with including plant nutrients. In this research, results indicate that summer snowflake was growth better in perlite+peat medium. Consequently, sawdust shows weak characteristics for a standard growing medium. In other words, enlargement and development of ornamental bulbous plants as like as *Leucojum aestivum* L, increased in substrate, which has got a better quality, such as porosity and water holding capacity.

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OVERVİEW OF DAHLIA BREEDING

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Abstract

Dahlias are popular ornamental plants cultivated in many countries. It is an important garden plant owing to its diversity in colours, size, shapes, forms and profusion of flowering. Dahlia,member of the Asteraceae family. This review describes and compares the conventional and molecular genetics methods being used for breeding. Dahlia is vegetatively propagated with tubers commonly. Breeding programmes have focussed on improving various characteristics to enhance ornamental values, including flower colour, size and form, and production quality. Although desirable traits have been introduced by classical breeding, there are limitations to this technique. Firstly, distant crosses may be limited by incompatibility or differences in ploidy level that is very common in dahlias. Secondly, characteristics such as uniform growth and synchronous flowering are polygenic. Thirdly, several viruses are known to infect dahlia. The dahlia breeding and its methods are described and discussed in this review. Alternative breeding methods will provide faster procedures. In the past, classical breeding approaches like introduction, hybridization, composite crossing, multiline, and backcross breeding were utilized for this purpose. However, each of these methods has advantages and disadvantages. Recent developments in plant biotechnology such as directed mutation, genomics and recombinant DNA technology were adapted by breeders to develop more improved cultivars of dahlias.

Key words: *Ornamental plant, landscape, Asteraceae, mutation breeding, hybridization.*

INTRODUCTION

Dahlias are popular ornamental plants cultivated in many countries. Dahlias are high-value flower crop in several countries in the world. *Dahlia* spp. are members of the *Asteraceae* family. The genus Dahlia's cultivated forms are known as either *D. pinnata* or *D. variabilis* (Sahar et al., 2009). It is popular ornamental plant owing to its diversity in colours, size, shapes, forms and profusion of flowering (De Hertegh, 1989).

Dahlias are allopolyploid with chromosome number 2n = 64 (Lawrence, 1929; Gatt et al., 1998). According to Sorensen (1969), the modern cultivars have 64 chromosomes and are now generally regarded as tetraploids (2n = 4x = 64), though they have also been classed as octoploids (2n = 8x = 64). Crosses between double and single flowered types produce a continuous range of form, indicating that doubleness is controlled by relatively few genes. However their interaction gives rise to great variation in colours (Phetpradap, 1992).

Because of high occurrence of polyploidy, *Dahlia* spp. exhibits various colors, sizes and flower shapes. In particular, dahlias exhibit a wide range of ray floret colors, such as ivory, red, yellow, pink and purple. The pigments accumulated in ray florets are flavonoids, butein, mainly anthocyanin's and flavones and their derivatives that produce yellow, red and ivory colours (Yamaguchi et al., 1999). It is grown as both annual and perennial plant. Many important diseases of dahlia are caused by fungal, bacterial and viral sources leading to diverse types of impairment (Bose and Yadav, 1989) and can be successfully eliminated using micro-propagated plant material (Sediva et al., 2006, Fatıma et al, 2007). It can be more susceptible to bacterial, fungal, and viral infections if is propagated by conventional vegetative methods.

Dahlia is a dicotyledonous plant and a leaf type is composite formed of 3-7 leaflets (Dole & Wilkins 1999). Dahlia plants reproduce vegetative tuberous roots and sexually by seed.

Dahlia is vegetatively propagated with tubers more commonly. Breeding programmes have focused on improving various characteristics to enhance ornamental values including flower colour, size and form (Rout et al., 2006). Although classical breeding contributed improved cultivars, limitations as discussed

below avoid speed of breeding methods. Firstly, crosses may be limited by incompatibility or differences in ploidy level. Secondly, characteristics such as uniform growth and synchronous flowering are polygenic. Thirdly, several viruses are known to infect dahlia. They are dahlia mosaic virus (DMV), cucumber mosaic virus (CMV), impatiens necrotic spot virus (INSV), tobacco streak virus (TSV), and tomato spotted wilt virus (TSWV) (Lobenstein et al., 1995; Pappu et al., 2005). Objective of this study are to summarize and discuss breeding methods of dahlia in relation to conventional breeding methods.

1. MUTATION BREEDING

For various reasons, mutation breeding has been especially successful in ornamentals as well as in many other horticultural plants such as citrus and apples. Firstly, the selection of mutations of directly perceptible characteristics including flower form or size, colour, is generally not difficult. The other reason is that a lot of cultivars are heterozygous that may allow extended variation through mutations and hybridizations. Moreover, *in vitro* or *in vivo* propagation methods frequently allow the successful production of mutants that can be recognized later (Broertjes, 1967). Ornamental bulb and tuber crops contain large, economically important varieties. As many of them indicate segregation after seed propagation, the majority of these crops is propagated vegetatively. This coupled with low speed of propagation, is one of the main obstacles to breeding. This low process cannot be speeded up since pruning, to support the growth of axillary buds, is often impossible. It is therefore not surprising that irradiation at the right moment is recognized as being of great significant; that is, irradiation should be carried out at the earliest possible stage of development, when a mutated cell has the largest feasible opportunity to make a substantial contribution to the genesis of the new plant, tuber or bulb.

In Dahlia, freshly harvested tubers were more suitable for irradiation. For many reasons *D. variabilis* must be considered as a promising species for mutation breeding. The high polyploidy and the great number of flower colour genes brings attention to this species (Broertjes, 1967). Flower colour and other distinguishable mutations ranging from dominant to recessive can be observed in the material due to the high degree of heterozygosity and vegetative propagation. Furthermore, genetic composition of a given cultivar is not altered significantly. Cross-breeding of a certain dahlia variety, on the other hand, will never result in a genotype which is identical except for recently introduced one like change in lower colour. For this reason, mutation breeding is important way of development in those varieties important (Broertjes, 1968).

Material selection is highly difficult due to complicated genetics and unknown genetic history of the current cultivars, which makes it impossible to choose cultivars. Thus mutation breeding is more promising than hybridization breeding (Broertjes, 1976).

It is of crucial importance to irradiate the buds at the earliest possible stage of development, for the purpose of give a mutated cell the best chance to take part in the formation of the shoot. Irradiation should therefore take place immediately after harvest, when no visible eyes can be detected on these so-called dormant tubers.

There are chemical (DMS, EMS, etc.) and physical (^{60}Co-gamma) reagents for mutation breeding. Although chemical mutagens usually cause point mutations (minor alteration in sequences), physical mutagens bear larger modifications in the chromosomes. List studies related to mutation breeding are reported in Table 1.

2. HYBRIDIZATION BREEDING

Hybridization remains significant component of a lot of plant breeding programs. Hybridization can include crosses between distinct species (interspecific hybridization), or crosses between genetically distinct individuals (selections, breeding lines, or cultivars) within a species (intraspecific hybridization) (Murray, 2003). Hybridization is generally significant for two main reasons: to transfer genes and therefore, the characters they control, from one plant to another; or to exploit the vigor that is

often observed when genetically distinct plants are crossed.

2.1. Intraspesific gene transfer

In general, modern dahlias have been developed through conventional breeding such as hybridizations and selections. Particularly, intraspecific hybridizations may have advantages due to less occurrence of complications such as incompatibility and early embryo losses. Breeders might expect lower incidence of chromosome imbalance within species crosses. Eriksen et al (2014) propose that multiple intraspecific hybridization events may have created especially potent conditions for the selection of a noxious invader, and may explain differences in genetic patterns among North and South America populations in *Centaurea solstitialis* L. (*Asteraceae*) inferred differences in demographic processes, as well as morphological differences previously reported.

2.2. Interspecific gene transfer

Interspecific hybridization, also named wide hybridization, is generally used when a specific character or group of characters is missing from a cultivated species. A research of related wild species is then needed to identify which of them may be beneficial as potential gene donors. Not all species are able to hybridize, and there can be remarkable variation in the ease of hybridization, even between closely related species.

Interspecific gene transfer through hybridization is possible among many dahlia cultivars and has occurred naturally. An extensive search of the relevant literature did not reveal evidence of gene transfer between dahlias and unrelated plant species yet. Thus gene transfer through wide hybridizations should be investigated.

3. MODERN APPROACHES

The molecular markers have contributed research on genetic variation among dahlia genetic resources. They are very useful identification of differences, germ plasm management and discriminating commercial cultivars for protecting breeders' rights (Ben-Meir et al., 1997; Rout et al. 2006).

In traditional breeding, selections were made on morphological bases that were extremely influenced by the environment. This created confusion in selection of creditable parents for breeding programs. However, the exploration of DNA based markers such as RAPD, AFLP, ISSR, SSR and SNPs linked to various economically significant traits has provided the opportunity to plant breeders to select their desired parents for further improving cultivars. (Hussain et al., 2012; Hussain, 2015).

Chebet et al. (2003) reported the use of biotechnological approaches to develop horticultural plant production especially the application of biotechnology on in vitro propagation of ornamental plants.

Many important diseases of Dahlia can be successfully eliminated using micro propagated plant material. In vitro culture is one of the key tools of plant biotechnology. Furthermore, micro-propagation of plants is a well-known strategy for effective production and propagation of the elite plant material. It helps in the improvement and rapid propagation of selected plants with requested characters in shortest possible time and new cultivars can also be developed by genetic modifications and protoplast fusion.

The regeneration of Dahlia plants has been reported either directly from explants without callus formation or indirectly through callus induction and regeneration. There are a few reports available on in vitro proliferation of *Dahlia sp.*. Fatima et al (2007) produced the largest number of dahlia plants by indirect organogenesis technique when used the cotyledon leaf, hypocotyls and shoot tip as explants. Salman et al (2010) reported similar results when they cultured the shoot tips on MS medium. Majid et al (2015) studied in order to produce large number of dahlia plants free of pathogens and matching the genetic traits of the mother plant by plant tissue culture technique.

List of relevant studies are given in Table 1.

Table 1: Studies conducted in the *Asteraceae* family

Study subject	Genus	Reference of study
Intergeneric hybridization	*Chrysanthemum naktongense x Chrysanthemum xmorifolium 'Aifen'*	Wu et al., 2015
DNA sequence analysis	*intergeneric hybrid (Argyrotegium mackayi x leucogenes leontopodium)*	Smissen et al., 2015
In suti hybridization	*Chrysanthemum*	Xiangyu et al., 2015
Hybridization and genomic in situ hybridization	*Tragopogon castellanus*	Mavrodiev et al., 2015
Intergeneric hybrids	*Anaphalioides bellidioides x Ewartiothamnus sinclairii and Leucogenes grandiceps x Raoulia eximia*	McKenzie et al., 2015
Hybridization	*Calendula maritima*	Plume et al., 2015
Natural hybridization	*Ligularia cymbulifera and L. tongolensis*	Yu et al., 2014
Intergeneric genomic shock	*Chrysanthemum morifolium x Leucanthemum paludosum*	Wang et al., 2014
Hybridization	*Cyanus triumfetti and C. montanus*	Olsavska et al., 2013
Hybridization	*Chrysanthemum nankingense x Tanacetum vulgare and C. crassum x Crossostephium chinense*	Wang et al., 2013
Karyotypic changes followıng hybrıdızatıon at the polyploid level	*Tragopogon mırus and T. mıscellus*	Lipman et al., 2013
Interspecific hybridization	Genus *Tolpis*	Gruenstaeudl et al.,2013
Hybridization	Members of the *Artemisia tridentata*	Garrison et al., 2013
Intergeneric hybridization	*Senecio sect. Crociseris*	Calvo et al., 2013
Interspecific and intraspecific polymorphisms	*Cichorium* L.	Bernardes et al., 2013
Natural hybridization	*Sphagneticola trilobata*	Wu et al., 2013
Intraspecific hybridization	*Artemisia tridentata*	Richardson et al., 2012
Hybridization	*Tithonia tubaeformis and T. rotundifolia*	Tovar-Sanchez et al., 2012
Intersectional hybridization	*Helichrysum orientale and Helichrysum stoechas*	Galbany-Casals et al., 2012
Natural hybridization and introgression	*Ligularia species*	Yu et al., 2011
Hybridization	diploid *Centaurea pseudophrygia and the* tetraploid *C. jacea*	Koutecky et al., 2011
Hybridization	Boltonia asteroides (L.)	DeWoody et al., 2011
Hybridization and genome duplication	*Secenio*	Hegarty et al., 2011
Intergeneric hybridization	*Chrysanthemum grandiflorum (Ramat.) Kitam. 'Zhongshanjingui' (female parent) and Ajania przewalskii Poljak. (male parent)*	Deng et al., 2010
Molecular, morphological, and experimental evidence for hybridization	The *Galapagos* endemic plants *Scalesia aspera, Scalesia crockeri,* and *Scalesia pedunculata*	Lindhardt et al., 2009
Molecular study of hybridization and homoploid hybrid speciation	*Argyranthemum sundingii*	Fjellheim et al., 2009
Mutation breeding by radiation technology	domestic and foreing ornamentals	Sup Song et al., 2005
Induced mutations	-	Ahloowalia et al., 2001
Applıcatıon of ın-vıvo and ın-vitro mutation techniques	-	Maluszynskı t.al.,1995
Mutations breeding	*Dahlia* spp.	Broertjes et al., 1966

CONCLUSIONS

This review describes and compares the conventional and molecular genetics methods being used for breeding. In the past, classical breeding approaches like introduction, hybridization, composite crossing, multiline, and backcross breeding were utilized for this purpose. However, these methods have low speed, and expensive. Furthermore, breakdown of resistance due to fast evolving pathogens could not be coped with using these time consuming methods. Therefore, molecular genetics approaches like mutation, genomics, recombinant DNA technology, were adapted by breeders to develop effective resistance in crop plants in a shorter time.

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EVALUATION *OF HELIOTROPIUM GREUTERI* FOR MORPHOLOGICAL CHARACTERISTICS AND POTENTIAL USE AS AN ORNAMENTAL PLANT

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Abstract

*Boraginaceae is an important family distributed worldwide that includes herbs, subshrubs, shrubs, or trees. The paper aims to introduce a potential ornamental plant in the Kayseri city of Turkey. Belonging to the family Boraginaceae, t*he plants of *Heliotropium greuteri naturally occur. In this study, 50 genotypes were examined for two morphological features (leaf size and flower diameter). The data obtained in the study were analysed with SPSS statistical software. Heliotropium gruteri genotypes indicated quite different characteristics studied in this region. Leaf size is highly variable (11- 42 cm) and leaf sizes of genotype 2, 9 and 17 are the largest; genotypes 37, 42 and 44 mm have smaller leaves. Looking at the flower top diameter, genotypes had values between 8.09 and 12.37 mm. Genotypes 25, 39 and 9 have the highest flower top diameters and genotypes 31, 10, 48 have the smallest flowers. No significant difference between genotypes in terms of flowers top diameter was detected. There was no correlation between leaf length and diameter of flower diameter. Thus, with fragrant flowers, it is suitable for use as a ground cover, which provides a decorative appearance to their environment, which can grow even in rocky areas, affected by harsh winds. They are able to tolerate moving and attracts honey bees as well. Because of the many features of H. greuteri such as drought tolerance and fragrance, it can have potential as ornamental plant. They can be suggested in parks, road sites, cemeteries and all low input areas as well as high input areas.*

Key words*: Heliotropium greuteri, Boraginaceae, ornamental plants*

INTRODUCTION

Boraginaceae family of *Lamiales* order has 100 genera and 2.000 species of tropical, subtropical and temperate regions. *Boraginaceae* is an important family distributed worldwide that includes herbs, subshrubs, shrubs and trees. Within the family *Boraginaceae*, *Heliotropium* species exhibit great variation in many features of biological interest including habitat preferences, physiognomy and morphological traits (Al-Turki, 2001). The genus *Heliotropium* is part of the *Heliotropieae* tribe and *Heliotropioideae* subfamily; it is a euryspecific genus with 250 species (Saad-Limama, 2005). South-West and Central Asia are major centers of diversity in the genus *Heliotropium*. Most species of *Heliotropium* grow in areas with an arid and semi-arid climate, mostly on dry soils, gypsum hills, sandy and gravelly deserts, disturbed soils, eroded slopes, as weeds in cultivated lands and wastelands, along riversides, and, rarely, around hot springs (Akhani, 2007)

The genus *Heliotropium* L., according to a recent survey, comprises nearly 300 species assigned to 19 sections (Al-Turki, 2001). Most species are distributed in tropical and temperate regions of both hemispheres in a variety of habitats, including drifting sands, hardened sandy plains, edges of cultivated or saline waste ground and steep rocky outcrops as high as 1.500 m (Collenette, 1999). Many researchers, in an effort to confirm the identity of *Heliotropium* taxa. *Heliotropium gruteri* that described by our study, is the new species described in Kayseri region. *Heliotropium gruteri*, seen as extensively in the region, have fragrant flowers. These plants are suitable for use in the refuges and ground cover plants. They are very attractive and actively grows up until the first frost by forming flowers. Research is needed for further information on the properties of this species.

MATERIALS AND METHODS

Kayseri province is natural habitat of *Heliotropium gruteri*. In this study, 50

genotypes were characterized for leaf and flower characteristics, two most important features of ornamental plants. Comparative morphological analyses were conducted at the sites where the plants naturally grow. The observation sites are in the elevation at 1000-1800 meters within Kayseri province of Turkey in Central Anatolia. Means of leaf sizes and flower diameters were analyzed with SPSS statistical software.

RESULTS AND DISCUSSIONS

Heliotropium greuteri belonging to *Boraginaceae*, is an annual and branched plant. The observations have been made in Kayseri region with hot summers and cold winters. The soil is mostly sandy-loam with desert pavement and coralline features. Flowering and fruiting occurs profusely from June to November. This study revealed valuable information on *H. gruteri'*s leaf and flower structure of 50 genotypes. *Heliotropium gruteri* genotypes had high level of variation in this region. Leaf sizes ranged between 11 and 42 cm. These properties are given in Table 1 below. While leaf size of genotype 2, 9 and 17 were the largest, genotypes 37, 42 and 44 had the smallest leaves. Looking at the flower top diameter, genotypes had the values between 8.09 and 12.37 mm. While genotypes 25, 39 and 9 having the highest flower diameter, genotypes 31, 10, 48 had the smallest flowers. These properties are shown in Table 2 below. No significant differences were detected between genotypes in terms of flowers top diameter. There was no correlation between leaf length and diameter of flower top.

Literally, *H. gruteri* are 20-60 cm high herbs, with spreading and retrorse hairs, herbs annual and much branched. Petiolate, up to 11.5-42.0 cm long , gradually reduced in upper portion of the stem; narrowly ovate, undulate, spreading and retrorsely bristly hairy; hairs of 2 to 3 types, the basal portion of the hairs on the midrib and veins extremely swollen. Infloresence terminal, of usually more than 2, spicate cymes, elongating in fruit. Flowers white, 5-10 mm long. Calyx 3 mm long, the lobes 2.5 mm long, densely bristly hairy, setose except at the bases. Corolla 5 mm long; tube slightly constricted near the mouth and below the stigma level; lobes obtuse, and spreading. Anthers 1 mm long, pointed, inserted at about the middle of the corolla tube. Stigma short, conical, apex obtusish, glabrous. Nutlets shortly winged, 3 mm long and wide including the wings, glabrous, with or without one or two spongy callosities on the outer surface. Shrubs of *H. gruteri* are given in Figure 1 below.

Figure 1. Pictures of shrubs of *Heliotropium greuteri* plants in August

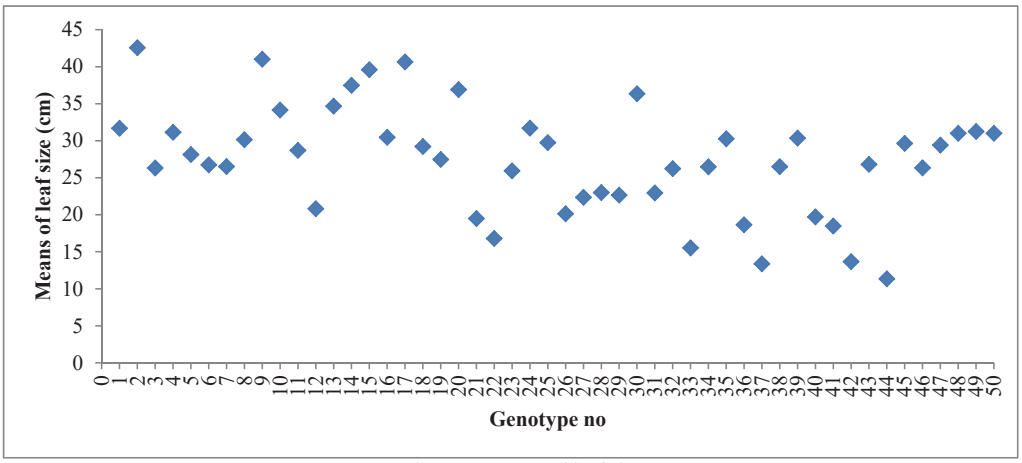

Figure 2. Means of leaf size

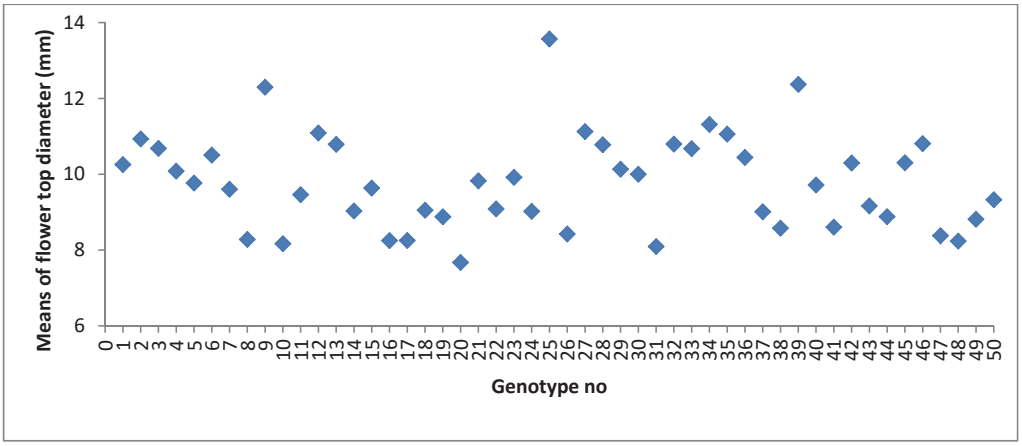

Figure 3. Means of flower top diameter

CONCLUSIONS

Turkey, because of its geographical location, high variable climates due to elevation differences, mountains ranging in short distance, geological, geomorphological structure, soil diversity and historical reasons, is one of the most important diversity centers in the world in terms of plant species.

As endemics of Turkey, *H. greuteri* may play a role in landscaping. We emphasis aesthetic value of natural species with their ornamental plant capacity. The usage of natural species play significant mission with ecological and providing identity for cities.

In this study, we indicated variation and potential of two important ornamentally important characteristics of 50 *H. greuteri* plants occurring in Kayseri, Turkey with elevation of 1200-1800 meters.

They are highly drought tolerant, attractive for fragrance, but having little size of flowers. Improving flower sizes may be better by breeding through selection, mutation breeding, etc.

Study results showed that *H. gruteri* have high potential for the use in landscape design.

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DIFFERENT APPROACHES ON BULBLET FORMATION WITH SCALING IN MADONNA LILY *(LILIUM CANDIDUM)*

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Abstract

The purpose of this study was, to determine the effects of different treatments on bulblet formation with scale propagation in 'Madonna lily' (Lilium candidum). The research was conducted in growth chamber at Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Horticulture in 2015-2016. Scales of Lilium candidum bulbs with 22-24 cm circumference, were used as a plant material. Effects of different incubation periods (10,12,14 weeks), incubation temperatures (10-15 °C, 20 -25°C), auxin (IBA 100 ppm, IBA 200 ppm) doses and scale positions (outer, middle, inner, center) on bulblet formation were investigated. The experiment was established according to randomised plot design with 3 replications. Some parameters like, bulblet formation ratio, bulblet number per scale, weight, diameter and height of bulblet, scale number, root number and root length of bulblet were also determined. As a conclusion, treatments have not any significant effect on bulblet formation ratio, but there were significant differences between the other parameters for some of treatments. In spite of this, incubation period of 14 weeks gives the highest average value for bulblet number per scale (1.467 piece) and bulblet height (19.105 mm). Also, the highest average value of bulblet weight (0.792 g) and bulblet diameter (13.282 mm) were measured in outer scales. While incubation temperature of 10-15 °C gives the best result for bulblet scale number (3.511 piece), the highest average value for root number of bulblet (3.900 piece) and root length of bulblet (11.224 cm) were measured in auxin dose of 200 ppm IBA.

Key words*: Lilium candidum, scale propagation, incubation, bulblet formation, ornamental plants*

INTRODUCTION

Turkey is very rich in terms of a plant diversity, in different three phytogeographic zones in the cross-point of Asia and Europe, along with the climatic change and soil properties. Turkey has about 12.000 taxa species and 3.750 of them are endemic (Avcı, 2005). Endemism ratio is 34.5 % (Uyanık et al., 2013). There are 1056 taxa geophytes and 424 of them are endemic (Özhatay, 2013). Geophytes, are the plants whose above ground parts such as stems, leaves and flowers dries and dies after completing their growth period and which in summer months live on thanks to their under earth storing parts such as bulbs, tubers and rhizomes, are also called natural flower bulbs. These are economically important in the sector of ornamental plants (Aksu et al., 2002; Zencirkıran, 2002). Some of these species have been exported over a hundred years. Bulb exportation of *Lilium candidum* are permited from only bulbs propagated in Turkey (Anonymous, 2016). *Lilium candidum* is globally known as "Madonna lily" or "white lily". It is a herbaceous, bulbous perennial plant belonging to *Liliaceae* family. It has fibrious roots and the roots are yellowish white colored. Its stem length is between 43-150 cm and has white flowers blooming between the end of May and the end of June, depending on the climate conditions (Özen et al., 2012). *L. candidum* can be propagated from seed and bulblets. However, propagation of seed takes five or more years from seed to develop plant capable of flower production. During the uprooting of the plant which takes such a long time to grow, newly sprouted, not ripened seedlings are also uprooted and therefore the damage increases more and more.

The objective of this study was, to develop some new methods with different approaches for scale propagation in *Lilium candidum* bulbs, which has been uprooted a lot from the nature and exported, also it was aimed on providing bulbs for exporting with these methods.

MATERIALS AND METHODS

This study has been conducted in 2015-2016 period in a growth chamber (Figure 1), where the temperature (^{o}C) and humidty (%) controlled by automatically, in COMU, Faculty of Agriculture, Department of Horticulture.

Figure 1. Inside of growth chamber

L. candidum bulbs with 22-24 cm in circumferences and 87.98 g in weight, were used as a plant material (Figure 2).

Figure 2. Bulb of *Lilium candidum*

The bulbs of *L. candidum* (Madonna lily) were provided from a firm exporting flower bulbs. The dry outer scales, any root remains and the bulb tip were all removed prior to scaling (Figure 3).

Scales were separated from basal plate of *L. candidum* bulbs (Figure 4), measured and calibrated for trials (Figure 5). Then washed with distilled water and sterilised with dilute alcohol.

Figure 3. *Lilium candidum* wihtout root

Figure 4. Scales separated from basal plate

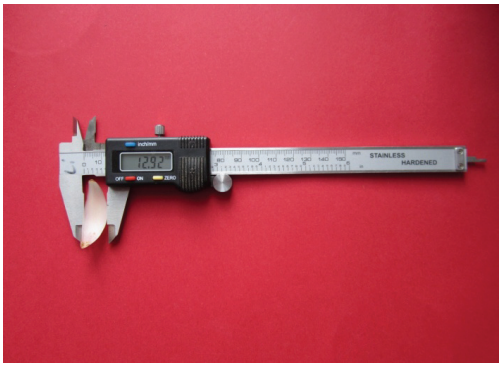

Figure 5. Calibration of lilium scales for trials

The average length of these scales is about 35.60 mm and the average width of the scales is 12.46 mm.

The scales were treated in 1% Captan and 0.5 % Mancozeb for 20 minutes to prevent fungal diseases (Figure 6), left in the shade for 10 minutes to remove the excess water, and kept in a cool place until planting.

Figure 6. Scales treated with fungicide

15 prepared bulb scales were mixed with 3 liters of damp perlite (Figure 7) and after the mixture was put to black polyethylene bags (5 L) (Figure 7). The bags were fastened tightly leaving some space on top (Figure 8,9), on October, 2nd 2015 (Aksu et al., 2002; Zencirkıran and Mengüç, 2002).

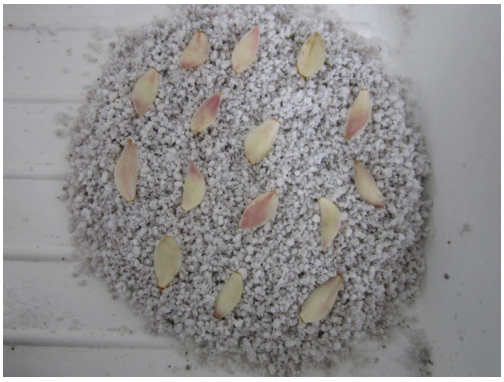

Figure 7. Perlite and scales

This research is consist of four different treatment such as incubation temperature, incubation period, scale position and auxin dose. In incubation temperature trial, the bags were incubated in a growth chamber at 10-15°C and 20-25 °C until January, 14th 2016 to form bulblets. Incubation period trial includes 10 weeks, 12 weeks and 14 weeks period. Scale position trial composed of outer, middle, inner, and center scales of Madonna lily. 100 ppm IBA, 200 IBA and control (without IBA) were used in auxin dose trial.

All experiment were established according to randomised plot design with 3 replications composed of 15 scales each.

Figure 8. Perlit and scale mixture inside of PE bag

After uprooting bulblets from bags, some parameters like bulblet formation ratio (%), bulblet number per scale (piece), bulblet weight (g), bulblet diameter (mm), bulblet height (mm), bulblet scale number (piece), root number of bulblet (piece) and root lenght of bulblet (cm) were measured.

The data were analyzed statistically by analysis of variance with SPSS 23. Separation of means was by the Duncan's multiple comparison test at $p = 0.05$.

Figure 9. Polyethylene bags in growth chamber

RESULTS AND DISCUSSIONS

According to Marinangeli and Curvetto (1997), bulblet formation or number of bulblets per scale were affected by some biotic factors such as cultivar, size, age, physiological status of the bulb, position of the scale in the bulb and by some abiotic factors such as, temperature, humidity, light, physiological and chemical treatments (Matsuo, 1987; Grassotti and Magnani, 1988; Magnani et al., 1988).

In this study it was determined that, incubation periods had a statistically significant effect ($p<0.05$) on only the bulblet number per scale. Maximum values of bulblet number per scale were obtained from 14 weeks of incubation period (1.467 piece).

On the other hand, there was not any significant difference between the three incubation period (Figure 10) (10,12,14 weeks) for the other bulblet characteristics (Table 1).

Figure 10. Scales and bulblets in incubation periodes

Table 1. The effect of incubation period on bulblet characteristics

Incubation Period	Bulblet Formation Ratio (%)	Bulblet Number per Scale (piece)	Bulblet Weight (g)	Bulblet Diameter (mm)	Bulblet Height (mm)	Bulblet Scale Number (piece)	Root Number of Bulblet (piece)	Root Lenght of Bulblet (cm)
10 Weeks	100.000 a	1.194 b	0.533 a	9.977 a	17.731 a	3.000 a	1.833 a	8.081 a
12 Weeks	96.970 a	1.067 b	0.629 a	10.912 a	18.158 a	2.938 a	1.806 a	9.483 a
14 Weeks	75.000 a	1.467 a	0.702 a	10.659 a	19.105 a	2.705 a	2.091 a	7.727 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$).

Many researchers stated that bulblet formation was also influenced by various plant growth regulators (Matsuo, 1972; Roh, 1990). In this research, similar results have been observed for auxin doses on some bulblet characteristics. Auxin doses had a statistically significant effect ($p<0.05$) on diameter and height of bulblet, bulblet scale number, root number and root length of bulblet (Figure 11).

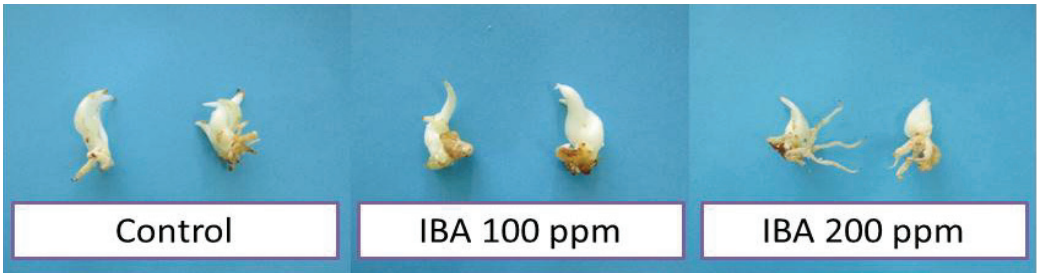

Figure 11. Bulblets in auxin doses and control

The highest value of bulblet diameter was 6.872 mm with 100 ppm IBA, while the lowest value of diameter was 5.446 mm with 200 ppm IBA (Table 2). Park (1996) reported that, generally the diameter of the bulblets was increased when the scales were treated with the 100 ppm IBA. With a value of 16.127 mm and 15.022 mm, 100 ppm IBA and control bulblets gives the best result for bulblet height. At the same time, the highest value was measured for bulblet scale number, at 100 ppm IBA (3.000 piece) and 200 ppm IBA (2.840 piece), respectively (Table 2).

Table 2. The effect of auxin doses on bulblet characteristics

Auxin Dose	Bulblet Formation Ratio (%)	Bulblet Number per Scale (piece)	Bulblet Weight (g)	Bulblet Diameter (mm)	Bulblet Height (mm)	Bulblet Scale Number (piece)	Root Number of Bulblet (piece)	Root Lenght of Bulblet (cm)
Control	22.932 a	1.083 a	0.268 a	6.398 ab	15.052 a	2.167 b	2.375 b	1.833 b
IBA 100 ppm	66.667 a	1.280 a	0.362 a	6.872 a	16.127 a	3.000 a	2.708 ab	9.152 a
IBA 200 ppm	48.889 a	1.300 a	0.249 a	5.446 b	11.356 b	2.840 a	3.900 a	11.224 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$)

Also, the highest value for root number of bulblets determined (3.900 piece) in 200 ppm IBA, similarly root length increased with auxin doses, thus, 200 ppm IBA gives the best result with an average value of 11.224 cm (Table 2).
Hence, growing structure of scales depends on their positions; it's an important factor for bulblet formation and characteristics. According to some researchers, scales from the outer and middle scales of lily bulb tended to produce more bulblets, which can be correlated with the total carbohydrate content in those scales (Matsuo, 1975; Park, 1996). Except bulblet formation ratio, all parameters affected significantly ($p<0.05$) by scale positions (Table 3). With 1.167 piece, the highest average value for bulblet number per scale was obtained from outer scales, other positions statistically acted at same level on bulblet number per scale. For weight, diameter, height, scale number and root number of bulblets, similar results observed in scale positions (Figure 12).

Table 3. The effect of scale positions on bulblet characteristics

Scale Position	Bulblet Formation Ratio (%)	Bulblet Number per Scale (piece)	Bulblet Weight (g)	Bulblet Diameter (mm)	Bulblet Height (mm)	Bulblet Scale Number (piece)	Root Number of Bulblet (piece)	Root Lenght of Bulblet (cm)
Outer Scale	83.335 a	1.167 a	0.792 a	13.282 a	18.319 a	3.500 a	2.273 ab	3.955 b
Middle Scale	80.000 a	1.028 b	0.517 b	9.473 b	15.824 b	2.765 b	2.355 a	10.468 a
Inner Scale	90.790 a	1.025 b	0.462 b	8.788 b	14.207 b	2.769 b	1.897 bc	10.000 a
Center Scale	79.840 a	1.000 b	0.236 c	7.107 c	12.036 c	2.000 c	1.500 c	8.813 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$).

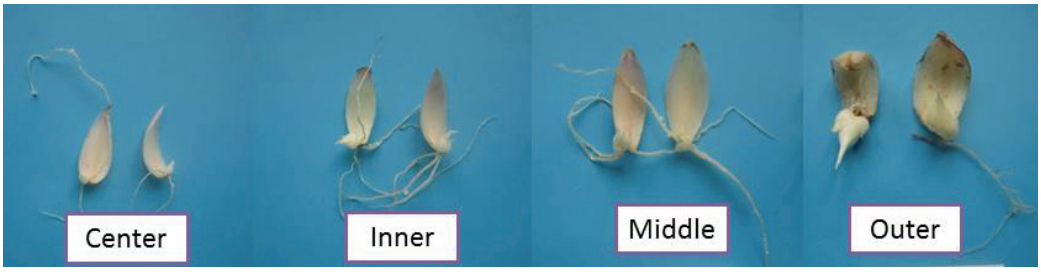

Figure 12. Scales and bulblets in scale positions

When the highest values measured in outer scales, center scales were give the lowest values, generally middle and inner scales statistically acted at the same level. So it was

clear that, there was an increase from center to outer scales. On the other hand, it was not investigated for root length of bulblet (Table 3). According to the results of Matsuo et al. (1987), larger scales produce more bulblets in Lily. Hanks (1985) reported that, the number and sizes of differentiated bulblets are influenced by the relative position of starting scales in Narcissus bulbs. Marinangeli et al. (2003) conclude that, middle scales are the best starting materials for experimental uses involving scale propagation and external scales must be included for production. Also, Padasht et al. (2006) reported that, outer and middle scales at 20 and 25 °C regenerated more bulblets with better properties than inner scales. Many researcher reported that, the growth and development properties of newly formed bulblets depends on the temperature during scaling (Van Tuyl, 1983; Aquettaz et al., 1990). Our results for *L. candidum* indicates that incubation temperatures had a statistically significant effect ($p<0.05$) on only the diameter and root length of bulblet (Table 4) (Figure 13).

Table 4. The effect of incubation temperature on bulblet characteristics

Incubation Temperature	Bulblet Formation Ratio (%)	Bulblet Number per Scale (piece)	Bulblet Weight (g)	Bulblet Diameter (mm)	Bulblet Height (mm)	Bulblet Scale Number (piece)	Root Number of Bulblet (piece)	Root Lenght of Bulblet (cm)
10-15 °C	95.553 a	1.089 a	0.410 a	7.933 b	17.279 a	3.511 a	2.178 a	5.658 b
20-25 °C	100.000 a	1.133 a	0.427 a	9.056 a	16.378 a	2.889 a	2.133 a	7.351 a
	ns	ns	ns	*	ns	**	ns	*

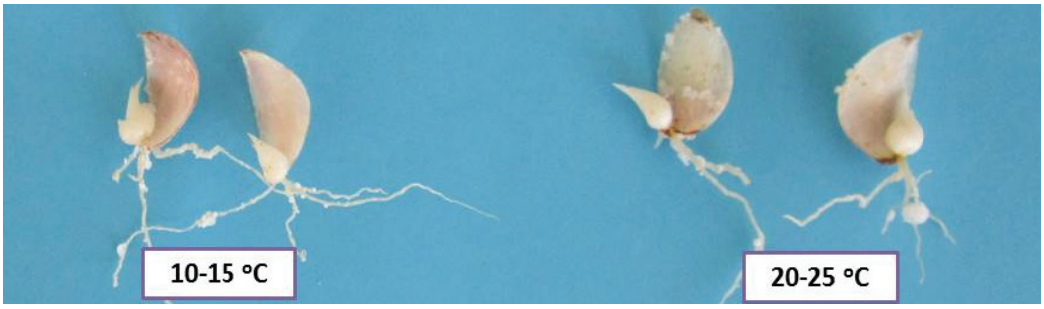

Figure 13. Scales and bulblets in incubation temperatures

The highest value for diameter of bulblet (9.056 mm) and root length of bulblet (7.351 cm) were taken from incubation temperature of 20-25 °C (Table 4). Similar results had been observed by Suh and Lee (2006), the number or bulblets produced per scale in both lilium varieties used in the trial, were not affected by incubation temperatures, however the diameter of 'Casablanca' bulblets were icreased as temperatures was increased from 20 °C to 30 °C. On the other hand, treatments have not any statistically significant effect on bulblet formation ratio, but there were only some quantitative differences between the treatments (Figure 14).

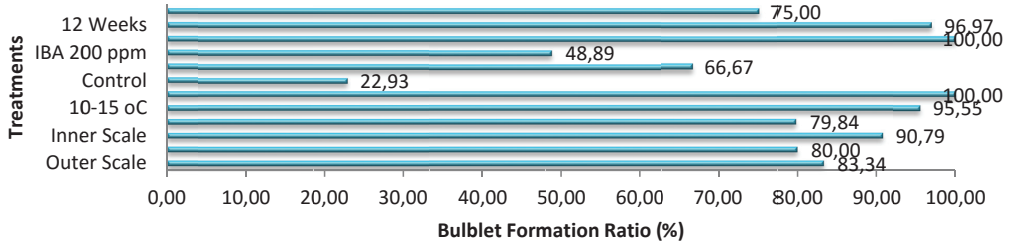

Figure 14. Changes on bulblet formation according to treatments.

The correlation coefficients related in bulblet characteristics are given in Table 5. According to this results, the strongest relationship (r=0.841) in *L. candidum* was found between the bulb diameter and bulb weight. In spite of this, there was a positive correlation between bulblet height (r=0.770) and bulblet weight and also between bulblet height (r=0.639) and bulblet diameter, while there was a negatively weak relationship (r=-0.189) between root length of bulblet and bulblet number per scale (Table 5).

Table 5. Correlation between the bulblet characteristics of *Lilium candidum*

	Bulblet Formation Ratio	Bulblet Number per Scale	Bulblet Weight	Bulblet Diameter	Bulblet Height	Bulblet Scale Number	Root Number of Bulblet	Root Lenght of Bulblet
Bulblet Formation Ratio	1							
Bulblet Number per Scale	-,008	1						
Bulblet Weight	,306	,100	1					
Bulblet Diameter	,247	,138	**,841****	1				
Bulblet Height	-,092	,108	**,770****	**,639****	1			
Bulblet Scale Number	,500	-,028	**,631****	**,609****	**,595****	1		
Root Number of Bulblet	,060	,016	**,448****	**,436****	**,291****	**,462****	1	
Root Lenght of Bulblet	,233	**-,189***	,180	,093	,040	,162	**,364****	1

*. Correlation is significant at the 0.05 level
**. Correlation is significant at the 0.01 level

Consequently, there were some positive relations too between bulblet scale number with bulblet weight (r=0.631), bulblet diameter (r=0.609) and bulblet height (r=0.595) respectively. Similar relations were found between root number of bulblet with bulblet weight (r=0.448), bulblet diameter (r=0.436), bulblet height (r=0.291) and also with bulblet scale number (r=0.462) respectively. It is seen on Table 5 that, there was an another relation observed between root length of bulblet with root number of bulblet (r=0.364).

CONCLUSIONS

This research was focused on the effects of different treatments, such as incubation period, incubation temperature, auxin doses and scale position on bulblet formation and characteristics with scale propagation in 'Madonna Lily' (*Lilium candidum*).

The overall results indicate that, treatments have not any significant effect on bulblet formation ratio, but there were significant differences between the other bulblet characteristics for some of treatments. Especially for the incubation period, 14 weeks gives the highest average value for bulblet number per scale and bulblet height. However, study results shows that 10 week is sufficient for an incubation period. We conclude that, the highest average value of bulblet weight and bulblet diameter were determined in outer scales. So, it was cleared that outer scales are the best propagating material, in addition to, middle scales could be used for propagation. While incubation temperature of 10-15 ^{0}C gives the highest value for bulblet scale number, 20-25 ^{o}C was more effective for bulblet diameter and root length of bulblet. Also, importance of auxins on rooting factors were understood once again with results of the study. As a conclusion it was seen that, different approaches and proper methods were necessary for scaling procedure in *L. candidum*. However, for geophytes there was not any specific information about formation, growth and development of new bulblets.

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LANDSCAPE DESIGN PROJECT OF HISTORICAL PLACES: CASE STUDY OF BIGA MUNICIPALITY

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Abstract

The army that frequently campaigned at the time of the Ottoman Empire performed the salaat at the open-air places founded for worship called Namazgah. Later, these places became the sea of points for the people who went on a pilgrimage or went to become soldiers, and used for isha or tarawih prayers, too. In this study, the stages of the project of Biga Municipality in order for Namazgah, which is a historical and cultural heritage from the Ottoman Era. For this purpose, having functional restoration and using the space close by as a recreation area have been explained. The detailed information about the project and its location have been obtained from the authorities of the Biga Municipality before designing this project. Furthermore, the expected problems and the main concept of the project have taken into consideration while running this project. In the results of this project, a new and restored landscape look has been given to the old Namazgah so that the residents of Hamidiye (Biga District) and its neighborhood could perform their worships easily after the completion of the project.

Key words*: Biga Municipality, Canakkale, Namazgah (Open-Air Prayer Place), landscape project*

INTRODUCTION

In past, many Namazgah and worshipping places have been built for the purpose to bring the common people together, share their daily activities as well as could perform their prayers and other religious duties. Such kinds of activities are still being continued from ancient age till today (Cayci et al., 2012). These venues have been constructed in the form of open as well as closed common places (Berktay and Atasoy, 2007)

The concept and shape of worship vary among religions. Churches and synagogues have been constructed for ritual pracitces and ceremonies in Christianity and Judaism (Dikme, 2012). In Islam, it is often mentioned about the importance of prayer in the Qur'an as well as the Hadith. That is why, places have been built intended for this worship (MRA, 2014). Muslims, from the first age, used every clean place for worship (Sems, 2012). The last Prophet Muhammad (P.B.U.H) also performed his prayers in clean open places. Sometimes, the Friday and Eid prayers were performed by the Prophet Muhammad (P.B.U.H) in open areas near the Masjid-e-Nabvi and later, these open places were converted into a Namazgah (Can, 2008). Islam was one of the dominant religion of the Ottoman Empire along with other monotheistic religion such as Judaism and Christianity, where their followers continued to live without any hardship through a proper public system. A speedy increase began in the number of worship places at the beginning of the 16th century by the time of Yavuz Sultan Selim as a result of transfer of caliphate to Ottoman Dynasty (Bas, 2011). Ottoman Sultans, guided themselves to the Prophet Muhammad (P.B.U.H) in many aspects and continued their Namazgah tradition and constructed Namazgah in open areas next to the closed worship places. The Ottoman army that frequently campaigned, constituted Namazgah at appropriate places so that they could perform the salaat. After that, these places became the sea of points for those people who went on a pilgrimage or army training, and such places were also used for isha or tarawih prayers. These places have been established by taking consideration to the cities, towns, caravan routes, picnic points and the major intersections of army shipment along with the premises that eliminate the needs of common people. Fountains, wells and ablution lavatories must be added next to these places for proper cleanliness which is a precondition of worship in Islam religion. In summers, the areas where

large trees were found have been selected as Namazgah because they needed to be cool for worshipers. But those trees are not found further more for providing shades to the worshipers (Kurum, 2007; Bayraktar, 2012). That is why, new trees were planted in those area with the help of this project.

Biga has mostly undergone a calm process during the Ottoman domination. It has become a transit route and also led an important strategical position of Ottoman forces while moving to Rumelia and Western Anatolia. During the death of Chalabi Mehmed, it was decided to assemble the Anatolian Beylerbeyi forces in Biga before moving to Izmir Beyi walking on the Cuneyd Bey, and the movement has been started from here. Anatolian Beylerbeyi forces have been gathered in the Biga Plain for moving to Rumelia both during participating in navy campaigns as well as in the campaign conducted for European countries. The army, accommodated in Biga, would prepare itself for the campaign and performed their salaat (prayers) in open places. The remnant of a Namazgah, established in ancient times, is still found today in Hamdibey District (Biga Municipality, 2014).

The first salaat, during the conquest of Biga (1344-1354-1364, the conquest of Biga is stated as different in various sources but the year of 1354 is largely being considered), was performed in this Namazgah due to none availability of Masjid in Biga. An original Mihrab which was made out of marble is found into the wall of Qibla. Until recently sound stairs of the Minbar (pulpit) couldn't get through to the present day. In Namazgah, a single decorated marble stone was found between Minbar and Mihrab which is inserted into the masonry wall. Having a water-filled pot onto it provided for the consumption of birds. As embossment, it is similar to a badge motif. In past, this stone has been brought and fixed there aimed to stop the steeling attempts into the territory of Namazgah. That stone has been placed upside and down before this project but it has been put in a correct and suitable place after the restoration of the Namazgah. After that the birds have lost the water supply functional setup (Anonymous, 2014).

The extant, this place carrying historical and cultural heritage characteristics has been destroyed, and it has lost the feature of a Namazgah. In this study, it is aimed to give information about the prepared landscape project that ensures the performance of the salaat worship again in this area by protecting the aesthetic and materialistic features of the area. Design phases of the project and its usage has been included in these phases when becoming a part of this project.

MATERIALS AND METHODS

The study area is located within 90 km from the center of Çanakkale province in Biga (Figure 1). The area, situated in Hamdibey District, is 0.5 km away from the center of Biga and 64 m above from sea level. The project area is surrounded by Hıdır and Namazlık Street in north, Şehitlik and Sümer Street in south, Yaprak Street in west and Asmalı Street in its eastern side.

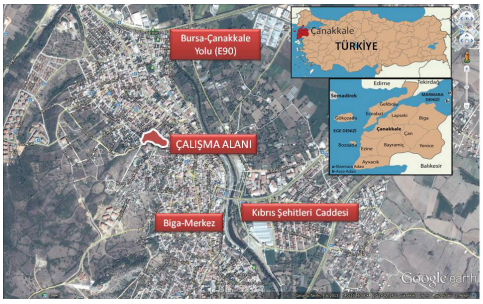

Figure 1. Location of the Study Area (Original picture taken by the author in 2014)

In this study; topographic data and coordinate information of the area have been obtained from the Housing Authority of Biga Municipality by measuring the field with a GPS station due to the insufficient slope and plan data related to the project area. The data relating to the previous history of the study area as well as the location of the Biga have also been examined. The documents relating to the construction features and the short histories of Namazgah were utilized. The information have been obtained by interviewing with the municipal authorities and the taken photographs related to project area were also used as materials. Autodesk, AutoCAD 2014

and Sketchup Pro 2012 programs were used during the creation of project.

The methodology of this study has consisted in observation, assessment, data collection and analysis phases. The studies regarding to our project have been elaborated with 6 subheadings, given below, taking help and benefits from the following studies: of Korkut (Korkut, 2002) landscape architecture studies have been done of Korkut (Korkut, 2002), landscape design process of Uckumbet Parki of Yilmaz and Yilmaz (Yilmaz and Yilmaz, 2009), outdoor spaces of the public offices and institutes examined of Atabeyoglu and Bulut (Atabeyoglu and Bulut, 2007), design of the parking and ceremonial areas of Tekirdag Governorship handled of Sisman et al (Sisman et al., 2008), design process of the Rector's House of Bartın University operated of Celikyay (Celikyay, 2011), the landscape design of the office of Erzurum Metropolitan Municipality of Turgut (Turgut, 2011), landscape designing in the gardens of primary education level schools of Kiper and Karakaya (Kiper and Karakaya, 2013), design of the campus of Social Sciences Vocational High School handled of Atabeyoglu (Atabeyoglu, 2014) and opportunities for design approaches in landscape planning described of Bartlett et al (Bartlett et al., 2014).

- Requirements determination of project's owner
- Site selection, objectives and identification of problems
- Data collection intended to determine the current situation (survey and literature studies)
- Legal status, problems originating from area, identification of limitations related to the construction and financial matters and sharing them with project's owner
- Creating functional schemes with analysis of area and its surroundings
- Application project (structural and planting design)

RESULTS AND DISCUSSIONS

The basic aim of this project work is to redesign and restart the activities of the Namazgah that is being found idle for a long time and facing a continuous destruction and open for looting by the unknown people. The different phases of this study are given below in detail:

Requirements determination of project's owner

It is required to re-arrange and regulate the Namazgah along with its surroundings where the first salaat was performed during the conquest of Biga, and also being used frequently for prayers in the Ottoman period. It is required that the properties of Namazgah should be adhered to its actual form and used materials till the formation of places during the restoration to its former function of this idle area.

Site selection, objectives and identification of problems

The most important limiting factor for a designer is the need of large openings to Namazgah. In the project area, it is not possible to create further place/structure fiction by giving patterns with ground materials. In case of excessive slope of some sections in the outside rest area of Namazgah causes difficulties. Also created sufficient area for the usage of common people. Although the high impact of view; transportation and wind factors were also found among necessary problems. And then put some solution to solve such problems. Investigations were carried out in the area prior to start the project work. For this purpose the requirements and desires of municipality have been taken into account. The major problems which have been faced during planning this project are given below:

- The inter-street access roads are narrow and winding because of the area is at a distance near to the city center. The crowd tries to reach this area for performing their daily worships collectively (that is called 'Cemaat' in Turkish).
- There is no sufficient space for parking around the area.
- Recreational area outside of the Namazgah, and the closed and semi-open places of this area must be constructed by taking into account the sloppiness of the area. This situation plays a limiting role in some points of the area.

- Some historical remnants are found with a portion of the boundary wall that needs to be preserved. Such a condition creates limitations while designing the area.
- Security vulnerabilities, like falling and slipping, are found in such recreational areas where the effect of view is much high.
- The necessity of leaving open space for salaat along with making pausing points, and the circulation will be created by new comers to the area along with the people waiting for salaat, and those who taking ablution and doing preliminary preparation for salaat inside the Namazgah create opponent interaction.
- It reveals the necessity of appropriate use of the project period, selection of natural materials and an area having historical features.
- It brings the necessity of making slope supporting walls in both areas (Namazgah and recreational).
- Creating terraces in the recreational area by getting benefit from slope in order to prevent the reduction of the effect of project site.
- Inadequacy of enlargement of the roads and other places found in project area and its vicinity.
- There would be bottlenecks related to the project i.e.; drainage and runoff caused by rain in the project area.

Data collection intended to determine the current situation (survey and literature studies)

Figure 2 shows the area where the data have been collected and also used as area of this project.

Figure 2. Study Area and Its Vicinity (Google Earth)

Access to area has been provided with the help of different street links. In spite of that, the structure and widths of the road by using one-way applications. Shrinking of different places created problems and made impossible the construction of maneuver of project area. Figure 3 shows the location of project area situated on a sloping hill that has been called as an "angle of the excellent". This location had been used by the army personnel for preparation before any war campaign during the Ottoman Period. Despite of slope, the Ottoman Empire army does not link with the information for the ground about its suitable characteristics. The location is with a constant breeze. In the present, lighting is very insufficient and security weaknesses are constituted.

Figure 3. A View from the Study Area (Original picture taken by the author in 2014)

Legal status, problems originating from area, identification of limitations of construction, financial matters and sharing them with project's owner

In the 6th Article of No. 2863 Law of Protection of Cultural and Natural Assets, the conservation required immovable and natural heritage are described as follows:

a) Immovable properties were made up to the end of the 19th century with conservation required natural assets,

b) Immovable properties were made after the determined data in terms of their importance and characteristics are required to be protected by the Ministry of Culture and Tourism,

c) Immovable cultural properties situated within a protected area,

d) Buildings and common places were considered as the great historical assets of the National Defense authority. The establishment of the Republic of Turkey along with those

historical houses related to the founder of Turkey, Mustafa Kemal ATATÜRK without the concept of time and registration. The measurements of our national history would be considered to be determined by the help of this project.

However, the architectural, historical, aesthetic, archaeological and other important features of the Namazgah and its vicinities are required to be protected under the terms of the decision of Protection Councils. They were not counted as conservation of required immovable cultural assets. Rock tombs, written, pictorial and embossed rocks, illustrated caves, mound, tumuli, historical sites, acropolis and necropolises; castles, fortresses, bastion, rampart, historical military posts, redoubt and barracks with fixed weapons found inside them; ruins, caravanserais, inns, baths and madrasahs; domes, shrines and inscriptions, bridges, aqueducts, waterways, cistern and wells; remnants of historical routes, milestones, perforated stones indicating the old boundaries, stone monument; altar, shipyards, docks; historical palaces, mansions, houses, waterside residences and villas; mosques, masjids, musallas, namazgah; public fountains, alms house, mint, hospital, place for timekeeper, dervish lodges; graveyards, ottoman bazaar, covered turkish bazaar, grand bazaar, synagogues, temples, covered markets, basilicas, churches, monasteries; mosque complexes, ancient monuments and ruin walls; wall paintings, embossments, mosaics, fairy chimneys and other similar properties are included among the examples of immovable cultural properties.

Historical caves, rock shelters; trees and group of trees that show some kind of special characteristics are from the examples of immovable natural heritages.

The municipal authorities were informed about the identified problems related to area along with the relevant legal regulations, and the necessary permissions intended for construction and restoration have been provided. Financial concerns and application based building difficulties have also been submitted to the relevant authorities according to the results of the survey work by considering the physical properties of the area.

Creating functional schemes with analysis of area and its surroundings

The usage areas will be created during the design phase that have been identified within the need-based program, in the light of results of obtained data from written documents and observations made on the spot. The utilizations, according to different needs, include the fitting solutions and the shaped place are given below:

- Flat and wide hard ground to be made inside of Namazgah for performing the salaat
- Fountain
- Mihrab
- Minbar (Pulpit)
- Minaret
- Restoration of the Namazgah boundary wall
- Waiting place before salaat
- Historical archway
- Planting intended for Shade
- Allowing the parking maneuver and parking for vehicles such as buses, those coming to the area, if necessary.
- Planting to reduce the effect of wind on the recreational area
- Semi-closed sitting and resting units
- Walking and strolling tracks

Application project

The application project has been prepared in order to ensure the protection of the historical and cultural texture of the revised Namazgah and elaboration with intended to the implementation of recreational areas that found on the sloping area next to Namazgah (Figure 4 & 5).

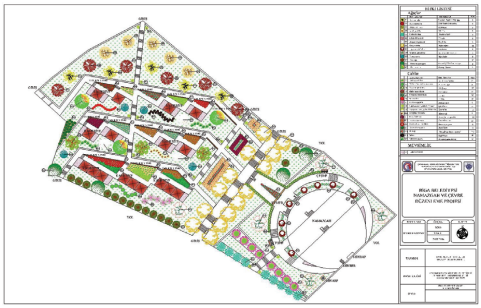

Figure 4. Application Project (by Authors)

Figure 5. Application Project 3D (by Authors)

A crescent-shaped axle has been designed in the Namazgah area consisted of 3027 m^2 for its internal transportation. This axle is connected to the junction of Sümer and Şehitlik Streets from its southwest and to the recreational area with an arched entrance from its northwest direction. The center of the crescent has been divided into 3 levels including obstructed ramp and stairs, and left as hard ground (Figure 6).

Figure 6. A View from the Area of Namazgah (by Authors)

A fountain was placed for ablution on the left side of the entrance to recreational area, and designed from marble and natural stone by considering the direction of Qibla and preserving the original locations of mihrab and minbar. A minaret has been placed beside the minbar symbolically. The walls with most of their destroyed parts and surrounding the Namazgah were constructed again over the entire area by protecting their original heights.
Ginkgo biloba (Maidenhair tree) is used on both sides and *Robinia pseudoacacia* (False acacia/Black locust) only on the left side of the arched door in transition to Namazgah from the recreational area (Figure 7). At the central part of the entrance of Namazgah an islet has been formed with the cultivation of the *Rosa sp.* (Rose) that is the symbol of affection, heard from the Prophet Muhammad (P.B.U.H) in Islam religion. *Buxus sempervirens* (Common box) was used as the boundary element along a line in the section of this part up to the first stairs. Roses (*Rosa sp.*) have been given place in between the *Lagerstroemia indica* (Crape myrtle) which planted inside of crescent without making narrow the area that is used for performing salaat.
Cedrus deodora has been used in its southwest entrance for the prevention of pollution and noise originating from vehicle transition as well as creating wind screen. *Cercis siliquastrum* was given place in the lower elevation of the same area along the row. *Cupressus mac. goldcrest* "Spiral" has been used in two points to highlight the entrance. *Magnolia grandiflora* was planted on the four corners of the area in order to create shadowed spaces, and grass fields were also created here.

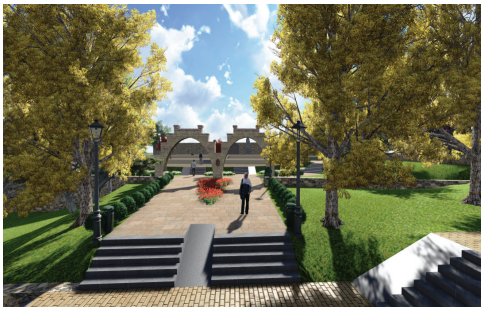

Figure 7. A View from the Area of Namazgah (by Authors)

Recreational area

The area allocated for the purpose of recreation is 5170 m^2. Four stages were made in the project area where the inclination of the area has been found into its peak. The first three stages were aimed to design for sitting and strolling and the last one is only intended for planting. A total of 17 arbors were placed in the area with the size of 4x4m, including 9 in the first stage and each 4 in the second and third stages (Figure 8).
The area is separated by a road that connects the Namazgah and the Sümer Street to Namazlık Street. Three connection paths have been created along this road for access to the

area. Plots containing *Gaura lindheimeri* (Gaura) and *Rosa sp*. (Rose) plants have been designed in rectangular form in the area found between these two roads. A single entrance was given to the area from its south. *Robinia pseudoacacia* (False acacia) has been added to the spaces along the street by protecting the existing pine species. Spaces found in the backside of arbors for sitting and strolling purposes have been arranged with different plant species by considering the differences of colors, textures and designs. In these spaces; *Berberis thunbergii atropurpurea* (Japanese barberry), *Cotinus coggygria* (Smoke tree/smoke bush), *Forsythia intermedia* (Lynwood gold), *Viburnum opulus* (Guelder rose), *Spiraea vanhouttei* (Pink ice), *Cornus alba* (Dogwood) shrubs and trees were planted by using spiral pattern. *Tilia tomentosa*, *Albizia julibrissin*, *Eleagnus angustifolia*, and *Picea pungens* trees were used as solitary (Figure 9). *Cupressus mac. goldcrest "Spiral"* has been placed reciprocally along the middle transportation road in the area. The area found in the lowest level was planted by using *Acer negundo*, *Acer platanoides* and *Betula pendula* tree species (Figure 10).

Figure 8. A View from Recreational Area (by Authors)

Figure 9. A View from Recreational Area (by Authors)

Figure 10. Plantation Design of Namazgah and its Surroundings (by Authors)

CONCLUSIONS

Our country possesses an important historical and cultural background and a state her roots are based on long years. Despite of hosting many historical legacies, some of them were not protected by showing the necessary importance, and have reached to the point of extinction. Namazgah, one of the historical entrust of our nation over the years, have also not been protected. The area of Namazgah that situated in Hamidiye District of Biga town in Çanakkale had being used for accommodation, performing the salaat and other religious activities in time of the Ottoman Empire; and then in spite of its use by the local people, it has undergone to various damages until today.

We aimed to start the activities of this area again and protecting its historical texture. The area, found beside it, has been designed by taking benefit from its incline feature, also supported with sitting units consisted from arbor systems.

Latest materials in combination with the old designs of the Namazgah is tried to restore in this project. The project materials have been selected in the lights of the directions of the Turkish Ministry of the Protection of History and Cultural Heritages without their least side effects to nature. The vicinities and neighborhood areas of the Namazgah was being faced many difficulties before this project. This project was aimed to eliminate such problems and difficulties in the lights of their structural solutions by taking help from the information related to the project location from the authority of the Biga Municipality.

Firstly, this project was prepared and secondly presented to the authorized personnel of the Biga Municipality of Canakkale Province. Consequently, the residents of the above mentioned municipality started their collective worships easily, particularly in religious holidays and special occasions, in the newly restored Namazgah after the completion of this project. Moreover, the open public places, park site and other recreational activities were started next to the surroundings of Namazgah with the help of this project.

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THE INFLUENCE OF PRECEDING PLANT CULTIVATION ON GROWTH AND PHYSIOLOGY OF AN *OCIMUM BASILICUM* L. CULTIVAR

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Abstract

The paper aimed to assess some morphometric and physiological parameters of a purple leaved Ocimum basilicum L. cultivar plants grown in substrates in which other plants (a green leaved Ocimum basilicum L. and Armoracia rusticana Gaertn. Mey. & Scherb.) were grown. The number of lateral stems and leaves and the mass of plants were positively influenced by cultivation after the other plants. Photosynthesis and transpiration rates of the same plants decreased, however the chlorophyll fluorescence parameters (Fv/Fm and ϕPSII) did not reveal a major influence on the photosynthetic apparatus. Chlorophyll and total phenolic contents decreased in plants grown after green basil and increased in plants grown after horseradish. The results show that basil can be grown in the same substrate used by the tested species with positive influences on growth.

Key words*: biometry, photosynthesis, chlorophyll fluorescence, substrate, horseradish.*

INTRODUCTION

Ocimum basilicum L. known as sweet basil belongs to *Lamiaceae* family, *Ocimum* genus, which includes approximately 60 species. It is a valuable plant species with multiple uses in medicine, cosmetics and gastronomy. Apart from the flavoring properties, the basil has antimicrobial, insecticidal, antioxidant, anti-inflammatory etc. activities (Putievsky and Galambosi, 1999). The properties of this species are mainly owed to the essential oils it synthesizes and to the large infraspecific variability with numerous phenotypes and also chemo-types (Grayer et al., 1996). More than 100 sweet basil cultivars exist that differ in leaf shape, color, height and odor. Originated in subtropical areas, where it can be grown either as an annual or perennial plant, basil is cultivated all over the world as annual plant. Due to its economic importance basil is cultivated on large areas and, thus, the basil crops have to meet the current requirements for agricultural sustainability. Also, as a medicinal and culinary herb, the value of the basil increases when cultivated under organic conditions. Such conditions include crop rotation, intercropping or organic fertilization. The cultivation parameters for basil have been largely investigated, with an emphasis on nitrogen, phosphorus and potassium requirements, time of seeding, distance between plants and rows, soil type, temperature, weeding strategies and pest control (Simon 1996; Meyers, 2003; Arabaci and Bairam, 2004). It is also known that the basil should be rotated once every 4 years and that the most suited preceding crops are cereals and legumes (Pârvu, 2002). However, the influence of the preceding crops on basil physiology and biochemistry has been less studied, rather the species for which basil can be a companion are known, such as tomatoes or marigold (Meyers, 2003; Tringovska et al. 2015).

The effects of preceding crops or intercropping species on other species are mainly due to the substances released in the soil by either leaf litter or roots. Such substances range from high molecular mass compounds such as proteins and polysaccharides, enzymes such as acid phosphatase, nucleases, invertases (Chang and Bandurski, 1964; Chhonkar and Tarafdar, 1981) to low molecular weight phenolic acids (Vaughan et al., 1994). The root exudates, when deposited in soil, a process also known as rhizodeposition, influence the microbiota of the soil and also the growth and development of surrounding plants. The exuded substances may have an effect on proximate plants physiological processes, protein synthesis or

enzyme functioning (Bertin et al., 2003; Farooq et al., 2011).

The present paper aimed to test whether basil grown on substrates following other plants is influenced at a physiological and biochemical level in its development. Prior to cultivating a purple leaved basil cultivar, another, green leaved basil cultivar or horseradish (*Armoracia rusticana* Gaertn. Mey. & Scherb.) were grown in the same substrates. *Armoracia rusticana* is a crop specie grown for culinary uses, due to its pungent taste which is given by glucosinolates and their transformation products such as isothiocyanates, but also for medicinal purposes, with proven in vitro anti-inflammatory activity (Yamaguchi, 2012; Marzocco et al., 2015). The specie presents an increasing demand on various markets and is also used in crop rotation for preventing potato and tomato crops pests (Filipović et al., 2015). Horseradish is a specie which is known to exert negative influences on the surrounding plants due to the root exudates it synthesizes (Dias and Moreiro, 1988; Itani et al., 2013). Several parameters were evaluated, to assess the opportunity of cultivating sweet basil in a culture comprising different basil cultivars or other spices which could influence the succeeding crops due to chemicals secreted in soil.

MATERIALS AND METHODS

Plant material

The tested specie was *Ocimum basilicum* L. cv. "Violet de Buzau", which was grown from seeds obtained from the Agricultural Research and Development Station at Buzau, Romania. The species grown prior to this cultivar in the substrate were *Ocimum basilicum* L. cv. "Aromat de Buzau", obtained from the same source and grown from seeds and *Armoracia rusticana*, planted as one year old roots obtained from local suppliers.

Experimental conditions

For the growth of plants, 4 L (15 cm height x 18 cm diameter) plastic pots were used. Pots were filled with 3 L of a mixture composed of commercial soil (60% v/v), peat moss (30% v/v) and perlite (10% v/v). For each variant, 3 pots were used.

Three experimental variants were set up: pots with purple basil seeded in substrate mixture alone (control plants), in substrate mixture in which green basil (4 plants per pot) was previously grown and in substrate mixture in which horseradish (4 plants per pot) was previously grown. Both the green basil cultivar and the horseradish plants were grown for approximately 3 months, period which, in the case of basil, coincided with the fruiting stage. After this period, the green basil and the horseradish plants were removed manually from the pots. Each pot was seeded with 10 purple basil seeds. The plants were thinned at 4 individuals per pot after 1 week.

The pots were kept at constant temperature regimes, between 22° C (night) and 25° C (day). Artificial light was supplied by 4800K fluorescent tubes for 14 h each 24 h. The atmospheric humidity was relatively constant, around 50%. Plants were irrigated twice a week with distilled water. The growth of purple basil lasted for 3 months, until the fruiting stage of plants.

Morphometrical assessments

For each variant, 12 individuals were assessed for stem height, number of leaves, number of lateral stems, number of inflorescences and mass of fresh plants.

Physiological measurements

The photosynthetic activity, transpiration rates and stomatal conductance were measured with an ADC Bioscientific LCi apparatus, on 3 leaves (from the lower, middle and upper regions of the plants) from 3 individuals each per treatment, 5 readings per leaf. Measurements were done during the light regime.

Chlorophyll fluorescence was evaluated by measuring F_0, Fm, Fv, Fv/Fm, Fs, Fm', ϕPSII on 5 leaves per treatment. Fv/Fm was measured following a 20 minutes dark adaptation of leaves using provided clips. ϕPSII was measured at normal light regime.

Biochemical parameters

Assimilatory pigments were extracted from grounded leaves (approx. 0.1 g) using 80% aqueous acetone. The absorbance of extracts were recorded at 470, 646 and 663 nm with a

Shimadzu UV 1240 spectrophotometer in 1 cm light path length glass cuvettes. Pigment contents were calculated using Wellburn (1994) equations.

The total phenolic contents were determined following the method described in Herald et al. (2012). An aliquot of 0.1 ml of 5% (w/v) extracts in 30% ethanol was mixed with Folin reagent, incubated for 5 min, Na_2CO_3 7.5% was added and incubated for further 90 min. Results were calculated using absorbance at 760 nm and expressed as gallic acid equivalents per gram fresh weight.

Total flavonoids were assessed in the same extracts using the $AlCl_3$ method and expressed as quercetin equivalents per gram fresh weight while antioxidant activity of extracts was determined according to the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method (Herald et al. 2012).

The statistical analyses conducted were represented by analyses of variance among treatments and the Tukey post hoc test at $p<0.05$, the results being expressed as means and standard errors.

RESULTS AND DISCUSSIONS

Morphometry

From a morphometric point of view, the analyzed parameters of the purple basil plants recorded certain significant variations. An increase in the number of lateral stems (Fig. 1) and leaves (Table 1) were recorded for plants grown after green basil. For the same plants, a significant decrease in height was recorded, thus the plants presented a more compact habitus.

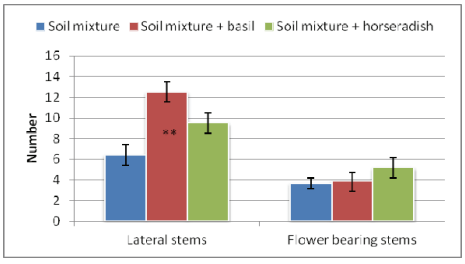

Figure 1. Number of lateral stems and flower bearing stems of purple basil plants
(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

Increases in mass and number of leaves, although not significant, were recorded also for plants grown after horseradish. The number of flower bearing stems was not significantly modified in any of variants.

Table 1. Morphometric indices of purple basil plants

Treatments / Parameters	Height (cm)	No. leaves	Mass (g)
Soil mixture	74.5±3.07	119.33±9.36	22.9±1.48
Soil mixture + basil	53.9±2.08**	188±14.11**	27.3±3.61
Soil mixture + horseradish	71.17±4.3**	133.83±9.19**	29.38±3.4

(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

Physiological analyses

The photosynthetic rate of purple basil plants significantly decreased when grown after the green basil cultivar and it remained within similar values with control plants when grown after horseradish plants (Table 2). Transpiration rates decreased significantly for both purple basil individuals grown after green basil and for those grown after horseradish plants.

Table 2. Physiological parameters of purple basil plants

Treatment/ Parameter	Photosynthesis rate (µmolls $CO_2/m^2/s$)	Transpiration rate (molls $H_2O/m^2/s$)	Stomatal conductance (molls/m^2/s)
Soil mixture	2.31±0.1	2.9±0.15	0.25±0.02
Soil mixture + basil	1.45±0.14**	1.67±0.15**	0.1±0.02
Soil mixture + horseradish	2.26±0.15	2.41±0.1*	0.35±0.22

(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

The stomatal conductance (Table 2), although not statistically significant, was lower in basil plants grown after green basil and higher when grown after horseradish plants compared to control plants.

Chlorophyll fluorescence measured in the light adapted state (ϕPSII) increased in a significant manner in both purple basil plants grown after green basil and also grown after horseradish (Table 3).

The higher values of ϕPSII occurred due to significantly lower values in both Fs and Fm' for the two variants compared to the control.

Table 3. Chlorophyll fluorescence parameters in purple basil plants

Treatments /Parameters	Soil mixture	Soil mixture + basil	Soil mixture + horseradish
F_0	22.4±1.87	23.8±5.11	28±2.35
Fm	246.4±10.92	236.6±27.88	255.8±27.86
Fv	224±9.59	212.8±24.74	227.8±25.89
Fv/Fm	0.91±0.01	0.9±0.02	0.89±0.01
Fs	300.5±17.99	113.5±10.2**	146.67±9.08**
Fm'	1461.08±90.93	623.58±40.27**	790.75±47.75**
PSII	0.79±0	0.82±0.01**	0.81±0*

(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

The Fv/Fm parameter, measured under a dark adapted state, recorded lower values, but not significantly, in treatments compared to the controls. These results are the effect of increased values of the F_0 parameter, although not significantly, in both variants compared to control plants.

Biochemical parameters

Among assimilatory pigments, the contents of chlorophyll *a* and carotenoids increased in purple basil grown after horseradish, while in the other cases, the contents remained similar compared with the controls (Fig. 2)

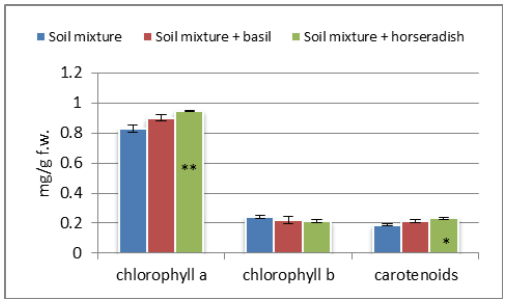

Figure 2. Assimilatory pigments contents in basil plants
(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

The total content of phenolic compounds and of flavonoids, determined in ethanolic extracts, increased in plants grown after horseradish compared to controls, with significance in the case of total phenolics. Purple basil plants grown after green basil registered a decrease of these parameters, significantly so in the case of flavonoids (Fig. 3).

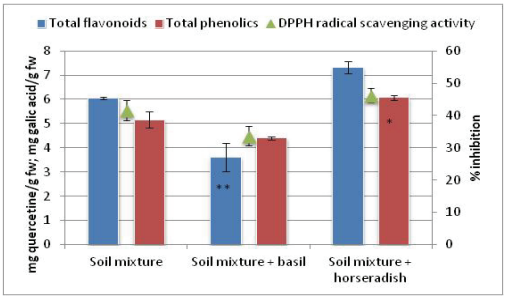

Figure 3. Total phenolics and flavonoid contents and free radical scavenging activity of purple basil plants 2.5% ethanolic extracts
(*-significant differences from control plants at $p < 0.05$; **-significant differences from control plants at $p < 0.01$).

These values were reflected also in the scavenging activity of the extracts, which was higher in plants grown after horseradish and lower in plants grown after green basil compared to control plants.

In crops, as in natural populations, the plants can influence each other through various processes. These include direct competition for light, water or nutrients, release of volatile compounds and soil mediated interactions. The soil represents a medium in which numerous substances are released, either by decomposition of plant material or by root exudates. These processes represent the basis for many agricultural practices that are included in organic farming systems, practices such as crop rotation, intercropping, mulching etc. (Farooq et al., 2011).

In the area around the roots, various substances can be found, originating from root exudates. These substances are represented by ions, oxygen, low molecular weight organic compounds (such as arabinose, glucose, oligosaccharides, amino acids such as arginine, asparagine, cysteine, glutamine etc. organic acids such as acetic, ascorbic, benzoic, ferulic, malic and phenolic compounds), as well as higher-molecular-weight compounds such as flavonoids, enzymes, fatty acids, growth regulators, nucleotides, tannins, carbohydrates,

steroids, terpenoids, alkaloids, polyacetylenes, and vitamins. Some of these compounds, especially the phenolics, influence the growth and development of surrounding plants and soil microorganisms (Bertin et al., 2003).

The basil roots are known, under certain conditions, to produce rosmarinic acid (Bais et al., 2002). Horseradish roots were proven to secrete, in the substrate, peroxidase (Willey, 2016), probably together with other compounds, as the horseradish root residues are known to be toxic to certain species such as lettuce (*Lactuca sativa*) (Dias and Moreira, 1986; Itani et al., 2013).

In the present study, the fresh mass of basil plants and the number of flowering stems increased (however not significantly), as did number of lateral stems and leaves (significantly) when cultivated after other plants, suggesting a better availability of nutrients in substrates. This type of effect is known to occur as a result of root exudates and biomolecules interacting with compounds present in soil. Root exudates can enhance the solubility of both anions and cations (the latter by the presence of organic acids), can release Al and Fe from compounds, can form nano precipitates and can alter the adsorption of toxic elements and nutrients on substrate particles (Violante and Caporale, 2015). It is considered that certain properties of root exudates can favor agricultural production by increasing nutrient availability (Gianfreda, 2015) and also that proper crop rotation can lead to a 20% increase in yield (Farooq et al., 2011).

A possible stress might have occurred in the case of plants grown after the green basil cultivar, as these plants had reduced height but the highest number of lateral stems and leaves.

Regarding the photosynthetic rates, the decrease in basil plants cultivated after the green basil cultivar may be attributed to the higher number of leaves, but with smaller area per leaf. This kind of effects can occur under the presence of certain organic compounds in substrates such as organic acids which can also determine reduced stomatal opening (Zhou and Yu, 2006), as was found in the present study. The same parameters were not significantly influenced in the case of basil plants grown after horseradish, suggesting that the composition of exudates does not affect the photosynthetic process. The reduced stomatal conductance values of plants grown after green basil may also explain the decrease in transpiration rates (Chapin, 1991). Although apparently the stomatal conductance increased in plants grown after horseradish, the large standard deviations of the mean value may explain the reduction in transpiration rates in the same plants.

Regarding the chlorophyll fluorescence, in our study, the basil plants recorded similar values among treatments for both Fv/Fm and ϕPSII parameters, although the slight increase in the latter was statistically significant. Fluorescence parameters are generally known to decrease under various types of stresses, including the presence in substrates of allelochemicals, as was recorded for *Cucumis sativus* after application of cinamic acid (Zhou and Yu, 2006) or for *Dactylis glomerata*, *Lolium perenne* and *Rumex acetosa* after application of benzoxazolin-2(3H)-one and cinamic acid (Hussain and Reigosa, 2011a) or *Lactuca sativa* when exposed to cinamic acid (Hussain and Reigosa, 2011b). However, other reports have shown that the effect on chlorophyll fluorescence of *Bidens pillosa* and *Lolium perenne* of applied plant leachates was concentration dependent (Rashid et al., 2010). Such concentration dependence is also known as “saw tooth effect” and is explained by the simultaneous influence of chemicals on various physiological processes and the interactions among these (Reigosa et al., 1999). Also, the effects of chemicals on physiological processes depend on the nature of the chemical and on the tested species. For instance, the same compounds (benzoxazolin-2(3H)-one and p-hydroxybenzoic acid) had no effect on the chlorophyll fluorescence of *Polygonum persicaria*, but significantly decrease the values for *Dactylis glomerata*, while ferulic acid had no influence in the case of both species (Reigosa et al., 2001). We could therefore assume that the treatments did not induce a significant stress in tested plants and the concentrations of potentially toxic compounds exuded were low.

Assimilatory pigment content of tested plants increased, significantly for chlorophyll *a* and carotenoids in plants grown after horseradish.

Chlorophyll content is known to increase with the availability of the nutrients as reviewed in Marschner (2011). This is the case for many species, such as wheat (Bojovic and Sojanovic, 2005), melissa (Sharafzadeh et al., 2016) and basil (Politycka and Golcz, 2004), thus further sustaining the idea of improved nutrient availability in the substrates.

The content of phenolic compounds in plants is subjected to biotic and abiotic factors such as light, nutrient, salt stresses or pathogen interaction (Waśkiewicz et al., 2012). Phenolic contents can increase under fertilization of plants, as was observed in the case of basil (Scagel and Lee, 2012). However, a role as growth regulators was suggested for certain phenolics, with positive correlations observed between the amount of phenolics in tissues and plant growth (Kefeli and Kutacek, 1977). Since the basil plants grown after horseradish registered higher amount of phenolics and also increased plant height, while plants grown after green basil registered lower amounts of phenolics and reduced stem height compared to controls, such a hypothesis should be further investigated. Also, the different contents of phenolics in the plants further suggest the different nature of chemicals present in the substrates and their differential effect on basil plants. As a consequence of the amount of phenolics, the antioxidant activity of basil extracts varied accordingly, with higher scavenging ability for plants grown after horseradish, a correlation between these two parameters being proved for many species, including for basil (Juliani and Simon, 2002).

Overall, the data obtained for the growth of purple basil in the present study, indicate that basil can be successfully grown both after other basil cultivars as well as after horseradish. The positive effects on the growth of basil can be attributed to the presence in the substrate of exuded organic compounds such as organic acids and enzymes. Organic acids can improve nutrient availability (Violante and Caporale, 2015) while enzymes such as peroxidase can use organic toxic compounds as a substrate, degrading them and thus decreasing toxicity in the substrate (Vaughan et al., 1994). Although some exuded substances may influence physiological processes (photosynthesis, transpiration, stomatal conductance), cell division and elongation, membrane fluidity, protein synthesis, enzyme regulation (Farooq et al., 2011), the cultivation of several species simultaneously or in succession can positively contribute to the content of organic matter and nitrogen in the soil, improve water and nutrient availability and suppress weeds (Tringovska et al., 2015). *Armoracia rusticana* is a specie already used in crop rotation systems, which protects other crops from pests (Filipović et al., 2015) and it may exert other types of beneficial effects.

CONCLUSIONS

Ocimum basilicum L. plants can be grown on substrates where other basil or horseradish plants were previously grown with some beneficial effects, especially in the case of the latter. Positive effects are represented by increased morphological parameters values and bioactive compounds content. Further analyses are required to determine the type and the concentration of compounds excreted in substrate by roots and a more complete assessment of agronomic, physiological and biochemical parameters of plants grown in succession.

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OPPORTUNITIES FOR *EX*-SITU CONSERVATION OF *CYCLAMEN COUM* MILL. IN SINITE KAMANI NATURAL PARK, EASTERN BALKAN RANGE, BULGARIA

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Abstract

The protected specie Cyclamen coum Mill. is found on the territory of Sinite kamani Natural Park in Ablanovo, Chukata and before Enyova bulka areas. Main threats for its populations in the Park are the anthropogenic impact and the difficult seedling regeneration. For their conservation and for stabilizing their state, according to the assessments made, it is necessary to include both in-situ and ex-situ conservation measures. The objective of the present study is to develop a technology for growing Cyclamen coum from ripe seeds under laboratory conditions. To implement the objective numerous literary sources have been studied and biannual terrain studies of the population in Sinite kamani Natural Park have been conducted. The developed technology is successful and totally compliant with the ecological conditions of the natural habitats of the species in the Park. By applying it the species has been successfully reproduced in the scientific laboratories of the Faculty of Agriculture at Trakia University from ripe seeds to replenish the populations. The technology can be used for replenishing other natural populations of Cyclamen coum in Bulgaria as well. To establish the effectiveness of the implementation of this measure for ex-situ conservation, we need to continue observations after introducing the plants grown in laboratory conditions in order to trace their adaptation and further development and, if necessary, to take care of their conservation and stabilization of their state.

Key words: *Cyclamen coum, ex*-situ preservation, Sinite kamani Natural Park.

INTRODUCTION

Sinite kamani Natural Park is situated on the southern slopes of Eastern Balkan range, Bulgaria. Regardless of its relatively small territory – 11,308.8 hectares, over 25% of the vascular plants in Bulgarian flora have found shelter in it. This study is part of a project, one of the aims of which is *ex*-situ conservation of protected and endemic species inhabiting the territory of Sinite kamani Natural Park. According to preliminary biannual investigations conducted, one of the species whose preservation in the park requires the application of *ex*-situ conservation measures as well, is *Cyclamen coum* Mill. Main threats to the populations are anthropogenic impact (trampling, unregulated picking of its beautiful flowers for bouquets, eradication of tubers for ornamental and medicinal purposes) and difficult seedling regeneration. The species is protected by the Biological Diversity Act in Bulgaria (2002) included in Appendix I of the Convention on the Conservation of European Wildlife and Natural Habitats - Bern Convention (1979) and Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora CITES (1973). *Cyclamen coum* was reported for the first for the territory currently comprising Sinite kamani Natural Park by Urumov (1906) in Chukata area. The field was confirmed by Andreev (1981), in relation to developing the forest management plan of the park. Grozeva et al., (2014) found in 2013 a total of 3 populations of the species in the natural park in Ablanovo, Cukata and before Enyova bulka areas and the best, according to the assessment of their state, is the one in Ablanovo area.

The objective of the present study is to develop a technology for growing *Cyclamen coum* from ripe seeds under laboratory conditions in order to replenish and stabilize the species populations in Sinite kamani Natural Park.

MATERIALS AND METHODS

The study was conducted in 2014-2015. To develop the technology for growing *Cyclamen coum* various literary sources have been used (Hayek, 1929; Yankulov, 1964, 2000; Medicinal plants, 2001; Grozeva et al., 2004) and the data from conducted observations and analyses related to the implementation of project "Restoration of habitats and preservation of biodiversity conservation in Sinite kamani Natire park, Sliven, Bulgaria". The morphological characteristics of the species conforms to the one given in Flora of the People's Republic of Bulgaria (Peev, 1982) and the data from the terrain studies conducted during the vegetation periods of 2014-2015.

Laboratory studies have been conducted in the scientific research laboratories of the Faculty of Agriculture at Trakia University – Stara Zagora.

Ripe seeds were taken from the species population in Ablanovo area after obtaining a permit from the Minister of environment and waters.

When preparing the soil for sowing the seeds the data from the conducted soil analyses for each population were used (Grozeva et al., 2015).

All activities in collecting ripe seeds, their growing in laboratory conditions and taking the plants grown back into the natural populations of the species in Sinite kamani Natural Park conform to the Protected Areas Act (1998) and the Biological Diversity Act (2002).

RESULTS AND DISCUSSIONS

Morphology and biology of the species

Perennial herb with tuberous rootstocks and fibrous roots (Figure 1). Tuber is round, smooth, fleshy, usually 3-5 cm in diameter. Leaves rounded to kidney-shaped, a wavy folded edge and unclearly serrated with 9-16 cm long, bare stems coming directly from the tuber. They develop before or during flowering. Leaf lamina 2-4.4 cm long and 3.7-6 cm wide, bare on the top, green, often with white concentric contour or stain, and brown grainy underneath. The flowers grow in spring. Pedicel long, usually coiling spirally in fruit. Calyx 5 lobed, brown grainy, 5-8 mm long and 1.5-2 mm wide. Corolla back ovate, bare, rolled back, pointed at the top and without ears at the base, red-purple, 6-23 mm long, 5-11 mm wide. Stamens 5, inserted at base of corolla. Staminal handles extended at the base, glandular fibrous; anthers rounded at the base. The fruit slightly elongated, bare, 7 to 12 mm long and 7 to 10 mm wide, opening with 5-8 short triangular teeth. Seeds light brown with a reddish tinge, ± extended; 1.4-2 mm long, 1.2-1.8 mm wide, slightly verrucose surface. The number of seeds in a box is between 14 and 26.

Phenology

Flowering from late January to March. Fruiting from March to April. Propagated by seeds and vegetatively - by daughter tubers.

Habitat

The species forms populations in the bushes and oak forests in North-Eastern Bulgaria, The Black Sea coast, The Balkan Range (Central and Eastern), Thracian Plane, The Tundja Hilly Plane, The Strandja (Peev, 1982; Delipavlov and Cheshmedzhiev, 2003; Asyov and Petrova, 2012).

Figure 1. *Cyclamen coum* Mill. – general view.

Requirements to environmental factors

Cyclamen coum according to data by Peev (1982), Asyov and Petrova (2012) prefers light deciduous forests at an altitude up to 500 m. On the territory of Sinite kamani Natural Park in Chukata area population of the species was registered at an altitude of 729 m. Frost-hardy plant. With stands low temperatures during the winter-spring period. The species is demanding

to soil moisture during flowering. In summer withstand drought. No special requirements to soil fertility as long as they have light mechanical composition with good permeability. Our studies (Grozeva et al. 2014, 2015) show that in Sinite kamani Natural Park *Cyclamen coum* develops on cinnamon forest and leached cinnamon forest soils with acidic to neutral reaction of the soil solution.

Activities in growing *Cyclamen coum* from seeds under laboratory conditions

To reproduce the species from seeds under laboratory conditions, at the end of flowering phenophase and early fruiting phenophase isolation bags were placed on well-developed, healthy and abundantly flowering plants from the population in Ablanovo area in order to collect ripe seeds (Figure 2A).

Due to the fragility of the flower handles the bags were tailored on site and were not tied but wrapped around the handles (Figure 2B).

Seeds were collected in full maturity, which in the conditions of Eastern Balkan Mountain, Ablanovo area at altitude of 537 m, they reached in the first half of April. The bags with ripe seeds were taken and transferred to the research laboratories of the Faculty of Agriculture, where the seeds are separated from the fruit, cleaned from mechanical impurities and stored in paper bags (Figure 2C,D).

To accelerate their period of rest, the seeds were placed in a refrigerator at temperature 2-5 °C for 28 days. At 5-day periods the packages were taken out and inspected. Seeds were reviewed in appearance: color, smell, glitter, etc. Their health status in relation to pest and disease attack was determined by using microscope equipment (Figure 2E).

During the rest of the seeds the soil for their sowing was prepared in the scientific research laboratories at the Faculty of Agriculture. Previously soil samples were taken from the three populations of the species. Two soil varieties were registered: Cinnamon forest soil in Chukata area and near Enyova bulka area and Leached cinnamon forest soil in Ablanovo area. According to data about chemical composition and reaction of the studied soils is was found out that *Cyclamen coum* develops in soil with reaction of the soil solution from acidic to neutral, with low to medium humus content, poorly stocked with absorbable forms of nitrogen, medium to lower stock of absorbable phosphorus and well stocked with potassium compounds.

Favorable environment for the development of the species are soils with light mechanical composition with good permeability and the presence of powdery structure or skeletal elements has no significant influence. Soil mixture of 2 parts beech leaves, 1 part peat and 1 part sand was prepared with a similar mechanical composition to the soil in the natural habitats. Although the species is not demanding in terms of soil fertility, putrid manure was also added to the prepared soil in soil fertilizer ratio 2:1. The so prepared manure soil mixture was sifted to remove mechanical impurities and larger lumps and poured into terrines (Figure 2F).

Seeds were sown on 20 May one by one into the prepared moist soil and covered with about 2-3 mm of the same soil, moistened and placed in a dark place at a temperature of 15-18 °C (Figure 3A). They were regularly watered with distilled water with temperature 2-3 °C higher than that of air. On the 25th day after sowing the first seeds germinated, on the 30th day - the last ones. After germination of all seeds, containers were placed in light (Figure 3B).

Care was taken at the beginning of their development the small tubers formed to be fully in the soil. In order to obtain healthy and vigorous plants, average temperature of 16-18 °C and air humidity around 50 % were maintained and the difference between the day and night temperature did not exceed 5 °C. Care for plants comprised regular watering to ensure moderate humidity, weeding, airing during the day in order to harden the plants, inspection for attack by diseases and pests.

Figure 2. *Cyclamen coum* Mill.: A,B-placing the isolation bags; C-separating the seeds from fruit; D-cleaning the seeds from mechanical impurities; E-checking the health condition of the seeds; F-preparing the soil for sowing

To supply the needs of plants for sufficient nutrients single foliar supplementation with combined NPK fertilizer was applied when first leaf was formed (Figure 3C,D). Economically significant diseases and pests were not found, but if necessary, appropriate insecticides and fungicides could be used. From late August until September 12, gradually all plants grown from seeds went into a period of rest. The soil was slightly moistened periodically, air temperature 20-23 °C was maintained.

The return of the plants grown into the territory of Sinite kamani Natural Park was done during the period 10-16 October. They were planted in selected appropriate sections of the populations in Ablanovo, Chukata and after Enyova bulka areas, the soil being very well treated and cleaned beforehand (Figure 3E). After planting the plants were watered and covered with mulch (Figure 3F). During the next vegetation period from the second half of January to 18 February development of 100 % of the plants imported in populations of Ablanovo area, 95 % of those imported in the population after Enyova bulka area and 92 % of those in Chukata area was recorded. These data give reason to believe that the used *ex*-situ measure for conservation of *Cyclamen coum* is successful. For complete stabilization of all three populations of the species it is imperative to continue the process of filling them with plants grown from seeds. The construction of a

scientific research laboratory on the territory of Sinite kamani Natural Park would help to promote the conservation of this and other protected rare and endemic plant species on the territory of the park.

Figure 3. *Cyclamen coum* Mill.: A-sowing the seeds; B-seed germination; C,D-forming a first leaf; E-soil preparation for the return of plants; F-planting the plants.

CONCLUSIONS

The developed technology for growing *Cyclamen coum* from ripe seeds until formation of young tubers in laboratory conditions is successful and can be used to replenish the natural populations of the species on the territory of Sinite kamani Natural Park, as well as other populations of the same species in Bulgaria. To establish the full effectiveness of the application of this measure for *ex*-situ conservation, at least 3-year observations of the plants imported in the populations should be made, trace their adaptation and further development and, if necessary, take care of their conservation and stabilization.

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COMPARATIVE STUDY ON GROWTH AND DEVELOPMENT IN *TAGETES* GENUS

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Abstract

Tagetes species are well known and grown both in cities and in villages. They are appreciated for their long flowering from early summer to late autumn. Improvement of technological links to the species Tagetes in solar is one of the main concerns of flowers growers that yields high quality products and low production costs. As a result of widening the range of Tagetes varieties is constantly necessary of their study in comparative cultures to see how expressing the genetic heritage in areas where are grown.

Key words*: Tagetes, technological links, solarium.*

INTRODUCTION

About the total area planted with flowers in Romania today it is hard to say. In 1987 there were about 135 ha planted with flowers, greenhouses belonging to the state sector. Other 1,500 ha were recorded with flower field crops and nurseries. Areas planted with flowers, both in protected crops and out, were much bigger if we consider the private sector that work in this area of activity, but these did not recorded in official statistics (Şelaru, 2008).

Currently, the trade balance is negative (import is increasing and exports almost nonexistent). The main causes are the production decline due to outdated technologies, overcome assortment and, above all, a favoured commercial policy.

For Romania, the main problem is to align external quality standards, recover old markets and break into new ones through diversification of the assortment, upgrading, using seedlings. Floriculture future depends on how are provided: quality, quantity and production continuity.

Tagetes genus includes about 30 species of which only three annual species shows more interest in ornamental plants culture (Drăghia and Chelariu, 2011). They are appreciated for their long flowering period from early summer to late autumn, but also to control and combat *Pratylencus* nematodes in vegetable gardens in terms of environmental technologies without the use of insecticides. The results show the possibility to fight *Pratylenchus* nematodes with 40-70% depending on the used species (Prohab and Borcean, 2009).

Improvement of technological links to the species Tagetes in solar is one of the main concerns of flowers growers that yields high quality products and low production costs. As a result of widening the range of *Tagetes* varieties is constantly necessary of their study in comparative cultures to see how expressing the genetic heritage in areas where are grown.

MATERIALS AND METHODS

The experience was conducted in solarium conditions, in 2015, in Pitesti area, Argeş county. As biological material were used seven *Tagetes* hybrids, that represented the experimental variants as follows:

V_1-*Tagetes patula nana „Aton Spry”* (Figure 1)

V_2-*Tagetes patula nana „Aton Flamed”* (Figure 2)

V_3-*Tagetes patula nana „Aton Bee”* (Figure 3)

V_4-*Tagetes patula nana „Aton Orange”* (Figure 4)

V_5-*Tagetes patula nana „Durango Bolero”* (Figure 5)

V_6-*Tagetes erecta „Antigua Yellow”* (Figure 6)

V_7-*Tagetes erecta „Antigua Orange”*(Figure 7)

No studies have been made on these hybrids of *Tagetes*. During the growing season, 16.04-04.06.2015, there were applied proper care works. Biometric measurements have been made weekly from planting to sale: the height of plants, number of leaves, leaf length and width, also the number of flowers.

Figure 1. V_1 — *Tagetes patula nana „Aton Spry"*

Figure 2. V_2 — *Tagetes patula nana „Aton Flamed"*

Figure 3. V_3 — *Tagetes patula nana „Aton Bee"*

Figure 4. V_4 — *Tagetes patula nana „Aton Orange"*

Figure 5. V_5 — *Tagetes patula nana „Durango Bolero"*

Figure 6. V_6 — *Tagetes erecta „Antigua Yellow"*

Figure 7. V_7 – *Tagetes erecta „Antigua Orange"*

RESULTS AND DISCUSSIONS

It was determined the rate of growth in height of plants by taking measurements weekly from planting to sale (Figure 8). Growth in *Tagetes* varieties was different from one variety to another. The best was developed as expected a representative of the *Tagetes erecta* species namely *Tagetes erecta* "Antigua Orange" -V7 with 26.3 cm and the weakest *Tagetes patula nana* "Aton Durango Bolero"-V5 with 20.7 cm.

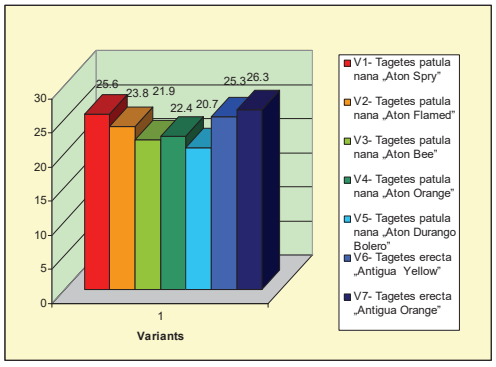

Figure 8. Growth in height of plants

The number of leaves per plant is shown in Figure 9.

At transplanting date (14.03.2015) all plants had the same number of leaves (2), but at the end of the measurements (4.06.2015) as we can see the number of leaves per plant varied depending on the variety.

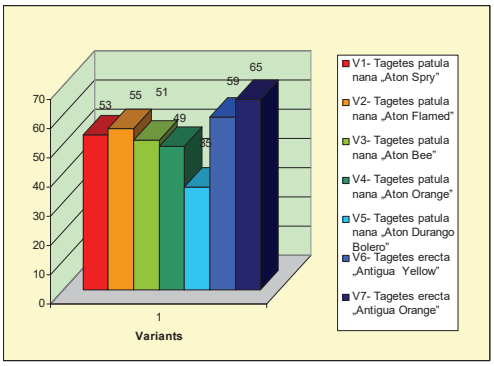

Figure 9. The total number of leaves per plant

The total number of leaves per plant was different from one variety to another and ranged between 35 leaves at V5- *Tagetes patula nana* – "Durango Bolero" and 65 leaves at V7- *Tagetes erecta* "Antigua Orange".

The number of flowers per plant varied from one variety to another (Figure 10). On 16.04.2015 flourished varieties of *Tagetes patula nana* and on 23.04.2015 flourished *Tagetes erecta* varieties, with flowers obviously higher than those of the species mentioned above.

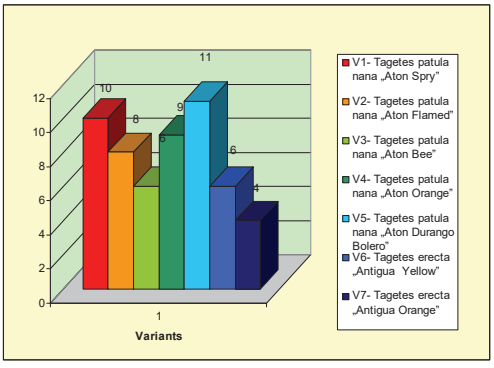

Figure 10. The number of flower per plants

The best results were registered for the variety *Tagetes patula nana* "Durango Bolero" by 11 flowers per plant, and the worst results were recorded in *Tagetes erecta* "Antigua Orange" with a maximum of 4 flowers per plant.

It is noted that representatives of the *Tagetes patula nana* had a larger number of flowers per plant compared with representatives of *Tagetes erecta*.

CONCLUSIONS

Comparative study of new varieties of *Tagetes* is required to recommend the most suitable varieties to growers according to their destination.

Rhythm of growth in plant height proves again that the species *Tagetes erecta* show higher growth compared to *Tagetes patula*.

It appears that at transplanting moment all plants have the same number of leaves, but at the end this character differs depending on the variety.

Antigua orange variety shows the best results in terms of this character, while the lowest values recorded in Durango Bolero variety.

The best results in case of flower number were registered for the variety *Tagetes patula nana* "Durango Bolero" by 11 flowers per plant, and the worst results were recorded in *Tagetes erecta* "Antigua Orange" with a maximum of 4 flowers per plant.

If you are looking for the highest plant with larger flowers we recommend the cultivation of the species *Tagetes erecta*, and if desired cultivation of varieties shorter but more abundant flowering we recommend the choice of the species *Tagetes patula nana*.

All varieties studied were characterized by good growth rate, high percentage of flowering and looked very attractive.

The study undertaken can confidently recommend cultivating of new varieties because they have high productivity, particularly commercial aspect and resistance to handling and transport.

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THE AESTHETIC CRITERION – COMPONENT FOR DESIGNING URBAN PUBLIC GREEN SPACE. STUDY BASED ON TWO ALTERNATIVES FOR IZVOR PARK, BUCHAREST

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Abstract

The design of urban green space is based on a series of principles, criteria and determinant factors for the quality of the urban public space. These criterion and principles are defined by multiple aspects: physical, functional, ambient, aesthetic and ecological, each of which are important in the configuration of the landscape arrangement project. The current work addresses the problem of the aesthetic criterion in landscape design by studying comparatively two project alternatives, each having a distinct compositional style and each representing a certain type of aesthetic vision for the organization of the physical space. These alternatives belong to a mixt composition style specific for the urban landscape design of the 20th century.

Key words*: urban green space, landscape design, aesthetic criterion*

INTRODUCTION

Izvor Park covers a surface of approximately 17 hectares and is located in the central area of Bucharest close to the People's Palace, while being framed by the roads: Splaiul Independenței, Izvor Coșbuc Street, B. P. Hașdeu Street and Mihai Vodă Street.
The park was partially developed between 1987 and 1988 as the first version of land vegetal "furnishing", followed by the selection of one of the 22 designing alternatives created for the park in the same period of 1988.
The 1989 revolution has brought to a stand the final design development and construction of the park. For the completion of the study alternatives, both the landscape function and the general compositional shape of space organization were considered, while the aesthetic criterion represented an essential component in designing this urban green entity. In landscape design the aesthetic criterion has direct final correspondence in the visual and ambient quality of the physical space. Therefore the importance of approaching the aesthetic criterion when developing a design is of maximum interest for the landscape architects.

MATERIALS AND METHODS

The research method used within the present work is that of comparative analysis of the two alternatives V_1 and V_2 in order to highlight the main aesthetic characteristics of both projects. These alternatives represent the actual study material of the work. It pursues the analysis of aesthetic composition elements which match two different aesthetic trends. Each of the two alternatives is the result of practical compliance within the project towards the landscape functionality attributed to this space.
The prevalent landscape function is that of promenade-rest, alongside the function of pedestrian transit and pedestrian connection between the adjacent roads of the site. The composition elements are alleys, water and vegetation, the latter being represented by ensemble arboreal vegetation, lawns, arboreal alignments and floral decorations (in alternative V_1). The park entrances and promenade alleys build the base compositional structure of the projects, which is further sustained by the other elements – water and vegetation – in the final aesthetic configuration of each alternative. In alternative V_1 water has a considerable presence, the surface of the water mirror holding approximately 40000 m^2 while

representing an important visual element of the designed space. In alternative V_2 water is present on much smaller surfaces in two circular basins located at alley intersections (small circuses); within this alternative water holds a surface of 630 m^2.

RESULTS AND DISCUSSIONS

Stylistically, the two study alternatives each belong to a distinct trend: alternative V_1 (Fig. 1) is composed of a geometrical style (Stănescu, 2011). All the elements – alleys, water, vegetation – take regular geometrical shapes, resulted from the association of straight lines as they highlight the main characteristics of this particular style (Mostaedi, 2004): tracks of straight alleys, alley intersections under 90° angles market with two decorative water basins which take circular shape. The surface of the water mirror is the dominant element of the composition and it also has rectangular shape with slightly smoothed edges. Vegetation is arranged wither in simple or double alignments along the alleys or in groups and bulks towards the outer areas of the park. Vegetation also includes floral decorations with geometrical shapes located alongside the main alleys of the park, which they enrich aesthetically and visually.

Alternative V_2 (Fig. 2) is composed of a free landscape style characterized mainly by network of alleys and the layout method for the vegetation (Stănescu, 2011). The alleys comprise of free shapes which are sinuous, comfortable, with a pleasant aspect; the two circuses representing the alley intersections are marked by two decorative water basins, circularly shaped, each having the surface of 315 m^2. The presence of water is much more discreet that in the other alternative, leaving more space for vegetation, which is the dominant characteristic of this design alternative. The central area of the park is reserved for wide spaces covered in lawn, while the outskirts are covered in groups and bulks of broadleaves and coniferouses (Kluckert, 2005). There are also present alignments in multiple sequences in order to create a real protection screen for the park against the rest of the urban area.

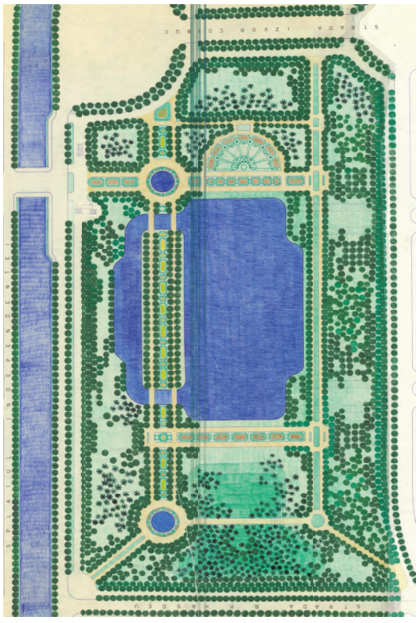

Figure 1. Alternative V_1

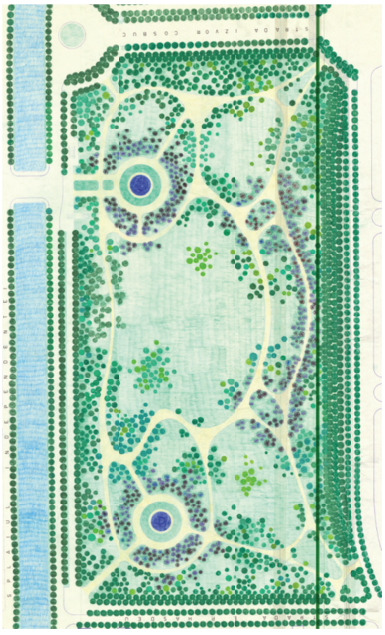

Figure 2. Alternative V_2

CONCLUSIONS

The current study was based on the analysis of two landscape design alternatives – V_1 and V_2. Alternative V_1 was considering the geometric architectural style specific for the 80' in this urban area of Bucharest, which is located near the People's Palace and is considered to be a representative area of the city. Alternative V_2 is characterized by a free landscape style which is much less drastic and strict.

It can be concluded that the compositional aesthetic principles applied in these projects prevail over the functional elements, but do not cancel them, but on the contrary sustain them.

The alternatives studied fall under two distinct style trends which are essential components of the aesthetic criterion in landscape design.

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EARLY MODERN GARDEN DESIGN ILLUSIONS AND DECEPTIONS. TWO DIFFERENT QUESTS FOR PARADISE - VILLA LANTE AT BAGNAIA AND VILLA ORSINI AT BOMARZO

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Abstract

Considered some of the most fascinating examples of Mannerist gardens in Italy, Villa Lante at Bagnaia and Villa Orsini at Bomarzo, were conceived in the late 16th century as a kind of landscape and architectural expressions of the Man's quest for the Lost Paradise. Although they were designed roughly in the same period of time, they differ completely in almost every sence, but, in the same time, they complete each other in many ways.
The following paper aims to compare the architectural layouts, planting designs and allegorical programmes of both gardens as to emphasize the way Paradise was seen in the late 16th century in Western Europe and to show two different landscape design mechanisms that try to mimic the quest for the (re)descovery of Eden.

Key words: *Paradise, garden philosophy, Mannerist, 16th century, Villa Lante, Bomarzo*

INTRODUCTION

If we can consider the garden to be an image – a figure, then, walking through the garden, through its paths may often be a real process of allegories - a process of figuration and most of all, especially in the Late Reneissance – a quest for finding God. Perhaps the most representative examples of this figurative process of some of the ideas the garden is trying to convey are the famous Italian Mannerist gardens of the sixteenth century; and out of these, two stand out - they both complement but also cancel each other; they intertwine, but are fundamentally different, they revolve around almost identical ideas and symbols, but convey different messages (apud. Lazzaro, 1980). The two gardens, Villa Lante from Bagnaia and Villa Orsini or Sacro Bosco from Bomarzo, build both on their own and together a universe translated into a microcosm of symbols, alchemical allegories and meta-linguistic messages – they tackle the theme of Man's return in Paradise but offer distinct solutions and variants:
"The Renaissance Universe is hierarchical, with God at the summit, human beeings in the center, nature below, and each part related to the other. The natural world was perceived in terms of its usefulness for human needs: plants and animals provide food and medicine. They also reflect human traits, virtues and beliefs and therefore serve as symbols – heraldic, moral, philosophical and religious. At the same time, the visible world correspondes with the divinely cosmos – the microcosmo reflects the macrocosm. To know this world is therefore to know God. " (Lazzaro, 1990)

MATERIALS AND METHODS

In order to understand the process of figuration and the quest to find the Lost Paradise that the two gardens promise, one must first closely observe all of the components of Villa Lante and of Villa Orsini. To this end, the following chapter aims to focus on reorganizing the gardens' elements in order to recompose the allegorical messages they intended to express when they were built.

Villa Lante and the Retrieval of Paradise

Villa Lante is probably the most interesting landscaping complex of the sixteenth century in Italy; it amazes through the architectural structure adopted in the garden, the use of slopes, water, by joining a *Hortus Conclusus* with a *bosco* etc. But the most spectacular part of this villa is precisely the philosophical story

it is trying to share through the arts, architecture and natural elements.

The landscaping complex brings together two complementary compositions – the mathematical garden or the tamed nature and the *bosco* or the wild nature – *"Nature, the very embodiment of ultimate divinity, was terrifying not only in its absolute power but also in its lack of content. Only mankind, the political animal, intended something"* (Scully, 1991). If the landscaping mathematics suggests divine reason and talks about a Heaven created by a mathematical God (*Deus geometer*) then the *bosco* or the forest represents the original chaos, the Fall and also the antithesis of Paradise.

The garden uses artistic metaphors and takes the visitors on an allegorical route that includes the main historical stages or key moments of the human life on its continuous path to absolute reason, to finding Paradise again – to illumination. This figural process starts from the chaos of the forest and ends in "geometry and perspective", but at the same time, it goes through several "checkpoints" that define the human condition from its appearance on earth until it reaches the supreme goal – reaching illumination or finding the lost Eden.

To be more exact, the route begins through a secondary gate and not at the primary (as one might think) that brings visitors face to face with the fountain of the mythical Pegasus. The image, through the legend that revolves around this sacred animal (Chevalier and Gheerbrant, 1995), suggests the fact that this place is the origin of all things and of the adventure that aims to obtain the ultimate divine illumination. The place where the winged horse's hoof hits the ground becomes the starting and the inspiration point that will help man find the realm of reason – the mathematical Heaven! (Don, 2008).

Located at the entrance of the complex, the fountain of Pegasus can be found right next to the very landscaping expression of Heaven – the mathematic garden; but the visitor is not allowed to get directly to it and neither does he know this garden exist. This garden takes the form of a *Hortus Conclusus* and a *Garden of paradise* at the same time – *"Originally, fences were a practical land symbolical necessity for enclosing sacred places, and indeed the very concept of the enclosure had been deeply rooted in the vocabulary of the garden ever since the medieval term Hortus Conclusus identified its special character. Enclosing even a small bed with a fence or even just a small border was a means of making it sacred, so that its symbolic value took priority over any practical purpose" (Vercelloni and Vercelloni, 2009)*.

The fountain has therefore a double role - invites the visitor to enter the garden, but also represents a hyphen which both separates and links the wild garden with the rational one.

Leaving the fountain of Pegasus behind, in front of the visitor's eyes appears the forest (symbol of the unconscious and of anti-reason, chaos) from which point he can choose many paths, each sending him to a new adventure and a new discovery. Among the most significant such points of the bosco one might remember a few elements such as: Il Barco, the maze, the beaver's fountain, the mascarons' fountain, the lions' fountain, the fountain of Bacchus etc.

Il Barco. Il Barco is actually a hunting annex built by Cardinal Ottaviano Riario Visconti, grandson of the original owner of the complex, the Cardinal Raffaele Sansoni Riario (Ehrenfried, 2007). The latter was the one who in 1498 closed the entire perimeter of Villa Lante with walls, making it a hunting park. The building, which still bears the symbols of Cardinal Ottaviano Riario Visconti (a snake coiled around a flower), was used as a hunting pavilion and later was incorporated into the bosco's structure (https://villalante.wordpress.com/il-barco/).

The lions' fountain. The Lions' fountain represents a statuary set, hidden deep in the woods, and it is made up of a circular dip in stone with 4 symmetrically displayed lions with water flowing out of their mouths. There is no known accurate information on the origin and/or its significance, but this element is present even in the sixteenth century famous plan of Villa Lante. (https://villalante.wordpress.com/il-barco/).

The mascarons' fountain. The mascarons' fountain is also hidden deep in the woods, and, just as in the Lions' fountain, there is not much information on its origin or meaning.

Moreover, in the centre of the rectangular basin that surrounds it, one can find an almost identical fountain with that on the right of the Diluvial Fountain on the upper terrace of the geometric garden (https://villalante.wordpress.com/il-barco/).
These parallels between the two distinct microcosms (the forest harden and the mathematic garden) seem to realize connections between them, joining the two separate parts of the villa both physically and symbolically, making them indissoluble.
Beaver's Fountain. This fountain also follows the same pattern, as it is not yet described in any of the researched documents. Perhaps it also aims to highlight the natural wilderness and it contributes to shaping that image and atmosphere of chaos.
The Labyrinth. Unlike the other elements mentioned above, this one no longer exists today. Given the fact that the symbolic significance of the maze is made up of a search process, it is perfectly plausible for this part of the garden to be located in the *bosco* just to emphasize the chaos and incessant search of the individual for a higher world. Perhaps its purpose was to entice visitors, making them believe that going through the maze would get them out of this forest with sacred powers, while it did nothing else but to bring man even more to the Fall, deceiving and tiring him. Moreover, the metaphor of the labyrinth was doubled by the metaphor of the forest (in itself a maze), the route becoming increasingly more difficult and dangerous through the bosco, the exit out of this place getting a much brighter aura.
Having escaped from the forest adventure, visitors can step into perhaps the most spectacular garden of the Italian Mannerism. This new landscaping construction suggests liberation from the terror of the unconscious and takes its visitors on a straight path that runs together with the tumultuous journey of a watercourse. So the water becomes the guide and leads man towards his ultimate goal.

"The Early Reneissance garden was primarily static and could be viewed in its entirelly from a fixed point of view. To borrow from literary terminology, it had a unity of space and time. The gardens after the 1520s consisted of a series of successive spaces, isolated from each other physically and visually. They could only be experienced through movement, and the relationship between spectator and garden became active rather than passive. " (Graafland, 2003 quoting MacDougall, ***)

The water, which must be followed closely, stems from the so-called "Fountain of the Diluvial" (Iliescu, 2014). It symbolizes the biblical flood that cleansed the world of "impurities" and that provides through purification the chance of a new order of life. This allegorical fountain separates therefore the garden of knowledge from the infamous *bosco*. It is composed of three major elements, each creating a new image and outlining a new symbol that completes the allegories of this ensemble. The fountain leads the way to thinking and reason, representing in the philosophical semantics of the garden, the "archaic harmony between man and nature" (http://www.lazio.dk/villa_lante_di_bagnaia.htm) – the relationship of interdependence and mutual respect between a rational being and his life environment.
So the main source of water comes from a cave, a symbol of the maternal womb and uterus, highlighting both the purifying role of the water and the symbolic role of the cave. This cave is flanked by two loggias that Cardinal Gambara adorned with paintings of the muses from the Greco-Roman mythology – thereby suggesting the divine role of the arts in the man's process of search and finding of the Paradise. From the roofs of these loggias spring up, in the spirit of Late Renaissance or Mannerism, a series of fine water jets designed to capture and sprinkle visitors – a typical farce of the sixteenth century.
The last piece of this fountain is composed of a container made of carved stone with four symmetrically arranged human figures on the outside. Perhaps, just like its counterpart in the *bosco*, this too forms a link between the human being and the route that he must follow in life and the entire vocabulary of symbols associated to number four (4 seasons, 4 cardinal points, 4 human states, 4 dominant winds, 4 sacred rivers of Paradise, 4 phases of the moon, 4 elements etc). Moreover, this element makes, through repetition, a new link

between the forest and the geometrical garden, pointing out the connections, the rivalry, but also the interdependence between the two seemingly antithetical components of the landscaping complex from Bagnaia – *"a garden manifests the rivalry between man and nature, not the victory of one over the other"* (apud. Lazzaro, 1990).

The allegorical route continues along a strong symmetry axis subordinated to a straight route of a water course. Following its course, water takes the form of a hexagonal fountain (the Dolphin Fountain- which incorporates the heraldic symbols of Cardinal Gambara) where, the path narrows and opens in a depth perspective, along a water staircase which opens and ends with one architectural item representing Cardinal Gambara's emblem – the crayfish. From here, the water flows further to the Giants' waterfall, an architectural waterfall flanked by the statues of the Tiber and Arno rivers. This, with its two statues arranged symmetrically as to the way water falls, symbolizes the friendship between the Papacy in Rome (Tiber) and the Medici family in Florence (Arno). From the allegorical fountain of the two rivers, the water keeps flowing through the Cardinals' table (object made out of stone that served as entertainment and dining space) (apud. https://villalante.wordpress.com/mensa-del-cardinale/).

According to Kluckert Ehnrenfried, the Giants'fountain and its layout on the central axis in the middle of the geometrical garden can be interpreted as a metaphor not only for the friendship between the Medici and Vatican, but also as a metaphor for the road from Bagnaia to Bomarzo (Ehrenfried, 2007), or from Searching for Heaven to Avoiding Eden.

From the Cardinals' Table, the water leads to an "Enlightenment" which is more and more visible. The course takes the form of a new waterfall – Waterfall of Lights, and this one, unlike the other decorative pieces, does not include in its design statues of human or animals figures, but terraces with pots of flowing water. However, although it doesn't incorporate human figures, statues of Greek and Roman deities, Venus and Neptune, can be found in caves arranged symmetrically to the central axis (Iliescu, 2014). The Waterfall of Lights can be seen as representing boiling water or the purifying force of water and fire combined (https://villalante.wordpress.com/fontana-dei-lumicini/).

Leaving behind this fine work of art, the view widens and opens up to the last two parts of the garden. However, before concluding the allegorical route, the visitor can observe the appearance of two identical buildings located on one side and the other of the watercourse: Pallazina Gambara and Pallazina Montalto.

Pallazina Gambara was the first building erected in the "Garden of knowledge/of reason". Built between 1568-1578 after a square plan, it incorporated the Gambara family symbol, the crayfish (symbol of the road to Salvation, symbol of loyalty), and a torch (symbol of martyr fire) and an inscription (SOL ALIIS) that can be interpreted as saying eighter "Only for others" - meaning that the project was conceived as a spiritual message addressed by the Cardinal to the visitors or "Light for others" - meaning that the sun, symbol often used by heretics and pagans, is for all and not just for the Church of Rome (https://villalante.wordpress.com/palazzina-gambara/).

Moreover, it seems that these symbols are related one way or another with a comet which coincided with the time the pavilion was constructed (https://villalante.wordpress.com/palazzina-gambara/); but so far, this information could not be verified. Inside, the building has painted scenes which almost always show ideas linked to Salvation and the (re)discovery of Paradise.

Palazzina Montalto, although it appeared in the original design of Cardinal Gambara, it was built between 1590 and 1612 by Alessandro Peretti Montalto Damascena, nephew of Pope Sixtus V.

The only difference between the two pavilions consists in symbols that decorate the exterior of the two buildings. Thus, in Pallazina Montalto's case, the building's frieze is adorned with the following symbols: mountains representing the Montalto dynasty, a branch with pears, referring to the Peretti dynasty, the star with eight corners - Christian symbol of the eternal salvation. On the other

hand, the pavilion's interior was decorated in the same iconographic spirit as Pallazina Gambara (https://villalante.wordpress.com/palazzina-montalto/).

Passing through the two pavilions that served as the owners and their servants' house in the past, the visitor ends the allegorical route of taming and of the absolute control of nature. This mastery of nature emerges as a geometric floor in which even water stops being wild, and it becomes subject to the human will. So, it is no longer churning and it no longer falls, but takes the form of a still water and stands in a geometric pool – the Fountain of the Moors.

This fountain is a reinterpretation of the Persian model of Heaven and is often perceived as embodying a calm sea that can be crossed in all directions. At its centre is not a piece of water or a pot with plants, as it happens in the Persian design and later in the Arabic and even Christian gardens, but we discover the statues of four young people (called Moors because of the dark color of the volcanic rocks they are made of) who support the heraldic symbols of the Montalto family (pears, mountains and the star with eight corners).

It seems that this reinterpretation of the Garden of Paradise is actually much more complex than it may seem. It appears that the perfect geometric shape of the garden was inspired by the legend of the martyrdom of St. Lorenzo. He was burned on a grill shaped like a grid because he refused to provide money from the Christian community to the prefect of Rome.

Moreover, Cardinal Gambara was apparently very attached to this saint because one might even see that, in his pavilion, there is a fresco depicting the martyrdom of St. Stephen and Lorenzo. It is considered by some authors that this legend became the main theme of the garden of reason - "*the grid is, in other words, the sacred symbol representing Saint Lorenzo, one of the most celebrated holy Catholic men, who devoted his entire life to Jesus Christ and the Cross*." (https://villalante.wordpress.com/graticola-di-san-lorenzo/).

Regarding the circular shape of the basin, it seems to have been inspired by the architecture of the church of Santo Stefano Rontondo in Rome and together with the floors with rich embroideries of buxus represented, in Gambara's view, symbols of absolute faith. Even the water gushing from the well was used as a metaphor of the road, purification and salvation that the visitor will receive when he will reach the heavenly Jerusalem (https://villalante.wordpress.com/fontana-dei-mori/).

In the four rectangular pools arranged symmetrically around the central statue, there are four stone boats, each containing a soldier carved in stone. These were intended to represent four soldiers throwing water at the heraldic symbols, suggesting both the Protestant attacks of the Latin Church and especially the Turks' attacks stopped by the fleet led by some members of the Gambara family at the Battle of Lepanto (https://villalante.wordpress.com/fontana-dei-mori/). At present, however, for unknown reasons, the water jet produced by the statues of the four soldiers goes the opposite direction - toward the ground floors with the box embroideries.

Beyond the geometric ground there is a narrow perspective that connects the central axis of the garden and the main street of Bagnaia. But to reach the city, the visitor must pass through a monumental gate disposed axially to the garden. This gate is present even in the sixteenth century plans, but its current configuration is most certainly due to the Lante della Rovere family (last owners of the Villa, XVII century) who decided to adorne this facade with their personal emblem. (https://villalante.wordpress.com/passeggiando-nella-villa/ingresso-foto/)

The allegorical route of this garden ends therefore, next to the central gate (Ingresso). It ends a process of figuration taking the visitor through a world that takes secular, mythological and biblical meanings, and that can talk to and relate both the divine purpose of man (retrieving Paradise) and his own passage through life. On the other hand, the same route can be travelled in the opposite direction, suggesting a path of retreat and meditation:

"We start in the town at the foot of the mountain: the good, proud, sharp-edged,

manmade town. From there, we enter a gate in a mythical wall. What can lie behind it? In fact, it opens upon a broad, geometric parterre with a deep basin of water lifted in its center, above which the stemma of the Montalto is flaunted in the air. Beyond the parterre, the villa is divided into two pavilions, one on the left, one o the right, eith the ramps of Praeneste and Tivoli mounting between them toward a dark, heavily wooded garden. This climbs the mountainside toward secret pools and springs an dis continued by a wooded park that climbs farther up the slopes – who knows, perhaps into the unknown heart of the mountain itself.

What a sinister courtesy the villa opens up to invite us into the wild. How dark it i sunder the trees. As we press onward, the shaggy shapes of the forest begins an driver gods, water-worn, emerge, half human, half animal, covered with moss. Human shapes are merging back into the nature, perhaps beyond the animal to the vegetable world. […] until suddenly the villa opens into its two parts once again, this time, miraculously, to let us aut, to let us see the civilised parterre, but more than that, far more than that, to show us the city out there in the light beyond the forest, the work of man, our refuge and our only hope. " (Scully, 1991)

Regardless of the route one takes, the landscaping from Bagnaia emphasizes the sanctity and divine meaning of the garden and creates through it, a microcosm which actually represents an Eden at a human scale, made posible through architectural and horticultural processes that mimic the human reasoning processes.

The garden being covered, and the message revealed, there is only one question left concerning the projection of the Villa Lante.

It is known that the Renaissance brought a series of physical and mathematical discoveries, including perhaps the most famous one, namely the linear perspective. This geometric construction will govern art, architecture, and gardens of the Renaissance, Mannerism and Baroque, becoming one of the main features of landscape design done in this period of time.

"The harmony of the universe was ehoed in the harmony created through huma nart in buildings, pictures, households, governments and gardens. […] The order in the garden, achieved through similar means (mathe-matics), likewise reflects and reproduces a cosmic order. […] Because the ordered microcosm reflects the macrocosm, the garden was the ideal vehicle to aquire knowledge of the divine order, a step by step process all things in the visible world were understood as links in a chain leading to the divine." (Lazzaro, 1990)

But if art, architecture and the gardens are subordinate to the perspective, how is it possible that the plan of the Villa Lante is drawn partly in a rising perspective and partly in elevation? The answer can be given by the general shape of the plan. Thus, by further abstracting its image, one may notice a certain similarity between the general outline of the plan and the shape of a human skull.

If we consider that the fundamental idea of Villa Lante is the retrieval of Paradise through reason, then, in terms of specificity and particularities of studies; especially those concerning the allocation of imagination, reason and memory in the human brain (apud. Clarke and Dewhurst, 1996) made in the Renaissance; through alchemy and through a complex hermeneutical approach, it can be deduced that the carefully drawn plan of the garden itself suggests the message that it wants to convey through the proposed routes – Retrieval/ rediscovery of lost Eden by mathematical reason. Moreover, if we compare the plan of the gardens from Bagnaia with Robert Fludd's drawings from *Utriusque cosmi maioris scilicet et minoris metaphysica, physica atqve technica historia*, we can notice that there are many correlations between design, theme and the garden.

Although it is probably not the best example, the correlation between the orriginal plan of Villa Lante and Robert Fludd's drawing is used as to emphasize the fact that the shape of the garden was itself a means of expressing the fact that this particular garden was designed as to represent the true path that leads to Paradise.

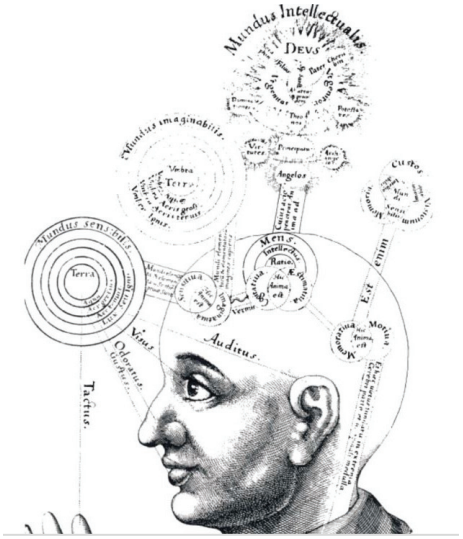

Figure 1. Utriusque cosmi maioris scilicet et minoris metaphysica, physica atqve technica historia
(Clarke E., Dewhurst K., 1996, p. 42)

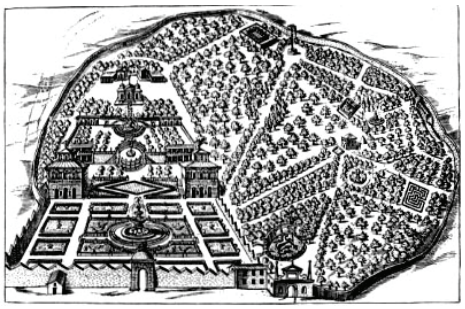

Figure 2. Villa Lante, 16th cenury plan
(www.gardenvisit.com)

In conclusion, Villa Lante can be considered an architectural landscaping microcosm which uses all the artistic means specific to the Italian Mannerism to convey a complex message that hides in fact an allegorical story of man searching for the lost Paradise. Unlike the Villa Lante, Villa Orsini from Bomarzo tries to trick visitors, guiding them through a sacred grove but which, unlike the *bosco* site at Bagnaia, leads the visitors in circles and offer them a single image - the image of the Fall. Villa Orsini does not offer a way out of the sacred forest and no route through which one can access the Lost Paradise, even if it gives the impression that it will reveal itself to man and that it will be decisive and strong to overcome all the wonders of the Tartarus found on the Villa's route.

" [...] an attempt to banish the melancholy by subjecting onself to cheerful stimuli (the case of Villa Lante). The other is to do the opposite, namely to surround oneself with sad and gloomy things, thus giving oneself a kind of homeopatic dose of melancholy to stimulate a counter-reaction (Villa Orsini)." (McIntosh, 2005)

Sacro Bosco or Paradise Lost

"Tu ch'entri qui con mente Parte a parte Et dimmi poi se tante Maraviglie Sien fatte per ingano O pur per arte – You who enter this place, observe it piece by piece and tell me afterwards whether so many marvels were created for deception or purely for art." (McIntosh, 2005)

Just like the message posted at the entrance to the garden says, Villa Orsini will introduce visitors to an unfamiliar world, in an abstract but real, kinetic and aggressive universe.

Unlike Villa Lante, although the Sacro Bosco from Bomarzo has the same subject - namely the route to the retrieval of Paradise, it offers a new vision of this initiation process. Most likely, the route was inspired by the visionary's own life experience, the garden being designed as a monument of commemoration of the tragedies he has surpassed.

Unlike Villa Lante, Villa Orsini was designed entirely as a complex maze (Ehrenfried, 2007). This structure enabled the visionary to use different registers of shapes, sizes and symbols to recreate a false initiation road where nothing that exists seem real, and nothing that is promised or expected will ever exist. In this garden, even nature is no longer subject to its own rules, it is strongly deformed and decomposed – *"In Bomarzo, the rules of the world are no longer valid, it is as if this garden wished to escape from the laws of nature."* (Ehrenfried, 2007)

As with Villa Lante, the Bomarzo garden offers visitors several possible routes through which they can discover both the universe

transformed through landscaping art and the promised Heaven. Irrespective but the chosen path, going through the labyrinth will never lead to the expected outcome.

"It is a sacred wood, full of disquieting monuments, some seeming to grow aut of the rock itself. The original entrance and axes of mouvement along its slopes can only be conjectured. The intended sequence of experiences is therefore not clear, perhaps was never meant to be so. The path through the modern entrance is perhaps as good as any other. It leads downward toward the trees, crosing a little watercourse leading farther into the depths of the forest. Directly ahead, standing in the light across a gentle ope field, a good, rationally abstract chapel can be seen, columned and domed, but the path does not lead toward it. Instead, it turne away from it down the darkening slope. Soon, hewn aut of the natural rock, Hercules rises before us, tearing the giant Cacus to pieces in his hands. He is guarding the garden for us but is markedly alarming, nonetheless. Finally, we come to the deepest part of the forest, and the darkest. There the stream runs into a cleft in the earth and disappears with a gurgling throat. The horrible gargle is surely enhanced by the whale's mouth, all teeth and gullet, into which the living rock at the mouth of the crevice has been carved.

Right there, looming over the cavern, an enormous round-eyed tortoise has been carved aut of the rock. On its back, the figure of a woman, apparently sounding a trumpett, is placed. We know from the emblem book of teh Cavaliere Ripa that she is an image of Fame – Fame lost down here in the depths of the wood, sounding her trumpet in the wild, while high above her the Orsini Castle can be seen shining in the sun. There is no connection between the two, no apparent route from his place tot hat. The effect is again bestial – the woman is, after all, right aut of the Apocalyptic Vision of St. John, mounted as if in ecstasy upon a beast. […] just beyond Fame, in the darkeness of the forest, a bright light gleams. It is Pegasus, the winged horse, symbol of hope, touched by a ray of sun, rearing up in the darkeness. He show sus the only route to follow aut of here. It is a sinister-enough path through the wood: Nel mezzo del cammin di nostra vita/ mi ritrovai per una selva oscura.

As we follow it upward, we are led further into dream. Goddeses of earth recline like Etruscan matrons in the rock, heavily and somnolent, bearing urns upon their heads. A house appears before us in a sunlit glade. It is leaning steeply into the hill. We mount ever higher beyond it. A war elephant looms up: a castle crowns its back, and a mahout sits upon its head. It is lifting the broken body of a soldier in its trunk. An enormous lizard flares beside it. We are climbing aut of the depths, but the images around us are becoming more alarming all the while. At last our dream, the guardianof our sleep, is broken by a figure o true nightmare: a colossal screaming face, as big as a house, demolishing the censor, awaking us as if to our own scream. And then, awake, we are aut, standing in the sunshine beside the mercefully abstract chapel we saw before and looking beyond it toward the palace, bathed now in clear white light, but remembering still the woman on the beast deep in the wood of our unaccountable yearning of fame. " (Scully, 1991)

From the description given by Vincent Scully, one can observe the mystical character of the garden and how it is translated into reality as a complex maze.

Although it belongs to the same historical period as Villa Lante, Villa Orsini at Bomarzo offers a new model for the use of the principles of construction of Mannerist gardens and although it approaches the same theme of rediscovering Paradise Lost, it uses many metaphors hidden in the carved stone, conveying a message which is different from the one in Bagnaia and opposite atmosphere to the balanced landscape of Bagnaia.

Seen by Christopher McIntosh as a complex landscape which combines several different themes: "part of it a memorial to Vicino's deceased wife, part therapy for melancholy, part autobiography in stone, part collection of alchemical symbols, part mannerist experiment" (McIntosh, 2005) Villa Orsini from Bomarzo must be seen as a whole, as a sum of elements that make up one story and one life experience - Orsini's tragedies.

RESULTS AND DISCUSSIONS

As already shown, both Villa Lante and Villa Orsini were designed as to represent the quest for Paradise. The final result of the allegorical processes that make up the two villas offer though two different views over what the road to Paradise and what Eden should look like.
Although very different in this aspect, they tend to be closely related to one another because they tend to use similar images and visual expressions as to emphasize the lanscape design philosophy.
To this end, both gardens use similar natural, mythological and/or philosophical themes and elements but interpret their images completely different:
Pegasus. While at Villa Lante, the mythological winged horse is inviting the visitor to walk the paths to Paradise and offers him a glimpse of the promised Eden, at Bomarzo, the same animal is placed probably only to annoy the visitor, offering him a false hope. Pegasus is placed in the *bosco* in both gardens, but his presence does not deliver similar messages.
The watercourse. Both gardens invite their visitors to walk their paths following a watercourse. At Villa Lante, the water is a phisical substitude for the human reason, its "taming" reflects the man's own road to illumination by following the path of reason. This path, and the water that makes it blends itself with a handfull of mythological, profane and religious elements, emphasizing the fact that achieving divine reason is a very complex and delicated process that covers all areas of sciences and humanism. On the other hand, the forever untamed watercourses at Bomarzo drive the visitor further and further away from what he hopes he will achive by following them.
The *Bosco.* While both gardens use woods as mazes and symbols of the chaos and the unknown, the Bagnaia *bosco* is part of the allegorical path from chaos to reason, thus being just part of a visitor's experience. At Bomarzo on the other hand, the forest represents the action itself. Here, the *bosco* is the perfect foreground for the entire spectacle of chaos and deceit.
The Paradise. The final expected outcome of the two villas is the (re)descovery of Eden. To this end, Villa Lante offers a more or less straight path that leads to the Paradise, while Villa Orsini only promises it and deceit its visitors by showing them a false hope under the image of the Pegasus.

CONCLUSIONS

The figuration processes described in the two examples discussed above, provides a comprehensive view of the role of the gardens in the sixteenth century, on the relationship between man and the divine in the same period of time, on the relationship between nature, art and science, etc.
Both Villa Lante and Villa Orsini represent two architectural and horticultural experiments belonging to the Mannerist era in Italy; extremely complex and delicate topics related to the history of art, the art of gardens. They address common themes and even common elements (the sacred forest, Pegasus' statue, etc.) but contain different actions and outcomes, regardless of how they are covered. The two gardens mimic the way to Paradise, but, depending on the chosen route, they can delight, disappoint or madden.

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THE CONSERVATION PROCESS ADAPTING THEORY TO A NEW CONTEXT

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Abstract

In Romania historical garden conservation is a new discipline. Often the professionals involved in this process have to rely on their own intuition or on case studies of projects undertaken in countries with a richer tradition in this field. It is obvious that a unitary and professional approach is needed. We propose in the present study a possible methodology for approaching the conservation process, based mainly on the rich experience of the English National Trust.
In the first part, this study will attempt to draw from this accumulated knowledge a set of principles, which is by no means exhaustive, and does not represent a guarantee for successful conservation. Rather, this is a list of procedures which have become widely established in England. They have been verified by experience, and can be adapted to a new context. The approach to conservation can and does vary, depending upon the subject of study, and its context as the practice of the National Trust proves. The second part of the study deals with the way these principles have been adopted, adapted and applied on a school project during the Historic Garden Restoration classes at the USAMV Landscape Architecture department. The methodology of working with the students on a conservation project involving the regeneration of the Floreşti Estate focused on those procedures which would help the students develop the basic skills needed when dealing with a heritage asset.

Key words*: heritage, historical gardens, garden conservation, restoration, regeneration.*

INTRODUCTION

When heritage is mentioned, most people would probably think about buildings and monuments, art collections, maybe literature and music. However, the value and importance of historical gardens[1] as part of the common heritage is increasingly being recognised, hence a new and unprecedented interest in garden protection, conservation and regeneration has emerged. In Romania **garden conservation** is a very new discipline, and the professionals pioneering this path often have to rely on their own intuition and common sense when dealing with it. Publications on this subject are few, the legislation is lacking, and there is little unity of approach. In this study 'garden conservation' refers to a much more complex process than the term 'conservation' seems to suggest. The Burra Charter defines conservation thus: 'Conservation means all the processes of looking after a place so as to retain its cultural significance' (Burra Charter, 1999, Article 1.4).[2] Simply put, cultural significance means 'aesthetic, historic, scientific, social or spiritual value for past, present or future generations' (Ibid, Article 1.2.). Thus, conservation has come to mean, especially for professionals, retaining the meaning and importance of a place, and not only preserving its physical matter, or fabric. Conservation can encompass a wide variety of interventions, ranging from maintenance to repair, restoration, reconstruction,[3] or more complex processes of regeneration.

[1] In this article a **historical garden** will be defined according to the Florence Charter: 'A historic garden is an architectural and horticultural composition of interest to the public from the historical or artistic point of view.' (Florence Charter, 1982, Article 1). It includes notions like private and public garden or park, country estate, etc.

[2] The definitions proposed by the charter have become established, at least in England, having been adopted by Historical England and other institutions dedicated to heritage protection; they also have the advantage of being simple and short. (Watkins and Wright, 2007);

[3] '**Maintenance** means the continuous protective care of the fabric and setting of a place, and is to be distinguished from repair. **Repair** involves restoration or reconstruction. **Preservation** means maintaining the

In countries like England, garden conservation has become a well-established practice, indeed, a tradition.

The National Trust, founded by some of John Ruskin's disciples at the end of the nineteenth century (Waterson, 1995), and at present the greatest owner of historical gardens in Europe (cf. National Trust website), has been a major pioneer of garden conservation, and its experience has helped define today's standards of good practice within this field. As the history of the trust illustrates, approaches to garden conservation have undergone many changes, from restorations 'in spirit,' or just creating 'appropriate' gardens for historical buildings, restoring to 'the last significant phase' according to thorough research, or the approach of 'conserve as found,' to ample regeneration projects (Cook, A., 2004).[4] Although approaches to garden conservation are likely to continue changing, this on-going process has led to the accumulation of a valuable mass of knowledge and practical experience, to the establishment of standards of good practice, and to the formation of a dedicated vocabulary.

In **the first part**, this study will attempt to draw from this accumulated knowledge a set of principles, which is by no means exhaustive, and does not represent a guarantee for successful conservation (understood in the wider sense stated above). Success depends on other factors as well, not least on the competence of all the people involved, from specialists to workmen, and their dedication and commitment. Rather, this is a list of procedures which have become widely established in England.[5] They have been verified by experience, and can be adapted to a new context. The approach to conservation can and does vary, depending upon the subject of study and its context, as the practice of the National Trust proves.

The second part of the study deals with the way these principles have been adopted, adapted and applied during the *Historic Garden Restoration* classes at the USAMV Landscape Architecture department.

The methodology[6] of working with the students on a conservation project involving the regeneration of the Florești Estate was based on the stages listed below, but focusing on those procedures which would help the students develop the basic skills needed when dealing with a heritage asset: site survey, documentary research, analysis and reconstruction of the site's design and history, assessing the present condition, and developing a project based on the results of their research.

Part 1. The conservation process

The aim of a conservation project is to retain the 'cultural significance' of the heritage asset, in this case the historical garden. This means understanding what is important and valuable about it, and deciding what to do in order to preserve it. After assessing the significance of the place, its current condition and the issues involving it, one should decide the level of intervention needed in order to preserve this significance.

Some well-preserved places might require only maintenance, others might be threatened by loss of significance due to decay, and might require works of repair and restoration. In other cases, revealing and highlighting the significance of the place might require reconstruction.

Often, historical gardens need to undergo a process of revitalisation and regeneration in order to be integrated into the contemporary context. This might mean being assigned new viable functions and uses, or allowing new development within the protected areas, which, whilst sensitive to preserving the character and

fabric of a place in its existing state and retarding deterioration. **Restoration** means returning the existing fabric of a place to a known earlier state by removing accretions or by reassembling existing components without the introduction of new material. **Reconstruction** means returning a place to a known earlier state and is distinguished from restoration by the introduction of new material into the fabric. **Adaptation** means modifying a place to suit the existing use or a proposed use. […] **Setting** means the area around a place, which may include the visual catchment**.** […] **Meanings** denote what a place signifies, indicates, evokes or expresses. Meanings generally relate to intangible aspects such as symbolic qualities and memories. **Interpretation** means all the ways of presenting the cultural significance of a place.' (Ibid, Article 1.4.-1.17.)

[4] For a brief account of the history of the changing approaches to garden conservation see also 'Hartwell House and Apafi Manor: Conservation through conversion' (Comanescu, 2013).

[5] See below footnote 7 and the accompanying text.

[6] See Materials and methods.

significance of the garden, will help bring it to life in the new environment. Most often, a number of kinds of intervention will be applied on the same site.

Thus, the stages of the conservation process could be surmised as follows[7]:

- **Understanding the site**: its complete history, what it is today, and its current condition.
- Assessing its **significance**: why is it important and for who?
- **Risks and opportunities**:
- Identifying issues and vulnerabilities: this should result from the above two stages. Of particular importance are the factors that may endanger the significance of the place.
- Defining a vision: aims and policies. Explaining what should be done; this section may include recommendations for procedures like maintenance, restoration or reconstruction, as well as setting out directions for more complex processes like regeneration or revitalisation.
- Developing a **project** and an action plan: this section details the proposed interventions, and sets out the stages in which the proposed work should be undertaken. It may include a master plan, a management programme, a maintenance checklist, etc.
- The **implementation stage**.

A. Understanding the site. Survey and research.

The first step when dealing with a heritage asset is understanding what it is. This means knowing as much as possible about its history, from the earliest times to the present date, about the people who contributed to its creation and subsequent evolution, about the ideas it might embody, about what it is today and the problems and issues which might threaten it. The first and absolutely necessary steps toward understanding the heritage asset are survey and research.

It is important to bear in mind that in the case of historical gardens surveying techniques will be a little different than for buildings. They include specific procedures like vegetation surveys, ecological assessments, hydrological and geological surveys, garden archaeology, as well as identifying each hard feature of the garden (paths, bridges, garden buildings, water features, etc.), mapping them and assessing their condition. The type and number of surveys undertaken will depend upon the site, its importance, complexity and state of preservation.

A. 1. Site visits

Site visits. When starting the survey and research stage, the first step is visiting the site, in order to form initial impressions, and to get a 'feel' of the place. Subsequent site visits will be needed for detailed surveys, and later on for confrontations with the results of documentary research. Important points to be kept in mind on site visits include: the coherence and unity of the place, or the lack thereof, the condition of the garden, the relationship between the house or other buildings and the garden, identifying significant features and their condition, views, blocked views, things that have a negative impact, planting, the condition of the trees, how the place is used and by whom, accesses, etc.

A. 2. Documentary research

The next important step is **documentary research**, which, combined with site survey, should result into a **history of the place**, a chronological, complete scheme of the site's development from the earliest times to the present day. It will also set the garden into a **wider context**, answering questions like: are there similar gardens? What are the other works of the garden's author(s)? Is it a rare or early example of a garden of this type? Documents to be consulted include: maps, design proposals, pictures, photos, aerial photos, drawings, descriptions, journals of the owners, chronicles, lists of materials and plants to be bought for the garden, and also already published studies

[7] This list is largely based on and adapted after recommendations by Historical England, the Heritage Lottery Fund and the National Trust on how to develop a Conservation Management Plan (CMP). The CMP is a widely used document, an instrument which in essence describes what a heritage asset is, its significance, its current condition, issues and vulnerabilities, and sets out long term management policies, as well as short term (3-5 years) prescriptions, including maintenance and restoration project works. The CMP is required for funding and development applications, and is extensively used by the National Trust in order to provide continuity of management for their properties. The CMP usually represents the bases for a project. See: National Planning Policy Framework, Heritage Lottery Fund, (Watkins and Wright, 2007, pp. 25-39);

about the place, articles, and other records. These are only some of the documents to be gathered and examined. There is desktop research, at the local and district town hall, at the records office, at libraries, archives, museums, private collections. Finding documents requires time and skill, and sometimes travelling. All these documents should be organised into an accessible data base, which should then be permanently updated with results of new research or records of new work. From this research, a history of the place will be built. It will help identify the main phases of the site's development, and divide the site in areas with a specific character. One of the most recommended procedures at this stage is map overlay and comparison. Documentary research will always be confronted with site surveys.

A. 3. Site surveys

Site surveys include identifying, assessing and mapping all the elements on site: buildings, garden buildings, water features, earthworks, terraces, paths, landmarks, walls, fences, vegetation, hydrological and geological surveys, tree surveys (besides mapping the existing trees, drawing up files for each outstanding specimen), ecological and wildlife surveys, where necessary archaeology and garden archaeology. Surviving and lost historical views and borrowed landscapes will be identified in order to explain the **local context**.

Surviving features assessments, as well as tree surveys specifying the species, age, condition, aesthetical value, and importance, are of particular importance. Site survey and documentary research are the basis for reconstructing the significant phases of the site's design, and for later works.

B. Assessing significance

It is essential to specify why the heritage asset is significant, for whom, and how this significance is linked to the actual fabric of the place. In some cases assessing the significance of a place can be pretty straightforward and simple. However, in more complex cases there can be many layers of significance, and all of them should be considered when proposing a project which might have impact upon them. Significance can refer to historical, evidential importance, artistic qualities, spiritual associations, importance due to association with an outstanding person or event, rarity, age, condition, superiority to objects of a similar kind (an outstanding example of a garden of a particular style, an impressive collection of rare trees), wildlife and ecology, archaeology, etc. The importance to the local community or other groups of people such as enthusiasts should not be forgotten.

C. Risks and opportunities

Once the significance of the place has been established, real or potential threats to it can be identified in view of the research previously done. Thus, **issues and vulnerabilities** concerning the site will be assessed. They might be related to decay, danger of loss of fabric, fragmentation, loss of character and meaning, danger from development, lack of finances to maintain the place, lack of visitors, or conflicts between different types of heritage, but also loss of authenticity, lack of sustainability, over-commercialization.

As the risks concerning the site are analysed, and solutions are sought, a certain **vision** will emerge. Thus certain **general aims and policies** will be established: the kind of interventions that are necessary in order for the significance of the place to be preserved, and, if possible enhanced. These interventions may range from works of maintenance and repair, to restoration, reconstruction, and the integration of new features such as cafes, souvenir shops, cultural centres, or others.

The general attitude towards change when dealing with heritage assets should be reserved; however in some cases the regeneration of a place requires a creative, but sensitive and respectful approach and it is always necessary to make the place functional, responding to contemporary needs.

D. The project

The project will be based on the above research and conclusions, and can include a **master plan**, an **action plan** with specifications regarding the **stages of the project**, how it should be implemented, which procedures have priority. The proposal should take into consideration things like how the project will be financed, what qualifications are required of the staff, once the main stages of the project are completed, how will the property be maintained and financed in the future.

Like the previous stages, the project will most likely be the result of collaboration between experts. At this point it is very important to make sure that all the participants have a clear understanding of the aims of the project viewed as a whole. The coordinator of the team, in particular, should integrate the input of other specialists into a coherent scheme, making sure that the resulting garden is a harmonious whole.

E. The implementation stage

As part of the conservation process, the implementation stage is of crucial importance for the success of the conservation project, and should be addressed, especially in Romania, where the staff employed for on-site works is usually not trained in work on heritage sites.

The manner in which the proposed interventions and works are executed is of great importance, and if inappropriately done, can ruin not only the project, but the historical garden itself. This is why it is recommended that the execution should be supervised by the person who was in charge of the project. Likewise, the staff and other professionals should be familiar with the aims of the project, the significance and character of the garden, with the specific terminology employed in garden conservation, and should have the skills and competences for this type of work. The same should be true of the people who will be in charge of the future maintenance and management of the garden.

Part 2. The conservation project

MATERIALS AND METHODS

For a thorough understanding of the methodology of historical garden conservation (in the broad sense specified above), we propose applying it to a specific, complex, and for many reasons significant case study: the Cantacuzino Estate in Florești, Prahova.

The subject of the regeneration of this site was addressed in a school-project during the 2014-2015 *Historic Garden Restoration* classes at the Landscape Architecture Department at the USAMV, Bucharest. The *Historic Garden Restoration* classes take place during the first semester of the 3rd year of study (14 weeks) and are usually organised in 2 taught course hours and 2 hours of practical activities per week. For the Florești case study, the students had 9 weeks for research and 5 weeks for project work.

The abovementioned methodology was adapted to the school-project, some of the points being necessarily omitted, being outside the sphere of tasks that the landscape architecture students could accomplish. The activity of the students was organised in two stages: research and project work. For the research stage, due to the multiple and diverse research directions which had to be covered, the students were organised into groups of two to five. For optimal involvement, they were given the opportunity to approach the directions of research of their choice, according to their own preferences. At the end of the first stage, an indispensable data base was created, comprised of the results of the research work. For the second stage, the students were organised into larger groups of eight to eleven members. Although the number of students in a group was determined by the professors, the members were not. We opted for this approach to favour good communication in each group. The groups were encouraged to develop different solutions for their projects. These would encompass various types of interventions, including: preservation, repair and restoration, reconstruction and, on a broader scale, regeneration. The projects were meant to organise the proposed interventions into stages, which would allow the concomitant use of the site for cultural, sportive or other activities, which in turn, would financially support the future works. To avoid mistakes, these stages of the school-project were closely guided by supervisors competent in the field of historical garden protection, conservation and restoration.

RESULTS AND DISCUSSIONS

The research stage was preceded by a presentation of the already known information about the Cantacuzino Estate in Florești, Prahova: a topographical survey, the surveys of the 'Little Trianon' palace, photographs from various historical periods, data on the original owner, about the architect of the palace and about the supposed designers of the garden, and a historical study of the palace.

In order to become directly acquainted with the object of study, the students' first activity was a site visit. The students were organised in groups after that visit. They were assigned tasks according to their own preferences regarding research directions. The students undertook research at the National Archives, at the Academy Library, at the History Museum of the Ploiești Municipality, at the Florești Village Hall. This endeavour was really successful. A whole archive of documents reflects daily life on the Florești estate, although these documents are apparently dry and uninteresting. Historical plans dating from before the construction of the present palace have been found, identifying the main areas of the estate: the pleasure grounds and the hunting park. The 1905 plan already shows a clear division into specific areas: the pleasure grounds, the hunting park with the mills' pond and the river meadow, as well as the Cap Roșu Park at the northern end of the estate (Figure.1).

Figure 1. Florești Estate plan, 1905, Detail
Source: Arhivele Naționale, Planuri, Județe lit. O-V, Inventar 2343, Cota 248.

At the National Archives, a 1906 'Boundray Book for the Florești Estate' has been found.[8] It encompasses a complete inventory of the estate, and shows a clear division into specific areas: the pleasure grounds, the hunting park with the mills' pond and the river meadow, as well as the Cap Roșu Park at the northern end of the estate.

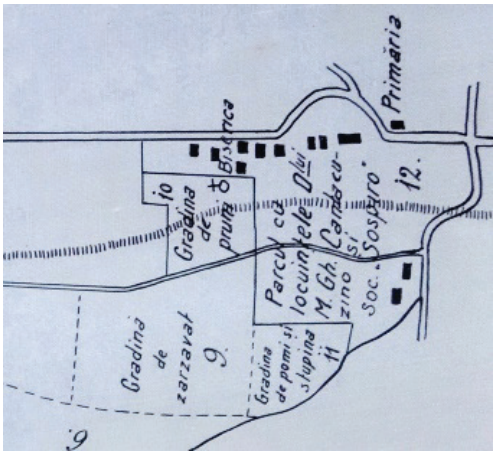


Figure 2. Florești Estate plan, 1924, Detail
Source: Arhivele Naționale, G.Gr.Cantacuzino, Inventar 1829, Cota 608.

A 1924 plan shows a plum tree orchard and a vegetable garden, bee hives in an orchard, a wilderness, hayfields, and poplar and alder woodland (Figure 2.). Other documents mention: buildings in the 'garden in the Park', two glasshouses, beehives and fruit bearing trees, a mill, a cattle farm and 'an orderly dairy.' The most important plan was found at the Central Archives of the Bucharest Municipality, and it represents a restoration proposal for the park, signed 'Pinard' and dated July 1912. Worthy of mention are the important views marked on this plan (Figure 3.).

Other students have elaborated a site survey recording all the trees and the built elements, like buildings, walls, bridges, ponds and other water features. Comparative studies regarding the wider context of the 'Petit Trianon' as archetype were also undertaken, by analysing places that are also named after and likened to the French original.

The studies showed that most of these places were situated in urban areas, with evident consequences upon the dimensions of the gardens. A comparative study on the Cotroceni ensemble highlighted a series of similarities concerning the decorative features, such as a rectangular pond and the balustrades from the

[8] Arhivele Nationale_Hotărnicii_inv.2473_Jud. PH_Cota 53 - "Cartea de Hotărnicie pentru moșia Florești" din 1906, publicata in 1908.

Palace garden, which were erected at the beginning of the twentieth century, like the ones at Floreşti estate.
The studies revealed the fact that the ensemble at Floreşti is a late example of a nineteenth century garden, with a geometrical area around the main building, transitioning into a landscaped pleasure garden, and then into the wider parkland. It should be underlined that, except for the 'Petit Trianon' itself, no other Versailles feature was used as model for the Floreşti estate. The eighteenth century gardens of the Petit Trianon present an idyllic view on village and pastoral life. They have no connection with the public parks of the nineteenth century, like Buttes Chaumont, Monceau or Montsouris, which, on the other hand, have a great number of elements in common with the pleasure grounds at Floreşti.

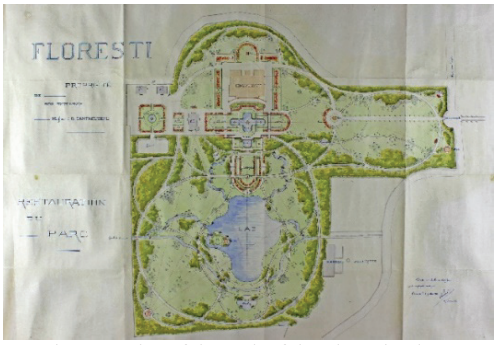


Figure 3. Plan of the park of the Floreşti Palace, belonging to I. G. Cantacuzino, no. 90, 1912
Source : Arhivele Centrale ale Municipiului Bucureşti, Inventar 2343 vol. II, Planuri O-V, Judeţul Prahova.

The most important and useful information about the site was found in two articles published in contemporary periodicals: 'A day at Floresti,' published in *România Ilustrată* magazine, (Antemireanu, 1905) and 'Disposition en terrasse. Aménagement d'un Jardin régulier, d'une large facture, à flanc de coteau, devant une demeure de style Trianon (Domaine de Floresti, au Prince Cantacuzène, Roumanie)', published in *La vie à la Campagne*, (Maumené, 1914). The first article describes the estate in detail, mentioning specific areas, and providing photographs. It also mentions the author of the first landscaped layout. 'The Pleasure grounds at the artfully crafted Floreşti estate date from around 1830. They were laid out, in their present form, by Meyer, the famous gardener who was also commissioned by General Kiselef to realise the eponymous, and most admirable boulevard in Bucharest, the most exquisite adornment of the Capital.' (Antemireanu, 1905). The plan published in 'La vie à la Campagne' shows the superior terrace, the geometrical garden around the palace, and also the link to the 'pleasure grounds,' to the edge of the lake (Maumené, 1914).
The current heritage legislation has also been studied, as well as the List of Historical Monuments, according to which the estate is a category A listed heritage asset, that is, of national importance. The Cantacuzino Estate (PH-II-a-A-16490) is an ensemble of national importance, which lies parallel to the Prahova River, from North to South, on a distance of 3 km. The main elements of the ensemble are: the buildings, the most valuable of which is the Palace called the 'Little Trianon' (PH-II-m-A-16490.01), built between 1910 and 1916, designed by the architect Ion D. Berindey, the water tower (PH-II-m-A-16490.02), built between 1910 and 1916, the enclosure wall (PH-II-m-A-16490.04), and the 'Holy Trinity' and 'Nativity' Church, with the Governor Grigore Cantacuzino's family crypt (PH-II-m-A-16491), 1887. The park (PH-II-m-A-16490.03) has naturally been the main object of our study.
The students surveyed the existing vegetation (Figure 4), and analysed the important views for the general composition and for emphasizing both the palace and the grounds.

Figure 4. Vegetation Survey of the formal gardens and the pleasure grounds.
Authors: the students from the third year of study

All this documentary research was corroborated with all the other information provided by

plans, other documents, and most importantly, the survey of the site. Other surveys such as excavations or other archaeological works, and ground investigation, which are in principle recommended, were not undertaken, this being a school-project. At present, the condition of the buildings in the ensemble is poor, the palace being in an advanced stage of degradation; it is a ruin in fact. The enclosing wall has also collapsed in various places. Some of the gates have disappeared, while the water tower needs to be consolidated and restored.

Assessing Significance

According to the Historical Monuments List (2010 and updated in 2015), the whole ensemble at Florești, as well as its main features are of national importance. Even some of the unlisted features, such as the buildings of the present sanatorium are important due to their association with the Governor Grigore Cantacuzino (1800-1849) and his wife Luxita Kretzulescu (Figure 5). He is also the founder of the Florești church (1826-1830), which was later rebuilt by his wife. It is said that within the present tuberculosis asylum buildings, previously the villas of Gh. Gr. Cantacuzino's children, there are murals by Gh. M. Tattarescu, who also painted the church built by Luxita Kretzulescu – Cantacuzino in 1887.

Figure 5. The Little Trianon mirrored in the lake. Inset: Vornicul Grigore Cantacuzino and Luxita Kretzulescu, the parents of Gh. Gr. Cantacuzino, called 'the Nabab' (Ion, 2010)

The estate has belonged to one of the most prominent and interesting figures of the beginning of the twentieth century, Gh. Gr. Cantacuzino (Figure 6), called 'the Nabab', due to his enormous fortune. He was one of the most appreciated political figures, Member of Parliament and Prime Minister. 'The Nabab' was renowned for his authentic patriotism, which is remarked upon in the article 'A day at Floresti,' (Antemireanu, 1905).

Figure 6. Mr. and Mrs. Gh. Gr. Cantacuzino in the park at Floreşti (Antemireanu, 1905, p. 259)

Even today, there are many legends about this charismatic man, with a strong but warm personality. The Florești estate has been a favourite place for many personalities, including King Mihai (Fabra Bratianu, 2012) (Figure 7).

Figure 7. King Mihai I, Ileana Brătianu and two cousins on the deck of the lake in the pleasure grounds. Source: (Fabra Bratianu, 2012, p. 103).

Apart from its association with the Cantacuzino family, and especially with Gh. Gr. Cantacuzino, the Florești estate is important due to the exceptional quality of the palace architecture by I. D. Berindey (Figure 8), and to its relationship with the designed landscape, which has survived to a great degree.

The site is associated both with W. F. C. Meyer, and with E. Pinard, two of the most prominent garden designers in Romania. Further site surveys are needed to determine

more precisely what input each of them had and how much of their designs survives.

Figure 8. Details of the 'Little Trianon' Palace
Photo: Mihaela Radu

Most of the parts of the ensemble have survived, including: the pleasure grounds, the hunting park, the villas, the utility areas, and various important features: earthworks, water features, the general planting scheme, and some of the main views. All these elements are still in place, and although deteriorated, they are identifiable and can be restored. Thus, the ensemble is valuable as an example of late nineteenth century and early twentieth century country estate.

Although this type of estate is fairly common in Europe, in Romania they have become rare, which adds to the site's importance on a national level. The refinement and luxury of Pinard's design for the formal terraces are underlined by Maumené, in his presentation of the gardens.[9]

Moreover, the reinforced concrete features testify to Emile Pinard's intervention, whose project for the terraces (Figure 10) was praised at the end of the article 'La vie à la Campagne'.[10] Pinard was familiar with the appreciation of the contemporaneous French landscape architects. The project of the Bibescu (today Romanescu) park in Craiova was awarded the Golden Medal at the 1900 Paris International Exhibition and its authors were Edouard Redont, Jules Redont and his brother, and Emile Pinard.

Although the pleasure grounds at Florești are not a veritable arboretum, they do accommodate a collection of rare trees, and a plane tree, remarkable for its age, dimensions and aesthetic value (Figure 9).

Figure 9. The Pleasure Grounds, October 2014
Photo: Andreea Soare

Risks and opportunities

The most noteworthy feature of the ensemble is the palace called the 'Little Trianon,' which is at present in a ruinous state, and in danger of collapsing. Urgent consolidation works are imperiously needed so that this most important element of the park should not be lost. The whole composition revolves around this central element, and depends upon its presence. Works undertaken in the immediate proximity of the palace can induce vibrations which may affect and further deteriorate the monument. As it is, major and irreversible deterioration of the palace's fabric has already taken place. The retaining wall, the staircases, the inferior pond are also in a poor state, while only dispersed fragments of the balustrade have survived.

The lack of funds for a complete restoration has led to the need to find alternative solutions: for the park maintenance works a contract between USAMV Bucharest and the Cantacuzino Florești Foundation was signed.

[9] « On descend sur la deuxième terrasse par des escaliers latéraux accompagnes, comme l'est le mur de soutènement, de balustrades qui ont été prolongées latéralement. Un nouveau bassin est dispos contre le mur de soutènement et sur toute la largeur de la partie saillante. Il est alimenté par l'eau du bassin supérieur. Le mur de soutènement de cette seconde terrasse, qui se retourne en pan coupé, sera maintenu bas avec des caisses à fleurs posées sur les pilastres, cela pour éviter la répétition de la balustrade supérieure ; dans les pans coupes s'encastreront des bancs de pierre, abrite chacun par un portique recouvert de plantes grimpantes. » (Maumené, 1914, p. 188).

[10] « Par la dominante de ses grandes lignes, sa facture sobre et élégante, son encadrement libre de massifs et de grands arbres, cet ensemble doit parfaitement s'harmoniser avec le Parc paysager dans lequels il s'encastre. Il est digne en tout point des créations de l'école française des Jardins contemporains, dont, en Roumanie M. Pinard est l'excellent représentant. » (Maumené, 1914, p. 188).

For the consolidation and restoration of the palace, an idea competition was organised, which will be followed by developing a project and applying for EU funding.

The Florești estate is full of life even in its present state. The international horsemanship competition, Karpatia Horse Trials is annually organised here and enjoys great popularity. Although it is a great opportunity to bring people on the site, it has some drawbacks too: a few huge trees from the hunting park have been cut, new land works were undertaken in order to build water obstacles for the horse races, and, not least, new works involving reinforced concrete were undertaken on the geometrical pond in front of the palace.

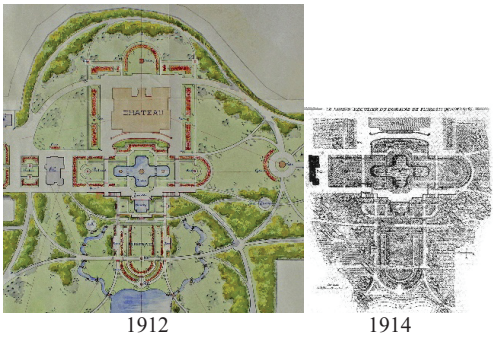
The grounds can be visited anytime. One of the main problems is that at present no effective security can be provided for the site. This leads to further deterioration of the built edifices, as well as of the poplar woodland, through uncontrolled cuts. This situation can lead to loss of authenticity.

Another threat is uncontrolled young tree growth. Thus, clearing works are needed, as well as maintenance works for old, rare and spectacular specimens. Likewise, the hard elements of the pleasure grounds should be restored: ponds, staircases, bridges, and water features. The research undertaken by the students revealed the fact that the area around the palace is situated on the crest of the *Florești Anticline*, on a salt massive, which can provoke landslides. This is important to know, because it will influence the types of future work which will be undertaken on the superior terrace, where the palace is situated.

For the conservation of an ensemble as complex and valuable as the Florești estate, a vast variety of interventions are required. Apart from the classical maintenance, restoration and revitalization works, a creative and sensitive approach will be needed in order to make sure that the ensemble will be functional in the future. This type of approach, called regeneration, allows for new functions to be introduced, and for new features such as: new accesses, parking lots, cabins for security staff, restrooms, resting places, belvederes, and event dedicated areas. These features should be integrated so as to affect neither the substance nor the spirit of the place.

The project

By studying the materials accumulated during the research stage, both documentary, and site surveys, we concluded that we have the possibility to elaborate a simplified classical conservation project. In the future these materials will be completed with archaeological surveys, which are needed for uncovering lost artefacts, as well as for finding the fragments of features that have been destroyed in time.



1912 1914

Figure 10. Florești Estate, *the geometric gardens*
Plans by Emile Pinard

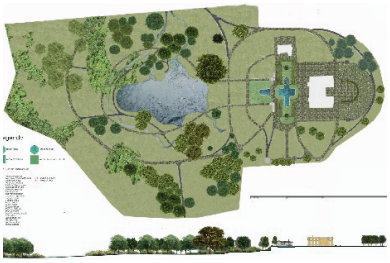
The owners have expressed a few requirements concerning the conservation project: they would like the restoration of the pleasure grounds to be as exact as possible, but with the addition of a parking area; the project should be sustainable and easy to implement; they are looking for proposals of activities which should take place both on the superior terrace, and on the pleasure grounds. These activities should bring in revenues which would then be used for further restoration of the park and palace. These sensible suggestions transform the project in something more than just revitalization. It will become a regeneration project, which involves not only a resuscitation of the place, but its rebirth. This is why the students have been organised in large groups of eleven, eight, and respectively ten members. As in the case of establishing the teams for the research stage, the preferences of the students were taken into consideration, keeping in mind a certain vision of the project. The supervisors adopted this attitude with the aim of obtaining the best possible results and of inducing the students the pleasure of working in this field. The students were encouraged to elaborate diverse projects, starting from the same data. Each group has had full access to the materials

resulting from research, as well as to the requirements of the owners. Each group has drawn their own set of conclusions, which led them to diverse solutions. The plans of each stage of the site's development were juxtaposed, in order to be examined and analysed, and on this basis a strategy of approach to the project was decided. Each group was encouraged to elaborate stages of the implementation of the project, so that the park would function continuously, bringing in revenue and attracting visitors.

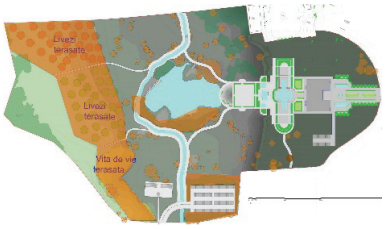
The aim of this school-project was helping the students develop the basic skills needed when dealing with a historical garden as a heritage asset. This includes: becoming familiarised with undertaking research at libraries, archives, etc. in view of understanding the asset and developing a project, organising a database with all the accumulated information, understanding the importance of a sensitive and sensible approach, adopting an 'in spirit' intervention, but avoiding pastiche, dealing both with teamwork and individual work, inducing a positive, empathic attitude toward the condition of heritage assets in general, and also the actual involvement in salvaging endangered assets.

CONCLUSIONS

The resulting three projects have many points in common, but they also present substantial differences. The spectacular trees will be retained and highlighted, while the valuable surviving features such as bridges and water features will be restored. The differences between the projects revolved mainly around the way the area around the palace was resolved, the connection between the palace and the gardens, and he connection between the terrace and the lake (Figure 11). Only the second team proposed a formal access from the east. This proposal was unfortunately not sustained by convincing arguments. The project work of the students has not been sustained financially either by the Cantacuzino Foundation or by the owners of the estate, but it has been facilitated by the convention between the University and the Foundation, which allowed all the students, from every year to conduct their practical activities on the site. The initiative of approaching this subject during the Historic Garden Restoration classes belonged to the professor of this subject.



Group 1



Group 2

Group 3

Figure 11. The projects of the students

The research stage has been difficult due to the distance of approx. 85 km to the site and also due to the unfavourable weather (October, November, and December). Another difficulty was linked to the students' timetable and the programme of the archives, libraries, museums where the research was undertaken. The students were given the opportunity to have intercourse with the owner of the estate and to participate in the 'Karpatia Horse Trials' event. During the project work the students have become affectively involved in their work, which has greatly contributed to the outcome of the projects. We strongly believe that a scholastic approach to conservation is less efficient, and cannot benefit from the same level of involvement, without which exceptional results are impossible.

ACKNOWLEDGEMENTS

This research work was carried out with the cooperation of the representative of the owners of the Florești estate, the students and their supervisors. We thank the students for the passion with which they got involved in the project and undertook the research work, which is like a veritable and fascinating detective work.

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MISCELLANEOUS


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STUDIES REGARDING THE EFFECTIVENESS OF PRE AND POST - HARVEST TREATMENTS, UPON THE SHELF LIFE OF SOME APPLE FRUITS VARIETIES STORED UNDER NATURAL VENTILATION ENVIRONMENT

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Abstract

The present paper show the research results of the pre-harvest treatments using the fungicides: Rovral 500 SC - 0,15 %, Magnate 50 EC - 0,09 % and Switch 62,5 WG – 0,1%, as well as post-harvest treatments: using Rovral 500 SC – 0,15% and Magnate 50 EC –0,09 %. The experiment was placed on the private farmers from Voinesti village – Dambovita region, well known regarding the tradition of apple growing, using the apple varieties Florina, Generos and Ciprian. From the obtained results it can be noticed that the pre-harvest and especially the post-harvest treatments, including packaging materials disinfection, are effective to control pathogens during storage period. The product Rovral 500 SC was more efficient than the fungicide Switch 62,5 WG, but less efficient than Magnate 50 EC product.

Key words*: treatments, rottenness, pre - harvest, post – harvest.*

INTRODUCTION

In the apple growth technology, one of the most important intervention is represented by the phytosanitary treatments performed in the orchard, as well as those carried out prior to the storage period (Boyette M. D. et al., 2008.). The losses due to the rottenness during the storage period are considerable, being up to 20-30% from the total yield.

Improving the cultural practices and to choosing the best varieties has an important contribution to the yield increase and to maintaining the fruit quality during the storage period.

Pre-harvest and post-harvest phytosanitary treatments represent an indispensable issue for the apple culture (Collins Mark et al., 2011).

Economical losses caused by the parasite fungus justify the phytosanitary treatments during the vegetation period, but at the same time, imply a special care to decrease the pesticide residues on fruits (Chira Lenuta, 2008).

During storage period, apples can be attacked by a high number of fungus pathogens that cause their rotting. Infection can begin from the orchard or during transport and the storage period, usually because of the poor hygiene of the packaging materials or of the storage environment.

MATERIALS AND METHODS

The experience has been organized in the Voinesti village area, in the private orchards of some members of the Dambovita Fruit Growing Association.

The purpose of this study was that of evaluating the apple fruits storage capacity and quality maintaining, following the phytosanitary treatments applied in the orchard and after harvest, in the autumn of the years 2014 and 2015. There were also analyzed samples of fruits, with a view to

appreciate the physical and chemical characteristics, at the end of the storage period, for Florina, Generos and Ciprian varieties.

It is necessary to mention that in the orchard the treatments have been performed on 1 ha/ farmer and after harvest the fruits were exposed to phytosanitary treatments, 100 kg fruits of each variety.

For the pre-harvest treatment the fungicides used were Rovral 500 SC - 0,15 %, Magnate 50 EC - 0,09 % and Switch 62,5 WG - 0,1%. These have been applied 20 days before harvesting and are recommended to prevent and to control the major apple fruits storage diseases produced by the fungus: *Penicillium sp., Botrytis cinerea, Monilinia fructigena* and *Gloeosporium album.*

The storage of the fruits has been performed in the store with a natural ventilation environment, with the following conditions: temperature 14-15^{o}C and air relative humidity 75-80 %.

For the post-harvest treatments in 2015 the following fungicides were used: Rovral - 0,15 % and Magnate - 0,09 % which have demonstrated to be the most effective on the orchard in the previous year. The two experimental variants were the treatment on fruits and the treatment on packaging materials (plastic material boxes) and fruits.

RESULTS AND DISCUSSIONS

As far as the pre-harvest treatment is concerned the data presented in Table 1 it can be observed that for all varieties, the best results have been obtained with the product Magnate 50 EC at 0,09 %. The attack rate was 6,0 % in the case of Florina; 6,4% for Generos and 5,8 % at Ciprian, but after different storage periods depending on the variety.

Table 1. The effectiveness of pre-harvest treatment on losses caused by rotting, during storage period

Variety	Variant	Concentration (%)	Storage period (days)	Rotted fruits
Florina	Control	-	90	17.7
	Rovral 500 SC	0.15	90	6.8
	Magnate 50 EC	0.09	90	6.0
	Switch 62,5 WG	0.10	90	7.4
Generos	Control	-	70	15.6
	Rovral 500 SC	0.15	70	7.2
	Magnate 50 EC	0.09	70	6.4
	Switch 62,5 WG	0.10	70	7.6
Ciprian	Control	-	105	16.8
	Rovral 500 SC	0.15	105	6.7
	Magnate 50 EC	0.09	105	5.8
	Switch 62,5 WG	0.10	105	7.8

Between the tested fungicides, the bad results have been obtained in the case of Switch 62,5 WG, apple fruits being attacked on a rate of 7,4 % - Florina; 7,6 % - Generos and 7,8 % - Ciprian. The product Rovral 500 SC was more efficient than the fungicide Switch 62,5 WG, but less efficient than Magnate 50 EC product.

We can underline that the treatments performed in the orchard before harvest period have had a major effect to reduce the percent of rotting fruits in the storehouse. The reduction was above 65 % in the case of Magnate 50 EC 0,09 %, in comparison with the untreated control.

Also, it was observed that in the case of Florina the main pathogen agent was *Penicillium* sp. which produces the moist rot, while for Generos the most important was the lenticular rot produced by the fungus *Gloeosporium album*. For the Ciprian variety mixed attack produced by *Penicillium* sp., *Botrytis cinerea* and *Monilinia fructigena* was observed.

If we consider the storage period, which was 90 days for Florina, 70 days for Generos and

105 days for Ciprian, we can say that the last variety had a very good behavior during storage, in relation with the major pathogens.
In regard to the post-harvest treatment, as can be observed in Table 2, these were more efficient than those performed during the vegetation period, with the same product and concentration. The Magnate 50 EC product in a concentration of 0,09 % was again remarked and it gave the best results. Ciprian variety had a less rate of rotting fruit, even if the storage period was longer.

Table 2. The effectiveness of post-harvest treatment on losses caused by rotting, during storage period

Variety	Variant	Concentration (%)	Storage period (days)	Rotted fruits (%)
Florina	V1- Control	-	90	11.4
	V2- Rovral (fruits)	0.15	90	5.4
	V3-Rovral (package + fruits)	0.15	90	2.3
	V4- Magnate (fruits)	0.09	90	4.5
	V5-Magnate (package + fruits)	0.09	90	2.0
Generos	V1- Control	-	70	10.4
	V2- Rovral (fruits)	0.15	70	4.8
	V3- Rovral (package + fruits)	0.15	70	2.9
	V4- Magnate (fruits)	0.09	70	4.4
	V5-Magnate (package + fruits)	0.09	70	2.2
Ciprian	V1- Control	-	105	10.0
	V2- Rovral (fruits)	0.15	105	4.8
	V3-Rovral (package + fruits)	0.15	105	2.8
	V4- Magnate (fruits)	0.09	105	4.2
	V5-Magnate (package + fruits)	0.09	105	2.4

As we can see, in the case of all varieties, the lower rotting percent has been registered for the variants where the fruits as well as the packaging materials were treated, so this is an very important issue for the farmers who store the apple fruits for a longer period. To prevent rotting, it is recommended to disinfect the packaging materials, because these are an important source of pathogen infection, mainly when they are reused for a longer period of time. Thus, in the case of the Florina variety - V3, the attack rate was only 2 %, as compared with the control – 11,4 %, or to the variant when there only fruits have been treated – 4,5 % , when the Magnate fungicide was used.
From the present data it can be noticed that the pre-harvest and especially the post-harvest treatments, including packaging materials disinfection, are effective to control pathogens during storage period.

CONCLUSIONS

The storage period under natural ventilation environment was 90 days for Florina, 70 days for Generos and 105 days for Ciprian; the last variety had a very good behavior during storage, in relation with the major pathogens.
For all varieties, the lower rotting rate has been registered at the variant in which both the fruits and packaging materials were treated because these are an important source of pathogen infection.
For the Florina variety, the principal pathogen agent was *Penicillium* sp. which produces the moist rot, while for Generos the most important was the lenticular rot produced by the fungus *Gloeosporium album*.
The product Rovral 500 SC was more efficient than the fungicide Switch 62,5 WG, but less efficient than the Magnate 50 EC product.

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TEMPERATURE AND PH INFLUENCE ON ANTAGONISTIC POTENTIAL OF *TRICHODERMA* SP. STRAINS AGAINST *RHIZOCTONIA SOLANI*

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Abstract

Species of the genus Trichoderma sp. are considered as potential biocontrol agents (BCA) for many plant diseases. The effectiveness of biocontrol agents depends on several parameters therefore their application showed consider climatic factor that could affect their biocontrol capacity.The present study examined the antagonistic potential of two Trichoderma sp. strains (Td85 and Td50) against Rhizoctonia solani depending on pH (4.5;5.5) and temperature (25°C, 30°C). Both strains of Trichoderma sp. studied inhibit stronger growth of Rhizoctonia solani at 30°C compared to 25°C. Also our results revealed that both strains of Trichoderma have maximum antagonistic ability to Rhizoctonia solani strain at pH=4.5.

Key words*: climatic factors, biocontrol, inhibition percent*

INTRODUCTION

Rhizoctonia solani is a soil pathogen that causes diseases in a wide range of hosts of agricultural, horticultural and ornamentals crops (Cundom et al., 2003). It can cause severe damage specially during the seedlings pre-emergence and post-emergence stages and is a limiting factor in the production of crops. Diseases caused by *Rhizoctonia solani* are difficult because this pathogen survives for many years as sclerotia or as mycelium in soil or organic matter under various conditions and has an extremely wide host range. (Osman et al., 2011).

Many important agricultural and horticultural crops worldwide are mostly affected by *R.solani* including tomato, bean, potato, strawberry, soybean, tobacco, tulip (Rahman et al., 2014; Seema and Devaki, 2012; Osman et al., 2011; Lahlali and Hijri, 2010, Grosch, 2007; Elad et al., 2006; Singh and Chand, 2006; Schneider et al., 1997; Tu et al., 1996).

Species of the genus *Trichoderma* sp. are considered as potential biocontrol agents (BCA) for many plant diseases (Galarza et al. 2015; Singh et al. 2013; Kohl and Schlosser 1989).

The effectiveness of biocontrol agents depends on several parameters, that include specific pathogen, soil texture, water content, pH, temperature and crop history (Berg et al., 2005; Kredics et al., 2003), therefore their application should consider the environmental stress that could affect their ability to maintain their biocontrol capacity.

Although there are several publication on the antagonistic activities of *Trichoderma* sp., there is little information on the effects of pH and temperature on its antagonistic proprieties against *Rhizoctonia solani* (Daryaei et al., 2016; Lahlali and Hijri, 2010; Montealegre et al., 2009; Santamarina and Rosello, 2006).

The goal of this work was to evaluate the *in vitro* effects of different environmental conditions (temperature and pH) on the antagonism of *Trichoderma* sp. towards *Rhizoctonia solani*.

MATERIALS AND METHODS

One *Rhizoctonia solani* strain and two *Trichoderma* sp. strains (Td85 and Td 50) obtained from RDIPP culture collection were used in this experiment. These fungal strains were maintained at 4°C on Potato Dextrose Agar (PDA) with periodical subculturing on the same medium at 25°C.

Trichoderma sp. strains used in this experiment were identified at species level as *Trichoderma*

asperellum according molecular analysis(Paica et al., 2015).

Fungal isolates of *Trichoderma* sp. were *in vitro* screened for their ability to suppress the mycelial growth of *R. solani* in dual culture assays (Morton and Stroube, 1955).

Mycelial blocks (5mm) were cutted from the periphery of 5 days old culture of both *Trichoderma* sp. and *R.solani*. Two mycelial blocks one from *Trichoderma* and other from *R.solani* were placed in a same time on PDA (Potato Dextrose Agar) plate in opposite directions and incubated at two temperatures (25°C, 30°C) for 4 days. The radial growth of each colony was measured at 48.72 and 96 hours interval.

PDA with different pH levels (4.5 and 5.5) were poured into Petri dishes and a 5 mm plug from the margin of actively growing colony of *Trichoderma* sp. strains and *R.solani* were placed in opposite direction and were incubated at 28°C for 4 days.

Controls were also set up with the pathogen alone so that a growth without interactions could be precisely measured. Three replicates for each antagonist-pathogen combination and for the controls were considered. Percent inhibition of mycelial growth of targeted fungal pathogen over control was calculated by following equation:

I% = C - T/C where:

I%-percent inhibition in mycelial growth

C-colony diameter of pathogen in control plates

T -colony diameter of pathogen in dual culture plates

RESULTS AND DISCUSSIONS

A broad range of temperature tolerance for growth and sporulation of *Trichoderma* sp. is a very interesting feature for suitability of the antagonism.

A clear zone of interaction between antagonist and pathogen was observed after 48 h of incubation. The mycelium of both *Trichoderma* sp. strains grew abundantly on *R. solani* after 4 days of incubation.

Both *Trichoderma* sp. strains were able to significantly decrease the radial growth of *R.solani* mycelium within 4 days at both temperature conditions. *Trichoderma* Td85 was more active against *R.solani* strain at 30°C compared to 25°C. Td85 limited gowth of *R.solani* mycelium more than 57% at 30°C (fig.1). Also, Td85 strain was more effective at 25°C with a inhibition percentage of 54.48% compared to Td50 with a percentage of 53.33% (fig.1 and 2). Our results are in agreement with Grosch et al, (2007) who reported that most strains of *Trichoderma* sp. studied by them showed better antagonistic activity against *R.solani* at higher temperature (30°C).

Also, our results are in conformity with those of Montealegre et al., (2009) who suggested that low and high temperatures (between 5°C and 22°C) do not changes the biocontrol capacity of different *Trichoderma* sp. strains on *R.solani.*

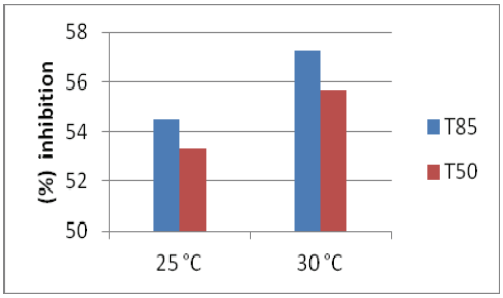

Figure 1. Effect of the temperature on the inhibition of the mycelial growth of *R.solani* in dual culture assay with *Trichoderma* sp. after 4 days of incubation

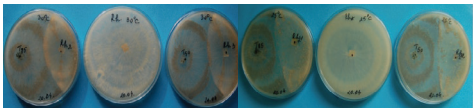

Figure 2 Antagonistic effect between *R.solani* and *Trichoderma* sp. at 30°C (left) and 25°C (right) after 4 days of incubation

Our results showed that both *Trichoderma* sp. strains were antagonistic to *R.solani* at both pH values although differences were found among strains. The data presented in fig.3 indicate that both *Trichoderma* sp. strains studied were effective in suppressing *R.solani* at pH=4.5 compared to pH=5.5.

At pH=4.5, no significant differences between the two antagonistic strains was observed regarding the percentages of inhibition (56%) of the mycelial growth (fig.3, fig.4). However at pH=5.5, Td85 strain had a slight increase of

inhibition procentage (53.72%) compared to Td50 (52.15%)

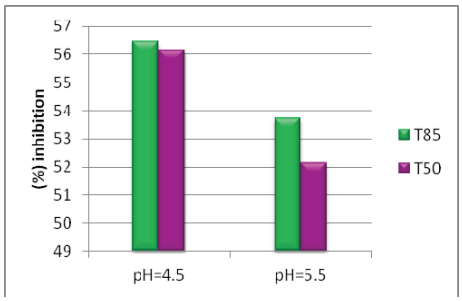

Fig. 3 Effect of pH on the inhibition of radial growth of *R. solani* in dual culture with *Trichoderma* sp. strains after 4 days of incubation

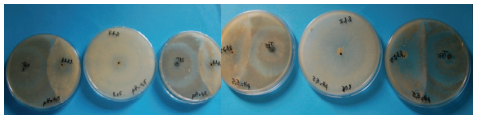

Fig.4 Antagonistic effect between *R.solani* and *Trichoderma* sp. at pH=4.5(left) and pH=5.5 (right) after 4 days of incubation

Results of Daryaei et al., 2016 suggest that at different pH values *Trichoderma atroviride* gave significantly various amount of inhibition and overgrowth activity against R.solani in dual culture assays with the strongest inhibition(76%) at pH=7.5. However Bagwan, 2010 reported that most favourable pH for maximum antagonistic potential of *Trichoderma viride* against *S. rolfsii* and *R.solani* ranged between 5.5 to 6.5.

CONCLUSIONS

The interaction between *Trichoderma* sp. and *R.solani* was dependent on temperature and pH. *Trichoderma* Td85 strain proved to be most effective with the highest percentage of inhibition at 30°C whereas Td50 strain showed lower inhibition at this temperature.

Our results supported that the most appropriate pH for maximum antagonistic potential of tested strains was 4.5.

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EFFECTS OF CHRONIC TOXICITY INDUCED BY CADMIUM ON THE GAMETOPHYTE OF TWO FERN SPECIES

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Abstract

The aim of the present study was to determine the effect of chronic cadmium (Cd) action on the germination of spores and gametophyte differentiation in species Athyrium filix-femina (L.) Roth *and Dryopteris filix-mas* (L.) Schott*, on different culture media (Knop solution, soil) for a period of 3 months. Cadmium was used in the following concentrations: C=0 mg Cd · L^{-1} Knop solution/kg^{-1} soil, V_1=25 mg Cd · L^{-1} Knop solution/kg^{-1} soil, V_2=50 mg Cd · L^{-1} Knop solution/kg^{-1} soil, V_3=100 mg Cd · L^{-1} Knop solution/kg^{-1} soil, V_4=150 mg Cd · L^{-1} Knop solution/kg^{-1} soil. The percentage of germinated spores was found to decrease with the increasing Cd concentration in the environment, while germination is delayed in time. Unlike the Knop solution variants, in the soil variants gametophyte development was not significantly affected; for V1-2 soil concentrations the sporophyte appears in Athyrium filix-femina, a stage that was also noted for the Knop solution control, for the same species. In the case of the variants grown on Knop solution, although the spores did germinate and the gametophyte began to differentiate, Cd-induced chronic stress cannot be compensated by the gametophyte, so that the cells lose their membrane integrity, and their survival is compromised.*

Key words*: heavy metals, Athyrium filix-femina, Dryopteris filix-mas.*

INTRODUCTION

Cadmium is one of the most important heavy metals, and it is usually encountered: on the International Agency for Research on Cancer. list due to its carcinogenic properties, on toxic substance list of Agency for Toxic Substances and Disease Registry (CAS ID #:7440-43-9), among the top 126 priority pollutants, according to the United States Environmental Protection Agency (Flora, 2014).

It is a transition metal (block d), which presents chemical similarities to zinc (Zn) – in fact, they both belong to the same group (12). These similarities can cause the toxicity of Cd: replacement of Zn, a trace element, by Cd affects metabolic processes (Wuana and Okieimen, 2011).

According United States Geological Survey, to estimate Cd reserves, Zn reserves are checked, while taking into account this aspect: Cd is approximately 0.003% of Zn ores.

Cadmium pollution is due to natural sources, the contribution of which varies between 10-50% of total emissions, and also to anthropogenic sources. For example, the mining of zinc is estimated to release approximately 6 million tones, as a byproduct of Cd (Raza et al., 2015). In order to present the main anthropogenic sources International Cadmium Association proposes a classification that takes into account the presence of Cd as impurity – non-Cd products: iron and steel, fossil fuels, cement, phosphate-based fertilizers, and, as a necessity: NiCd batteries, pigments, Cd alloys, electronic Cd compounds, etc.

Determining Cd toxicity on living beings is performed using acute and chronic toxicity tests. Acute toxicity refers to short-period exposure of an organism to the action of one or more toxic agents. Within this framework, lethal concentration (LC_{50}) is determined, i.e. the concentration that leads to the death of 50% of the test organisms. In nature, most pollutants manifests their action after a long time, and pollution is usually in non-lethal concentrations. Chronic toxicity is the "capacity of a substance or a solution to induce adverse effects for a long time, after repeated or continuous exposure, sometimes over the whole lifetime of an organism" (United States Environmental Protection Agency).

The best-known and most severe form of chronic exposure to the action of Cd is that occurring in Japan: consumption of rice contaminated with Cd leads to the disease

called "Itai-Itai", which is characterized by kidney damage and disorders of the bone system (osteomalacia and osteoporosis) (Nordberg et al., 2015).
According to Pavlik (1997) 90% of the Cd taken up by plants comes from the ground, and only 10% from the atmosphere, as the main paths of penetration are the roots and leaves.
Catalá et al. (2011) recommended using ferns in toxicity tests, both acute and chronic, because the results can be extrapolated to wild plants or cultivated plants, they are found in different habitats (ecological or organic relevance), and growing spores and development gametophyte can be made on different media (solution, soil, etc.).
In order to know pteridospore sensitivity in a chronic toxicity testing of different substances and environmental samples should be used (Catalá and Rodriguez-Gil, 2011).
The aim of this paper was to determine the chronic effect of the action of Cd on the germination of spores and gametophyte differentiation in species *Athyrium filix-femina* (L.) Roth (*Aff*) and *Dryopteris filix-mas* (L.) Schott (*Dfm*) on different culture media.

MATERIALS AND METHODS

In order to obtain the spores of the two species, the author took several study trips along the Vâlsan Valley over the period August 2015. Mature leaves were collected from several individuals in different sites in order to ensure genetic diversity. After releasing the spores in the sporangia, there followed their collecting and preserving in a refrigerator at 4°C.
Testing media:
Two test media were used: Knop solution [$Ca(NO_3)_2$:1.00 $g \cdot L^{-1}$; $MgSO_4$: 0.25 $g \cdot L^{-1}$; KH_2PO_4: 0.25$g \cdot L^{-1}$; KNO_3: 0.25$g \cdot L^{-1}$] and flower earth Florisol obtained by processing from the deposit in Dersca-Dorohoi, with a pH between 6.5-7, humidity 60-70% N: 410 ppm, P: 192 ppm, K: 1350 ppm; organic substance min 70% dry product. The soil was sterilized at 60°C.
Tested substance: The substance tested was Cd acetate in various concentrations; reporting was done per L for the samples in Knop solution, and per kg for the soil variants: Control (C)=0 mg $Cd \cdot L^{-1}$ kg^{-1}, V_1=25 mg $Cd \cdot L^{-1}$ kg^{-1}, V_2=50 mg $Cd \cdot L^{-1}$ kg^{-1}, V_3=100 mg Cd L^{-1} kg^{-1}, V_4=150 mg $Cd \cdot L^{-1}$ kg^{-1}.
To ensure optimal conditions for development, the culture vessels were kept in growth chamber at 25°C in the daytime, and 15°C at night, with constant humidity and illumination (photoperiod: 16 hours of light, and 8 hours of dark). The soil variants were placed in Petri dishes and periodically watered with distilled water.
The experiment had 3 repeats. For the Knop solution variants quantitative determinations were made: the percentage of germinated spores was determined, and to do the statistical interpretation the SPSS program, version 16 was used, with which the average and the standard deviation were calculated. Comparisons were made using Duncan's test. To monitor the differentiation of the gametophyte in all variants, observations were made at regular intervals, and photomicrographs were made under an OPTIKA B275 microscope with an A630 Canon Power Shoot camera and under a OPTIKA stereo-microscop.

RESULTS AND DISCUSSIONS

Germination of spores is influenced by a number of factors such as light, phytohormones, ions of metal, temperature (Suo et al., 2015).
As far as the cultures of spores are concerned, which used the Knop solution, Cd significantly affected germination, primarily by reducing the percentage of germinated spores. All experimental variants were affected, except V_2Cd *Dfm*, where there were 7 percent more spores than in the controls, and in *Aff* – between V_3Cd and the control there were no significant differences (see Table 1). Also, spore germination was delayed in time in the V_4Cd variant, in both species: germination was reported after a month compared to the control. Also, in this variant the lowest percentage of spores germinated was obtained: 4 for *Dfm* and 15 for *Aff.*
Time delay and a lower percentage of spores germinated due to the presence of various concentrations of Cd were also reported by Gupta and Devi (1992), and Biswas et al. (2015) in several species of ferns.

Gupta et al. (1992) found that, in concentrations of 2.5 and 5 mg Cd L^{-1} spore germination was inhibited, and the development of the gametophyte was discontinued at the stage of prothallium blade in the species Ceratopteris thalictroides.

Table.1. Influence of heavy metals on the germination of spores

Species	**C**	**V_1Cd**	**V_2Cd**	**V_3Cd**	**V_4Cd**
Percentage of germinated spores (mean ± standard deviation)					
Athyrium filix-femina	90.3±2[a]	26.6±5[c]	45±1[b]	92.3±1[a]	15±1[c]
Dryopteris filix-mas	81.6±2[b]	75.3±4[c]	89.3±5[a]	54±3[d]	4±1[e]

Legend: The values are the means of 3 replicates ± standard deviation; a, b, c, d, e – the results obtained from the Duncan test: the comparisons were made between control and V_{1-4} for each metal.

In Table 2 and Table 3 the gametophyte differentiation after one month is shown, both on the soil and on the Knop solution, in both species.

Table 2. Gametophyte differentiation of *A. filix-femina* (one month after experiment initiation)

Variants	Knop solution	Soil
Control	blades differentiation, antheridia	young chordate prothallia, antheridia
V_1Cd	filaments differentiation, germinated spores	prothallium blade, antheridia
V_2Cd	prothallium blade, three-dimensional cell masses, antheridia	chordate prothallia
V_3Cd	prothallium filament, three-dimensional cell masses	chordate prothallia, rare prothallium blade, antheridia
V_4Cd	filaments differentiation	prothallium blade, antheridia

Table 3. Gametophyte differentiation of *D. filix-mas* (one month after experiment initiation)

Variants	Knop solution	Soil
Control	prothallium blade	young chordate prothallia
V_1Cd	damaged filaments and blades differentiation, short rhizoid, three-dimensional cell masses	chordate prothallia
V_2Cd	blades differentiation, three-dimensional cell masses	chordate prothallia
V_3Cd	filaments, blades differentiation	prothallium blade and filament
V_4Cd	germinated spores	prothallium blade and filament

It was found that gametophyte development was much faster in the soil-grown variants, where the following stages were noted: chordate prothallia in the controls of both species (Figure 14, 17) V_2Cd (Figure 23) and V_3Cd in *Athyrium*, V_1Cd and V_2Cd in *Dryopteris*, blade in V_1Cd (Figure 20) and V_4Cd in *A. filix-femina*, and for the second species, filaments in V_{3-4}Cd. In *Athyrium*, antheridia with viable antherosoids were observed, in all cases, except V_2Cd. In the Knop solution variants the most advanced stage of development was the prothallian blade one, which occurred in the controls of the two species (Figure 1, 4, 5) and in V_2Cd *Athyrium* (Figure 7). The filament stage usually occurred at high concentrations V_{3-4} (Figure 10).

Due to the influence of Cd, the gametophyte development was affected: the filaments (V_1Cd in both species) and blades were partially damaged (V_2Cd in *Dryopteri*s - Figure 12) and three-dimensional cell masses were formed (Figure 8). The abnormal growth of the prothallium blade was reported by Gupta and Devi (1994), as well, in the species *Pteris vittata*, where the gametophyte is much more sensitive to Cd action than the sporophyte.

Table 4. Gametophyte differentiation of *A. filix-femina* (three months after experiment initiation)

Variants	Knop solution	Soil
Control	chordate prothallia with sporophyte	chordate prothallia with archegonia
V_1Cd	damaged filaments, germinated spores	chordate prothallia and sporophyte with embryonic leaf
V_2Cd	damaged prothallia	chordate prothallia and sporophyte
V_3Cd	damaged filaments	mature chordate prothallia with archegonia, young chordate prothallia with antheridia, fecundation
V_4Cd	damaged filaments, germinated spores	prothallia, antheridia

Table 5. Gametophyte differentiation of *D. filix-mas* (three months after experiment initiation)

Variants	Knop solution	Soil
Control	young elongated prothallia	chordate prothallia, antheridia
V_1Cd	damaged filaments	chordate prothallia,
V_2Cd	damaged filaments and blades	chordate prothallia, archegonia
V_3Cd	germinated spores, damaged filament	young chordate prothallia
V_4Cd	few germinated spores	young chordate prothallia (small)

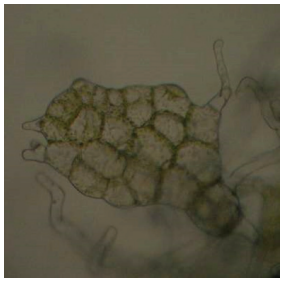


Figure 1. *Dfm,* C, one month (x100).

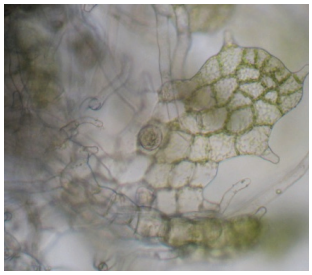


Figure 2. *Dfm,* M, 3 months (x100).

Figure 3. *Dfm,* C, 3 months (x400).

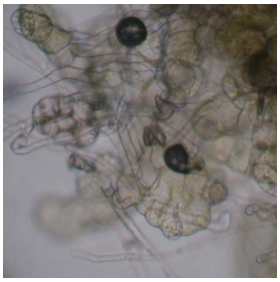


Figure 4. *Aff* C, one month (x100).

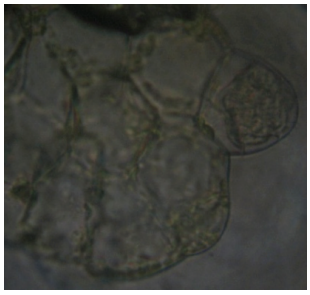


Figure 5. *Aff* C, one month (x400).

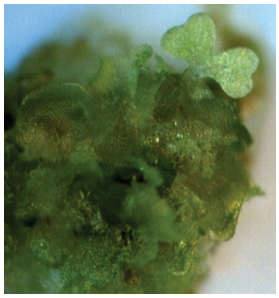


Figure 6. *Aff* C, 3 months (x10).

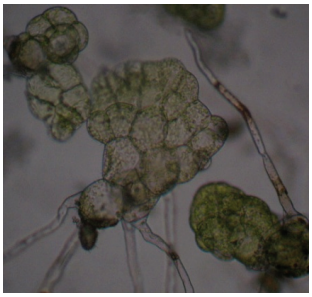


Figure 7. *Aff* V_2, one month (x100).

Figure 8. *Aff* V_2, one month (x100).

Figure 9. *Aff* V_2, 3 months (x100).

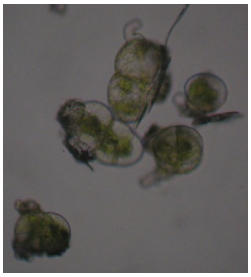


Figure10. *Dfm* V_3, one month (x100).

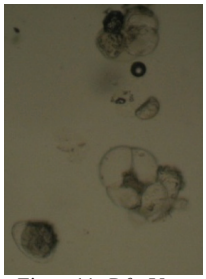


Figure11. *Dfm* V_3, 3 months (x100).

Figure12. *Dfm* V_2, one month (x100).

Figure13. *Dfm* V_2, 3 months (x100).

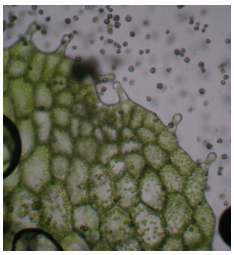


Figure14. *Dfm* C, soil, one month (x100).

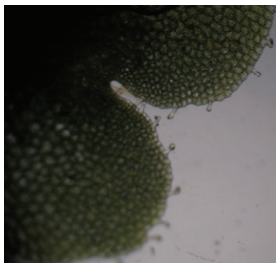


Figure15. *Dfm* C, soil, 3 months (x40).

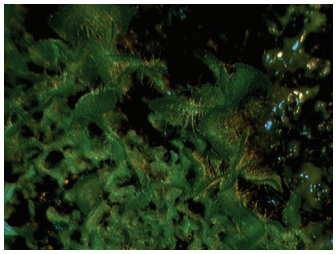


Figure16. *Dfm* C, soil, 3 months (x10).

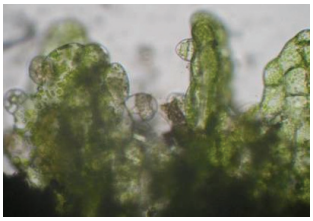


Figure17. *Aff* C, soil, one month 10x10 (x100).

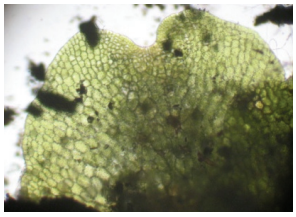


Figure18. *Aff* C, soil, 3 months (x40)

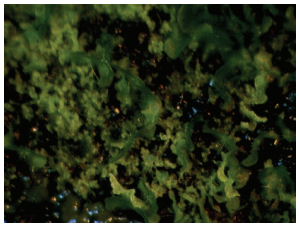


Figure19. *Aff* C, soil, 3 months (x10).

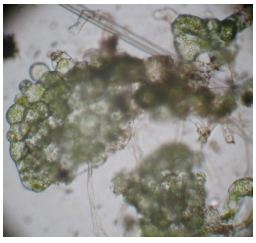


Figure 20. *Aff* V_1, soil, one month (x100).

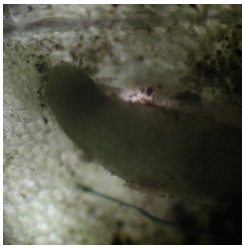


Figure 21. *Aff* V_1, soil, 3 months (x100).

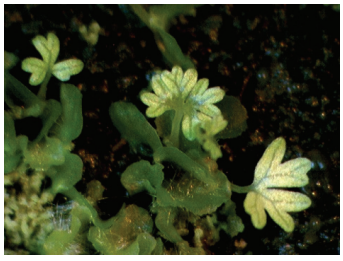
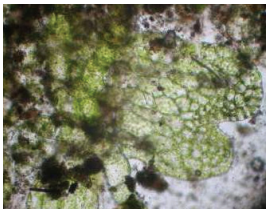
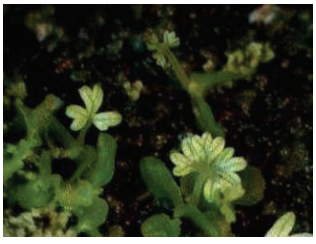


Figure 22. *Aff* V_1, soil, 3 months (x10).





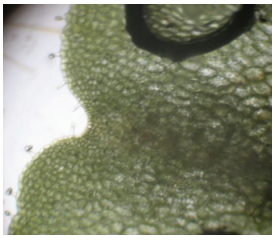


Figure 23. *Aff* V_2, soil, one month (x100). Figure 24. *Aff* V_2, soil, 3 month (x10). Figure 25. *Dfm* V2, 3 months (x40).

After 3 months from the initiation of the experiment, in *A. filix-femina* as control, the Knop solution variant, the sporophyte was formed (Figure 6), while *D. filix-mas* was still in the gametophyte stage: prothallium blade (Figure 2) with antheridia (Figure 3). According to Table 4 and Table 5, regardless of species and experimental variant, after 3 months the gametophyte development was stopped in the stage of chordate prothallium (Figure 9), with damaged blades and filaments (Figure 11, 13). For low concentrations (V_{1-2}), in the soil-grown variants, Cd stimulated the development so that within 3 months the sporophyte was formed on the gametophyte (Figure 22, 24), in which the juvenile leaves can be noted (Figure 21). In the remaining Cd variants, and also in the control, the

gametophyte was still in the stage of heart-shaped prothallium of different sizes (Figure 15, 16, 19) with/without antheridia and/or archegonia (Figure 18, 25).

CONCLUSIONS

Cd affected the percentage of spores germinated and gametophyte development in the experimental variants grown on Knop solution.

The influence of Cd on gametophyte development was far more significant in the solution variants (damaged blades and chordate prothallia) as compared to those grown on soil, and between species, in *Dryopteris filix-mas* as compared to *Athyrium filix-femina*.

In V_{1-2} concentrations for soil-grown *Athyrium* the occurrence of the sporophyte is noted. In the case of the variants grown on Knop solution, although the spores did germinate and the gametophyte began to differentiate, Cd-induced chronic stress cannot be compensated by the gametophyte, so that the cells lose their membrane integrity, and their survival is compromised.

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POSTHARVEST HANDLING OF FRUIT AND VEGETABLES IN TURKEY

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Abstract

Turkey is the unique country that has land in both Europe and Asia. Turkey has approximately 75 000 000 population and is an agricultural country. In this context Turkey has great fruit and vegetable production potential. Turkey is one of the biggest producers of apples, pears, quinces, peaches and apricots, citrus (as fruit species) and tomatoes, peppers, melons, watermelons (as vegetable species). However, the amount of cold stores and packinghouses is deficient for its potential. Moreover the export rate is 5-10% of production. In addition the rate of postharvest losses of these crops is approximately 35-40%. On the other hand, the usage of controlled atmosphere (CA) stores and new postharvest technologies debouched rapidly. As a result increasing the amount of CA stores and modern packinghouses, arranging the errors in cold chain and improving the reproducing conditions will make Turkey an important fresh perishable exporter. Besides, the potential and capacity of production will be validated by minimizing the postharvest losses. On the other hand, cave storage will be improved by some modifications such as temperature, humidity and air circulation conditions. Their numbers, and hence capacities are steadily increasing. Eventually, the cave stores have impressions as being the predominating stores for citrus, potatoes and onions.

Key words*: Turkey, horticultural production, postharvest losses, postharvest technology.*

INTRODUCTION

STATUS OF HORTICULTURAL SECTOR

Turkey is blessed in the production of wide range of fruits and vegetables of which many are indigenous to the area such as pears, quinces, cherries, plums, grapes, walnuts, hazelnuts and pistachios. Of the total cultivated land, fruits and olives trees including vineyards occupy 3.012 million ha (11.0%) whereas vegetables 0.663 million ha (2.4%). It is estimated that 75% of vegetable fields and probably 40-50% of fruit, vine, olive orchards are irrigated. Although the land has nearly remained stable over the last decade the total production of horticultural produce increased from approximately 24 million tons to over 45 million tons (1990-2010). This may have been largely due to increase in large bearing orchards, and for vegetables, improved cultural techniques and high yielding varieties. Table 1 and Table 2 show figures on main fresh fruits and vegetables. Total annual fruit production was 9 million tons in 1990 and is probably around the 15-16 million tons in recent years. Total annual vegetable production was around 22 million tons in 1990 and is probably 30 million tons in recent years. Estimated export rate of 5-7% for fruits and vegetables reveals 1.5 million tons fruits and 1.8 million tons of vegetables are exported. Intensive production of fruits and vegetables is concentrated in the Mediterranean, Aegean, and Marmara Region as well as the central plateau, hazelnuts in the Black Sea Region, and pistachio in the Mediterranean and Southeastern Anatolia Region. Protected cropping is concentrated in microclimatic zones along the Mediterranean coast and Aegean Region. In these areas 90% of the total protected culture is used for vegetables, 7% for cut flowers and indoor plants, and 3% for fruits yield (strawberries, bananas, peaches).

The objectives of the Government for the development of horticultural sector have been mainly concentrated on: a) Modernizing production techniques to increase productivity and growers income, b) Producing highest quality of fruits and vegetables as possible, c) Meeting the food requirements of persistently increased population, d) Promoting horticultural exports as a result of oversupply.

In fact, many economic policies and reforms initiated by the Turkish government in the early

1990's and reviewed in 2002 have encouraged growers, private sector and market forces as well as exporters, as a result of which overall horticultural production has reached 45 million tons a year.

Table 1. Fruit production in Turkey (tons).

Year	Apple	Pears	Quince	Louquats	Peaches	Plums	Apricot	Cherries	Sour cherries	Cornel	Olive
1990	1900000	413000	79000	9000	350000	188000	240000	143000	90000	17000	1100000
2000	2400000	380000	105000	11500	430000	195000	530000	230000	106000	12000	1200000
2010	2600000	380003	121085	12112	539403	240806	450000	417905	194989	12517	1352827

Year	Almonds	Hazelnuts	Walnuts	Chestnuts	Pistachios	Mulberry	Pomegranates	Strawberries	Persimmon
1990	46000	375000	115000	80000	14000	80000	50000	51000	10000
2000	47000	470000	116000	50000	75000	60000	59000	130000	12000
2010	55938	600000	178142	59171	128000	75096	208502	299940	26277

Year	Oranges	Mandarin	Lemons	Grape fruits	Kiwi fruits	Bananas	Figs	Grapes	GrenTea Leaves
1990	735000	345000	357000	33000	-	36000	300000	3500000	608440
2000	1070000	560000	460000	130000	1400	64000	240000	3600000	758038
2010	1710500	858699	787063	213768	26554	210178	254838	4255000	1305566

Table 2. Vegetable production in Turkey (tons).

Year	Tomatoes	Cucumber	Pepper	Eggplant	Okra	Squash	Pumpkin	Melon	Water melon	Pea (green)
1990	6000000	1000000	900000	735000	22000	294000	57000	1650000	3300000	37000
2000	8890000	1825000	1480000	924000	27500	260000	72000	1865000	3940000	48000
2010	10052000	1739191	1986700	846998	36748	314340	89368	1611695	3683103	90191

Year	Bean (green)	Calavence	Cowpea	Broad beans	Cabbage	Lettuce	Artichokes	Celery	Cauliflower	Broccoli	Spinach
1990	430000	31000	-	62000	699000	186000	10000	12000	68000	-	160000
2000	514000	41000	12000	45000	725000	333000	24500	16500	90000	-	205000
2010	587967	70614	16591	41929	693012	419298	29070	1534	158579	26493	218291

Year	Cultivated mushroom	Onion	Garlic	Leek	Carrots	Radish	Purslane	Parsley	Mint	Dill	Rocket	Cress
1990	-	1736000	95000	340000	168000	71000	4000	-	-	-	-	-
2000	-	2428000	102000	308000	235000	167500	2250	40000	5000	1700	1150	1250
2010	21559	2065478	98170	244812	533253	155673	4936	56332	11772	2978	4058	2380

These measures and programs have an "open-end" potential (increases 100%, 200% or more) in contrast, however to "close-end" potential defined as postharvest technology and handling.

After realizing that estimated losses of horticultural crops running at 25-30% in Turkey more emphasis has been given to the postharvest handling systems in the last two decades.

POSTHARVEST SYSTEMS AND HANDLING PRACTICES

Postharvest systems and handling was first introduced in Turkey in the early 1960's by Prof. L. Lary Claypool from the University of California Davis. Now many people working at the Universities and Ministries have specialized in the postharvest field and carrying out research and training programs on horticultural crops.

In Turkey, most fruits and vegetables the higher temperature during the growing period the earlier the time of harvest. Long hot summers especially predominating in the South create sunburn problems on produce, thus detracting from quality. Sunburned crops may show symptoms of sun scald if they are stored too long like apples, pears, tomatoes, peppers etc. As a result some pome fruit varieties on dwarf rootstocks are growing with shading net systems recently. Russeting is another environmental problem in coastal regions. If leaf overlapping cannot avoided, tomatoes and many fruits as well as citrus, pome and stone fruits are highly affected for quality. Frost and hail damage cripple the yield and quality on many fruits and vegetables in some years.

Overhead sprinkling and mixing overhead fans are used for late spring cold injury especially.

More recently, Ministerial and some private organizations have development laboratories and mobile analyses units to help growers solve nutritional problems. But some postharvest physiological disorders connected with nutritional imbalances have a big problem still especially for pome fruits storage. All the calcium deficiency related disorders which appear during storage and during marketing have nearly been prevented. Foliar application of their salts has been a common practice by the growers on apples, pears, quince, tomatoes and pepper.

At the moment, extensive research studies conducted by the universities as well as by the Ministries Institutes have well established the use of growth regulators to increase produce quality. But, the use of growth regulators may have limited use due to harmful effects on human health, in practice. Also there are well established toxicology laboratory in some regions, for residue analyses on exporting fresh crops.

Many research studies are in progress relating preharvest cultural practices to the postharvest produce quality. Variety evaluations, dwarfing rootstocks, effects of pruning and thinning, mulching, soil cultivation and pollination are some areas being explored and the results are disseminated to growers and extension personnel for useful implementations.

Today, in Turkey, as in the past this is achieved through the hand harvesting in all horticultural crops except in the processing industry. There is no doubt that humans can accurately select for maturity, allowing accurate grading and multiple harvest, can handle the commodity with a minimum damage. Mechanical harvesting is practiced to great deal on sweet corn, potatoes, onions, olive, tomato paste production, juice and canning production.

Postharvest losses occurring quantitatively and qualitatively in all phases of post-production cycle (cold chain) are in the vicinity of 35-40%. Fruits and vegetables are generally only cooled by packers and exporters when products are to be transported over long distances. Cooling operations are nevertheless simple and carried out mostly in cold – rooms and far from being technologically development. Since much of the produce is locally sold, cooling is not commonly practiced, because it is expensive and losses are not considered excessive. The cold chain from producer to consumer is frequently interrupted due to lock of efficient facilities such as packing houses, cold stores, cold transport and cool market operations and distributions. Following the harvest fruits and vegetables in Turkey are destined for either storage or market. Preparations for both destinations are mostly done in the field, in the packing shed or in a covered area. Preparations include, receiving, cleaning, trimming, sorting, hand grading, sizing and packing (Figure 1).

Figure 1. Classical fruit cold storage, handling and packaging for apple

Only small production goes through the modern packinghouse operations where the sequence of operations varies with different crops packing houses were mostly in operation nearby big consumption center and also founded nearby shipping port. Major provinces were İzmir, Antalya, Mersin and Istanbul which nearly equally shared the total by 40% each. In general, sorting and sizing of products are made according to their physical properties such as diameter and weight. Some of them have been graded objectively the produce by color. So, many modern packinghouses mostly handling vegetables are operative in Marmara Region. They are equipped with modern machinery and annexed with cold storage. They are mostly owned and operated by the exporting companies. Many cold stores and packinghouse build to handle 5 000 – 10 000 tones in during the last 10 years, especially to handle postproduction phase of pome and stone fruits.

Some forms of deterioration, such as sprouting, water loss, storage disorders, insect manifestations and fungal rots can be minimized with chemical treatments before storage or marketing. Antitranspirants, surfactants and other skin coating agents and ethylene absorbents such as 1-methylcyclopropene (1-MCP), aminoethoxy vinyl glycine (AVG) are being investigate by research laboratories and their commercial use is to be spread. Such studies implicated plant nutrition studies and research efforts have yielded results of practical implementations. Field applications are widely practiced but their postharvest use before storage is limited due to lack packinghouse operations or simple machinery which can be used in packing sheds after harvest. Along this line, commercially scald is significantly reduced by the postharvest dip in diphenylamine (DPA) especially Granny Smith apples and domestic pear cultivar Deveci, Anjou and Abbe Fetel. Nevertheless, the growers in Turkey are showing keen interest on pre-storage treatments with chemicals including the fungicides, since fungal rots contribute to postharvest losses at the highest rates.

Hydrocooling and forced-air cooling systems are used by several modern cold stores in Turkey (Figure 2). Turkey could gain great advantage their upon reduce postharvest losses especially during marketing if wholesale and regional markets as well as ever increasing supermarket chains are furnished with compact ice-bank cooling units.

Figure 2. Hydrocooling for cherry and DCA (Dynamic Controlled Atmosphere) atmosphere storage for apple

Curing of potatoes, onions and garlics is done in the field in Turkey where harvest time is characterized by hot and sunny days with concurrent low humidity levels. It is reasonable to believe that such in-field curing may create problems resulted from excessive heat, lack of

aeration, soil-born diseases and field rodents and other pests. These factors obviously shorten the storage life and contribute the high rates of postharvest losses likely to occur in these crops at the vicinity of 35-40%. Proper temperature, humidity and air rate levels required for curing of onion and garlic have been obtained but these are limited practically.

The purpose of fresh fruits and vegetables storage in Turkey is not different than anywhere else in the world. Storage of fresh fruits and vegetables prolongs their consumption and in some cases even improves their quality. Even using the most modern cold stores, expectations of growers or handlers in Turkey sometimes are crippled due to high rates of storage losses because they ignore the fact that each member ring of the cold chain should be tightly bound to the next one and storage alone, as a separate ring, cannot improve the condition unless other former rings i.e., preharvest factors, harvesting and handling practices, precooling, packaging and hauling have been orderly and properly fulfilled.

Mechanically refrigerated stores capacity is about 750.000 tons in Turkey. Controlled atmosphere stores are a new concept for Turkey but it is progress because of storage quality, prolongs their consumption and price of crops at off-seasons. Only apples are stored in CA for 9 months (Figure 2).

The simplest of the alternative storage source but least practiced in Turkey is keeping the produce on the plant or, in situ for few months after they attain maturity. This is particularly applied on few citrus species, persimmon, grapes in certain areas, and among the vegetables, potato, carrot and garlic. Grapevines are individually covered with clear plastic as protection from early frosts.

Usually, some vegetables are stored in pits and trenchers by covering their surface with soil. Pits are used for storing potatoes, carrots, turnips and lesser extend on cabbage.

In some areas insulation with straw, hay or even manure is practiced when cold winter prevails. These varieties are mostly local and have no commercial value.

Cave storage can play an important role in storing some durable fruits and vegetables in regionally “Cappadocia Valley” which is historical place (Figure 3).

Their numbers, and hence capacities are steadily increasing.

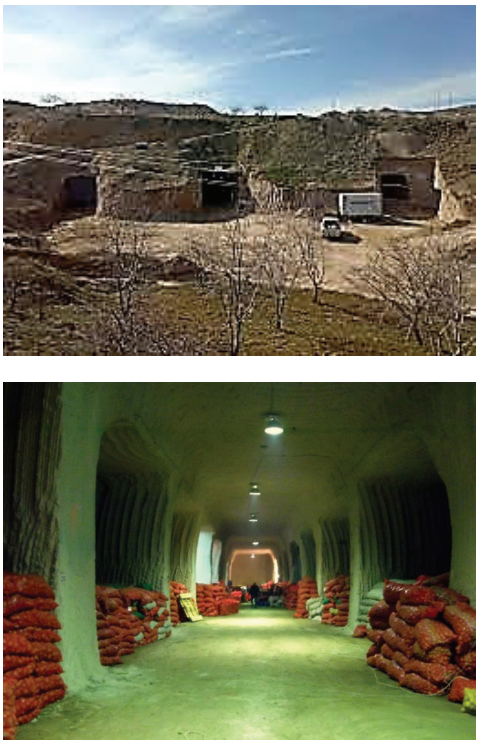

Figure 3. Cave stores in Cappadocia Valley for potatoes storage

Eventually, the cave stores have impressions as being the predominating stores for citrus especially for lemons.

Much of the produce of lemons, oranges and grape fruit grown in the Mediterranean Region is shipped out to the area for subsequent storage. It is customary the hose the surfaces inside the store with water to provide extra humidity apples are to be stored. The floor is also watered occasionally. Potatoes and onions are kept in rather less humidified caves in different dimensions. Walls are usually 1.0-1.5 meters wide. Aeration is achieved by natural convection. The doors are opened early in the morning when the ambient air is cold and entering air flowing around the stacks of produce, leaves the cave through the pipe flues. Temperature inside the cave should not deviate so much and probably stays near the average annual ambient temperature. The overall storage capacity of these caves is near 700.000

tons all located within the provincial borders of Cappadocia Region To reduce overall postharvest losses in these cave stores some modifications appear to be essential. A refrigeration, heating unit and mechanically ventilation and inside circulation of air are indispensable.

The present situation of the postharvest system in Turkey is as follows: a) Small scale production mostly on fragmented land involves high physical handling, transportation and transaction costs, b) Postharvest losses are as high as 30%, c) Too many intermediate agencies in marketing the produce each demanding payment for their services, handling small lots generate overall high cost, d) Unstable market demands and prices create significant risks for market oriented produce.

CONCLUSIONS

Recommendations for future strategies should be as follows: a) The Government at first sight should make an extensive international market survey, implicating the foreign marketing research agencies. European Community (EC) countries, Middle East and Near East, Russia and Turkic States could constitute potential markets, b) Growers will orient themselves to produce high quality crops whose postharvest handling will demand extreme care and involve modern concepts of postharvest treatment, processing storage and transportation, c) In Turkey usually apples, pears and table grapes are stored in cold stores. It should be cold stored in commonly different fruits and vegetables such as quince, peach, melon, cabbage, tomatoes, pepper, onion and potato etc. And store owners to modify their old stores into CA systems.

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ACTIVE FUNGAL ENDOPHYTES AGAINST PHYTOPATHOGENIC FUNGI- DWELLERS OF ROMANIAN AND CANARIAN *ARTEMISIA* SPP.

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Abstract

Endophytic fungi have been isolated from Artemisia austriaca (isolates HRO184, HRO183, HRO169 and HRO115), A. vulgaris (isolate HRO158) and A. thuscula (isolates HLP7, HLP26, HLP27, and HLP44) in Romania and La Palma, Canary Islands, Spain. The strains were studied for their bioactivity against phytopathgenic fungi (Alternaria alternata, Alternaria dauci, Alternaria brassicicola, Fusarium oxysporum, Fusarium solani, Fusarium moniliforme, Sclerotinia sclerotiorum, Botrytis cinerea, Phoma sp., Geotrichum sp. and Cladosporium sp.) using dual culture, agar dilution and volatile compounds (VOC) techniques. In dual culture assays, all Canarian isolates were strong antagonists of at least one pathogen and two Romanian isolates, HRO169 against S. sclerotiorum and HRO158 against A. brassicicola. In VOC assays, a difference was observed between slow growth isolates (1-2 cm/week) and normal growth (4-5 cm/week), therefore three moments of fungal pathogen inoculation were used: after three and five days from the endophyte inoculation. The highest inhibition gradually produced by the volatile compounds was observed with HLP44 isolate against Cladosporium sp. (%I = 31.5) at 7th day. Solvent extracts were obtained from HRO169, HRO158, HLP44, HLP27 and HLP7 isolates and further two of them (HRO158 and HLP44) were fractionated using vacuum-liquid chromatography eluted with n-hexane:EtAc:MeOH gradient to give seven fractions. Extract obtained from HLP44 isolate strongly inhibited A. brassicicola (%I = 44), F. moniliforme (%I =52.78) and S. sclerotiorum (%I = 50.4), at 0.1mg ml^{-1}. Ethyl acetate fraction was the most active against A. brassicicola, F. solani and B. cinerea (%I = 42.68, 59.17 and 49.36, respectively, at 0.1mg ml^{-1}) followed by the EtAc:MeOH (90:10) fraction which also inhibited A. brassicicola and F. solani (%I = 35.83 and 39.53, respectively).

Key words*: Artemisia*, *bioactivity*, *endophytic fungi*, *phytopathogens*

INTRODUCTION

Fungal endophytes are microorganisms that live in the intercellular spaces of stems, petioles, roots and leaves of plants without causing observable manifestation of their existence (Strobel and Long, 1998). Same substances can be produced by both endophytes and plants (Stierle et al, 1993; Strobel et al, 1997; Lee et al, 1995; Kusari et al, 2008). According to Strobel (2002), plants with medicinal value or unusual longevity, plants that survive under extreme conditions often harbour potential fungal endophytes that produce bioactive metabolites. Medicinal plants are revealed as host endophytes which in turn provide protection from infectious agents (Strobel et al, 2002). A cavalcade of endophytic species dwelling in medicinal plants revealed bioactivity features in various studies (Li et al, 2005; Raviraja et al, 2006; Chowdhary and Kaushika, 2015; Purwantini et al, 2015). The symbiotic relation between the endophyte and its host is considered 'defensive mutualism' id est the resistance of the host to pathogens, phytophagous insects and environmental conditions increases; secondary metabolites are involved in most cases (Gonzalez-Coloma et al, 2016). An array of compounds belonging to various chemical groups are metabolized by endophytic fungi such as phenols, steroids, flavonoids, quinones, terpenoids, xanthones, peptides, alkaloids, aliphatic compounds, phenylpropanoids, isocoumarins, benzopyranones, tetralones, cytochalasines and

enniatines (Schulz et al. 2002, Rocha et al. 2011, Schulz and Boyle 2005, Aly et al. 2010, Santos et al, 2003). New compounds were isolated from endophytes inhabiting *Artemisia annua* (Ge et al. 2010; Lu et al, 2000) and *Erythrina crista-galii* (Weber et al, 2004). The production of antioxidant compounds by plants, like phenolic acids and their derivatives (Huang et al., 2007), isobenzofuranones (Strobel et al., 2002), isobenzofurans (Harper et al., 2003), as well as mannitol and other carbohydrates (Richardson et al., 1992), is attributed to the presence of reactive oxygen species (ROS) generated by endophytes (Gonzalez-Coloma et al, 2016). Volatile compounds (VOCs) are also produced by fungal endophytes among other microorganisms, but less is known about the pathways in which they are produced. Many of them are either metabolic transformation products of lipids, proteins, heterocyclic metabolites or other components of living tissues or degradation end-products ('waste products') of fungal catabolic pathways (Bennett et al, 2012). Species of endophytes produce antifungal and antimicrobial VOCs like *Muscodor albus* (Strobel et al, 2001), *Oxyporus latemarginatus* (Lee et al, 2009) and *Gliocladium* sp. (Stinson et al, 2003), respectively. *Artemisia* is a wide studied genus of plants for its medicinal and bioactive properties (Soylu et al, 2005; Brudea, 2008; Dancewicz and Gabrys, 2008; Garcia et al, 2015; Abad et al, 2012) and recently its endophytic fungi communities have been taken into observation looking for bioactivity features (Haniya et al, 2013; Qian et al, 2014; Purwantini et al, 2015, Cosoveanu et al, 2016). The present study selected several species of fungal endophytes previously isolated from *Artemisia austriaca*, *Artemisia vulgaris* and *Artemisia thuscula* to evaluate their bioactive potential. It is noteworthy to mention that is the first notification on fungal endophytes isolated from *A. austriaca* and *A. thuscula* and their bioactivity.

MATERIALS AND METHODS

Plant sampling and isolation techniques

Plant samples were collected and processed in 2012 in Romania: *A. austriaca* from Tuzla, Murighiol, Babadag Lake; *A. vulgaris* from Mahmudia; and in La Palma Island (Spain): *A. thuscula* from La Galga and Tigalate. A surface sterilization method was used in order to suppress epiphytic microorganisms from the plant samples. Briefly, plants were immersed first in sterile H2O, followed by 1min in EtOH 70%, 1min in sodium hypochlorite 15%, 1 min in EtOH at 70% and finally washed with sterile H2O (changed from Schulz et al., 1993). The isolation procedure was performed according to Cosoveanu et al. (2014). In order to analyse the fungal diversity, each replicate obtained from distinct stem fragments was registered. When an endophyte was acquired in pure culture it was preserved (Czapek, T=5°C and Glycerol 20% DI H_2O, T= -30°C), bioactively tested and identified.

Dual culture assays

Dual culture technique was employed to find endophytic fungi that produce metabolites which inhibit *S. sclerotiorum*, *F. oxysporum*, *F. moniliforme*, *F. solani*, *A. brassicicola*, *A. dauci*, *A. alternata*, *Phoma* sp. *Geotrichum* sp. and *Cladosporium* sp. mycelial growth *in vitro*. PDA plates were incubated at 25ºC in darkness for 7 days and observed daily; plates were left for a further week to check the stability of the interaction. The following criteria based on Kusari et al. (2013) were used to interpret the results:

0-Mycelia grow until making contact with each other
1-Mutual inhibition (both mycelia stop growing at a certain distance)
2-Mycelia grow until making contact with each other and in the area where the contact is produced morphological changes occur/ the growth is stopped in a convex form
3-Pathogen growth is detained at a certain distance from the endophyte (<2 mm)
4-Pathogen growth is detained at a certain distance from the endophyte (>2 mm)
RDP- Rapid development and parasitism of the endophyte
RD- Rapid development of the endophyte
RDL- Rapid development of the endophyte and lysed mycelia of the pathogen
L- Opponent fungus presents lysed mycelia
P- Endophyte displays parasitism on pathogen

It should be noted that also the pathogen may respond similarly to criteria 3, 4, RDP, RD, RDL, L and P (further, the results for this case are

noted with *). However, this study focuses only on the endophyte response towards pathogen.

Fungal isolates

Fungal isolates (endophytic and pathogenic strains) were maintained on PDA, T=25°C, in darkness. Endophytes were selected based on their results in preliminary assays of antagonism. Pathogens were chosen due to their different interactions with the host and their high economic importance: *Alternaria alternata*, *A. dauci*, *A. brassicicola*, *Fusarium oxysporum*, *F. moniliforme*, *F. solani*, *Sclerotinia sclerotiorum, Botrytis cinerea, Phoma* sp, *Geotrichum* sp. and *Cladosporium* sp.

Biometric agar dilution assays

Tests were carried out to determine the biological activity of extracts using biometric agar dilution method. The extracts were incorporated into PDA as follows: 1, 0.5 and 0.1 mg ml^{-1}. The final percentage of ethanol in the media was adjusted to a concentration of 1% (v/v). Plates containing the solvent (ethanol) were used as negative control. Each pathogen was spot - inoculated at eight equidistant points to PDA media amended with the fungal extracts at tested concentrations. Three replicates were used per treatment. For each extract and concentration, inhibition of radial growth (%I) compared with the control was calculated after 72 hours of incubation at 25ºC, in the dark. The radial growth was measured with an image - processing software Image J -Wayne Rasband (NIH).

Kruskal-Wallis Test and Mann Whitney U were performed using IBM SPSS Statistics 21.0.

Volatile compounds assays (VOC)

The VOC assays analyse the activity of volatile compounds produced by the endophytes on phytopathogens. The assays were done in Petri plates, using only the plate bases with nutritive media for the fungal inoculation as following: plate base with endophyte + plate base with pathogen and the controls: base without pathogen + base with endophyte and correspondently for the pathogen. Plates were incubated for a week at T=25°C, in darkness. Assays were performed in triplicates. Measurements were made daily, calculating an average of two measurements of the same inoculum diameter. In the case of slow growth endophytic fungi three different moments for the pathogen inoculation were chosen allowing to the endophytic fungus to develop more as follows: inoculation of both fungi at the same time (Moment 0), inoculation of the pathogen after three days from the inoculation of the endophyte (Moment 1) and inoculation of the pathogen after five days from the endophyte's inoculation (Moment 2).

The following criteria were used to interpret the results:

A = E<CE: The pathogen inhibits the endophyte
B = P<CP: The endophyte inhibits the pathogen
C = CE<E: The pathogen enhances the endophyte's growth
D = CP<P: The endophyte enhances the pathogen's growth
* CE: Control of endophyte; CP: Control of pathogen; E: Endophyte in interaction; P: Pathogen in interaction

High scale cultivation of endophytic fungal isolates

Five of nine fungal isolates showing antagonistic activity were further explored for bioactivity of their crude extracts by multiplication on rice media. Rice medium was prepared in 500ml Erlenmeyer flasks containing 120g of rice grains with 40ml H_2O (autoclaved). The flasks were inoculated with 4-6 disks of endophytic fungus mycelium (25°C, darkness for three weeks).

Chemical solvent extraction of endophytic fungal isolates (crude extract and fractionation)

120ml of ethyl acetate (Sigma Aldrich) was poured on the rice grains covered with the fungus mycelium and kept aside for 24h. The content was filtered under vacuum using a Buchner funnel and the solvent extraction was repeated thrice. The collective extract was dried up with vacuum rotary evaporator under reduced pressure at 50°C and used as crude extract for further evaluation. Two of the crude extracts were selected to be fractionated. The extracts were previously subjected to partitioning between n-hexane and MeOH (V/V) to remove the fatty acids, twice. The MeOH fraction was chromatographed on a SiO_2 vacuum-liquid chromatography column (VLC) eluted with n-

hexane:EtAC:MeOH gradient to give seven fractions (three times the volume of the column per solvent fraction). Fr-1 (n-hex. 100%), Fr-2 (n-hex:EtAc 90:10), Fr-3 (n-hex:EtAc 75:25), Fr-4 (n-hex:EtAc 50:50), Fr-5 (EtAc 100%), Fr-6 (EtAc:MeOH 90:10), Fr-7 (MeOH 100%). Fractions were further treated as the crude extracts.

RESULTS AND DISCUSSIONS

In dual culture assays, isolate HLP44 inhibited the development of six pathogens with a higher distance than 2mm (criterion 4) and three with a distance of 2mm (criterion 3) out of 11 pathogens (Table 4). Isolates HLP7 and HLP26 were also strong inhibitors of four and three pathogens, respectively. Also HLP27 and HRO169 isolates impeded the development of one pathogen each. In the agar dilution assays, HRO169 (extract code-1090) inhibited (%I= 47.70, at 0.1mg/ml) only *F. oxysporum* although the results from dual culture did not predict it (criterion 1). The crude extract of HLP7 (code- 1114) inhibited *A. brassicicola* (%I= 32.55, at 0.1mg/ml) but not *Cladosporium* sp. (%I= 9.7, at 1mg/ml) although in dual culture the observed reactions were similar for both pathogens (criterion 4). Despite the interesting activity in dual culture (criterion 3), the extract was not active against *A. alternata* and *A. dauci*. (%I= 15.5 and 5.02, respectively; at 1mg/ml). HLP44 extract (code- 1092) was the most active and therefore it was fractionated. 1092 inhibited with interesting values seven of the nine tested pathogens (Table 3). Although all three species of *Alternaria* reacted in a similar manner in dual culture assay, the response of the extract in dilution agar assays variated (vs. *A. alternata* %I= 38.6, vs. *A. brassicicola* %I= 41.7. vs. *A. dauci* %I= 19.5, at 1mg/ml). As for the three species of *Fusarium*, results of inhibition were similar (Table 3). The bioactivity of this extract was disjoined, having various active fractions with different pathogens (Table 3). Briefly, the fraction which most inhibited the mycelial growth of tested pathogens with interesting values was the one eluted with ethyl acetate (vs. *A. brassicicola* %I= 42.7, vs. *F. solani* %I= 59.2, vs. *F. moniliforme* %I= 43.2 and vs. *B. cinerea* %I= 49.4, at 0.1mg/ml). Ethyl acetate and methanol extracts of an endophytic *Chaetomium globosum* isolate were more effective than hexane extract against *S. sclerotiorum* (Kumar et al, 2013). Yet, the non-polar fractions (L0 and F2, eluted with hexane and n:hexane-EtAc 90:10, respectively) strongly inhibited *B. cinerea* (L0- %I= 35.1; F2- %I= 43.4, at 0.1mg/ml). Previous reports on hexane extracts obtained from *Colletotrichum globosum* showed antifungal properties against *B. cinerea* (Nakashina et al, 1991). Fungal endophytes were tested for their VOCs bioactivity and separated in two groups due to their type of growth: regular growth and slow growth. It was hypothesized that the more mass of endophytic mycelia is produced, the more VOCs would be generated a posteriori in the interaction with the pathogen. In the slow growth group the endophytes were left to develop in the absence of the pathogen for three and five and five days. The highest percentage of inhibition, reached gradually, (%I= 31.5, at Moment 3) was calculated for the interaction between *Cladosporium* sp. and HLP44, confirmed by Kruskal Wallis test ($p= 0.027$). Overall there were no significant differences between the inoculation moments, but neither interaction with high percentages of inhibition. In the group of regular growth, the interactions between the same pathogen and two endophytes were compared. No significant difference resulted ($p<0.05$), therefore no difference in the sensibility of the pathogen exposed to more than one reputed bioactive endophytes, was found. The structural groups of VOCs detected in different individuals of *A. vulgaris* collected from various countries and regions show similarities as the main groups belong to monoterpenes followed by sesquiterpenes (Zhigzhitzhapovae et al, 2016). *Artemisia* spp. essential oils have antimicrobial properties (Baykan Erel et al, 2010), antiparasitic and cytotoxic activity (Martinez-Diaz et al, 2015) so the community of harboured endophytes seems likely to be also producing bioactive VOCs, as previously shown (Strobel et al, 2011).

Table 1. VOC interaction between pathogens and endophytes with regular growth: percentages of inhibition (expressed as average and Standard deviation STD) and criteria of interpretation

	%I pathogens- AVG (STD)			%I endophytes- AVG (STD)			CRITE-RIA
Interaction	Day 1	Day 4	Day 7	Day 1	Day 4	Day7	
A. alternata &HLP26	20.74 (3.03)	2.86 (6.92)	2.33 (5.58)	-3.33 (4.71)	10.56 (2.66)	17.53 (3.91)	
A. alternata & HRO158	19.16 (4.43)	9.65 (4.50)	9.32 (3.05)	40.99 (14.62)	44.18 (39.66)	6.07 44.92)	
A. brassicicola & HRO158	7.69 (10.88)	-8.11 (8.63)	19.21 (5.33)	6.78 (12.04)	0.86 (5.17)	-3.15 (6.07)	B
A. brassicicola & HLP26	-18.59 (4.53)	-18.62 (19.19)	2.61 (11.18)	0.00 (0.00)	21.07 (10.44)	16.96(4.67)	
A. dauci & HRO169	3.42 (2.42)	5.12 (5.13)	0.49 (6.85)	3.04 (2.15)	-7.91 (7.22)	-15.64 (6.22)	
A. dauci & HLP26	-2.41 (15.29)	-1.50 (8.77)	-2.07 (6.39)	0.00 (0.00)	5.25 (3.84)	11.36 (2.09)	
F. oxysporum & HLP26	-30.37 (35.09)	1.53 (1.24)	17.16 (3.74)	3.33 (4.71)	28.82 (3.09)	44.33 (4.99)	A
F. oxysporum & HRO184	-10.74 (34.91)	10.27 (1.98)	9.07 (5.64)	-0.67 (8.26)	9.60 (5.00)	27.69 (3.17)	A
F. moniliforme & HRO158	-4.22 (9.71)	-0.11 (3.04)	2.55 (8.48)	-7.77 (14.87)	-5.47 (18.68)	-18.49 (19.38)	
F. solani & HLP26	-3.92 (7.02)	0.02 (3.74)	5.94 (1.38)	-20.00 (0.00)	9.06 (1.69)	22.19 (4.93)	A
F. solani & HRO184	-1.96 (5.11)	-2.06 (4.03)	7.15 (2.90)	-8.08 (18.24)	-7.23 (8.23)	8.31(7.22)	
B. cinerea & HRO158	27.23 (15.87)	-16.94 (14.07)	-21.89 (14.24)	6.66 (24.97)	-11.05 (30.42)	-15.70 (17.65)	
B. cinerea & HRO183	10.65 (11.44)	-7.38(10.86)	-10.03 (12.71)	-2.04 (6.73)	10.04(1.41)	7.47 (4.82)	
S. sclerotiorum & HRO158	-39.91 (60.20)	-20.20(28.57)	-20.20 (28.57)	4.40 (14.92)	10.42(26.87)	17.22 (58.66)	
S. sclerotiorum & HRO169	-33.07 (65.05)	-18.18 (25.71)	-18.18 (25.71)	6.73 (11.59)	6.29 (1.45)	2.36 (3.61)	

Table 2. VOC interaction between pathogens and endophytes with slow growth: percentages of inhibition (expressed as average %I and standard deviation STD), moments of inoculation of pathogen and criteria of interpretation.

		%I pathogen			%I endophyte			
Interaction	Moment	Day 1	Day 4	Day 7	Day 1	Day 4	Day7	CRITERIA
A. brassicicola	M0	15.0 (13.2)	17.1 (4.8)	6.8 (15.3)	0.0 (0.0)	15.4 (8.2)	-4.4 (16.1)	
& HLP44	M1	-0.8 (8.9)	36.2 (1.5)	33.2 (4.4)	13.5 (8.2)	2.7 (9.9)	-0.3 (9.5)	B
	M2	-3.0 (5.2)	35.3 (11.8)	24.1 (4.1)	2.4 (14.8)	-0.3 (9.5)	-1.9 (12.9)	B
A. brassicicola	M0	20.1 (1.3)	23.0 (7.6)	-1.2 (5.9)	0.0 (0.0)	4.5 (5.1)	5.8 (3.3) a	
& HLP27	M1	-2.3 (26.6)	24.3 (9.5)	8.7 (24.8)	-1.2 (4.0)	3.9 (3.8)	15.2 (3.1)b	
	M2	-18.2 (18.2)	16.7 (3.7)	25.2 (1.5)	-8.6 (8.1)	-9.3 (5.4)	-6.6 (8.3)c	B
A. dauci	M0	23.3 (2.9)	15.9 (9.6)	2.6 (20.9)	0.0 (0.0)	5.7 (5.6)	-0.7 (9.0)	
& HLP44	M1	16.7 (3.6)	19.4 (3.6)	5.6 (6.3)	9.6 (8.9)	12.8 (4.8)	12.4 (5.0)	
A. dauci	M0	6.7 (11.5)	1.5 (10.3)	6.5 (4.7)	0.0 (0.0)	-92.5 (62.3)	-77.8 (79.1)	
& HLP7	M1	20.8 (7.2)	-4.7 (6.4)	-3.0 (3.9)	-92.3 (60.1)	-73.0 (68.4)	-68.7 (72.7)	
	M2	9.1 (0.0)	8.4 (1.8)	9.8 (4.2)	-104.5 (83.5)	-119.1 (94.2)	-131.1 (109.0)	
B. cinerea	M0	18.5 (29.4)	2.5 (5.4)	2.5 (5.4)	-8.3 (14.4)	-4.7 (4.1)	-5.2 (1.2)	
& HLP27	M1	2.2 (3.8)	6.5 (12.3)	4.4 (12.7)	-1.2 (4.0)	-2.0 (5.3)	13.5 (4.7)	
	M2	8.9 (10.2)	3.6 (6.4)	1.8 (6.4)	-3.3 (5.0)	-8.7 (6.2)	-11.2 (10.2)	
Cladosporium sp.	M0	16.2 (16.7)	8.3 (15.7)	3.0 (10.5)a	0.0 (0.0)	4.2 (7.2)	-5.6 (20.6)	
& HLP44	M1	2.5 (13.1)	17.7 (7.7)	17.1 (4.8)b	1.5 (12.4)	3.0 (5.2)	4.7 (4.6)	
	M2	-18.6 (25.2)	15.1 (2.0)	31.5 (3.2)c	10.3 (13.7)	13.8 (7.6)	15.0 (6.2)	B
Cladosporium sp.	M0	-11.9 (33.0)	0.0 (23.8)	-5.5 (13.1)	0.0 (0.0)	-18.1 (28.5)	16.2 (22.3)	
& HLP7	M1	14.1 (4.4)	11.0 (1.7)	6.6 (12.2)	-88.3 (80.6)	-103.5 (116.9)	-122.8 (137.8)	
	M2	-12.8 (23.4)	2.0 (10.1)	16.2 (21.4)	-30.2 (62.1)	-46.6 (85.5)	-45.0 (86.2)	

Values with different letter have statistical difference P<0,05, U Mann Whitney Test

Table 3. Dilution agar assays with crude extract and fractions versus fungal pathogens- %I (STD)

Extract	[C]	A. a.	A. b.	A. d.	F. o.	F. s.	F. m.	S. s.	B. c.	Clad.
972	1 mg/ml	20.8 (5.8)						12.2(9.4)		
1090	1 mg/ml	29.8(4.1)	12.8(5.2)		45.8(3.3)a	23.3(7.8)a	16.2(7.4)	14.8(5.8)		
	0.5 mg/ml				57.0(5.2)b	31.2(6.0)b				
	0.1 mg/ml				47.8(4.8)a	6.3(7.8)c				
1091	1 mg/ml		44.5(4.7)	-6.6(8.9)				2.6(9.9)	1.4(8.6)	
1114	1 mg/ml	15.5(2.6)a	42.3(8.0)a	5.0(4.6a						9.7(12.5)a
	0.5 mg/ml	18.7(2.8)a	41.2(5.7) b	11.7(4.6) b						13.1(17.1)a
	0.1 mg/ml	18.0(2.8)a	32.6(4.3) b	4.8(4.6)a						-16.4(29.5) b
1092	1 mg/ml	38.6(1.6)a	41.7(6.1)a	19.5(4.1)a	37.9(10.2)a	34.3(5.5)a	42.5(7.2)a	71.5(8.0)a	45.2(2.5)a	
	0.5 mg/ml	29.6(1.2) b	46.0(5.3)a	13.8(*3.0)b	26.6(6.5) b	18.4(4.2)b	28.7(6.9)b	67.9(8.8)a	51.1(5.0)b	13.4(10.9)a
	0.1 mg/ml	17.2(2.0)c	44.0(5.3)a	3.5(*2.1)c	16.9(7.5) c	-1.5(4.3) c	52.8(7.8)c	50.4(33.7)a	18.6(8.8)c	25.5(19.0a
1092LO	0.1 mg/ml	-1.0(11.6)	9.2(7.5) *			5.4(6.7)	6.8(7.7)		35.1(11.9) *	
1092F2	0.1 mg/ml	4.8(6.7)	29.3(4.9) *			15.3(5.1) *	2.7(10.2		43.4(5.4) *	
1092F3	0.1 mg/ml	-4.6(4.9)	7.6(4.8) *			7.5(9.2)	0.5(6.8)		14.4(7.1) *	
1092F4	0.1 mg/ml	4.5(5.2)	27.2(4.8) *			34.0(4.5) *	21.2(9.1) *		19.6(2.8) *	
1092F5	0.1 mg/ml	21.5(2.8) *	42.7(3.8) *			59.2(0.9) *	43.2(5.5) *		49.4(3.1) *	
1092F6	0.1 mg/ml	3.2(9.2)	35.8(5.2) *			39.5(5.4) *	10.8(10.7)		32.9(1.6) *	
1092F7	0.1 mg/ml	2.0(5.4)	19.3(3.6) *			24.7(5.3) *	-17.0(6.6)		19.1(5.7) *	

U Mann Whitney was applied in the case of the assays with fractions and pathogens to check the statistical difference between control and treatment (values marked with * have p<0.05) and between treatment at different concentrations (values with different letter have statistical diference p<0.05). A.a.= *A. alternata*, A.b.= *A. brassicicola*, A.d.= *A. dauci*, F.m. = *F. moniliforme*, F.s.= *F. solani*, F.o.= *F. oxysporum*, S.s.= *S.sclerotiorum*, B.c.= *B. cinerea*, Clad= *Cladosporium* sp.

Table 4. Dual culture assay: fungal endophytes and pathogens (interaction criteria)

	HLP7	HLP26	HLP27	HLP44	HRO115	HRO158	HRO169	HRO183	HRO184
F. o.	0	3, P*	2*	3, P*	0	2	1	0	4
F. m.	0	0, P*	2*	3, P*	2	0	0	0	P*
F. s.	0	3, P*	2*	3, P*	0	2	1	0	4
B. c.	4	4	4	4	0	0	2	3, P	2*
S. s.	4	2	2	4	2	2	4	RD, 1	RD*P*
A. a.	3	3	0	4	0	0	3	1	0
A. d.	3	4	P	4	P	3	1	P	2
A. b.	4	4	3	4	P	3, P	3*	P	0
Phoma	4*	4*	4*	1	RD, L	4*	4*	4*, RD	4*
Geot.	4*	4*	4*	1	4*	4*	4*	4*, RD	nt
Clad.	4	1	1	4	P	0	1	P, RD	4*

A.a.=*A. alternata*, A.b.= *A. brassicicola*, A.d.= *A. dauci*, F.m. = *F. moniliforme*, F.s.= *F. solani*, F.o.= *F. oxysporum*, S.s.= *S.sclerotiorum*, B.c.= *B. cinerea*, Clad= *Cladosporium* sp. , Geot= *Geotrichum* sp. 0-Mycelia grow until making contact with each other; 1- Mutual inhibition (both mycelia stop growing at a certain distance); 2- Mycelia grow until making contact with each other and in the area where the contact is produced morphological changes occur/ the growth is stopped in a convex form; 3- Pathogen growth is detained at a certain distance from the endophyte (<2 mm); 4- Pathogen growth is detained at a certain distance from the endophyte (>2 mm); RD- Rapid development of the endophyte; L- Opponent fungus presents lysed mycelia; P- Endophyte displays parasitism on pathogen. *= the pathogen is evaluated with the correspondent criteria; the rest of the cases are applied to the action of the endophyte on the pathogen.

CONCLUSIONS

Our study has shown the potential of two fungal endophytes against important plant pathogens. The most interesting fungal endophyte is HLP44 isolate (from *A. thuscula*), as its valences are multiple being a spring of active compounds against *A. brassicicola*, *F. solani* and *B. cinerea* found mainly in the ethyl acetate fraction but also in the hexane fraction. More, this isolate strongly inhibited *B. cinerea*, *S. sclerotiorum*, *A. alternata*, *A. dauci*, *A. brassicicola* and *Cladosporium* sp. in dual

culture and *Cladosporium* sp. in VOC assay which converts it into a tool for biocontrol. Further studies will be carried out to identify the active compounds responsible for the inhibition of the mycelium growth of pathogens. One good candidate for *in vivo* further assays would be HRO158 isolate (from *A. vulgaris*) which inhibited the growth of the mycelium of *A. brassicicola* and *A. dauci* in dual culture assay.

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