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SUMMARY

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VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARHITECTURE

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QUALITY ASSESSMENT OF 'SOUTHLAND' CULTIVAR PEACHES ACCORDING TO CERTAIN TECHNOLOGICAL FACTORS OF CULTURE AND STORAGE

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Abstract

Because of the great taste attributes, aspect and specifically flavor, plus their importance in nutrition, peaches occupies an important place in consumption in both, fresh and processed condition. Their superior dietary attributes are determined by the content of vitamins, minerals, cellulose, acids and pectin. This paper aimed to present the impact of some production (fertiliser regime) and valorisation (preserving conditions) factors upon the quality and its maintaining capacity during nectarines preserving. The organoleptic appreciation, fruits' firmness, biochemical compose, quantitative and qualitative loses were determinated. The 'Southland' cultivar (created in 1946, America), is very much appreciated all over the world for its great fruits (200-220g) and productivity (30-35 kg/tree). It was provided from the experimental plots of the Research Station for Fruit Growing Constanta, fertilized in different manner, with organic and chemical fertilizers, applied on soil and/or foliar. The peaches were stored at RDIPMHP-Bucharest in three variants: in the ambient temperature (T=26-28^{0}C), in cold conditions (T = 2-4°C) and cold + modified atmosphere conditions, during 7, 28 and 35 days, respectively. The best results were obtained in case of the fertilisation with chemical fertilisers applied on soil + foliar, which induces the best quality and maintainance during stored. The results also indicate the superiority of fruit storage in the modified atmosphere comparing with the others methods.

***Key words**: loses, quality, storage capacity, temperature*

INTRODUCTION

The peaches are extremely perishable fruits, which raises serious problems concerning the maintaining of their quality during the valorisation process, from the moment which they are harvested until they reach the consumer (Alexe et al., 2013).

The realisation and maintenance of fruit quality depends on an ensemble of factors which intervene in all the technological links related to culture and valorisation, from the choosing of the cultivar and the maintenance of the culture to harvesting, conditioning and shipping. The peach tree cultivars display different characteristics as concerns their storage and valorisation over a longer period of time (Jamba and Carabulea, 2002). The storage capacity of peaches depends on the quality of the raw material meant to be stored, as well as on the conditions during storage. The chemical composition of peaches, which determines the level of biochemical processes during storage and therefore, the storage capacity is highly influenced by the fertilisation regime. The dosage in which the organic or mineral fertilisers is applied influences the chemical composition of the fruit, having an important effect upon the storage capacity

(Salunke, 1974; Ion, 2004).
Within valorisation technologies, it is recommended that certain technological processes regarding storage be applied, so that they determine the inhibition of physiological and biochemical processes within the fruit, leading to the maintenance of their commercial value for a period which as long as possible (Burzo, 1986; Lill and King, 1999; Burzo et al., 2005;).
The purpose of this paper is to evaluate the obtaining and maintenance of fruit quality of the peaches of the 'Southland' cultivar (American cultivar, created in 1946, appreciated in all world up to now for its great fruits and productivity), according to the fertilising regime of the culture and the storage conditions, after harvesting.

MATERIALS AND METHOD

The peaches were harvested from the experimental plot of R.S.F.G. Constanta. The 'Southland' (Figure 1 and 2) is an semi-late cultivar, the maturity period of fruits are in the third decade of the July and first decade of August. The fruit have a big weight (200-220 g) and a spherical shape, flattened laterally and ventral furrow is shallow.
The skin is thick, hairy and adherent to flesh, color is yellow-orange, covered with red-purple spots on the sunny side of fruit.

Figure 1. Branch with fruit of the Southland

The pulp is golden-yellow, with slight red seepage around the kernel, with great taste and flavor, relatively crisp, sweet-acidified, succulent. The stone is medium size, ovoid, with inlay in the form of pockets and grooves, dark brown and non adherent to the pulp. The tree is auto-fertile, vigorous, with a very high yield potential (30-35 kg/tree), is resistant to frost, drought and disease, except *Taphrina deformans.*


Figure 2. Peaches of the Southland cultivar

Fruits are utilised for direct consumption and processing. They present resistance to handling and transport.
In ochard, the trees was conducted in 4 different fertilisation variants:
- V1 – control (unfertilised)
- V2 – organic fertilisation (fermented manure)
- V3 – chemical fertilisation of the soil (NPK complex fertilisers, in relation to 15:15:15.)
- V4 – chemical fertilisation of the soil + foliar fertilisation (on soil with NPK complex fertilisers, in relation to 15:15:15 and of the plant with the foliar fertiliser Murtonik 20:20:20 + microelements: Mn, Fe, Cu, Zn, Bo, chelation form).

At the Research and Development Institute for Processing and Marketing of the Horticultural Products (RDIPMHP Bucharest), the fruits were stored in three storage variants:
- ambient temperature (T = 26-28°C, RH = 65-70%), in 1kg packaging - warm;
- refrigeration room (T = 2-4°C, RH = 83-87%), in packs of 1 kg covered with perforated polyethylene film - cold storage;
- refrigeration room (T = 2-4°C, RH = 92-96%), in 1 kg hermetic packages, so that the composition of the atmosphere inside has modified, by the reducing of the O_2 content and the increasing the CO_2 content and also of air

relative humidity-storage in modified atmosphere - MA.

The storage duration (days) varied according to the technological storage variant as follows:

- warm storage: 7
- cold storage: 28
- AM storage: 35

Immediately after harvesting, before the beginning of the storage and at the end of this period, certain determinations were carried out concerning the fruits' firmness, as well as organoleptic observations and biochemical analyses of the main components (soluble dry matter, soluble carbohydrates, titratable acidity). In addition, losses were quantified, both the quantitative (concerning the weight) and the qualitative ones (by depreciation), losses which occurred during the storage.

The firmness was determined by means of a OFD weight penetrometer, which measures in penetrometric units (1 PU = 0.1 mm) the depth to which the conic needle is able to penetrate the pulp of the fruit (length = 24 mm, diameter at its basis = 4 mm). The measurements were performed upon a number of 25 fruits/variant, each fruit being penetrated in four points in the equatorial area.

The appreciation of the organoleptic quality was carried out by means of a sensorial testing of a fruit, the evaluation method being a scale with point from 1 to 100. Tasting sheet were used which comprises three criteria: aspect, firmness, taste. Each of these three criteria weighs differently within the general grading, according to their importance: the aspect represents 15%, the firmness 35%, while the taste represents 50%. Taking into account the total score, there are five quality categories as follows:

Grade (quality category)	**Points**
Very good	80-100
Good	60-79
Acceptable	40-59
Mediocre	20-39
Unsuitable	0-19

The methods used in order to determine the biochemical components were:

- refractometry, using an ABBE refractometer, to determine the soluble dry matter;
- the Bertrand titrimetric method to determine the soluble carbohydrates;
- the titrimetric method for the determination of the titratable acidity.

Throughout the storage, the thermo-hydric factors from the cold room were checked on a daily basis, in order to ensure the fact that the best conditions for maintaining the quality were respected. At the same time, the capacity of the maintain fruit quality was evaluated, including the occurrence and development of different storage diseases.

RESULTS AND DISCUSSIONS

a. Organoleptic quality

The results obtained following the organoleptical testing (Figure 3) reveals the fact that at harvest, the peaches obtained a high score (96.08 points), because of their attractive aspect (12.75 points), good firmness (35.00 points) and pleasant taste (48.33 points).

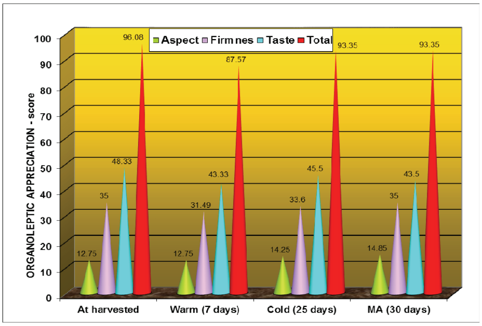

Figure 3. Organoleptic appreciation of 'Southland' cultivar

The grade, according to the obtained points, was "very good" for the fresh peaches - at harvest (Table 1).

Table 1. The organoleptic appreciation of peaches at harvest and after storage

Moment of determination	Grade
At harvest	very good
After warm storage	very good
After cold storage	very good
After AM storage	very good

After 7 days of storage in an ambient temperature, the organoleptic properties of the peaches remained in good parameters (87.57

points) so that, although the score dropped a little due to the diminishing of the firmness (31.49 points) and taste (41.33 points), the final grade was also "very good".
After cold storage for 28 days, the peaches of the 'Southland' cultivar maintained their beautiful aspect, good firmness and pleasant taste and, following the organoleptic testing, obtained a high score (93.35 points) and the grade "very good". After storage under conditions of modified atmosphere for 37 days, the peaches also maintained their firmness (35.00 points) and pleasant taste (43.50 points), but increased their beautiful aspect (43.50 points) and obtained the grade "very good", having a score of 93.35 points.

b. Firmness

At harvest time, the values of the firmness range between 21.56 PU at the fruit from the V1 variant and 63.89 PU at the fruit from the V3 variant, the average per cultivar being of 40.08 PU (Table 2).
During warm storage, the peaches lose easy their firmness, because of the quick ripening (175,08 PU). When the peaches are stored in cold conditions, the intensity of the ripening process diminishes, so that the fruit maintain their structural and textural firmness for a longer period of time (107,06 PU after 28 days of storing).
By enriching the atmosphere in the storage space with carbon dioxide, the metabolic processes become even slower and the peaches maintain their firmness (106,94 PU) for a longer period of time (35 days).

Table 2. The firmness of peaches of the 'Southland' cultivar at harvest time and after storage period

Variant	Penetration value – PU			
	at harvest	after storage		
		warm	cold	MA
V1	21,56	200,11	124,78	105,22
V2	43,44	166,89	93,33	101,33
V3	63,89	151,11	92,33	114,33
V4	31,44	182,22	117,78	106,89
Average per cultivar	**40,08**	**175,08**	**107,06**	**106,94**

c. Biochemical composition

The content of 'Southland' peaches, at harvest time (initially), in main biochemical components, is presented in Table 3. The results show that the values of biochemical indicators from the fruits vary according to variant of fertilization applied to peach culture.
Analyzing the soluble dry matter content on variants, it occurred that the V1 variant had the lowest value of this indicator (10.64%), and V4 variant had the highest value (11.18%), closely followed by V2 variant (11.14%).
The soluble carbohydrate content presents the highest value for the V2 variant (8.76%) and lowest in V1 variant (8.15%).

Table 3. The main chemical components of 'Southland' cultivar peaches at harvest time

Variant	Solub. dry matter (^{0}R)	Solub. carbo-hydrates (%)	Titratable acidity (malic acid/ 100g)
V1	10.64	8.15	0.81
V2	11.14	8.76	0.77
V3	10.68	8.21	0.77
V4	11.18	8.64	0.76
Average variety	**10.91**	**8.44**	**0.78**

In case of titratable acidity the differences between the four variants are small, however the V4 variant has the lowest content in malic acid (0.76%), and V1 variant, the highest content (0.81%).
The biochemical modifications occurred during storage in the three variants are shown in Tables 4 to 6.
The high temperature during storage favors the deployment with a great intensity of biochemical processes in fruits, such as after 7 days of cold storage the content in soluble dry matter increased much, whereas the soluble sugars, respectively malic acid fell much, compared with the others storage methods (Table 4).

Table 4. The main chemical components of 'Southland' cultivar after warm storage

Variant	Solub. dry matter (^{0}R)	Solub. carbo-hydrates (%)	Titratable acidity (malic acid/ 100g)
V1	12.11	6.35	0.73
V2	12.09	7.17	0.64
V3	12.00	5.93	0.68
V4	12.09	7.12	0,63
Average	**12.07**	**6.64**	**0.67**

The lower temperature during cold storage leads to the slow rhythm of these biochemical processes, such as soluble dry matter increased with 6.32% from the time of harvest and the content in soluble carbohydrates and titratable acids decreased with 2.13% , respectively 2.56% (Table 5).

Table 5. The main chemical components of 'Southland' cultivar after cold storage

Variant	Solub. dry matter (^{0}R)	Solub. carbo-hydrates (%)	Titratable acidity (malic acid/ 100g)
V1	11.57	8.11	0.76
V2	11.80	8.51	0.76
V3	11.24	8.00	0.74
V4	11.81	8.43	0.76
Average	**11.60**	**8.26**	**0.76**

The cold effect is more pronounced during the change of the gaseous composition of the air, by increasing the concentration of the carbon dioxide in storage space, such as during storage in modified atmosphere, the soluble dry matter content of the peaches has grown by only 1.55% against initial moment, and titratable acidity decreased with only 1.28% on the same time, as evidenced in Table 6.

It was found that in case of storage in normal temperature - warm (ambient temperature) for 7 days, total losses are higher in all 4 types of fertilization, due to both mass (weight) losses, but especially those by depreciation.

Table 6. The main chemical components of 'Southland' cultivar after storage in M.A

Variant	Solub. dry matter (^{0}R)	Solub. carbo-hydrates (%)	Titratable acidity (malic acid/ 100g)
V1	10.73	8.19	0.76
V2	11.36	8.51	0.78
V3	10.77	7.85	0.80
V4	11.46	8.39	0.76
Average	**11.08**	**8,23**	**0.77**

d. Qualitative and quantitative losses

The losses of the peaches recorded during the storage are presented in Table 7.

Table 7. Losses recorded during the storage of peaches under different technological conditions

Losses - %	Variant				
	V1	**V2**	**V3**	**V4**	**Average**
Warm: - total	43.11	23.07	32.54	18.85	29.39
- weight	14.42	13.12	14.24	12.09	10.97
- depreciation	28.69	9.95	18.30	16.76	18.42
Cold: - total	15.05	6.65	7.41	4.91	8.50
- weight	1.49	1.45	2.10	1.61	1.66
- depreciation	13.56	5.20	5.31	3.30	6.84
AM: - total	7.90	0.22	3.35	0.19	2.91
- weight	0.20	0.22	0.32	0.19	0.23
- depreciation	7.70	-	3.03	-	2.68

At the V1 variant – control we recorded the highest losses (43.11%), and the V4 variant - chemical fertilization in soil + foliar feeding, the lowest (18.85%). But skipping the variant of the fertilization, the total losses recorded during the warm peaches storage of the Southland are 29,39% (10.97% weight losses and 18.42% by depreciation losses).

The fruit impairment, in case of peaches, are due to late infections caused by fungi *Monilinia laxa* and *M. fructigena* before harvest, when they are too little visible. After harvest, during transport and storage, the attack rapidly evolves (depending on temperature) and the entire fruit rots. Moreover, during the storage can lead to the rotting of the surrounding healthy fruit, mycelium penetrating into them directly or through injuries almost invisible.

The fruits can also be infected through wounds, blows or pressure produced during harvest and handling by the molds *Rhizopus stolonifer* and *Botrytis cinerea*.

By using cold storage of peaches, losses were recorded both quantitative (weight) and qualitative (depreciation), much smaller than warm keeping. Thus, the values found at the 'Southland' cultivar, were: mass losses = 1.66%, depreciation losses = 6.84% and total losses = 8.50%.

V4 variant is also remarkable, with 4.91% total losses (1.61% mass losses + 3.30% qualitative losses), followed by V2 variant (losses: 6.65%, 1.45% and 5.20%, respectively). On last place, with total losses of 15.05% ranks V1 variant.

The losses determined after 35 days of storage in modified atmosphere conditions were significantly lower values compared with the others methods of storage.

The 'Southland' cultivar recorded total losses of 2.91% (0.23% mass losses + 2.68% by depreciation losses) only. The V4 variant of fertilization was not reported losses of quality and the quantitative losses were almost zero (0.19%). A similar situation is found in the V2 variant too, with total losses of 0, 22%. Even in the V1 variant the losses were significantly reduced, those being of 7.90%, 0.20% and 7.70% respectively.

From experimental data results that the fertilization variant of orchard peaches, the best results in terms of losses during storage, from all the three technological methods, is V4 variant, followed by V2 variant, and the worst results were obtained in V1 variant.

CONCLUSIONS

The peaches of the 'Southland' cultivar are appreciated from an organoleptic point of view, given the fact that they obtained a fairly good score upon their organoleptic testing at harvest. The evolution of the quality during storage depends on the conditions in the storage environment and especially on the temperature and gaseous composition.

From the point of view of the firmness, the best results were obtained by the peaches which were fertilised with foliar fertilisers. It was noticed that the speed of metabolising pectin substances and the decrease of the fruit's firmness differs according to the culture's fertilising regime but especially to the temperature and air composition in the storage room.

Contents of peach fruit in main biochemical indicators (soluble dry matter, soluble carbohydrate, organic acids) varies depending on crop fertilization system. In terms of biochemical, peaches from the culture fertilized with organic fertilizers and those from culture fertilized with chemical fertilizer incorporated into soil and foliar fertilization gave the best results. The biodegradation of organic acids and carbohydrates is influenced by storage temperature. As the storage temperature is higher, the biodegradation is even more pronounced. The chemical composition of the air in storage space is also an important factor.

The ability to maintain the quality of peach fruit varies also depending by the fertilization system, and storage conditions of the environment and especially by temperature and gaseous air composition. The V4 variant (chemical fertilization on soil + foliar feeding) induces the best storage capacity, with the lowest quantitative (weight) and qualitative (depreciation) losses. The most efficient methods of fruits storage is refrigeration room + modified atmosphere in which were recorded the lowest losses during storage.

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FROST RESISTANCE OF SOME SWEET CHERRY CULTIVARS IN THE BUCHAREST AREA

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Abstract

Sudden low temperatures could occur in the spring when some of sweet cherry cultivars are more susceptible to freeze and floral buds injuries. These are delicate moments for growers year by year. In this study, differences in cold hardiness and lethal temperatures were observed during the same phenological stage (bud burst) for the same cultivar as well as among tested cultivars ('Katalin', 'Kordia', 'Burlat', 'Rivan' and 'Regina'). It was used the frost induced method for determining cold hardiness of cherry buds (-7 ^{0}C/0,5h; -7 ^{0}C/1h; -1,5 ^{0}C/0.5h; -1,5 ^{0}C/1h; -2,5 ^{0}C/1 h). The injuries observed were highly dependent on the stage of development of flower buds. Progressive vulnerability of the bud to low temperatures was observed. During 2012-2014, it was noticed that the cultivars were sometimes sensitive, semi-sensitive or hardiness, varying with the amount of active temperatures over 7^{0}C. Although the determination of the maximum hardiness is genetically attainable, the description of changes in hardiness is possible; more years of observations are needed as a result of the strong climatic influence.

Key words*: sweet cherry, phenology, temperature stress, hardiness, cultivars*

INTRODUCTION

Nowadays, sweet cherry is getting more and more importance due to their nutritional and fresh fruits quality (Budan and Gradinariu, 2000), new orchards being setting up in different geographic areas.
As part of the Prunoideae group, *P. avium* L. in one of the species with medium cold hardiness (Snyder and de Melo-Abreu, 2005). Tree resistance (buds, flowers, woody tissue, roots) depends on the cultivar traits respectively age, water content of the tissues, rootstock as well as of environmental conditions (thermal oscillations, amplitude from autumn till spring, frost/defrost processes, intensity and freezing duration).
Intense frostbite occur when sudden transition from autumn to winter happened, in November-December or after repeated defrosts in January-February, when flowering buds are the end of the dormant period.
Reproductive buds of cherries are more susceptible to freezing injury than vegetative buds, while the blossoms of the growing cherry trees are extremely susceptible to frost damage (Asanica et al., 2013). Frost resistance varies within the tree itself and in the same extent within orchard cultivars, flowers of cultivars in the same phenological stage. The resistance of the flower bud to low temperatures changes rapidly and predictably in response to both temperature and the stage of floral development (Proebsting, 1982).
Differences in cold hardiness and lethal temperatures were observed during different developmental stages for the same cultivar as well as among cultivars. Differential thermal analysis (DTA) is an effective method for determining cold hardiness of dormant cherry buds (Melba R. Salazar-Gutierrez, 2014).
The aim of this study was to determine the critical temperatures for some sweet cherry cultivars in the South-East part of Romania at specific phonologic stage of the trees.

MATERIALS AND METHODS

The research was carried at the Faculty of Horticulture Bucharest and the biological material was collected from the Experimental Field of the Fruit Growing Department, located in the geomorphological unit Romanian Plain, subdivision Vlasiei to 44^{0}29´50˝N and 26^{0}15´26˝E.

The climate is temperate-continental with warm, sometimes hot and frequent droughts and cold winters, with large amounts of snow. The springs are short, with big jumps in temperature from month to month with significant variations between day and night; autumns are distinguished by thermal moderation and slow transition to winter. The annual rainfall is between 500 and 600 mm, the maximum occurring in the period May-July.

Five sweet cherry cultivars with different harvest time were tested: 'Rivan', 'Katalin', 'Burlat', 'Kordia' and 'Regina', all grafted on *Prunus mahaleb* L. The experimental temperatures and the duration of induced frost were designed as follows:

Variant	Temperature (induced freeze) (^{0}C)	Exposure time (h)
V1	-7^{0}C	1/2 h
V2	-7^{0}C	1 h
V3	-1,5^0	1/2 h
V4	-1,5^0	1 h
V5	-2,5^{0}C	1 h
Control	field tested resistance	

The phenological stage for cold hardiness test of the cultivars was bud burst, corresponding to 5th of April in 2013 and 12th of March in 2014. The temperature controller used for inducing frost was the refrigerator TENAK LT300.

In order to start the experiment, it were collected 30 fruiting branches for each cultivar splitted in five samples for every experimental variant. The branches were quickly worked out and the order in which the samples were exposed to induced frost was V2, V1, V5, V4 and V3.

Immediately after pulling out the refrigerated branches it were made transversal sections and was noted the viability of the floral buds on the branches. For the control samples (not freeze) the sections through the floral buds was previously made in the lab.

For fruit trees, the effects of the winter frost could be emphasized based on the agrometeorological index respectively cold units, represented by the amount of negative average air temperatures ($\sum$Tav <0°C = cold unit, XI-III). This reflect the degree of harshness and intensity of cold for the entire cold season (XI-III). This index characterizes the overall vegetation conditions in autumn and winter season, given the possible of sporadic February heating periods *("hot windows")* that may result in reloading and boost of early spring vegetation (Mateescu, 2004).

<u>Hardiness degree / winter type:</u>
<u>cold units ($\sum$T av < 0 ºC, XI-III):</u>

< 100° very low intensity / warm winter
101-200° reduced intensity / soft winter
201-300° moderate intensity / regular winter
301-400° high intensity / cold winter
> 400° huge intensity / very harsh winter

<u>Hardiness degree / winter type:</u>
<u>freezing units ($\sum$T av < -15 ºC, XII-II):</u>

< 10 reduced intensity / soft winter
11 - 30° moderate intensity / regular winter
31 - 50° high intensity / cold winter
51 - 100° very high intensity / very cold winter
>100° huge intensity / very harsh winter

The flowering period and fruit maturation were determined by distinct stages of each phenophase. The amounts of accumulated temperatures were calculated by summing, for each cultivar, based on daily temperature corresponding trigger data and fruiting performance of each phase separately. The amount of the average active temperatures over 7^{0}C for the bud burst and blossoming start phenophases were summed during the dormancy (end of December-start of January).

RESULTS AND DISCUSSIONS

From the current period analysed (2012-2014) results that the cultural years 2012-2013 and 2013-2014 accumulated an index of 128ºC CU (cold units) respectively 132ºC CU. These are correspondingly with soft winter and with reduce intensity.

From the hardiness point of view, an index of -32ºC was calculated for the winter 2012-2013 which means a regular/moderate winter and one of -64ºC for 2013-2014 meaning a very cold winter (Table 1).

Table 1. The degree of winters harshness based on the cold and freezing units

Agrometeorological index	2012-2013	2013-2014
Cold units (^{0}Tav < 0^{0}C, XI-III	128	132
Freezing units (^{0}Tmin < -15^{0}C, XII-II	-32	-64

The average winter monthly temperatures for both periods were lower with -1.6^{0}C in December 2012 and with -0.6^{0}C in December 2013 comparative with the multiannual average of -0,1^{0}C.

The warming trend is observed from the monthly average temperatures increase of January (1,7^{0}C in 2013 and 2.2^{0}C in 2014), February (2.3^{o}C in 2013 and 2.4^{o}C in 2014) and March (normal for 2013 - 3.7^{o}C and 3,7^{0}C in 2014) versus the average annual temperatures (Figure 1, 2).

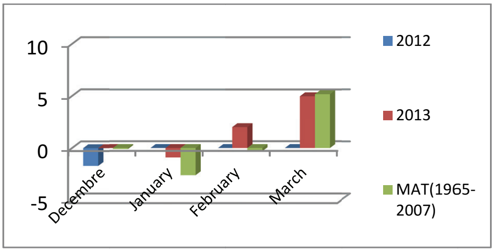

Figure 1. The monthly average temperatures of the 2012-2013 winter versus multiannual average temperatures (Bucharest)

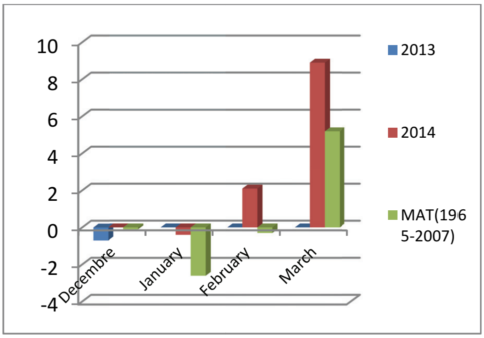

Figure 2. The monthly average temperatures of the 2013-2014 winter versus multiannual average temperatures (Bucharest)

During January and February, floral buds of the studied cherry cultivars have suffered visible injuries under the binocular magnifier. December 2012 was marked by negative minimum temperatures between -17.4^{o}C and 1.7^{o}C, with 3 consecutive days of low temperatures between -4.4^{o}C and -17^{o}C (January, 13-15) and with maximum negative temperature 4 days consecutive by -3.6^{o}C and -4.7^{o}C (January, 19-22); the significant differences compared to the values of multiannual minimum of -3^{o}C and maximum of 2^{o}C.

Significative fluctuations of daily temperatures occurred also in January occurring the minimum -12^{o}C and -14^{o}C over three consecutive days (January, 8-10) and positive maximum temperatures of 10-13^{o}C (January, 21-23) which led to a slight decrease of buds resistance (determined at the of field resistance for control variant) and a delay of the vegetation start.

During the 2013-2014 winter, it was recorded higher values than the annual thermal average, sometimes registering proeminent negative temperatures of -15^{o}C (11.XII), -14^{o}C (14.XII) and -17^{o}C (28.XII). February begins with temperatures somewhat lower than normal period with minimum temperatures between -6^{o}C and -11^{o}C (1 8.II) continuing 15 days with consecutive maximum between 8^{o}C to 19^{o}C, at which buds flowering are rushing to get in vegetation. On 10th of March, they were in the swelling phase and at the end of March the flowering stage already begun.

In general, resistance of cherry to winter frosts depends on tree evolution condition and the evolution of lower temperatures in order to produce frost. Depending on hereditary characteristics of cultivars, age, weather conditions accompanying frosts, the duration and intensity of freeze, it were found different degrees of injuries. Regarding the role of air temperature during the out of dormancy period and subsequently on the development of vegetation and fruiting phenophases they are determined by the cumulative action of daily average temperatures that exceed the amount of 7^{o}C. There is a level of optimum temperatures for each phenophase, along with other factors which ensure normal growth and development of the trees and is required for knowing in time the disturbances that occur in the growth and fructification cycle.

In Tables 2 and 3 are presented the main fruiting phenophases studied and temperature requirements of each cultivar for swelling bud stage, early flowering, late flowering and fruit maturation. From 2012-2013 and 2013-2014 winters characterization it was revealed a soft winters and with low intensity in terms of harshness winter (2013 - 2014) which signify it was moderate and rough winter in case of 2013-2014 winter (Table 1).

In terms of phenological dates, 2013 was a normal cherry year (Table 2) and a very early one in 2014. Bud swelling was triggered between 5.04-10.04 (2013) during 6 days and between 12.03-16.03 during 5 days (2014).

The cultivars accumulated active temperature above 7ºC meeting their individual needs based on the order of ripening in the Bucharest area. Early cultivars 'Rivan' and 'Burlat' accumulated between 199.2ºC and 224.5ºC in 2013 and 78ºC respectively 88ºC in 2014, while the cultivar with late ripening 'Regina' garnered 256ºC and 111ºC. The differences between the early and the late cultivar was of 56ºC in 2013 and 33ºC in 2014. For early flowering phenophase in 2013, 269.5ºC and 352ºC, the difference between maturation groups was of 82.5ºC. In 2014, limits of 164.5ºC and 229.5ºC concerning the temperatures accumulated till flowering counted a 65ºC difference.

Blossoming period lasting between 5-7 days (in 2013) and 5-6 days (in 2014) and the amount of degrees of temperature during flowering ranged from 35.5ºC to 85.5ºC and 46ºC to 57.5ºC under the influence of big temperature variations from respective periods (Table 2, 3). In the Bucharest-Ilfov area, given the above conditions, the studied cultivars have accumulated between 70 to 96ºC (in 2013) and 86 to 118.5ºC (in 2014). Budan and Gradinariu (2000) mention a need of 202 to 310ºC from the swelling buds to the blossom phenophase in the Pitesti-Maracineni region. The importance of air temperature decreases as relevance from blossom time to stone fortification and returns in importance during fruit maturation. From this point of view, the early cultivars (mid early) had accumulated between 799ºC ('Rivan') and 879ºC ('Burlat') medium season cultivars between 867.5ºC ('Katalin') and 912ºC ('Kordia') and late maturity cultivar 'Regina' 1125.5ºC according to the warm demand established by Kolesnikov (1959).

Table 2. Development of fruiting phenophases in 2013 at sweet cherry cultivars in the Bucharest area

Cultivar	Bud swelling	∑º active temp. above 7º C till bud burst	Start of blossom	∑º active temp. above 7º C till blossom	∑ºt from bud burst to blossom	End of flowering	Blossom period (days)	∑ºt during the blossom	Fruit ripening	∑ºt from bud swelling.	Growth and develoment stage (days)	∑ºt from blosoom to fruit maturation
Katalin	10.04	242	15.04	328	86	19.04	5	48	05.06	1374,5	48	867,5
Kordia	10.04	242	15.04	328	86	20.04	6	61	08.06	1432	50	912
Burlat	08.04	224,5	13.04	300,5	76	18.04	6	69	05.06	1374,5	49	879
Regina	11.04	256	17.04	352	96	21.04	5	35,5	18.06	1660,5	59	1125,5
Rivan	05.04	199,5	11.04	269,5	70	17.04	7	85,5	30.05	1283,5	45	799

Table 3. Development of fruiting phenophases in 2014 at sweet cherry cultivars in the Bucharest area

Cultivar	Bud swelling	∑º active temp. above 7º C till bud burst	Start of blossom	∑º active temp. above 7º C till blossom	∑ºt from bud burst to blossom	End of flowering	Blossom period (days)	∑ºt during the blossom	Average temp. limits (ºC)
Katalin	16.03	111	03.04	197	86	08.04	6	46	4-13,5
Kordia	16.03	111	05.04	212	101	10.04	6	51,5	4-16,5
Burlat	12.03	88	01.04	174	86	05.04	5	47,5	7-15
Regina	16.03	111	08.04	229,5	118,5	13.04	6	56,5	8-16,5
Rivan	10.03	78	31.03	164,5	86,5	05.04	5	57,5	7-13,5

In order to test the hardiness of buds flowering, fruit-bearing branches samples were placed in a freezer at different temperatures and exposure times (ET).

Flowering buds of the control variant, in the bud burst stage suffered minor injuries in 2013 ranging between 0.9% at early cultivar 'Burlat' and 8.7 % at late ripening cultivar 'Regina'. In 2014, it weren't recorded losses of buds in any of the cultivars studied (Table 4).
Exposure time (ET) for one hour significantly affect fruit buds, for each of negative temperatures used: -7°C (V2), -2.5°C (V5) and -1.5°C (V4). V2 registered losses between 100% at 'Katalin' and 'Burlat', 73.3% at 'Kordia', 50% at 'Regina' and 36.6% at 'Rivan' in the 2013 experiment. Smaller percentages of losses were recorded in the same variants in 2014, between 29% at 'Katalin', 26.4% at 'Kordia', 25.9% at 'Rivan', 24.2 % at 'Regina' and 9.6% at 'Burlat' cultivar.

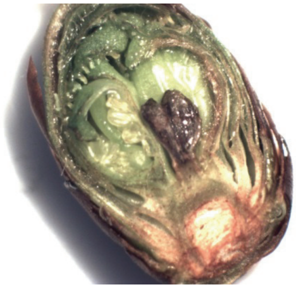

Figure 3. Cross cutting section of the flower bud ('Rivan', 2013)

V3 in the spring of 2014, which consist of -1.5°C temperature and exposure time of half an hour freeze has not encounting problems regarding the viability of flowering buds, which confirms that hardiness of the flowering buds belongs to specie/cultivar genetic structure (Table 3).
The two years study indicate 'Rivan' as the cultivar which recorded the biggest loss of flowering buds (34%) at V5 (-2.5°C/1h) with significant differences of percentages between years as well. He gathered 199.5^0C in 2013 (Table 3) and injuries of 18.4% (Table 4) and 78^0C in 2014 (Table 3) with 34% losses (Table 4).
Significant deviation recorded in 2013 compared to temperature variations and exposure times greater in V4 (-1.5^0C/1h) and 50.9 % bud losses.
Similar proportions of flowering buds losses was found at 'Burlat' cultivar with 10.3% (2013) and 10.4% (2014) when they acquired 88^0C and 224.5^0C in 2014. From the data presented in the Table 4, we found that at the same experimental variant V5, percentages were close enough at the late ripening cultivar 'Regina' (16.1% in 2013 and 10.7% in 2014) comparing to 'Katalin' or 'Kordia'.

Table 4. Bud losses in the field cold hardiness and induced freeze conditions at some sweet cherry cultivars (2013-2014)

Variant	Lost buds (%)									
	'Katalin'		'Kordia'		'Burlat'		'Regina'		'Rivan'	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Control (field)	6,8	0	7,1	0	0,9	0	8,7	0	7,3	0
V1 (-7°C/1/2h)	48	16,6	37,5	2,2	18,1	0	19,6	0	24,2	12,5
V2 (-7°C/1h)	100	29	73,3	26,4	100	9,6	50	24,2	36,6	25,9
V3 (-1,5°C/1/2h)	13,6	0	23,8	0	32	1,8	12	0	22,5	0
V4 (-1,5°C/1h)	20	2,9	47	0	58,7	5,6	17,7	0	50,9	0
V5 (-2,5°C/1h)	27,2	5,1	27,2	5,1	10,3	10,4	16,1	10,7	18,4	34

From the two years analyses it results that the most sensitive cultivar was 'Rivan' at the temperatures of -2.5^0C/1h in 2014.

CONCLUSIONS

Looking at the 2012-2014 period, it was noted that 2012-2013 winter time accumulated 128ºC cold units and the 2013-2014 period 132ºC which correspond to the soft winters with reduced intensity.

Regarding the hardiness, for 2012-2013 period the index of -32ºC matching the regular winter time and for the 2013-2014 period, the index of -64ºC indicate a very cold winter.
The phenology of 2013 year has recorded a normal developing phases and the 2014 year a very earliness stages.

Knowing the different amount of temperatures above 7^0C for each cultivar, the sweet cherry growers could estimate and predict the development of each phenophase and choose the right management for technological measures.

Induced freeze of -7^0C for 1 hour in the bud burst stage produce 100% injuries at 'Katalin" and 'Burlat' cultivars and important losses for the rest of cultivars too. Also 1 hour of -2.5^0C exposure time indicate 'Rivan' as the most sensitive cultivar in 2014 and 'Kordia' & 'Katalin' in 2013.

Doubling the exposure time of -1.5^0C produced an increase of buds losses at all cultivars, the biggest injuries being recorded by 'Burlat' and 'Rivan' cultivars (over 50%).

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THE GROWTH AND FRUCTIFICATION OF CHERRY TREES DEPENDING ON CUTTING SYSTEMS

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Abstract

It were studied the growth and fruiting of sweet cherry trees (Cerasus avium L.) from cv. 'Valerii Cicalov' and cv. 'Record', grafted on mahaleb rootstock (Cerasus mahaleb Mill.) in relation with pruning system. The orchard was established in 2003 with planting distance of 6 x 5 m. The trees have been formed after natural ramification with high volume. The staggered pruning of semi skeleton branches was made during the vegetation period and complementary in wood for 3-4 years. This variant advantages the formation of semi skeleton and fruit branches, compared to pruning production during the dormancy period and vegetation period. The fruits harvested in 2012-2013 weight 20,1-33,4 kg/tree at 'Valerii Cicalov' cultivar and 18,9-26,4 kg/tree at 'Record'. The staggered cutting of semi skeleton branches during the vegetation period garnishes the crown with fruiting branches and provides an increase of fruits production with 32% to cv. 'Valerii Cicalov' and with 21% to cv. 'Record' comparing to pruning production during the dormand period (control).

***Key words**: branches of fruit, cherry, growth branches, cutting reduction, variety*

INTRODUCTION

The continuous improvement process of pruning by introducing new technological sequences, boosts the existing crop technologies, but they require modeling to capitalize the biological potential of trees in the modern orchards.

The maintaining of branches after their formation at the necessary optimal volume to create an favorable report between the growing and fructification period, is achieved by implementing maintenance and fruiting pruning (Mitre et al., 2007; Balan, 2012).

To determine the optimal level of trees pruning it must be taken into account the biological characteristics of the cultivar, the reaction of the different types of prune. In other words, the level of maintenance and fruiting pruning degree is determined differently, depending on cultivar and pruning system. The rational pruning contributes to precocious fruit trees and obtain high and qualitative fruits, hastens the redemption of invested capital in plantation which increases the economic efficiency of the fruit growing (Balan et al., 2001; Simion et al., 2004).

The pruning of cherry in fruiting period involves improvement works of the light regime and ventilation, a limitation of height and horizontal branches extension.

The cutting is also applied to semi skeleton branches exceeding 4-5 years age, to obtain branches from buds with high biological potential, appropriated to differentiating the productive shoots each year with high value and high quality fruits (Budan and Gradinariu, 2000).

In order to identify some effective methods of maintenance and regeneration of cherry trees by applying the cutting reduction during the vegetation and dormancy period, in 2011 "Videx-Agro" company organized a practical research experience.

MATERIALS AND METHODS

The research was conducted in the cherry orchard (Cerasus avium L.) of "Vindex-Agro" company, planted in 2003 of the unincorporated village Malaiești, Orhei district. The biological material was represented by cv. 'Valerii Cicalov' and cv. 'Record', grafted on the mahaleb seedlings (Cerasus mahaleb Mill.). The planting distance was of 6 x 5 m. The trees have been formed after the natural ramification improved with high volume. The crown consists of a basal level with 3 branches above there are 3-4 embranchments inserted on the shaft spiral spaced at 35 cm one from each other.

To achieve the expected goal it were investigated the following variants:

V1 - cutting production (maintenance and fructification), during dormant period (control);

V2 - cutting production (maintenance and fructification), during the vegetation period;

V3 - staggered cutting of semi skeleton branches during the dormant period in wood for 3-5 years;

V4 - staggered cutting of branches during the vegetation period in wood for 3-5 years.

The experience was organized in randomized blocks; each variant includes four repetitions of each 8 trees. To record the effect of cutting reduction it were effectuated biometric measurements according to the methods used in horticulture. The average length of branches, number and density of fruiting formations determined from 3 typical trees, but the fruits were harvested from 32 trees using statistical methods of calculating (Мойсейченко et al., 1994). During the vegetation period, in the orchard it were made maintenance of soil and protection of plants as it is stipulated in the intensive technology culture of cherry.

RESULTS AND DISCUSSIONS

The regeneration cuttings become dominant for cherry since fruiting period and it is one of the main methods of growth control and load of fruit trees. (Stefano et al., 2009; Babuc 2012).

Figure 1. Branches from spigots at cv 'Valerii Cicalov' in the 4th leaf

These cuttings can be made during the dormant period as well as during the vegetation period, having the advantage of reducing tree vigor and training of young semi skeleton items distributed, proportionally, uniformly in adapted system ramification. (Donica, 2005).

The number of branches formed from dormant buds differs on shortened branches age (Figure 1).

Based on the results, it we observed that staggered cutting causes the rejuvenation of the ramifications.

The forming of spigots and sequenced cutting of semi skeleton branches cause favorable conditions to form well developed sprout and subsequently they hold fruit formations, they contribute at the growth of cherry tree productivity and fruit quality.

Forming spigots on wood for 3-4 years gave results, having from 4-6 sprouts for cv. 'Valerii Cicalov' and 2-3 sprouts for 'Record'.

Depending on the position and spigot length in ramification, it forms 3-4 sprouts unevenly distributed. In some case they were formed on mother skeleton branch and not on the sprout. The sprouts in the age of 5 years for cv. 'Record' did not form sprouts.

The results prove that the staggered cutting of semi skeleton branches during the vegetation period in wood for 3-5 years (V4), favor the formation of fertile branches and younger semi skeleton compared to the staggered cutting of semi skeleton branches during the dormant period in wood for 3-5 years (V3) and cutting production during the dormancy (V1) and during vegetation (V2) period (Balan and Ivanov, 2012).

The average length and total annual branches for cherry tree were influenced by the studied factors.

The presented data shows that the annual branches length formed on the spigot from bugs is correlated with the age and their number. So, older spigot, fewer formed sprouts; if the length of these are bigger then it is due to the location and to the nutrition.

Analyzing the growth of annual branches on the spigot, it can be mentioned that the average length of annual branches on the spigot for cv 'Valerii Cicalov' was between 43 cm and 61 cm in 2012 and 33 cm till 50 cm in 2013.

For cv. 'Record' the values of annual increases on the spigots were situated between 22 cm and 50 cm (Figure 2).

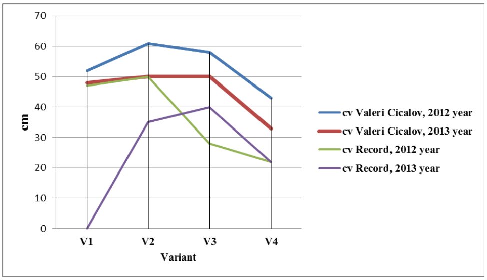

Figure 2. Average length of annual branches on the spigot according to cutting system

The summed length of annual branches formed from bugs was influenced significantly by the cultivar and by the pruning system. In the first year, after cutting, trees for cv 'Valerii Cicalov' the summed length of annual branches in V3 were of 2,21 m/spigot but the trees of the same cultivar pruned during vegetation period (V4) the value of this index was reduced, constituting 1,55 m/spigot (Figure 3). The lower values were recorded in the cultivar where cuts were applied during the dormant period. In 2013 the summed length of annual branches for cv 'Valerii Cicalov' also differ according to the cutiing system and it was from 0,96 m/spigot till 1,66 m/spigot. Irrespective of the cutting system the summed length of annual branches from cv 'Record' has lower values than cv 'Valerii Cicalov' and varied from 47 cm/spigot in V3 at cutting reduction in wood for 5 years. In 2012, were of 150 cm/spigot in V2 at cutting reduction in wood for 4 years. In 2013 the increases were of 67 cm.

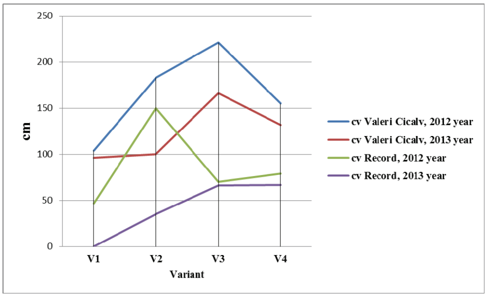

Figure 3. Summed length of annual branches on the spigot according to cutting system

The trees ability to generate quantitative and qualitative fruits is close related to the length of annual increases which is determined by environmental factors, soil and plantation maintenance.

The number of fruits formations, formed on spigot branches varies depending on the system and cutting period (fig. 4, 5). We observe at both cultivars a considerable increase of the number of fruit formations in 2013 to 2012. This is due to the age of the branch on the spigot. The number of spur bunches varied from 29 in 2012 till 367 in 2013. Conducting the sequenced cutting as well as the procedures for maintenance of ramification during the vegetation period contributed positively to the filing of fruit branches formations. Cv 'Record' characterized by slower growth compared to cv. 'Valerii Cicalov', generated also a lower number of fruit formation, having the values from 12 in 2012 till 247 spurs in 2013 in V3.

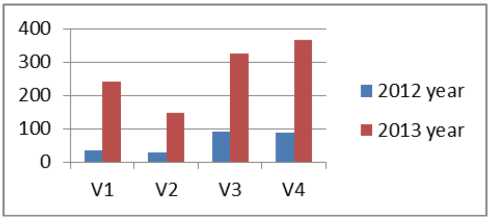

Figure 4. Number of fruit formation for cv 'Valerii Cicalov' on the spigot formed branches, according to cutting system

The strategic direction of this study is directed towards the exploitation of growth potential of trees according to fruiting potential of each cultivar. To obtain high, qualitative and stable yields, it is necessary to maintain the physiological balance among branches of different age and the filling with floral buds.

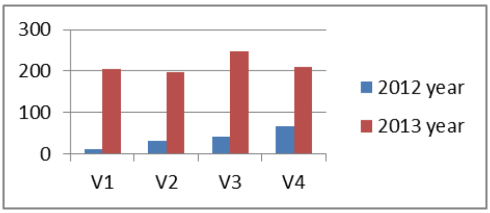

Figure 5. Number of fruit formation for cv 'Record' on the spigot formed branches, according to cutting system

Over time, the fructification cutting led to the creation of differences between the growth and formation of spurs because spurs bring fruits 8-12 years but medium and long branches have a slow evolution.

The carried research (Table 1) revealed that the fruit harvest was influenced by the system and period of cutting trees. In 2012 the fruit harvest for cv 'Valerii Cicalov' was 20,1-23,4 kg/tree but for cv 'Record' of 18,9-22,3 kg/tree. In 2013 it was an increase of fruit harvest in V4 where was applied the sequenced cutting of semi skeleton branches during the vegetation period, having 33,4 kg/tree or more than 32% in V1. Cv. 'Record' is less receptive at applied cutting it had a lower increase of fruit quantities but the quality of these fruits is net superior. The difference between control (V1) and sequenced cutting version of semiskelet branches during vegetation period (V4) for cv. 'Record' in 2013 is 21,1%.

The productivity of cherry trees increases significantly compared to the control so for cv. 'Valerii Cicalov' as well as for cv. 'Record' in V4 where it was applied the staggered cutting of semi skeleton branches during vegetation period. In 2012-2013 the cherry yield was about 9,46 t/ha for cv. 'Valerii Cicalov' and 7,52 t/ha for cv. 'Record'.

Table 1. The harvest of cherry trees depending on cultivar and cutting system

Cutting system	Productivity kg/tree		Productivity t/ha	
	2012 year	2013 year	2012 year	2013 year
cv Valerii Cicalov				
V1	20,1	25,3	6,69	8,42
V2	22,7	27,9	7,56	9,29
V3	21,8	30,2	7,26	10,06
V4	23,4	33,4	7,79	11,1
M	22,0	29,2	7,33	9,72
cv Record				
V1	20,1	21,8	6,69	7,26
V2	22,3	23,7	7,42	7,89
V3	19,2	25,7	6,39	8,56
V4	18,9	26,4	6,29	8,79
M	20,1	24,4	6,70	8,13

CONCLUSIONS

The capacity to form sprout on the spigot is bigger when the reductive branches are made on wood of 3-4 years. Older is the wood less is the number of the sprout formed. Staggered cutting of semi skeleton branches during vegetation period provides the garnishing of the semi skeleton with medium and vigorous length fruit branches.

The cuttings will be made during the harvest or after harvest. The large wounds will be disinfected with $CuSO_4$ solution and then they are protected with mastic.

The fruit harvest in 2012-2013 was of 20,1-33,4 kg/tree for cv. 'Valerii Cicalov' and 18,9-26,4 kg/tree for cv. 'Record'.

Staggered cutting of semi skeleton branches during vegetation period favors the garnishing of semi skeleton with fruit branches, having an increase of the fruits about 32% for cv. 'Valerii Cicalov' and about 21% for cv 'Record' compared to productive cutting during the dormant period.

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RESEARCH CONCERNING THE INFLUENCE OF DIFFERENT STORAGE CONDITIONS ON THE PRESERVATION CAPACITY OF SOME NEW APPLE VARIETIES

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Abstract

The preservation of apples for a longer period in order to assure the extension of the scope of the consumption as fresh fruit represents a major scope of the producer and merchandiser, which produce end export such fruits. The research performed in this field with the apple varieties 'Luna', 'Redix', 'Jonaprim', 'Goldrush', 'Florina', 'Rubinola' and 'Sirius', stored in different storage conditions, emphasized the difference between cultivars because of the different time of maturation. In the case of the same cultivar, the time of preservation was shorter in the ambient conditions (60 days for 'Sirius'), reaching 120 days in the modified atmosphere condition, under refrigeration. 'Florina' and 'Redix' were preserved for 90 days in the ambient condition and for 140 days in the modified atmosphere, under pre-refrigeration. The weight losses were lower for all cultivars in modified atmosphere (3,8% for 'Florina', 4,2% for 'Redix' and 4,6% for 'Sirius'). At the beginning of the storage period, and at the end of the preservation period, the main physico-chemical characteristics were also analyzed. The obtained results show that 'Florina' and 'Jonaprim' were the best, for example regarding the firmness values, at the end of the storage period.

Key words: *capacity, conditions, maturation, preservation, varieties*

INTRODUCTION

Specialists of producing and exporting apples countries have focused their attention on the long period apple storage problem, so that the apple could be sold, consumed in a staggered way.
The apple storage duration and the apple storage economic efficiencies are eventually determined by the loss of weight value by the depreciation due to rottenness (Chira L. et al., 2003).

MATERIALS AND METHODS

The research material was represented from seven new apple cultivars: 'Luna', 'Redix', 'Jonaprim', 'Goldrush', 'Florina', 'Rubinola' and 'Sirius', cultivated in Fruit Research Center of USAMV Bucharest.
The fruits were harvested at the harvesting ripeness time, when the main qualitative properties were analysed, afterwards, apple were stored in different storage conditions (Chira A., et al., 2002) which represent the experimental variants:
V1-ambient conditions; T=20°C; U.R.=65%
V2-refrigerating conditions; T=2°C; U.R=75%
V3-refrigerating storage and modified atmosphere (T=2°C, U.R.=90%).
The apple have been weight both when they were introduced for store and at the end of storage period, so that specialists could find out the differences of weight. At the same time, at the end of the storage period, the depreciation due to rottenness, the origin main pathogen agents and the main fruit physic-chemical and organoleptic properties of the best variant were evaluated.

RESULTS AND DISCUSSIONS

As we can see in the table 1, the ambient storage period conditions was shorter. The longer period was for V3. The fruits were stored in a refrigerating space and semi permeable plastic bags. In this way, it was ensured a higher relative air humidity, a modified gaseous composition, enriched with CO_2 (5-6%) and rarefied of O2.

These conditions preserved the fruits very good.

The losses of weight was grater at the fruits stored in ambient storage conditions. This happened due to the higher temperature and to the lower relative air humidity.

The least losses were registered at V3 – as the fruit transpiration has been diminished.

Table 1. The storage capacity of the apple fruits

Variety	Storage condition	Optimal storage period -days-	Weight Losses -%-	Rot Losses -%-	Total Losses -%-
LUNA	V1	70	6,2	9,0	15,2
	V2	100	4,4	6,2	10,6
	V3	120	3,6	4,0	7,6
REDIX	V1	90	6,8	7,6	16,4
	V2	120	4,8	5,4	10,2
	V3	140	4,2	3,8	8,0
JONAPRIM	V1	70	6,6	8,4	15,0
	V2	90	5,2	6,2	11,4
	V3	110	4,0	4,8	8,8
GOLDRUSH	V1	90	6,2	10,2	16,4
	V2	110	5,4	7,4	12,8
	V3	130	4,8	4,6	9,4
FLORINA	V1	90	5,8	6,8	12,6
	V2	120	4,0	5,0	9,0
	V3	140	3,8	3,2	7,0
RUBINOLA	V1	90	5,6	10,0	15,6
	V2	120	4,8	8,2	13,0
	V3	140	4,4	5,8	10,2
SIRIUS	V1	60	6,0	8,2	14,2
	V2	90	5,2	6,8	12,0
	V3	120	4,6	4,0	8,6

V2 – Ambient condition (T= 20°C, U.R. = 65%)
V2 – Refrigeration condition (T = 2°C, U.R. = 75%)
V3 – Modified atmosphere under refrigeration (T = 2°C, U.R. = 90%)

We can point out rather similar values after loss of weight at the seven tested apple cultivars, but the best results were registered for 'Goldrush'. The problem in the dropping is more significant that other cultivars, because of the thin epidermal skin.

The depreciation due to rottenness and physiological disturbs presented higher values in V1 case, while in V3 we can find the lowest values. Different cultivars registered different reaction. Thus, for 'Goldrush', the percentage of rotten apples was of 10,2% after a 90 days storage period (V1) in comparison with 'Florina' – 6,8% after 90 days storage period (V1).

In 'Goldrush' case, the presence of the pathogen agents that caused fruit depreciation has been influenced by the storage as follows: the *Gloeosporium album* has developed better in low temperature conditions and high relative humidity (V2, V3) in comparison with V1. *Botrytis cinerea* has manifested itself stronger at a high temperature (V1).

It has been observed that the 'Jonaprim', in V3, the *Penicillium sp*. attack was stronger.

Actually, in all cases for 'Jonaprim', the main attacking pathogenic agent was *Penicillium sp.*

The quality of the apples has been tested both during their harvesting period and at the end of the storage period.

In table 2 we show the results only for V3. This variant has proved to be the best – from the storage capacity point of view.

Regarding the average weight of the apples at harvesting, 'Sirius' was the biggest (250,7 g). 'Redix' weighted 184 g and 'Rubinola' 150 g. During the storage period, these values diminished because of the water loss by transpiration.

The firmness determined immediately after harvesting by Effegi penetrometer has the following values: 'Florina' – 6,4 kgf/cm^2 and 'Goldrush' - 5,6, kgf/cm^2.

These values decreased during the period due to pectin substance solubilisation and to the transformation of the substances into soluble pectin because of the pectinmetilesteraze enzyme. The values (at the end of storage period) were between 5,2 – 'Florina' and 4 kgf/cm^2 to 'Goldrush'. The soluble dry substance content and the titratable total acidity were two biochemical indicators of great interest. The soluble dry substance content had the following harvesting values: 'Goldrush' – 15,9 % and 'Florina' – 13,7%.

The soluble sugars value evaluation is as follows: at harvesting the content was between 12% at 'Sirius' and 15,9 at 'Jonaprim' and 'Goldrush'.

The hydrolysis process of the starch and the accumulation of the soluble sugars continued during the storage period. At the end of the period, the values increased till 13,8% to 'Sirius' and 16,8% to 'Goldrush'.

The titratable total acidity expressed in malic acid values is as follows: 'Jonaprim' – 0,40% (at harvesting) – 0,3% (end storage period) and

'Sirius' – 0,3% (at harvesting) and 0,18% (at the end of storage period).
The other analysed biochemical components are also shown in table 2.
This show us the advantage of storing apples in refrigerating storage using semipermeable plastic bags that can assure the best humidity conditions and a modified gaseous composition, favourable for fruit storage.

Table 2. The evolution of the mainly qualitative characteristics during apple storage period

Variety	The analysis period	Firmness Kgf/cm2	Water content -%-	Total dry Matter -%-	Soluble dry Matter -%-	Titerable acidity -%- acid malic	Ascorbic acid (mg/100g)
LUNA	At harvest	6,0	82,6	17,4	14,1	0,31	12,65
	V3- end storage	4,6	79,4	20,6	15,4	0,24	11,20
REDIX	At harvest	6,2	82,4	17,6	14,5	0,35	11,40
	V3- end storage	4,4	79,0	21,0	15,6	0,24	10,60
JONAPRIM	At harvest	5,8	83,0	17,0	15,9	0,40	12,80
	V3- end storage	5,0	79,7	20,3	16,6	0,30	11,40
GOLDRUSH	At harvest	5,6	83,2	16,8	15,9	0,26	12,20
	V3- end storage	4,0	79,2	20,8	16,8	0,20	10,80
FLORINA	At harvest	6,4	82,6	17,4	13,7	0,35	12,65
	V3- end storage	5,2	79,3	20,7	15,0	0,28	11,10
RUBINOLA	At harvest	6,0	83,0	17,0	15,0	0,30	11,80
	V3- end storage	4,8	79,3	20,7	15,8	0,20	10,60
SIRIUS	At harvest	6,2	82,8	17,2	12,0	0,30	12,40
	V3- end storage	4,8	79,0	21,0	13,8	0,18	11,00

CONCLUSIONS

The fruits storage period ranges from 60 days for 'Sirius' (in ambient conditions) to 140 days for 'Florina' and 'Redix' (in refrigeration under modified atmosphere).
The loss of weight during the storage period were greater in ambient conditions (16,4% 'Redix' and 'Goldrush') and lower in refrigeration modified atmosphere (7% to 'Florina' and 8% to 'Redix').
The main microbiological pathogens developed during storage period were *Gloeosporium album* for 'Goldrush' and *Penicillium sp*. for 'Jonaprim'.

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THE BEHAVIOUR OF SOME PEACH CULTIVARS PERTAINING TO THE PEACH WORLD COLLECTION IN PEDOCLIMATIC CONDITIONS OF TIMISOARA AREA

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Abstract

Peach represents one of the most appreciated fruit tree variety of the temperate climate, which in the last 30 years has benefited of a special attention, fact that has led to the expansion of cultivated areas with this variety and to diversification of the assortment. Researches carried out in USA, Canada, France, Italy, Spain, even in our country had led to obtaining a large number of varieties with remarkable agro-productive characteristics. In the present work were studied 9 cultivars of peach pertaining to the Peach and Nectarine World Collection introduced and multiplied in Romania by Acad. Dr. Vasile Cociu. The cultivars originating from all continents have been planted in Timisoara in 2007 with the purpose of being tested in culture and naturalizing in Romania of some new foreign varieties. Regarding the fruit weight were evidenced 'Yinquing', 'Giala di Roma', 'Tardiva', 'Eureka' and 'Piros Magdalena'. In terms of % kernel were highlighted 'Giala di Roma Tardiva', 'Marqueen' and 'Gold Dust' cultivars with less than 7% kernel %. Concerning dry substances, the highest sugar content was registered in the fruits of following cultivars: 'Marqueen', 'Eureka', 'Yinquing' and 'Giala di Roma Tardiva'.

Key words: *Peach, Peach and Nectarine World Collection, fruit weight, percent kernel, sugar content*

INTRODUCTION

Peach represents one of the most appreciated fruit tree variety of the temperate climate, which in the last 30 years has benefited of a special attention, fact that has led to the expansion of cultivated areas with this variety and to diversification of the assortment. Researches carried out in USA, Canada, France, Italy, Spain, even in our country had led to obtaining a large number of varieties with remarkable agro-productive characteristics. In the present work were studied 9 cultivars of peach pertaining to the Peach and Nectarine World Collection introduced and multiplied in Romania by Acad. Dr. Vasile Cociu.

MATERIAL AND METHOD

The experience was conducted in the didactical plantation of Fruit Growing Department of USAMVBT.

The biological material was constituted of 10 peach cultivars pertaining to the Peach and Nectarine World Collection founded in Timisoara in the year 2007.

The cultivars originating from all continents were multiplied at SCDP Băneasa from where they were purchased afterwards being planted in the pedoclimatic conditions of Timisoara.

The planting distances were 4x2 m and the crowns were conducted in free palmette system.

Nine less known cultivars were considered for this study respectively 'Marianna', 'Sun Hun Hui', 'Yingquing', 'Piros Magdalena', 'Gold Dust', 'Eureka', 'July Elberta', 'Giala di Roma Tardiva', 'Marqeen' and 'Spring Gold' as experimental control.

It were followed aspects linked to fructification of plants as fruits dimensions, large diameter, small diameter and fruit height, average fruits weight, kernel weight and pulp dry substance content. For each element 20 fruits were analyzed. Fruits were measured with calipers

and weighed with high accuracy balance Kern Pes. Dry substance was determined with the refractometer Hanna Instruments.
All the data were statistically processed using variance analysis.

RESULTS AND DISCUSSIONS

The obtained results regarding the fruits weight in the experimental years 2012-2013 are presented in Table 1, Table 2.

Table 1. Weight of the fruits studied in 2012

Cultivar	Average weight (g)	Relative value %	Difference to the control	Significance
Marianna	49.67	133.04	12.33	X
Sun Hui Hun	45.33	121.43	8.0	-
Yinquing	93.0	249.11	55.67	XXX
Piros Magdalena	51.67	138.39	14.33	X
Gold Dust	39.33	105.36	2.0	-
Eureka	51.33	137.5	14.0	X
July Elberta	41.0	109.82	3.67	-
Giala di Roma Tardiva	62.67	167.86	85.33	XXX
Marqueen	34.0	91.07	-3.33	-
Spring Gold	37.33	100	0	control

DL5% = 11.24 DL1% = 15.19 DL0,1% = 20.23

The highest value of the fruits weight in the year 2012 was registered on 'Yinguing' cultivar (93.0 g) and 'Giala di Roma Tardiva' (62.67 g), the difference to the experiment control being very significant, positive. Values superior to the control were obtained also on the fruits of the 'Piros Magdalena' (51.33 g) and 'Marianna' (49.67 g), both being significant positive to the control.
The lowest value of the fruits weight in the experimental year 2012 was registered on the 'Marqueen' cultivar (34.0 g) however without significance, the value being close enough to the experiment control.

Table 2. Weight of the fruits studied in 2013

Cultivar	Average weight (g)	Relative value %	Difference to the control	Significance
Marianna	53.29	129.11	12.01	X
Sun Hui Hun	49.46	119.84	8.19	-
Yinquing	99.21	240.37	57.94	XXX
Piros Magdalena	54.93	133.08	13.65	X
Gold Dust	43.18	104.61	1.90	-
Eureka	55.35	134.10	14.07	X
July Elberta	44.70	108.30	3.43	-
Giala di Roma Tardiva	67.21	162.84	25.94	XXX
Marqueen	37.71	91.37	-3.56	-
Spring Gold	41.27	100	0	control

DL5% = 10.92 DL1% = 14.76 DL0,1% = 19.66

In the experimental year 2013, the highest values of the fruits weight were registered as well on 'Yinquing' and 'Giala di Roma Tardiva' cultivars, the differences to the experiment control being very significant positive. Values superior to the control were registered on 'Eureka', 'Piros Magdalena' and 'Marianna' cultivars, all being significant positive to the experiment control. The lowest value of the fruit weight was registered likewise in 2012 on 'Marqueen' cultivar, value which vas not statistically insured.
The experiment results regarding the large diameter of the fruits on the peach cultivars studied in 2012-2013 are presented in the Table 3, Table 4.

Table 3. Large diameter of the fruits studied in the year 2012

Cultivar	Large diameter (average)	Relative value %	Difference to the control	Significance
Marianna	46.0	108.66	3.67	-
Sun Hui Hun	42.33	100	0	-
Yinquing	51.33	121,26	9.0	XX
Piros Magdalena	45.0	106.30	2.67	-
Gold Dust	40.0	94.49	-2.33	-
Eureka	46.67	110.24	4.33	-
July Elberta	43.67	103.15	1.33	-
Giala di Roma Tardiva	49.17	116.14	6.83	X
Marqueen	39.33	92.91	-3.0	-
Spring Gold	42.33	100	0	control

DL5% = 5.25   DL1% = 7.09   DL0,1% = 9.44

Table 4. Large diameter of the fruits studied in the year 2013

Cultivar	Large diameter (average)	Relative value %	Difference to the control	Significance
Marianna	39.77	92.74	-3.11	-
Sun Hui Hun	42.67	99.49	-0.22	-
Yinquing	52.39	122.15	9.5	X
Piros Magdalena	46.0	107.26	3.11	-
Gold Dust	40.33	94.05	-2.55	-
Eureka	47.07	109.75	4.18	-
July Elberta	44.44	103.62	1.55	-
Giala di Roma Tardiva	50.0	116.59	7.11	-
Marqueen	40.0	93.27	-2.89	-
Spring Gold	42.89	100	0	control

DL5% = 8.16   DL1% = 11.03   DL0,1% = 14.69

Table 5. Small diameter of the fruits studied in the year 2012

Cultivar	Small diameter (average)	Relative value %	Difference to the control	Significance
Mariana	43.33	107.44	3.0	-
Sun Hui Hun	40.67	100.83	0.33	-
Yinquing	50.33	124.79	10.0	XXX
Piros Magdalena	42.67	105.79	2.33	-
Gold Dust	38.33	95.04	-2.0	-
Eureka	45.67	113.22	5.33	X
July Elberta	42.0	104.13	1.67	-
Giala di Roma Tardiva	45.0	111.57	4.67	-
Marqueen	37.0	91.79	-3.33	-
Spring Gold	40.33	100	0	control

DL5% = 5.09   DL1% = 6.87   DL0,1% = 9.16

In the year 2012, the biggest value of the large diameter of the fruit was registered on the 'Yinquing' cultivar (51.33 mm) this being distinctly significant positive to the experiment control. A value superior to the control was registered by the fruits of 'Giala di Roma Tardiva', this one being significantly positive to the experiment control. With the exception of the 'Gold Dust' cultivar, all the others cultivars had fruits large diameters superior to the control but close in value. In the experimental year 2013, the only cultivar statistically insured regarding the large diameter of the fruit was 'Yinquing', difference to the control being significant positive. Three cultivars, respectively 'Marqueen', 'Gold Dust' and 'Sun Hun Hui' had registered values inferior to the control, but they were not statistically insured.

The experiment results regarding the small diameter of the fruits on the peach cultivars studied in 2012-2013 are presented in the Table 5 and Table 6. In the year 2012 the highest value of the small diameter of the fruit was registered on 'Yinquing', difference to the control being very significant positive. A superior value, statistically insured, was registered as well on the 'Eureka' fruits, this one being significantly positive versus the studied parameter. Values inferior to the control were registered on the fruits of the 'Marqueen' and 'Gold Dust' cultivars, but they were not statistically insured.

Table 6. Small diameter of the fruits studied in the year 2013

Cultivar	Small diameter (average)	Relative value %	Difference to the control	Significance
Marianna	44.0	104.22	1,78	-
Sun Hui Hun	41.67	98.70	-0.55	-
Yinquing	50.72	120.14	8.50	XX
Piros Magdalena	43.0	101.86	0.78	-
Gold Dust	39.0	92.38	-3.22	-
Eureka	46.0	108.96	3.78	-
July Elberta	42.5	100.66	0.28	-
Giala di Roma Tardiva	45.67	108.17	3.45	-
Marqueen	37.67	89.22	-4.55	-
Spring Gold	42.22	100	0	control

DL5% = 5.42 DL1% = 7.32 DL0,1% = 9.75

Table 7. Height of the fruits studied in the year 2012

Cultivar	Height of the fruits (average)	Relative value %	Difference to the control	Significance
Marianna	45.0	100.75	0.33	-
Sun Hui Hun	47.0	105.22	2.33	-
Yinquing	57.33	128.36	12.67	XXX
Piros Magdalena	45.33	101.49	0.67	-
Gold Dust	40.67	91.04	-4.0	-
Eureka	45.67	102.24	1.0	-
July Elberta	42.67	95.52	-2.0	-
Giala di Roma Tardiva	47.0	105.22	2.33	-
Marqueen	39.33	88.06	-5.33	0
Spring Gold	44.67	100	0	control

DL5% = 4.07 DL1% = 5.47 DL0,1% = 7.32

Table 8. Height of the fruits studied in the year 2013

Cultivar	Height of the fruits (average)	Relative value %	Difference to the control	Significance
Marianna	45.78	100.74	0.34	-
Sun Hui Hun	48.0	105.63	2.56	-
Yinquing	58,0	127.67	12.56	XXX
Piros Magdalena	46.33	101.97	0.89	-
Gold Dust	41.67	91.70	-3.77	-
Eureka	46.0	101.23	0.56	-
July Elberta	43.39	95.48	-2.05	-
Giala di Roma Tardiva	47.5	104.53	2.06	-
Marqueen	41.33	90.96	-4.11	0
Spring Gold	45.44	100	0	control

DL5% = 4.03 DL1% = 5.44 DL0,1% = 7.25

In the year 2013 the only statistically insured cultivar was 'Yinquing', the difference to the experiment control being distinctly significant positive. Values inferior to the control were registered as well by 'Marqueen' and 'Gold Dust' cultivars. The experiment results regarding the fruits height on the peach cultivars studied in 2012-2013 are presented in the Table 7 and Table 8.

In the experiment year 2012, the highest value of the fruits height was registered on the 'Yinquing' cultivar, difference to the control being very significant positive.

The lowest value of the fruits height was registered on 'Marqueen' cultivar, difference to the experiment control being significantly negative. All other cultivars had close values, not being statistically insured.
Also in the year 2013 the highest value of the fruit height was registered also on the 'Yinquing' cultivar, being very significant positive to the control experiment. The fruits of the 'Marqueen' cultivar had registered the lowest value of the fruit height, difference to the experiment control being significant negative. Values superior to the control were registered to 'Giala di Roma Tardiva', 'Piros Magdalena', 'Sun Hun Hui' and 'Marianna' cultivars but no one was statistically insured.
The experiment results regarding the kernel percentage on the peach cultivars studied in 2012-2013 are presented in the Table 9 and Table 10.

Table 9. Kernel percentage of the fruits studied in the year 2012

Cultivar	Kernel % (average)	Relative value %	Difference to the control	Significance
Marianna	13.76	125.44	2.79	XX
Sun Hui Hun	16.47	150.18	5.50	XXX
Yinquing	8.46	77.17	-2.50	00
Piros Magdalena	8.01	73.04	-2.96	00
Gold Dust	7.79	71.06	-3.17	000
Eureka	7.49	68.30	-3.48	000
July Elberta	8.37	76.35	-2.59	00
Giala di Roma Tardiva	6.29	57.33	-4.68	000
Marqueen	7.57	69.03	-3.40	000
Spring Gold	10.97	100	0	control

DL5% = 1.68 DL1% = 2.26 DL0,1% = 3.02

In the experiment year 2012 the highest kernel % was registered on 'Sun Hun Hui' cultivar (16.47%), difference to the control being very significant positive and on 'Mariana' cultivar (13.76%) difference to the control being very significant positive. On the opposite, the smallest kernel % was registered on 'Giala di Roma Tardiva' (6.29%), 'Eureka' (7.49%), 'Marqueen' (7.57%) and 'Gold Dust' (7.79%) cultivars, all being very significant negative to the experiment control.

Table 10. Kernel percentage of the fruits studied in the year 2013

Cultivar	Kernel % (average)	Relative value %	Difference to the control	Significance
Marianna	13.31	126.14	2.88	X
Sun Hui Hun	16.11	146.04	5.08	XXX
Yinquing	8.23	74.58	-2.80	0
Piros Magdalena	9.80	88.88	-1.23	-
Gold Dust	7.79	70.60	-3.24	00
Eureka	7.39	67.0	-3.64	00
July Elberta	8.32	75.46	-2.71	0
Giala di Roma Tardiva	6.45	58.45	-4.58	000
Marqueen	7.43	67.36	-3.60	00
Spring Gold	11.03	100	0	control

DL5% = 2.17 DL1% = 2.93 DL0,1% = 3.90

In the year 2013 the highest kernel % was registered on the same cultivars, respectively 'Sun Hun Hui' and 'Marianna'. The lowest % was registered on 'Giala di Roma Tardiva' cultivar which as well in the year 2013 obtained differences very significant negative to the experiment control. 'Eureka', 'Marqueen' and 'Gold Dust' cultivars also had low kernel %, difference to the experiment control being distinctly significant negative.

CONCLUSION

Regarding fruits weight in the year 2013, the values were superior to the year 2012, the climatic conditions from Timisoara being favorable to a good growth and development of peach and acumulated with a weaker attack of Taprina sp.

In both experiment years was evidenced the asian cultivar 'Yinquing' whose fruits were close to 100 g.

Good results were obtained on the 'Giala di Roma Tardiva' and 'Piros Magdalena' cultivars, which got big and constant values in both experimental years.

Also regarding the fruits size represented by the large diameter, small diameters and height were evidenced 'Yinquing', 'Giala di Roma Tardiva' and 'Eureka' cultivars which registered big and constant values in both years.

The lowest values of the studied parameters in both experimental years and in pedoclimatic conditions of Timisoara were registerd on 'Marqueen' and 'Eureka' cultivars.

The fruits with lowest kernel percentage in both experimental years were 'Giala di Roma Tardiva', 'Eureka', 'Marqueen' and 'Gold Dust'.

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THE BEHAVIOUR OF SOME HAZELNUT VARIETIES IN THE SOUTH AREA OF TIMIS COUNTY IN TERMS OF EXTERNAL FEATURES OF THE FRUITS

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Abstract

In our country the hazelnut is spread in the hill areas especially in Vâlcea, Gorj, Maramureș, Hunedoara counties. In cultures may be found only in some fruit growing resorts like Vâlcea, Fălticeni, Târgu Jiu. The Romanian hazelnut production it is generated mostly from the spontaneous flora where in can be detected valuable biotypes, but also from the newly established cultures with imported varieties. Nevertheless, the Banat area is disposing by a large germplasm on this important tree species, germplasm which deserve to be studied in order to identify some precious genotypes which may be isolated and multiplicated, thus contributing to improvement of Corylus avellana variety, which unfortunately in Romania is rather limited. The present work which is a part of a larger study on local hazelnut germplasm, is following some aspects of external features of the fruits: weight of the fruit, weight of the kernel, % kernel, large diameter of the fruit, small diameter of the fruit, fruit height. The studied biotypes were found in the peoples gardens from Ciacova, Ghilad, Jebel and Pădureni. In terms of the fruit weight were evidenced Jebel and Ciacova biotypes with 1.83 g respectively 1.73 g versus the experiment average with a value of 1.66 g. However, the kernel percentage on the above biotypes was smaller than the experiment average in both cases, on this parameter were distinguished the other two biotypes respectively Ghilad and Pădureni on which the percent kernel has exceeded 50%, a fact to be considered.

Key words: *hazelnut varieties, diameters and height of fruit, weight, per cent kernel*

INTRODUCTION

Hazelnut it is a quite important tree species for its fruits quality, decorative value of some of the species, stabilize and consolidate the lands, minimizing erosion.
Hazelnuts are rich in nutritional substances and are used as such, or in sweets and pharmaceutical industry. Hazelnut oil it is appreciated in painting, varnishing and cosmetics industry (Iordănescu, 2011).
Due to their high nutrition value and rich contribution in vitamins B (B_1, B_2, B_3, B_5), nicotinamide and especially vitamin E (tocopherol), hazelnuts are used in human food as fresh fruits or processed in a multitude of products (cakes, ice-creams, salads, candies, chocolate) (Cociu, 2003).
Hazelnut tree may be found in regions with wet oceanic climate, in bright oak forests, forest margin, or in bushes on the farm roadsides. In our country the hazelnut is spread in the hill areas especially in Vâlcea, Gorj, Maramureș, Hunedoara counties.
Unfortunately is slightly cultivated in Romania except some fruit growing resorts like Vâlcea, Fălticeni, Târgu Jiu, although its quality and economic importance determined it to be cultivated on large surfaces in a lot of countries.
In recent years in the hilly area of Banat were established hazelnut plantations by foreign investors, especially Italians, who have opted for planting Italian varieties, less known in our country.

MATERIAL AND METHOD

Hazelnut biotypes from which samples were collected, were found in the back gardens of people from Ciacova, Ghilad, Jebel and Pădureni, localities situated in the south of

Banat. Each biotype studied was named after the village of origin.

Average samples consisting of 25 fruits were conducted, on which were studied the following aspects: weight of the fruit, weight of the kernel, % kernel, large diameter of the fruit, small diameter of the fruit, fruit height.

Fruits were analyzed in the Fruit Growing Department laboratory of USAMVBT as follows:

- Initially the fruits were weighed with high accuracy balance, calculating the average weight for each biotype followed by husking and kernel weighing and subsequently kernel% calculation
- Large diameter of the fruit, small diameter of the fruit, fruit height were determined using electronic calipers, calculating average values for each biotype.

All the data were statistically processed using variance analysis, as the experiment control being used the varieties average.

RESULTS AND DISCUSSIONS

The obtained results regarding the external features of the hazelnut fruits on the studied biotypes are presented in Table 1, Table 2 and Table 3.

Concerning the large diameter of the fruits, the highest value was registered on Ciacova biotype (1.53cm), the difference to the experiment control being very significant positive. Another value superior to the control was registered on Pădureni biotype, but without registered significations due to the fairy close values.

Values under the experiment control were registered on Jebel and Ghilad biotypes (1.41 respectively 1.42 cm), both being significant negative to the control (Table 1).

Table 1. External features of the fruits on the studied biotypes (large diameter of the fruits)

Variety	Large diameter (cm)	Relative value %	Difference to the control	Significance
Variety average	1.45	100	0	Control
Ciacova	1.53	105.52	0.08	XXX
Ghilad	1.42	97.93	-0.03	0
Jebel	1.41	97.24	-0.04	0
Pădureni	1.47	101.15	0.02	-

DL5% = 0.04 cm DL1% = 0.06 cm DL0,1% = 0.08 cm

Regarding the small diameter of the fruits, highest value was registered on the Ghilad biotype (1.50 cm), followed by Jebel biotype (1.48 cm), both being very positive to the control. The lower value of the small diameter was registered on Pădureni biotype (1.31cm), difference to the control being very significant negative (Table 2).

Table 2. External features of the fruits on the studied biotypes (small diameter of the fruits)

Variety	Small diameter (cm)	Relative value %	Difference to the control	Significance
Variety average	1.42	100	0	Control
Ciacova	1.42	100	0	-
Ghilad	1.50	105.63	0.08	XXX
Jebel	1.48	104.23	0.06	XXX
Pădureni	1.31	9..25	-0.11	000

DL5% = 0.03 cm DL1% = 0.04 cm DL0,1% = 0.05 cm

The highest value of the fruits height was registered on Pădureni biotype (2.2 cm), difference to the control being distinctly significant positive. The other biotypes registered values below the control and they were not statistically insured (Table 3).

Table 3. External features of the fruits on the studied biotypes (height of the fruits)

Variety	Fruit height (cm)	Relative value %	Difference to the control	Significance
Variety average	1.89	100	0	Control
Ciacova	1.77	93.65	-0.12	-
Ghilad	1.80	95.24	-0.09	-
Jebel	1.80	95.24	-0.09	-
Pădureni	2.2	117.46	0.33	XX

DL5% = 0.20 cm DL1% = 0.27 cm DL0,1% = 0.36 cm

Average weight of the fruit, % kernel and weight of the kernel on the studied biotypes are presented in the Table 4, Table 5 and Table 6.
The highest value of fruit weight was registered on Jebel biotype (1.83 g), difference to the experiment control being very significant positive. Another value superior to the control was registered by the Ciacova biotype fruits, difference being distinctly significant positive. The smallest values of the fruits weight were registered on Pădureni biotype (1.55 g) and Ghilad (1.56 g) both being distinctly significant negative to the experiment control (Table 4).

Table 4. Average weight of the fruits for the studied biotypes

Variety	Average weight (g)	Relative value %	Difference to the control	Significance
Variety average	1.66	100	0	Control
Ciacova	1.73	104.22	0.07	XX
Ghilad	1.56	93.98	-0.10	000
Jebel	1.83	11.24	0.17	XXX
Pădureni	1.55	93.17	-0.11	000

DL5% = 0.05 g DL1% = 0.07 g DL0,1% = 0.09 g

The highest kernel percentage was registered on Ghilad biotype (61.17%), difference to the experiment control being very significant positive. Value above 60% kernel was registered also on Pădureni biotype, difference to the experiment control being distinctly significant positive. The lowest kernel percentage was registered on Jebel biotype (43.53%), difference to the experiment control being very significant negative (Table 5, 6).

Table 5. Kernel percentage of the fruits for the studied biotypes

Variety	Kernel percentage %	Relative value %	Difference to the control	Significance
Variety average	54.4	100	0	Control
Ciacova	51.70	95.04	-2.70	-
Ghilad	61.17	112.44	6.77	XXX
Jebel	43.53	80.02	-10.87	000
Pădureni	60.47	111.15	6.07	XX

DL5% = 3.55% DL1% = 4.80% DL0,1% = 6.39%

Table 6. Kernel weight of the fruits for the studied biotypes

Variety	Kernel weight (g)	Relative value %	Difference to the control	Significance
Variety average	0.90	100	0	Control
Ciacova	0.90	100	0	-
Ghilad	0.96	106.67	0.06	-
Jebel	0.80	88.89	-0.10	00
Pădureni	0.95	105.56	0.05	-

DL5% = 0.07 g DL1% = 0.09 g DL0,1% = 0.12 g

CONCLUSION

In terms of the fruit weight were evidenced Jebel and Ciacova biotypes with 1.83 g respectively 1.73 g versus the experiment average with a value of 1.66 g.

However, the kernel percentage on the above biotypes was smaller than the experiment average in both cases, on this parameter were distinguished the other two biotypes respectively Ghilad and Pădureni on which the % kernel has exceeded 50%, a fact to be considered.

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STUDY ON STARCH IODINE TEST FOR DETERMINING MATURATION STAGE AT SEVERAL APPLE CULTIVARS IN CORRELATION WITH CLIMATIC FACTORS AT SCDP BISTRITA USING A ONE YEAR MODEL BY MEANS OF IMAGE ANALYSIS IN IMAGEJ

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Abstract

The objective of the present study was to evaluate the possibility to determine the starch index based on the starch iodine test and subsequent image analysis, subsequently to study the changes of the main fruit quality parameters (starch index, soluble sugars, weight, diameter) and the description of the ripening process according to climatic factors. Elaboration of a research model regarding maturation stage and ripening process differentiation among cultivar groups (autumn and winter cultivars) is critical in fruit growing. Results showed that at autumn cultivars 'Auriu', 'Starkprim', 'Ionaprim', 'Auriu de Bistrita') in 2013 starch hydrolysis begun slowly in early September (04.09.2013) presenting incipiently lower level values between 2.1-2.8 starch index and after a month these values increased to 6.2-6.6 due to the increase of mean average temperatures along with genetic factors. At the winter cultivars ('Golden delicious', 'Ionathan', 'Salva', 'Starkrimson') at harvest time it has been observed a small hydrolysis, the values being situated between 1.1-1.2 and after 30 days the values increased slowly to 2.1-2.8, the hydrolysis process being thus much slower when compared with autumn cultivars. The soluble sugar content varied between 12.6-18.4 °Brix at autumn cultivars, the maximum being registered at 'Ionaprim' cultivar (18.4 °Brix), followed by 'Aura' (16.3 °Brix), data registered at harvest day. At 30 days after the harvest day (04.09.13) the soluble sugar content varied between 13.8-18.6 °Brix with a small increasement. At winter cultivars it has been observed a lower degree of starch hydrolysis and implicitly a reduced content of soluble sugars situated between 14.2-15.2 °Brix at harvesting time. At 30 days after harvest day the soluble sugar content varied between 14.6-16.3 °Brix. The ripening processes is primary genetically determined but it is influenced also by the environmental factors, thus an appropriate modeling is a key step in order to interpolate the maturation stage with the sum of active temperatures.

Key words: *starch, iodine solution, apple, image analysis, climatic factors, soluble solids*

INTRODUCTION

The starch content from apple fruits accumulate during the vegetation period and it is hydrolyzed in simple sugars in the last period of maturity and development. The starch hydrolysis takes place in the center of fruits and progresses toward to exterior (Philipps and Poapst et al., 1952) or based on recent researches this process has 4 different typology (A type - from the core to the exterior, B - in star shape, C type - little patches, D type – in concentric forms, Szalay et al., 2013).

In order to assess the maturity stage of fruits, the starch from the cut slices of fruit samples when put into reaction with a iodine solution it produces a dark blue-black staining.

Based on the distribution, development and the intensity of coloration Davis and Blair (1936) have elaborated a test guide for the evaluation of starch content for the 'McIntosh' cultivar. The iodine solution test was used in other investigations also (Hesse and Hitz 1938; De Haas and Wennemuth, 1964).

Some researchers (Philipps and Poapst et al. 1952) have affirmed that this test should be done in the early morning because starch content may vary during the day, and probable this test it is not suited for late ripening cultivars because at the harvest it is recorded just a small grade of hydrolysis of the starch. Smock (1948) concluded that the distribution of the starch is irregular in the fruit flesh and Blanpied (1960) affirmed that the starch content and the conversion into sugar is influenced by the climate, yield per tree and the cultural techniques.

Despite the fact that there are a series of contradictions in these research conclusions regarding this test, it is widely used in the fruit growing due to its simplicity in the practice, it is a rapid test and is the most important parameter in the monitoring process of maturation (Szalay et al., 2013).

Later these starch staining tests were completed for other cultivars also like Red Delicious, Northern Spy (Smith, 1974) and nowadays it is used also a German system (ART System-Apple Ripening Test). The value of the starch index is situated between 1 and 10 in the German system and 1 to 8 in the American system. Note 1 represents the hydrolysis of the lower grade and note 10 represent the most intense hydrolysis.

During the maturation process, the starch is converted to simple sugars. In the starch test the starch granules from the fruits are binding with iodine and it is formed the dark blue-black staining. If the dark blue-black staining is more intense means that the hydrolysis is in its incipient phase and if the dark blue coloring is more shifted to light blue-white-transparent state than maturation process is in its developed phase (starch converted to simple sugar molecules). In the southern zone of the USA it is used the starch index elaborated by Blanpied and Silsby (1960). In our studies we compared our results with the German system of estimation of starch hydrolysis due to the fact that this system allows the percent evaluation of the maturation grade, thus results being more accurate.

MATERIALS AND METHODS

Starch index quantification

In 04.09.2013 there have been sampled 10 fruits/variant at the 8 studied cultivars (4 autumn and 4 winter cultivars) grafted on rootstock M26 ('Aura', 'Starkprim', 'Ionaprim', 'Auriu de Bistrita', 'Golden delicious', 'Ionathan', 'Salva', 'Starkrimson', Fig. 1) and these were cut transversally in order to perform the staining procedure with a iodine solution (iodine in iodine potassium medicinal solution). The cut apple surfaces were impregnated with the iodine solution and placed on a filter paper for 10 minutes. Subsequently there have been effectuated photos with the transverse colored apples and stored on the PC. Colored images were transformed to binary images (black and white – black coloration meaning the starch area not hydrolyzed from the fruit, white meaning unstained fruit flesh area) using Image J software. Quantification of the starch percent not hydrolyzed (black) meaning the fruit surface occupied by the not hydrolyzed part or contrary the amount of white area was visually compared with the ART System chart in order to determine a proper starch index note.

Soluble sugars determination

In order to perform the dry matter determination it has been used a manual prism refractometer which measures the soluble sugar content in Brix grade.

RESULTS AND DISCUSSIONS

At the late ripening winter cultivars (Fig. 1) sampled in 04.09.2013 at harvest day, the starch index was of the lesser grade among the studied variants situated between 1.0-1.2, these cultivars having the highest starch content (Table 1). In Fig. 2 it is presented the variation of the starch index at four autumn apple cultivars with early maturation (‘Aura’, ‘Starkprim’, ‘Ionaprim’, ‘Auriu de Bistrita’), respectively 4 late ripening winter apple cultivars (‘Golden delicious’, ‘Ionathan’, ‘Salva’, ‘Starkrimson’) at the early phase of maturation (04.09.2013) and after 30 days after the first day of harvesting. In the case of the autumn cultivars at the harvest date 04.09.2013 the starch index was relatively reduced, but the hydrolysis process was much more advanced in comparison with the winter cultivars. The value of the starch index in the case of the winter cultivars varied between 2.1-2.8 (Table 1).

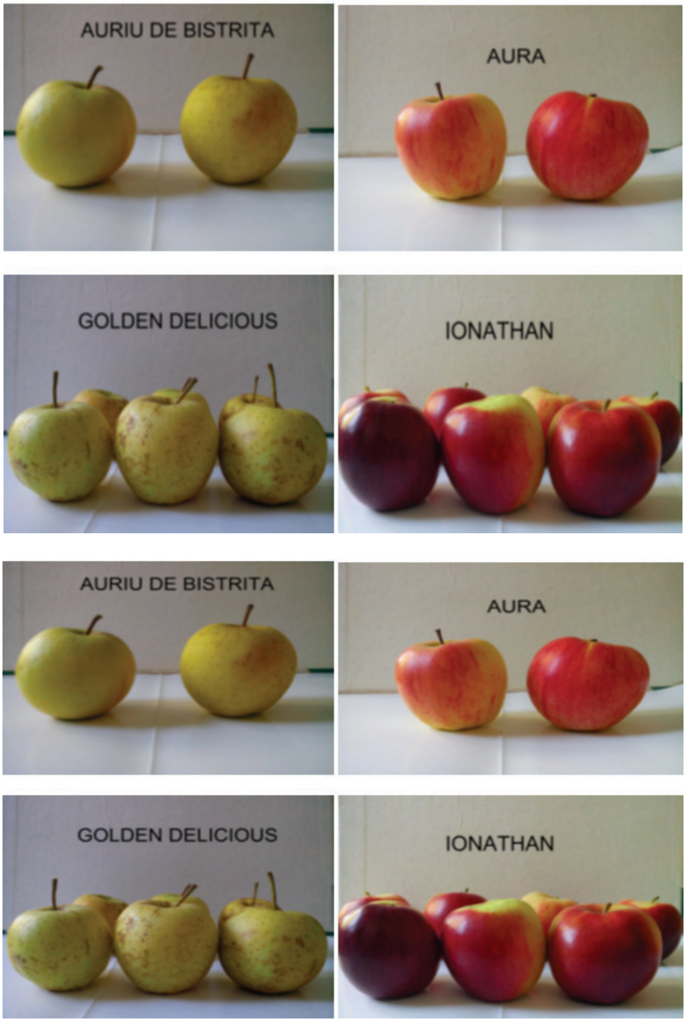


Figure 1. Autumn cultivars and winter cultivars studied in the experiment
(Photo: Jakab-Ilyefalvi Zsolt, 2013)

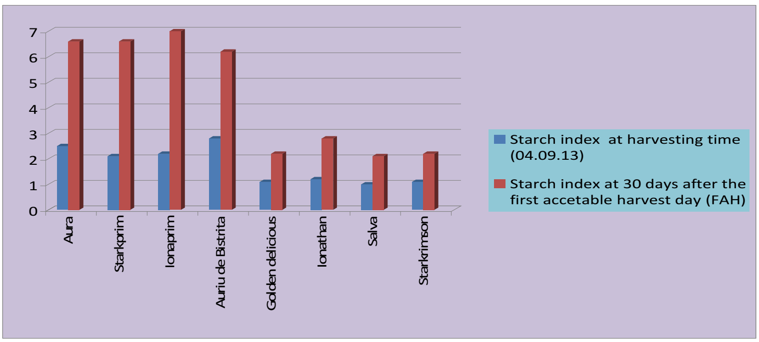


Figure 2. Variation of starch index of 4 autumn cultivars and 4 winter cultivars at the harvesting time and after 30 days of storage in classical storage house

Table 1. Pomologic characteristics and total soluble sugars of the studied apple cultivars at 30 days after harvest - SCDP Bistrita 2013

Cultivar	Mean weight (g)	Diameter (mm)	Total soluble solids (°Brix)	Starch Index
Aura	162	82	16,3	6,6
Starkprim	155	81	12,6	6,6
Ionaprim	110	73	18,4	7
Auriu de Bistrita	170	90	15,7	6.2
Golden delicious	125	86	14,2	2,2
Ionathan	110	76	15,2	2,8
Salva	130	82	14,5	2,1
Starcrimson	92	68	14,2	2,2
Treatment Average	131,8	79,8	15,1	4,5
Standard deviation	28,0	7,1	1,7	2,3

At 30 days after the first harvest date (04.09.13) fruits were again sampled from the same trees. In the case of the autumn cultivars ('Aura', 'Starkprim', 'Ionaprim', 'Auriu de Bistrita') it has been observed a more progressed maturation process and thus a much more advanced hydrolysis, the starch index was higher being situated between 6.2-7.0 (Fig. 3). At the winter cultivars ('Golden delicious', 'Ionathan', 'Salva', 'Starkrimson') it has been observed a smaller hydrolysis, the values being situated between 2.1-2.8.

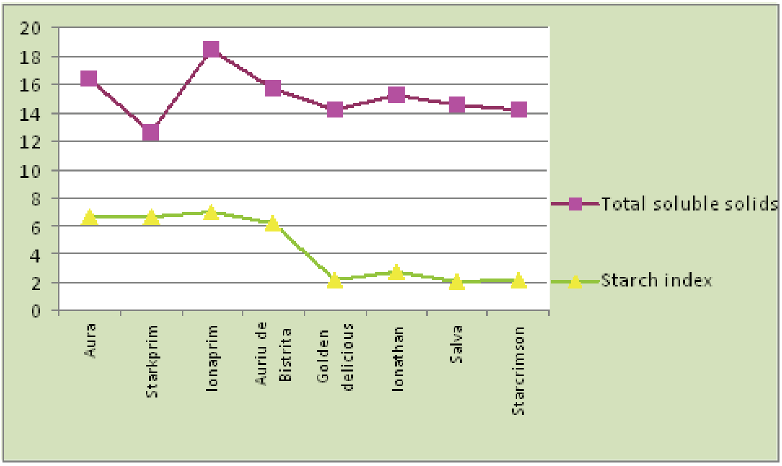
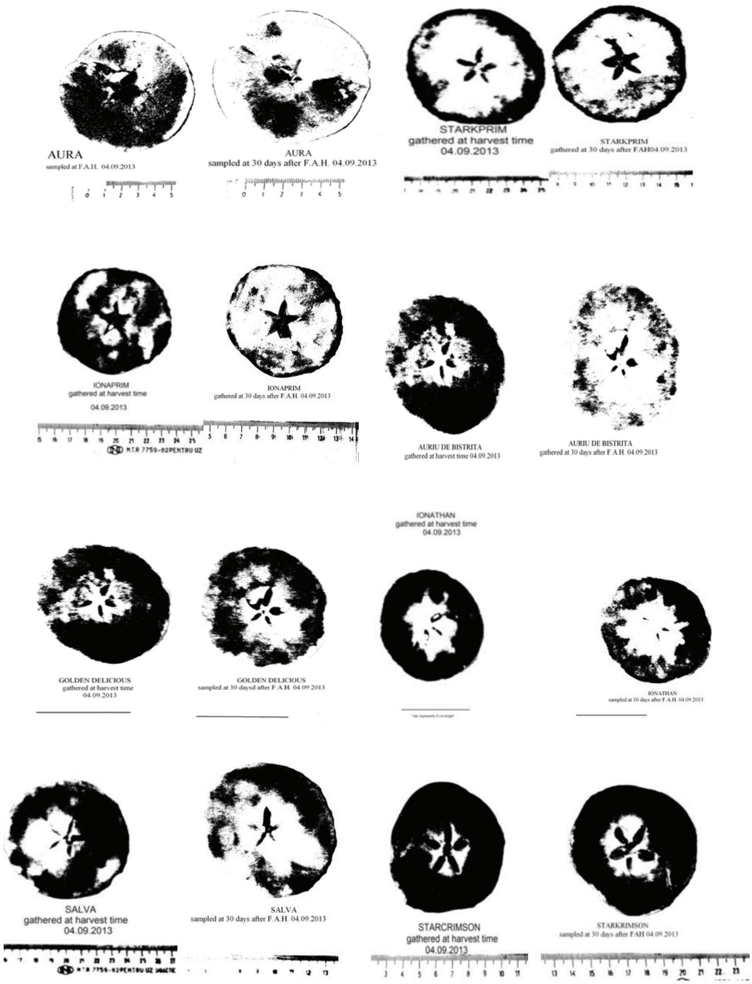


Figure 3. Total soluble sugars, Starch index variation of the studied apple cultivars at 30 days after harvest - SCDP Bistrita 2013

The data from Table 2 shows that there is a correlation between the area percent of the hydrolyzed starch (starch colored in dark blue-black) and the USA and the German noting system. The German system seems to be more sensible with fine starch changes, showing more subtle modifications of starch hydrolysis, although there are little differences between the two noting systems.

Table 2. Variation of starch index at 8 cultivars (image analysis by Image J), by means of USDA Cornell University starch index (1-8 index) and Art System (1-10)

Cultivar	Area of the fruit surface (cm^2) ImageJ	% area of hydrolised starch fruit surface ImageJ	% area of iodine colored fruit surface ImageJ	USDA Cornell starch index scale 1-8	Apple Ripeness Test Art System 1-10
Aura	55,06	82,10	17,90	6.8	6.6
Starkrpim	57,50	72,87	27,13	6.0	6.6
Ionaprim	46,72	78,12	21,88	6.7	7
Auriu de Bistrita	55,06	82,10	17,90	6.8	6.2
Golden delicious	41,05	42,05	57,95	2	2.2
Ionathan	55,11	36,47	63,53	3	2.8
Salva	55,95	43,49	56,51	3	2.1
Starkrimson	41,35	22,82	77,18	2	2.2



Digital image: Image J, Jakab-Ilyefalvi Zsolt, 2013

Figure 4. Starch index determination of apple cultivars by means of image analysis by Image J

There have been studied also the variation of the soluble sugar content of the 8 cultivars at the harvest day 04.09.2013 and after 30 days after the first acceptable harvest day (FAH) (Fig.4). In the case of the autumn cultivars harvested in 04.09.2013 there have been registered a soluble sugar content between 12.6-18.4 Brix, the maximum being registered at 'Ionaprim' cultivar (18.4 Brix), followed by 'Aura' (16.3 Brix) (Table 1). At 30 days after FAH the starch hydrolyzed stepwise, the soluble sugar content varied between 13.8-18.6 Brix. At winter cultivars it has been observed a lower grade of starch hydrolysis and implicitly a reduced content of soluble sugars situated between 14.2-15.2 Brix at harvesting time. At 30 days after FAH the soluble sugar content varied between 14.6-16.3 Brix.

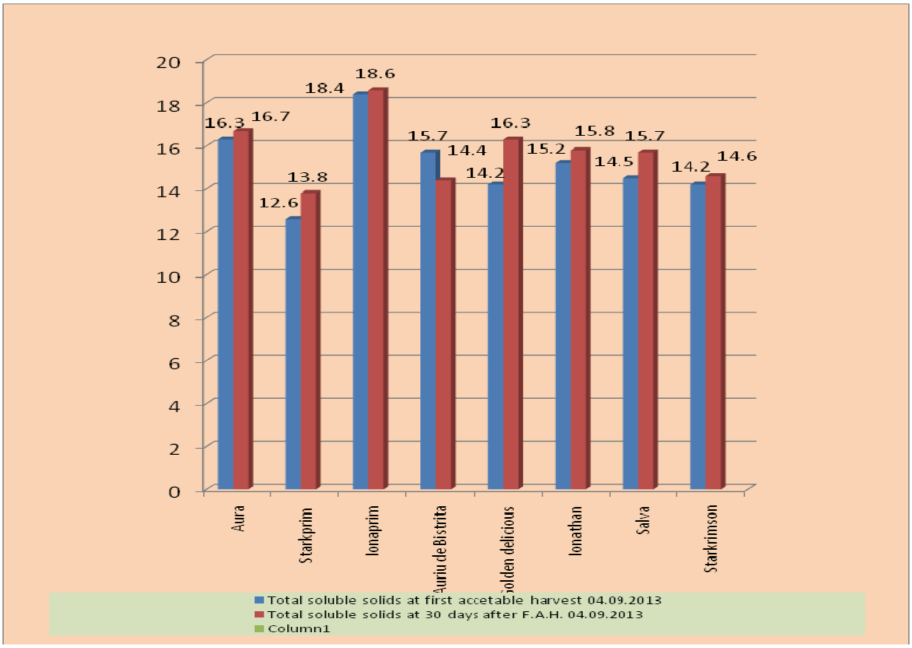


Figure 5. Variation of total soluble solids (Brix) at the harvesting time 04.09.2013 and after 30 days of storage in classical storage house

Studying the pomologic characteristics (Table 1) average weight, diameter it has been observed a higher dimension of fruits at autumn cultivars (73-90 mm) when compared with the winter cultivars (68-86 mm) respectively the weight parameter shows the same tendency (155-170 g).

As first observation we can conclude that the petal fall phenophase did not occurred differentiated as specific for every cultivar probable due to more special climatic conditions of the past years (climatic changes) oppositely almost it occurred in the same time frame during two-three days having as central petal fall day 05.05.2013 with a total sum of active temperatures of 326^0C [(Tmax+Tmin)/2-Tbase (5^0C)]. It is clearly evident that it is taking place a fine tuned shifting of the phenophases, at the end of flowering. At the first day of harvest 04.09.2013 the sum of active growing degree days (active temperatures, Table 3) was 1774^0C and at the second harvest session in 01.10.2013 and at circa a month after the first acceptable harvest day this sum totalized 2003^0C. At the first day of harvest 04.09.2013 the sum of active growing degree days (active temperatures, Table 3) was 1774^0C and at the second harvest session in 01.10.2013 and at circa a month after the first acceptable harvest day this sum totalized 2003^0C.

Table 3. Sum of active temperature degrees (GDD-growing degree days) between petal drop phase (05.05.2013) and first acceptable harvest date (FAH) 04.09.2013

Cultivar	Sum of active temperature degrees Petal drop 05.05.2013	Sum of active temperature degrees FAH 04.09.2013	Sum of active temperature degrees 01.10.2013	Starch index 04.09.2013	Starch index 01.10.2013
Aura	326	1774	2003	2.5	6.6
Starkprim	326	1774	2003	2.1	6.6
Ionaprim	326	1774	2003	2.2	7
Auriu de Bistrita	326	1774	2003	2.8	6.2
Golden delicious	326	1774	2003	1.1	2.2
Ionathan	326	1774	2003	1.2	2.8
Salva	326	1774	2003	1	2.1
Starkrimson	326	1774	2003	1.1	2.2

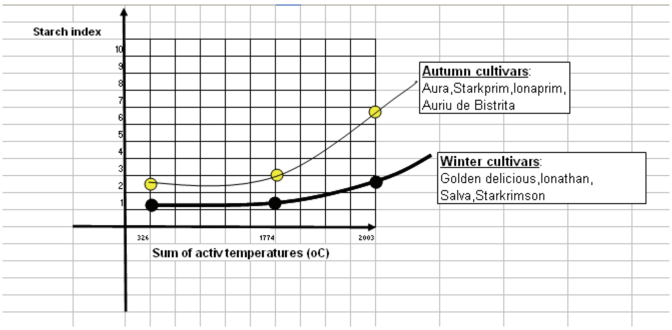


Figure 6. Variation of starch index maturity notes according to GDD

The chart from Figure 6 shows an ascendant trend of the hydrolysis of starch into soluble sugars (2.5-2.8 starch index) and after one month the starch index realized values between 6.2-6.6 the accelerated tendency of hydrolysis of the autumn cultivars being evident.

CONCLUSION

Research results distinguish clearly the fact that autumn cultivars (‘Aura’, ‘Starkprim’, ‘Ionaprim’, ‘Auriu de Bistrita’) must be gathered at optimal harvest date, when starch hydrolysis is in its incipient phase because fruits need to have firmness. The ripening processes is primary genetically determined but it is influenced also by the environmental factors, thus the using of the present results should be handled with precautions, when taking into account the optimal harvest because the study is a one year model, further multi-annual researches will be effectuated in order to evaluate the ripening stage of the apple cultivars in the northern zone of Bistrita hills region and the starch hydrolysis process in the harvest window.

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THE QUANTITY OF WOOD ELIMINATED THROUGH CUTTING ACCORDING TO THE CULTIVAR AND THE SHAPE OF THE HEAD AT CERTAIN NECTARINE TREE CULTIVARS FROM THE R.S.F.G CONSTANŢA

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Abstract
The purpose of this paper is to highlight the manner in which the annual growths influence the quantity of wood eliminated through cutting, taking into account the cultivar and the shape of the head as far as the nectarine tree is concerned. Due to the fact that the planting distances and density of the trees per hectare varies in accordance to the shape of the head it is highly important that we know the volume of work as well as the quantity of wood eliminated through cutting. The research took place at the RSFG Constanta over a period of 4 years and the studied nectarine cultivars were the following: Cora, Delta, Romamer 2 and Crimsongold; there were 4 shapes of the head and 4 planting distances: Tatura 6/2 m (833 trees/ha), Vertical cordon 4/1.5 m (1666 trees/ha), Veronese Vase 4/3 m (833 trees/ha) and Improved Vase 4/3.5 m (714 trees/ha). The study revealed the following: taking into account the shape of the head, the largest quantity of wood eliminated through cutting was recorded by the Vertical cordon in all the studied years (2008-2011); the quantity of eliminated wood is directly correlated with the Veronese Vase and the Improved Vase shapes in the sense that these two shapes are the most advantageous for the four cultivars, whereas the Vertical cordon requires extensive cutting works. The statistical analysis of the quantity of wood eliminated through cutting (t/ha) over the 4 studied years revealed the fact that the Cora, Delta, Romamer 2 and Crimsongold cultivars with the Vertical cordon shape of the head ensured a positive significance, while the other shapes ensured a negative significance from a statistical point of view.

Key words: *cutting, technological links, vigour, Prunus persica*

INTRODUCTION

Taking into account the year 2000, as far as crop systems were concerned, there were more and more discussions across Europe regarding the typology and productive efficiency of tree plantations as well as the realisation of an ideal tree model which intercepts and fully valorises the incident light, irrespective of the fact that the planting density would grow up to 20,000 trees/ha (Cepoiu, 2006). The same author states that this kinds of plantations names “full field” or “tuto campo” include cultivars with compact heads, short sprouts and thick leaves, rich in mesophyll and chlorophyll which ensure an increased productive efficiency as compared to standard cultivars from current intensive and super-intensive orchards. Romania’s pedo-climatic diversity offers favourable conditions to a wide variety of tree species, but the global climatic changes bring forward new criteria for the zoning of species and elements which are to be applied. In this context the choosing of the cultivar-parent stock combination, of the adequate shapes of the head, of the planting distances, of the technology of maintaining and fertilisation of the soil and tress and of the applied phyto-sanitary treatments must be a major preoccupation (Lespinasse et al, 1998). The extension of summer cuttings, the development of nectarines and of certain peach tree cultivars with highly pigmented fruit (Fideghelli et al, 1991) as well as other factors have determined a genuine race between specialists (both researchers and farmers) concerning the realisation of various shapes of the head which would correspond the environmental and socio-economic demands. The purpose of this paper is to highlight the manner in which the annual growths influence the quantity of wood eliminated through cutting, taking into account the cultivar and the shape of the head of certain nectarine tree cultivars and the number of branches remaining in every tree after cutting in the studied years 2008-2011.

MATERIAL AND METHOD

The research took place at the Research Station for Fruit-Growing Constanta (RSFG) in the period 2008-2011 and the biological material consisted of the Cora, Delta, Romamer 2 and Crimsongold cultivars. In the spring of 2002, when the trees were in full ripening period (year VI since planting) an experience was organised at the RSFG, experience based on two experimental factors: Factor A – the cultivar, with 4 categories: a1 = Cora, a2 = Delta, a3 = Romamer 2, a4 = Crimsongold and Factor B – the shape of the head and the planting distance considered together, with 4 categories: b1 = Tatura, 6/2m = 833 tress/ha, b2 = Vertical Cordon, 4/1.5m = 1666 trees/ha, b3 = Veronese Vase, 4/3m = 833 trees/ha, b4 = Improved Vase, 4/3.5m = 714 trees/ha. Given the fact that the region is semi-arid, the nectarine tree culture developed under an irrigated regime. The experience was performed on a calcareous chernozem (CZka), with a claylike texture, a low alkaline pH (8.2) in its entire profile. As far as the technology that has to be applied to the nectarine tree is concerned, there were no differences; it was applied in the same manner irrespective of the cultivar, the shape of the head or the planting distance.

The performed determinations focused on the quantity of wood eliminated through cutting which was calculated by means of weighing the wood for each variant and was expressed in kg/tree. An analysis of the variance of the vigour of the trees was carried out, expressed through the quantity of the wood eliminated through cutting in kg/tree and t/ha. Given the fact that the planting distances and the density of the trees per hectare vary according to the shape of the head, it is important to know the workload as well as the quantity of wood eliminated per hectare.

RESULTS AND DISCUSSIONS

According to the shape of the head, the largest quantity of wood eliminated through cutting was recorded by the Vertical Cordon in all studied years (2008-2011), followed by the Tatura, while the lowest values were recorded by the Vases. Each cultivar recorded significant variations according to the shape of the head. The Veronese Vase and the Improved Vase are advantageous shapes for all four cultivars, whereas the Vertical Cordon requires a large cutting volume in order to be maintained in the limits of the shape (Figure 1).

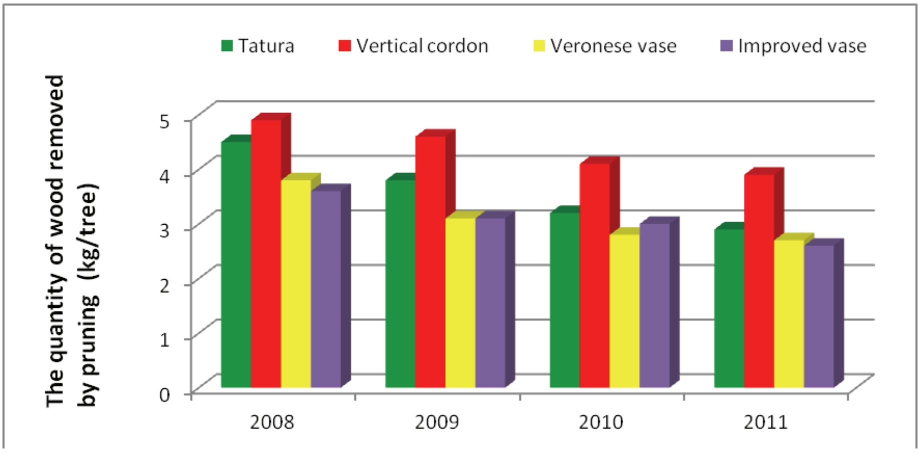

Figure 1. The quantity of wood removed by pruning according to the shape of crown the period 2008-2011

During the four studied years, the largest quantity of wood eliminated through cutting was recorded by the Cora cultivar, Vertical Cordon shape and the Crimsongold cultivar, Vertical Cordon shape (5.10 kg/tree), while the lowest value was recorded by the Romamer 2 cultivar, Improved Vase shape (3.20 kg/tree) (Figure 2).

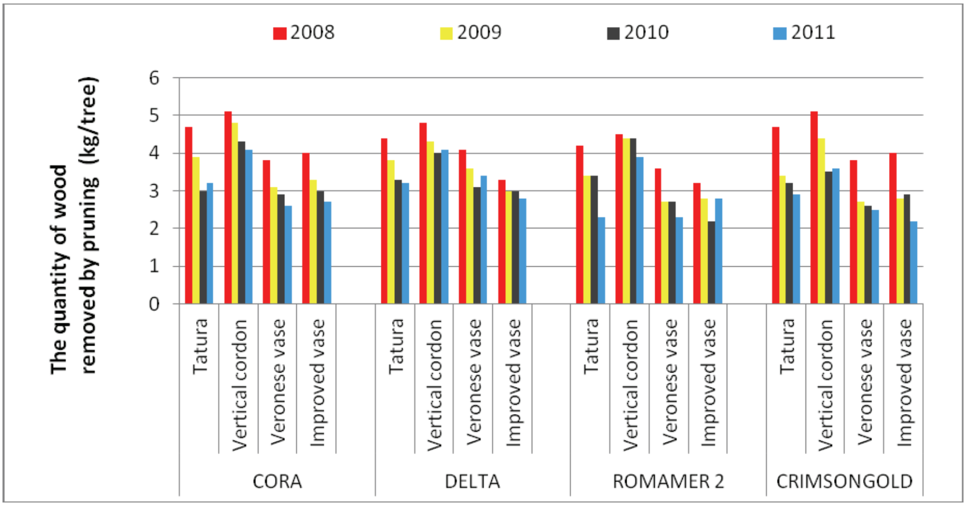

Figure 2. The quantity of wood eliminated through cutting according to the cultivar and the shape of the head, 2008-2011

The vigour of the trees expressed through the quantity of wood eliminated through cutting (kg/tree)

Following the variance analysis carried out regarding the vigour of the trees (expressed in the quantity of wood eliminated through cutting – kg/tree) during the four studied years, significantly positive differences were recorded by the Cora cultivar, Vertical Cordon shape (all four studied years, 2008-2011), the Crimsongold cultivar, Vertical Cordon (2008, 2009) and the Romamer 2 Vertical Cordon shape (2010, 2011) (Table 1). The Vertical Cordon recorded a significantly positive difference in comparison to the other shapes in the years 2009, 2010 and 2011, while in 2008 the Vertical Cordon shape was distinctly significantly positive.

In 2008 the Delta and Romamer 2 cultivars, Improved Vase shape of the head displayed significantly negative differences; the same happened in 2009 and 2010. The Romamer 2, Veronese Vase and Improved Vase were highly significantly negative, while in 2011 the Romamer 2, Tatura and Veronese Vase shapes and the Crimsongold cultivar, Improved Vase displayed distinctly significantly negative differences. The other cultivars and shapes of the head presented significantly negative differences.

The vigour of the trees expressed through the quantity of wood eliminated through cutting (t/ha)

Following the variance analysis carried out regarding the vigour of the trees expressed through the quantity of the wood eliminated through cutting (t/ha) during the four studies years, significantly positive differences were displayed by the Cora cultivar, Vertical Cordon shape and the Delta cultivar, Vertical Cordon shape. The Crimsongold cultivar, Vertical Cordon shape displayed significantly positive differences in the years 2008 and 2009, while the Romamer 2 cultivar, Vertical Cordon shape presented the same type of differences in the years 2009 and 2010 (Table 2). The other cultivars and shapes of the head displayed significantly negative differences. The Vertical Cordon displayed a significantly positive difference in comparison to the other shapes in the years 2009, 2010 and 2011.

Table 1. The vigour of the trees expressed through the quantity of wood eliminated through cutting (kg/tree) in the period 2008-2011

Variant	Shape of the head	2008			2009			2010			2011		
		kg/tree	Diff. comp. to the average	Significance	kg/tree	Diff. comp. to the average	Significance	kg/tree	Diff. comp. to the average	Significance	kg/tree	Diff. comp. to the average	Significance
CULTIVAR													
a1. CORA													
a1b1	b1.Tatura	4.7	+0.5	*	3.9	+0.3	-	3.0	-0.2	-	3.2	+0.2	-
a1b2	b2.Vertical Cordon	5.1	+0.9	***	4.8	+1.2	***	4.3	+1.1	***	4.1	+1.4	***
a1b3	b3.Veronese Vase	3.8	-0.4	o	3.1	-0.5	o	2.9	-0.3	-	2.6	-0.4	-
a1b4	b4.Improved Vase	4.0	-0.2	-	3.3	-0.3	-	3.0	-0.2	-	2.7	-0.3	-
	Average	**3.4**	**-0.8**	**ooo**	**3.8**	**+0.2**	**-**	**3.3**	**+0.1**	**-**	**3.2**	**+0.2**	**-**
a2. DELTA													
a1b1	b1.Tatura	4.4	+0.2	-	3.8	+0.2	-	3.3	+0.1	-	3.2	+0.2	-
a1b2	b2. Vertical Cordon	4.8	+0.6	**	4.3	+0.7	**	4.0	+0.8	***	4.1	+1.4	***
a1b3	b3. Veronese Vase	4.1	-0.1	-	3.6	0.0	-	3.1	-0.5	-	3.4	+0.4	-
a1b4	b4. Improved Vase	3.3	-0.9	ooo	3.0	-0.6	o	3.7	-1.0	*	2.8	-0.2	-
	Average	**4.2**	**0.0**	**-**	**3.7**	**+0.1**	**-**	**3.5**	**0.0**	**-**	**3.4**	**+0.4**	**-**
a3. ROMAMER 2													
a1b1	b1.Tatura	4.2	0.0	-	3.4	-0.2	-	3.4	0.0	-	2.3	-0.7	oo
a1b2	b2. Vertical Cordon	4.5	+0.3	-	4.4	+0.8	**	4.4	+0.3	***	3.9	+0.9	***
a1b3	b3. Veronese Vase	3.6	-0.6	oo	2.7	-0.9	ooo	2.7	-0.6	o	2.3	-0.7	oo
a1b4	b4. Improved Vase	3.2	-1.0	ooo	2.8	-0.8	oo	2.2	-0.3	ooo	2.8	-0.2	-
	Average	**3.9**	**-0.3**	**-**	**3.3**	**-0.3**	**-**	**3.2**	**-0.1**	**-**	**2.8**	**-0.2**	**-**
a4. CRIMSONGOLD-													
a1b1	b1.Tatura	4.7	+0.5	*	3.9	+0.3	-	3.2	0.0	-	2.9	-0.1	-
a1b2	b2. Vertical Cordon	5.1	+0.9	***	4.8	+1.2	***	3.5	+0.3	-	3.6	+0.6	*
a1b3	b3. Veronese Vase	3.8	-0.4	o	3.1	-0.5	o	2.6	-0.6	oo	2.5	-0.5	o
a1b4	b4. Improved Vase	4.0	-0.2	-	3.3	-0.3	-	2.9	-0.3	-	2.2	-0.8	oo
	Average	**4.4**	**+0.2**	**-**	**3.8**	**+0.2**	**-**	**3.1**	**-0.1**	**-**	**2.8**	**-0.2**	**-**
SHAPE OF THE HEAD													
b1	b1.Tatura	4.5	+0.3	-	3.8	+0.2	-	3.2	0.0	-	2.9	-0.1	-
b2	b2. Vertical Cordon	4.9	+0.7	**	4.6	+1.0	***	4.1	+0.9	***	3.9	+0.9	***
b3	b3. Veronese Vase	3.8	-0.4	o	3.1	-0.5	o	2.8	-0.4	o	2.7	-0.3	-
b4	b4. Improved Vase	3.6	-0.6	oo	3.1	-0.5	o	3.0	-0.2	-	2.6	-0.4	-
		X=4.2 DL 5% =0.4 DL1% =0.6 DL0.1% =0.8			**X=3.6 DL 5% =0.5 DL1% =0.7 DL0.1% =0.9**			**X=3.2 DL 5% =0.4 DL1% =0.6 DL0.1% =0.8**			**X=3.0 DL 5% =0.5 DL1% =0.7 DL0.1% =0.9**		

Table 2. The vigour of the trees expressed through the quantity of wood eliminated through cutting (t/ha) in the period 2008-2011

Variant	Shape of the head	2008			2009			2010			2011		
		t/ha	Diff. comp. to the average	Signif.	t/ha	Diff. comp. to the average	Signif.	t/ha	Diff. comp. to the average	Signif.	t/ha	Diff. comp. to the average	Signif.
A. CULTIVAR													
a1. CORA													
a1b1	b1.Tatura	3.91	-0.49	-	3.25	-0.63	-	2.50	-0.97	-	2.66	-0.60	-
a1b2	b2.Vertical Cordon	8.49	+4.09	***	7.99	+4.11	***	7.16	+3.69	***	6.83	-3.57	***
a1b3	b3.Veronese Vase	3.16	-1.24	-	2.58	-1.30	-	2.41	-1.06	-	2.16	-1.10	-
a1b4	b4.Improved Vase	2.85	-1.55	-	2.35	-1.53	-	2.14	-1.33	-	1.92	-1.34	-
	Average	**4.60**	**+0.20**	**-**	**4.04**	**+0.16**	**-**	**3.55**	**+0.08**	**-**	**3.39**	**+0.13**	**-**
a2. DELTA													
a1b1	b1.Tatura	3.66	-0.74	-	3.16	0.72	-	2.75	-0.72	-	2.66	-0.60	-
a1b2	b2. Vertical Cordon	7.99	+3.59	***	7.16	+3.28	***	6.66	+3.19	***	6.83	+3.57	***
a1b3	b3. Veronese Vase	3.41	-0.99	-	2.99	-0.89	-	2.58	-0.89	-	2.83	-0.43	-
a1b4	b4. Improved Vase	2.35	-2.05	o	2.14	-1.74	o	2.64	-0.83	-	1.99	-1.27	-
	Average	**4.35**	**-0.05**	**-**	**3.86**	**-0.02**	**-**	**3.65**	**+0.18**	**-**	**3.57**	**+0.31**	**-**
a3. ROMAMER 2													
a1b1	b1.Tatura	3.50	-0.90	-	2.83	-1.05	-	2.83	-0.64	-	1.91	-1.35	-
a1b2	b2. Vertical Cordon	7.49	+3.09	**	7.33	+3.45	***	7.33	+3.86	***	6.49	+3.23	**
a1b3	b3. Veronese Vase	2.96	-1.41	-	2.25	-1.63	-	2.25	-1.22	-	1.91	-1.35	-
a1b4	b4. Improved Vase	2.28	-2.12	o	1.99	-1.89	o	1.57	-1.90	o	1.99	-1.27	o
	Average	**4.06**	**-0.34**	**-**	**3.60**	**-0.28**	**-**	**3.49**	**+0.02**	**-**	**3.07**	**-0.19**	**-**
a4. CRIMSONGOLD													
a1b1	b1.Tatura	3.91	-0.49	-	3.25	-0.63	-	2.66	-0.80	-	2.41	-0.85	-
a1b2	b2. Vertical Cordon	8.49	+4.09	***	7.99	+4.11	***	5.83	+2.30	**	5.99	+2.73	**
a1b3	b3. Veronese Vase	3.16	-1.24	-	2.58	-1.30	-	2.16	-1.31	-	2.08	-1.18	-
a1b4	b4. Improved Vase	2.85	-1.55	-	2.35	-1.53	-	2.07	-1.40	-	1.57	-1.69	o
	Average	**4.60**	**+0.20**	**-**	**4.04**	**+0.16**	**-**	**3.18**	**-0.29**	**-**	**3.01**	**-0.25**	**-**
B. SHAPE OF THE HEAD													
b1	b1.Tatura	3.74	-0.66	-	3.12	-0.76	-	2.68	-0.79	-	2.41	-0.85	-
b2	b2. Vertical Cordon	8.11	+3.71	**	7.61	+3.73	***	6.74	+3.27	***	6.53	+3.27	***
b3	b3. Veronese Vase	3.18	-1.22	-	2.60	-1.28	-	2.35	-1.12	-	2.24	-1.02	-
b4	b4. Improved Vase	2.58	-1.82	o	2.20	-1.68	-	2.10	-1.37	-	1.86	-1.40	-
		X=4.40 **DL 5% =1.70** **DL1% =2.36** **DL0.1% =3.25**			X=3.88 **DL 5% =1.70** **DL1% =2.36** **DL0.1% =3.25**			X=3.47 **DL 5% =1.49** **DL1% =2.06** **DL0.1% =2.84**			X=3.26 **DL 5% =1.49** **DL1% =2.06** **DL0.1% =2.84**		

Table 3. The quantity of wood removed through cutting (kg/tree) and the number of branches remaining in the tree after cutting in the studied years 2008-2011

CULTIVAR	Shape of the Head	2008		2009		2010		2011	
		Quantity of wood eliminated through cutting (kg)	No. of branches remaining in the tree after cutting	Quantity of wood eliminated through cutting (kg)	No. of branches remaining in the tree after cutting	Quantity of wood eliminated through cutting (kg)	No. of branches remaining in the tree after cutting	Quantity of wood eliminated through cutting (kg)	No. of branches remaining in the tree after cutting
CORA	Tatura	4.70	69	3.90	79	3.0	79	3.20	83
	Vertical Cordon	5.10	98	4.80	90	4.30	93	4.10	85
	Veronese Vase	3.80	87	3.10	89	2.90	92	2.60	87
	Improved Vase	4.0	85	3.30	91	3.0	87	2.70	96
Average/cultivar		**3.45**	**85**	**3.77**	**87**	**3.3**	**88**	**3.15**	**88**
DELTA	Tatura	4.40	70	3.80	86	3.30	89	3.20	88
	Vertical Cordon	4.80	88	4.30	92	4.0	92	4.10	87
	Veronese Vase	4.10	91	3.60	87	3.10	89	3.40	79
	Improved Vase	3.30	89	3.0	94	3.70	78	2.80	85
Average/cultivar		**4.15**	**85**	**3.70**	**90**	**3.52**	**87**	**3.40**	**85**
ROMAMER 2	Tatura	4.20	82	3.40	96	3.40	96	2.30	83
	Vertical Cordon	4.50	93	4.40	112	4.40	113	3.90	101
	Veronese Vase	3.60	88	2.70	97	2.70	97	2.30	79
	Improved Vase	3.20	92	2.80	86	2.20	89	2.80	93
Average/cultivar		**3.87**	**89**	**3.32**	**98**	**3.17**	**99**	**2.82**	**89**
CRIMSONGOLD	Tatura	4.70	69	3.90	79	3.20	83	2.90	87
	Vertical Cordon	5.10	98	4.80	90	3.50	96	3.60	88
	Veronese Vase	3.80	87	3.10	89	2.60	79	2.50	83
	Improved Vase	4.0	85	3.30	91	2.90	94	2.20	79
Average/cultivar		**4.4**	**85**	**3.77**	**87**	**3.05**	**88**	**2.8**	**84**
Average/shape of the head	**Tatura**	**4.5**	**73**	**3.8**	**83**	**3.2**	**87**	**2.9**	**85**
	Vertical Cordon	**4.9**	**94**	**4.6**	**96**	**4.1**	**99**	**3.9**	**90**
	Veronese Vase	**3.8**	**88**	**3.1**	**91**	**2.8**	**89**	**2.7**	**82**
	Improved Vase	**3.6**	**88**	**3.1**	**90**	**3.0**	**87**	**2.6**	**88**

The number of branches remaining in the tree after cutting

According to the shape of the head, in the studied years 2008-2011, the largest number of branches remaining in the tree after cutting was recorded by the Vertical Cordon shape of the head. The second place was occupied by the Veronese Vase and Improved Vase shapes, while the lowest number of branches remaining in the tree was recorded by the Tatura shape (Figure 3, Table 3).

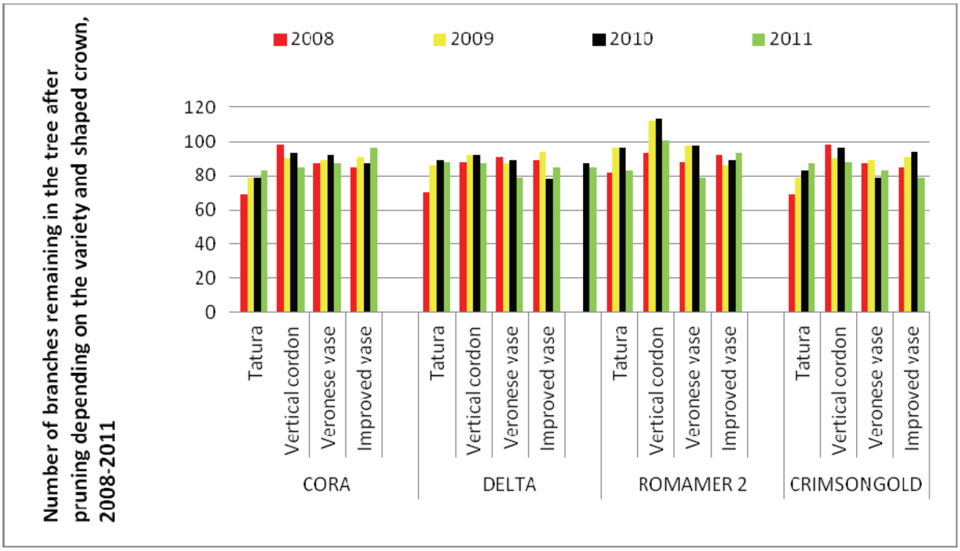

Figure 3. The number of branches remaining in the tree after cutting according to the cultivar and the shape of the head, 2008-2011

In the studied years 2008-2011 the number of branches remaining in the tree after cutting according to the cultivar varies from one year to another, the Romamer 2 cultivar displaying a larger number of branches in comparison to the other cultivars (Figure 4).

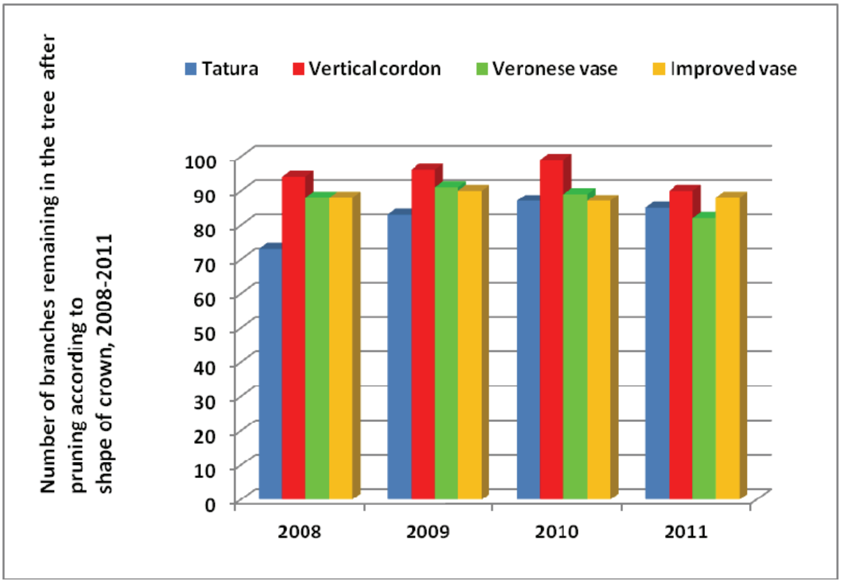

Figure 4. Number of branches remaining in the tree after pruning according to shape of crown, 2008-2011

CONCLUSIONS

The largest quantity of wood eliminated through cutting per tree was recorded by the Vertical Cordon in all four studied years (2008-2011), while the Veronese Vase and Improved Vase displayed lower values.

As far as the cultivars are concerned, the Cora and Crimsongold cultivars, Vertical Cordon shape of the head displayed superior values, followed by the Romamer 2 cultivar.

The quantity of wood eliminated through cutting per surface unit was larger at all four cultivars having the Vertical Cordon shape of the head.

The number of branches remaining in the tree after cutting was larger at the Romamer 2 cultivar in comparison to the other cultivars. As far as the shape of the head is concerned, larger values were recorded by the Vertical Cordon shape, followed by the Veronese Vase and the Improved Vase. The lowest number of branches remaining in the tree was recorded by the Tatura shape.

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THE BEHAVIOUR OF CERTAIN PEACH TREE CULTIVARS TOWARDS THE ATTACK OF THE MAIN PATHOGEN AGENTS

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Abstract

The purpose of this paper is to highlight the cultivars which are the most resistant to disease and pests and which have adapted the most efficiently to the pedo-climatic conditions of Dobrogea. The research took place during the period 2008-2011 at the RSFG Constanta in a peach tree culture planted in 1991 and it involved the following cultivars: 'Springold', 'Springcrest', 'Cardinal', 'Collins', 'Redhaven', 'Jerseyland' and 'Southland'. Observations were recorded concerning the attack of several fungi (Taphrina deformans, Cytospora cincta, Monilinia laxa and Monilinia fructigena) following the application of phyto-sanitary treatments with insecticides and fungicides administered at the right moment and in optimum quantities. Among the studied pathogens, the blistering of the leaves caused by the Taphrina deformans Berk et Tull. fungus is by far the most damaging foliar pathogen agent. Its attack has serious consequences both upon fruit production as well as upon the physiological balance of the trees, resulting in their degradation. Within the pedo-climatic conditions of the south-eastern part of the country, this disease is quite frequent in cultures which causes severe damage. The majority of the studied cultivars manifested and elevated resistance towards the attack of the Taphrina deformans pathogen. In 2011, the 'Springold' and 'Springcrest' cultivars were sensitive towards this pathogen agent. As far as the latter is concerned, we must mention the fact that the sensitivity of the cultivar is also influenced by the environmental conditions specific to each studied year. The most resistant cultivar (basically, without attack) towards the Cytosphora Cincta is 'Redhaven', while the most sensitive was 'Collins'. As far as the Monilinia laxa and Monilinia fructigena fungi are concerned, all the studied cultivars proved to be tolerant. Following this study, the most valuable genotypes can be extended in demonstrative allotments and commercial plantations.

Key words: *resistance, blistering, natural infection, Prunus persica*

INTRODUCTION

In our country, Dobrogea is the region that offers the most favourable conditions for the peach tree culture. Unfortunately, however, this area is also favourable for the development of certain extremely harmful pathogen agents, such as: *Taphrina deformans*, *Cytospora cincta*, *Monilinia laxa* and *Monilinia fructigena*. The diseases caused by the attack of pathogens are frequent when spring is cold and humid and cloudy for long periods of time. Under these conditions the attack is strong and the quality of the production diminishes considerably.

The peach tree is the third most import fruit-growing species in our country, after the apple tree and the plum tree and is cultivated in warmer areas, with an average annual temperature of 10-11.5 °C. The soil needs to be deep, aerated, with a pH varying between 5.7 and 7.5; also, the content of active limestone must not surpass 7% when using the franc parent stock and 15% when the parent stock is an almond tree (Stănică and Braniște, 2011).

The peach tree is widely spread, in Europe (43% of the annual production, 18,000,853 t in 2008), Asia (23%), Central and North America (17%), South America, Africa, Oceania. 4,203,800 t were produced in Europe in 2008, the most productive country being Italy (1,720,000 t), followed by Spain (1,149,000 t), Greece (700,000 t), France (401,000 t), etc. Romania produced 16,400 t in 2008 (Stănică and Braniște, 2011). Both in our country as well as worldwide the aim is to obtain fruits of a superior quality, with an increased and constant productivity of the trees which should be resistant and/or tolerant to the main diseases specific to the species. The importance of knowing the pathogen agents and the pests which could attack the peach tree is correlated with the practical necessities which occur in the orchard and storage period, taking into account the fact that the quality standards of the material for cultivation whose purpose is the creation of new plantations, as well as the quality standards of the fruits can be achieved only if the pathogens and pests are known so that their attacks can be controlled.

Trandafirescu (1998, 2007) has studied the resistance to the *Taphrina deformans* within the peach tree and nectarine tree species, detailing the research for each cultivar from the national peach tree collection planted in 1981.

The purpose is to highlight the cultivars which are the most resistant to diseases and pests in order to be extended in demonstrative allotments and commercial plantations, thus occupying a good place in the structure of the peach tree assortment from the South-Eastern region of Romania.

MATERIAL AND METHOD

The research took place in the period 2008-2011 at the R.S.F.G. Constanţa, in Valu lui Traian, Constanţa district, in a peach tree plantation planted in 1991, the planting distance is 4/4m (625 trees/ha) and the shape of the trees is a free flat palmette. The studied cultivars have different ripening periods and are the following: 'Springold', 'Springcrest', 'Cardinal', 'Collins', 'Redhaven', 'Jerseyland' and 'Southland'. Given the fact that the region is semi-arid, the peach tree culture developed under an irrigated regime. The soil is a calcareous chernozem (CZka), with a claylike texture, a low alkaline pH (8.2) in its entire profile. The behaviour of the peach tree cultivars towards the attack of pathogens (*Taphrina deformans*, *Cytospora cincta*, *Monilinia laxa* and *Monilinia fructigena*) was observed under conditions of natural infection. From a technological point of view, 6-8 insecticide and pesticide treatments were applied against the main disease and pests. The system concerning the maintenance of the soil was black ground between rows and herbicided on the rows of trees (a strip of 50 cm). Observations were carried out concerning the behaviour of certain peach tree cultivars towards the attack of the main pathogen agents, as well as observations regarding the phenology of the studied cultivars (the swelling of the flowering buds period and the ripening time). Observations were also carried out concerning the evolution of the disease on the leaves, fruit and sprouts following the applying of phyto-sanitary treatments with insecticides and fungicides, treatments administered at the optimal moment and dosage.

The research was carried out under conditions of natural infection, according to the test created by Crossa (1968). The evaluation technique consisted in assessing the frequency of the attacked organs and the intensity with which the symptoms manifested themselves, these being the aspects according to which the behaviour was assessed.

The observations were performed by ranking the intensity of the attack on a scale of 0-4, as follows:

W.A.= cultivars without attack (F%=0 and I=0)

T=tolerant cultivars (F%=0.1-5% and I=0 $^{\underline{\pm}}$ +)

We.A.=weakly attacked cultivars (F%=5.1% - 10% and I=+)

M.A.=moderately resistant cultivars (F%=10.1%-25% and I=+)

S=sensitive cultivars (F%=25.1–50% and I=+ $^{\underline{2}}$ 4)

V.S.=very sensitive cultivars (F%=50.1%-100%, I=+ $^{\underline{4}}$ 4)

In order to determine the development of the main vegetative stages under the conditions of the R.S.F.G. Constanta, phonological observations were carried out concerning the trees both in the resting period, as well as during the vegetative phase.

Fructification phenophases

As far as the genesis of the flowers is concerned, the start of vegetation in spring represents the continuation of the process of flower creation which has been interrupted by the biological resting period.

- The swelling of the flowering buds;
- The beginning of blossoming.

Similar to the other vegetative stages, the blossoming of the trees is influenced by the evolution of the climatic conditions. The beginning of the blossoming in the period 2008-2011 was recorded for all the studied cultivars.

Intensity

The intensity of the blossoming is appreciated on a scale of 0 to 5, the specific moment of appreciation being the mass blossoming stage. The grade 0 is considered when the cultivar has no blossoms whatsoever, while the grade 5 is when the cultivar displays a large amount of blossoms.

- The ending of the blossoming;

The blossoming can last a longer or shorter period of time depending on the maximum temperatures during the day and the intensity of the wind, correlated with the degree of differentiation between the trees (meaning the quantity of blossoms per tree).

- The hardening of the core;

The hardening of the core is determined by piercing the fruit with a needle at regular intervals, usually of two days. When the needle has difficulty piercing the fruit it is considered that the hardening of the core has begun. The operation was performed in a progressive manner, according to the calendar, in the same day for all studied cultivars.

- The ripening of the fruits;

The ripening of the fruits is highly influenced by a series of climatic and agro-technical factors, such as the temperature, the drought, the quantity of fruit per tree, the shape of the crown, the density of the trees, etc.

Taking the calendar into account, each ripening period has large variation limits from one year to another, depending on how the climatic factors influence the vegetation to be early, late or extra-late.

RESULTS

In the period 2008-2011, the phenophase of the flowering buds swelling at the peach trees (Table 1) underwent between the following limits:

Between 20.03 and 29.03 at the 'Springold' cultivar;

Between 22.03 and 29.03 at the 'Springcrest' cultivar;

Between 24.03 and 30.03 at the 'Collins' cultivar;

Between 18.03 and 30.03 at the 'Cardinal' cultivar;

Between 22.03 and 03.04 at the 'Redhaven' cultivar;

Between 03.03 and 29.03 at the 'Southland' cultivar;

Between 22.03 and 03.04 at the 'Jerseyland' cultivar;

Basically, the swelling of the flowering buds occurred between 03.03 and 03.04 (one month).

The beginning of the blossoming

Similar to the other vegetative stages, the blossoming of the trees is influenced by the evolution of the climatic conditions. For all the studied cultivars the beginning of the blossoming in the period 2008-2011 was recorded, the differences between cultivars being very small (a few days), meaning that mutual pollination was ensured. Thus, the limits for this stage were 26.03 and 10.04.

The duration of the blossoming depends on the maximum temperatures during the day and the intensity of the wind, correlated with the degree of differentiation between the trees (meaning the quantity of blossoms per tree).

The scaling was carried out when the petals of the last blossoms fell from the trees. The duration of the blossoming (average for the four studied years, 2008-2011) expressed in number of days (Table 1) varied between 8 days ('Springold', 'Redhaven' and 'Southland' cultivars) and 22 days ('Springcrest', 'Collins' and 'Southland' cultivars in 2011).

The intensity of the blossoming

In 2010 a weak intensity was recorded by the 'Springold' and 'Springcrest' cultivars (2 and 3, respectively, upon blossoming). The 'Collins', 'Cardinal', 'Redhaven', 'Southland' and 'Jerseyland' cultivars displayed a significant intensity upon blossoming, of 4 and 5.

The hardening of the core

This phenophase occurred in the first ten days of June (04-16.06).

The harvesting maturity

Its variation limits were June 21st ('Springold') and August 10th ('Southland'), this phenophase being genetically influenced.

At Valu lui Traian the pathogens of economic importance for this specie under the conditions of the studied period 2008-2011 were the following mycoses: *Taphrina deformans* Berk et Tull (blistering of the leaves), *Cytospora cincta* Sacc (perennial cancer of the sprouts), *Monilinia laxa* and *Monilinia fructigena* Aderh Ruhl Honey (rotting and mummification of the fruit).

Table 1. The development of the main fructification phenophases at the peach tree in the period 2008-2011

No.	CULTIVAR	Year	Swelling of the flowering buds	Blossoming			Intensity	Hardening of the core	Harvesting maturity
				Beginning	Ending	Duration (days)			
1	SPRINGOLD	2008	20.03	28.03	18.04	21	5	04.06	29.06
		2009	25.03	08.04	23.04	15	5	10.06	27.06
		2010	29.03	5.04	12.04	8	2	12.06	21.06
		2011	27.03	02.04	10.04	9	4	07.06	29.06
		Limits	**20.03-29.03**	**28.03-08.04**	**12.04-23.04**	**8-21**	**2-5**	**04.06-12.06**	**21.06-29.06**
2	SPRINGCREST	2008	22.03	29.03	16.04	19	5	04.06	03.07
		2009	29.03	08.04	30.04	22	4	08.06	07.07
		2010	20.03	05.04	16.04	12	3	10.06	09.07
		2011	28.03	07.04	17.04	11	5	09.06	12.07
		Limits	**22.03-29.03**	**29.03-08.04**	**16.04-30.04**	**12-22**	**3-5**	**04.06-10.06**	**03.07-12.07**
3	COLLINS	2008	24.03	29.03	12.04	18	4	04.06	08.07
		2009	29.03	08.04	30.04	22	5	10.06	11.07
		2010	30.03	09.04	21.04	13	4	07.06	12.07
		2011	26.03	07.04	18.04	12	5	16.06	17.07
		Limits	**24.03-30.03**	**29.03-09.04**	**12.04-30.04**	**13-22**	**4-5**	**04.06-16.06**	**08.07-17.07**
4	CARDINAL	2008	18.03	26.03	10.04	16	5	04.06	13.07
		2009	26.03	08.04	23.04	15	5	10.06	18.07
		2010	30.03	10.04	18.04	9	5	08.06	15.07
		2011	25.03	04.04	15.04	12	5	05.06	19.07
		Limits	**18.03-30.03**	**26.03-10.04**	**10.04-23.04**	**9-16**	**5**	**04.06-10.06**	**13.07-19.07**
5	REDHAVEN	2008	22.03	28.03	16.04	19	5	04.06	21.07
		2009	28.03	08.04	23.04	16	5	10.06	28.07
		2010	03.04	10.04	17.04	8	4	07.06	23.07
		2011	27.03	05.04	16.04	12	5	05.06	16.07
		Limits	**22.03-03.04**	**28.03-10.04**	**16.04-23.04**	**8-19**	**4-5**	**04.06-10.06**	**16.07-28.07**
6	SOUTHLAND	2008	24.03	29.03	13.04	14	5	05.06	04.08
		2009	29.03	08.04	30.04	22	5	08.06	10.08
		2010	03.03	10.04	17.04	8	5	07.06	07.08
		2011	25.03	09.04	20.04	12	5	14.06	09.08
		Limits	**03.03-29.03**	**29.03-10.04**	**13.04-30.04**	**8-22**	**5**	**05.06-14.06**	**04.08-10.08**
7	JERSEYLAND	2008	22.03	28.03	16.04	19	5	07.06	20.07
		2009	28.03	08.04	23.04	16	5	10.06	18.07
		2010	03.04	10.04	19.04	10	4	07.06	13.07
		2011	26.03	06.04	20.04	15	5	04.06	15.07
		Limits	**22.03-03.04**	**28.03-10.04**	**16.04-23.04**	**10-19**	**4-5**	**04.06-10.06**	**13.07-20.07**

The resistance to diseases is one of the most important problems of the peach tree culture. Research have shown that, 20 years after planting, certain cultivars display a resistance towards the attack of pathogen agents (Table 2).

The analysis of the data in the table reveals the fact that most of the studied cultivars manifested an increased resistance towards the attack of the harmful pathogen *Taphrina deformans*, the exception being the 'Springold' and 'Springcrest' cultivars in the years 2010 and 2011 (Figure 1), years in which they proved to be sensitive.

In the case of this pathogen we must mention the fact that the sensitivity of the cultivar is also influenced by the environmental conditions of each studied year. Among the pathogens which constituted the object of this paper, the blistering of the leaves caused by the *Taphrina deformans* Berk et Tull. fungus is undoubtedly the most harmful foliar pathogen agent. Its attack has serious consequences both upon the fruit production as well as upon the physiological balance of the trees, causing their debilitation.

The perennial cancer of the peach tree caused by the *Cytospora cincta* Sacc fungus is, together with the blistering caused by the *Taphrina deformans*, the most important pathogen which diminishes fruit production.

Figure 1. The 'Springold' cultivar attacked by the *Taphrina deformans* in May 2011

According to the intensity (I) of the attack the studies cultivars were classified as follows (Table 2):

Taphrina deformans - 6 resistance classes

- Cultivars without attack (W.A.) – two cultivars, the intensity (I) of the attack being zero: 'Redhaven' and 'Cardinal' (in the studied years 2008, 2009, 2010).
- Tolerant cultivars (T) – 'Collins', 'Jerseyland', 'Southland';
- Weakly attacked (We.A) – no cultivars;
- Sensitive (S): 'Springold' and 'Springcrest';

None of the studied cultivars entered the moderately attacked and very sensitive classes.

As far as the attack of the *Cytospora cincta* Sacc is concerned, the observations revealed the fact that the sensitivity and the resistance of the cultivars towards it depend exclusively on the soil. The studied cultivars were classified as follows:

- Cultivars without attack (W.A.) – only 'Redhaven';
- Tolerant cultivars (T – 'Cardinal' (2008, 2010, 2011) and 'Springold';
- Weakly attacked (We.A) – 'Southland' (2009, 2010, 2011), 'Springcrest' (2008, 2009, 2010) and 'Jerseyland';
- Sensitive (S) – 'Collins'.

None of the studied cultivars entered the moderately attacked and very sensitive classes.

As far as the attack of the *Monilinia laxa* and *Monilinia fructigena* Aderh et Ruhl Honey fungi is concerned, all studied cultivars proved to be tolerant (T), both the frequency (F%) as well as the intensity of the attack (I) being graded with zero.

The introduction within cultures of peach tree cultivars with an increased resistance towards the attack of the most harmful pathogen agents has numerous economic advantages, such as, among which the diminishing of production losses and the reduction of expenses regarding pesticides and fuels. These are correlated with the protection of the environment – reduction of the battering of the soil due to the fact that the tractor rarely crosses the orchard, the reconstruction of the soil’s structure, the reduction of the pollution of both the environment and the fruit, as well as the protection of the consumers’ health.

Table 2. The behaviour of the peach tree cultivars towards
the attack of the main pathogen agents in the period 2008-2011

No.	Cultivar	Year	*Taphrina deformans* Resistance class Intensity of attack (grades*)						*Cytospora cincta* Resistance class Intensity of attack (grades*)						*Monilinia* Intensity of attack (grades*)	
															laxa	*fr.*
			WA	T	We A	S	MA	VS	W A	T	We A	S	MA	VS	T	T
Cultivars planted in 1991																
0.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10	11	12	13	14	15.	16.
1.	SPRINGOLD	2008	■	■					■	■					■	■
		2009	■	■					■	■					■	■
		2010	■	■	■	■	■		■	■					■	■
		2011	■	■	■	■	■		■	■					■	■
2.	SPRINGCREST	2008	■	■					■	■	■				■	■
		2009	■	■					■	■	■				■	■
		2010	■	■	■	■	■		■	■	■				■	■
		2011	■	■	■	■	■		■	■					■	■
3.	CARDINAL	2008	■						■	■					■	■
		2009	■						■	■					■	■
		2010	■						■	■					■	■
		2011	■	■					■	■	■				■	■
4.	COLLINS	2008	■	■					■	■	■	■			■	■
		2009	■	■					■	■	■	■			■	■
		2010	■	■					■	■	■	■			■	■
		2011	■	■					■	■	■	■			■	■
5.	REDHAVEN	2008	■						■						■	■
		2009	■						■						■	■
		2010	■						■						■	■
		2011	■						■						■	■
6.	JERSEYLAND	2008	■	■					■	■	■				■	■
		2009	■	■					■	■	■				■	■
		2010	■	■					■	■	■				■	■
		2011	■	■					■	■	■				■	■
7.	SOUTHLAND	2008	■	■					■						■	■
		2009	■	■					■	■	■				■	■
		2010	■	■					■	■	■				■	■
		2011	■	■					■	■	■				■	■

* Grade Intensity of the attack

CONCLUSIONS

- Most of the studied cultivars manifested an increased resistance towards the attack of the harmful pathogen agent *Taphrina deformans*, being either without attack (W.A.) or tolerant (T).
- The 'Springold' and 'Springcrest' cultivars proved to be more sensitive.
- As far as the attack of the *Cytosphora cincta* fungus is concerned, the sensitivity and the resistance depend exclusively on the soil. The 'Collins' cultivar proved to be sensitive, whereas the 'Redhaven' cultivar proved to be resistant.
- As far as the attack of the *Monilinia laxa* and *Monilinia fructigena* fungi is concerned, all studied cultivars proved to be tolerant (T).
- The cultivars from Valu lui Traian which manifested an increased resistance towards the attack of pathogens of economic importance will be recommended for usage in works of improvement, as well as for extension in production.

These cultivars which are resistant to the *Taphrina deformans* will simplify the peach tree's crop technology for small areas and will ensure a biological production towards which all cultivators aim.

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INFLUENCE OF THE DRIP IRRIGATION ON THE PHYSICAL AND CHEMICAL PLUMS CHARACTERISTICS

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Abstract

The premise of scientific and technical experiments was to evaluate the behaviour during storage of the 2 cultivars of plums ('Stanley' and 'Anna Spath'). Fruits were held after the harvest period of about 7-45 days, depending on cultivar, storage spaces, storage of the resort, where the experiments were subsequently taken and kept in cold conditions (t = 3-5 ^{0}C; UR-95%) over a period of 30 days in cold storage of Belciugatele Teaching Station, located inside of Moara Domnească farm. After removal from storage were made determinations regarding: the recorded quantity and quality of the losses of fruit during storage; the chemical evolution of the main components of plums during storage; The drip irrigation was started from March 20, water was given 4 hours per day. The amount of water needed are between 300 and 700 m^3/ha. Among the tested cultivars, Anna Spath performed best, which was ranked on the first place with 2,26 % total loss without loss by spoilage.

Key words*: chemical, drip irrigation, losses, storage, varieties*

INTRODUCTION

Making a modern fruit growing cannot be conceived without providing a corresponding hydric regime that fruit species need (Cohen D.,1993). Currently, irrigation is necessary to be understood, the principles of integrated fruit growing, highly effective as a tool for regulating the activity of vegetative and productive trees (Iancu M., 2006). It is known that a slight water stress amplification phenomenon has the effect of fruit bud differentiation (Popescu M., Godeanu I., 1989). Also, reducing splashing and the excessive vegetative growth of shoots directing the assimilated to the fruits (Gherghi A., 1983).
Influence of drip irrigation on production quality is the effect on the nutritional and food value of fruits (Atkinson D., 1977). Nutritional and food value of the fruits represents the purpose for growing fruit trees, fruit bushes and strawberries as supplements of vitamins, sugars, minerals, acids and energy (calories) needed for proper body growth and development (Botu I., Botu M., 2003).
Plums in controlled storage conditions can prolong the storage period of 2 months without losing its qualities. Storage and consumption during the same organoleptic characteristics depend on the terms offered and the particular variety. It is known that during storage, the fruits suffer structural-textural changes that are produced slower or faster depending on the characteristics of varieties and storage conditions (Gherghi A. et al., 2001).

MATERIALS AND METHODS

The content of this paper is based on research, observations, experiments, extensive research of issues prioritized and data processing including the whole range of issues, starting from the foundation of the application irrigation necessity. As a result, the entire work is based almost exclusively on their research findings on the effect of irrigation on apple and plum at Moara Domnească farm conditions. The elaborations of the researches for this paper were carried out in 2011-2012.
Locality is included in the relief of the Romanian Plain, the Vlăsiei Plain subdivision in the area of transition from steppe to forest area. The general appearance of the landscape is flat, with small bumps and depressions many called depression of different shapes and sizes.

Groundwater is at different depths from 6 m to 10 m depending on relief. In 2011, the accumulated rainfall was 288 mm, and 501.6 mm in 2012. All values are under the multiannual average on 50 years, of 548.0 mm. The soil belongs to Moara Domnească reddish brown type (preluvosol), subtype softness.

To establish the ability to maintain quality fresh plums taken 2 more spread cultivars in the current assortment in our country, 'Stanley' and 'Anna Spath'. The premise of the scientific and technical experiments was to assess behavior during winter storage of the 2 varieties of plums introduced in the experimentation. Fruits were kept after harvest a period of about 7-45 days, depending on cultivar, in the storage spaces of the resort, from where they were then taken for experiments and refrigerated in storage conditions (t=3-5°C, RH = 90-95%) in cold storage of Belciugatele Didactic Station, located inside the Moara Domneasca Farm. The experiments were conducted in 6 different comparative variants based on variety and storage conditions (Table 1).

After removal from storage, it were made determinations regarding the following:

- the quantitative and qualitative losses recorded by the fruit during storage;
- evolution of the main chemical components of the apples during storage.

Existence of optimal flow experience across storage-keeping with reference to standardized packaging units, space conditioning, refrigeration thermostatic cell, organoleptic testing laboratory, equipment and devices for measurements and analyzes provided made it possible to achieve this goal.

During storage it has been made the daily examination of the thermal-hydric factors in the refrigeration room, for ensuring that optimal conditions to maintain the quality. Also we proceeded to assess the ability to maintain fruit quality by findings the appearance changes occurred regarding dehydration, the appearance and evolution of different storage disease. Taking into account the high degree of maturity of the fruit during storage and quality changes occurring during storage it was estimated that during cold storage the limit is 45 days. After removing plums from the storage space the determinations were performed on the table and impairment losses (spoilage), biochemical analysis of the main components. Determination of mass loss and spoilage during storage products was done by weighing samples of fruit resulted, respectively the fruit impaired (spoiled) during storage, compared with the initial amount deposited, the results being expressed as a percentage.

Tests for the main chemical components (dry substance, total sugar, titratable acidity) were performed by standard laboratory methods as follows:

- dry substance was determined by refractometry method using ABB table refractometer with results expressed in %:
- total sugar by Berthrand method with results expressed in %;
- acidity by titrimetric method with results expressed in % of malic acid;

Table 1. Experimental variable

Cultivar and storage conditions	Temp ^{0}C	CO_2	Storage period (days)
Stanley/hemibioza	28-30	-	7
Stanley/fizioanabioza	3-5	-	14
Stanley/chimioanabioza	3-5	4	21
AnnaSpath/hemibioza	28-30	-	7
AnnaSpath/fizioanabioza	3-5	-	14
AnnaSpath/chimioanabioza	3-5	4	21

RESULTS AND DISCUSSIONS

Influence of drip irrigation on plums chemical and physical characteristics

The results of the quantitative and qualitative losses recorded by the fruit during storage at the experimental variants are presented in Table 2.

After 7 days of plum storage at ambient temperature, it was registered weight losses between 10.74% and 20.02% and by spoiling from 2.20% to 26.67 %, depending on cultivar.

Total losses during storage are between 12.94% at 'Stanley' and 46.67%, at 'Anna Spath' cultivar. 'Stanley' registered total losses 4 times lower than 'Anna Spath', because of lower weight losses and very low damage losses.

After 30 day of cold storage, plums registered 11.71% - 15.15% weight losses and spoiled losses between 0 and 6.60%, depending on cultivar.

Table 2. Loss during preservation at plums

Cultivar	Weight %	Damage %	Totals %	Storage period days
Stanley-hemibioza	10.74	2.20	12.94	7
Stanley-fizioanabioza	11.71	-	11.71	30
Stanley-chimioanabioza	0.49	-	0.49	45
Anna Spath-hemibioza	20.02	26.67	46.69	7
Anna Spath-fizioanabioza	15.15	6.60	21.75	30
Anna Spath-chimioanabioza	0.58	6.20	6.78	45

Total losses were by 11,71% at 'Stanley' cultivar and by 21.75% at 'Anna Spath'. 'Stanley' cultivar registered the lowest level of weight losses and no damage losses.

After 45 days in chimioanabiosis conditions plums presented weight losses between 0.49 % and 0.58%. Spoiled losses were between 0-6.20%, depending on cultivar.

'Stanley' cultivar registered a total losses percent by 0.49% and 'Anna Spath' registered 6.78%. 'Stanley' cultivar recorded a very low total losses percent because of a low level of weight losses and no damages during the storage. The evolution of the main chemical components during storage are presented in Table 3.

Table 3. Chemical components evolution on plums

Cultivar	Dry soluble substance %		Titrable acidity %		Total sugar %	
	initial	final	initial	final	initial	final
Stanley-hemibioza	15.57	17.02	0.77	0.77	9.70	11.33
Stanley-fizioanabioza		19.33		0.49		11.97
Stanley-chimioanabioza		19.77		0.59		11.80
Anna Spath-hemibioza	22.57	24.22	0.92	0.95	10.45	11.97
Anna Spath-fizioanabioza		15.33		0.70		10.95
Anna Spath-chimioanabioza		16.65		0.78		11.02

Initial dry substance content was between 15.57% and 22.57%, titratable acidity values were between 0.77 and 0.92% and total sugar content was 9.70% and 10.45%, depend on variety.

'Anna Spath' cultivar presents an initial dry substance content, titratable acidity and total sugar higher than 'Stanley' cultivar.

The plums storage for 7 days at ambient temperature had a dry soluble substance content ranged from 17.02%-24.22%, titratable acidity ranged from 0.77%-0.95% and total sugar ranged from 11.33%-11.97%, depend on cultivar.

In hemibiosis conditions, the 'Anna Spath' plums cultivar presents a higher content in dry substance, titratable acidity and total sugar than 'Stanley' cultivar. The tendency of main compounds was to increase dry substance and total sugar content and stationary initial titratable acidity content.

The plums storage in refrigerated conditions during 30 days, have a dry substance content between 15.33-19.33%, titratable acidity between 0.49-0.70% and total sugar content between 10.95-11.97%, depend on cultivar.

The fruits from 'Stanley' cultivar had a higher dry substance and total sugar content than 'Anna Spath' fruits.

The tendency of main chemical components was to increase dry substance and total sugar and decrease titratable acidity comparative to initial values. An exception is 'Anna Spath' variety where the dry substance content decrease comparative with initial value.

After 45 days by storage in chimioanabioza conditions, plums registered a content in dry substance between 16.65-19.77%, a titratable acidity content between 0.59-0.78% and 11.02-11.80% content in total sugar, depend on cultivar.

The fruits from 'Stanley' cultivar presents a higher dry substance and total sugar content than fruits from 'Anna Spath' cultivar.

Comparative with initial values, plums presents an increase of dry substance and total sugar content and a decrease of titratable acidity content.

Table 4. Phisical features of plum cultivars

Cultivar	Fruit weight average-g	% of kernel	Form index
Stanley	30.8	6.5	1.30
Anna Spath	34.1	14.4	1.04

Regarding some physical characteristics, 'Stanley' cultivar has a weight of the fruit by 30.8 g, lower than 'Anna Spath' weight were the kernel percent is bigger and decrease the fruit weight (Table 4.)

CONCLUSIONS

Conclusions on losses

The total losses registered by plum varieties, during storage depending on variety and storage conditions.
Among the cultivars tested best behavior was 'Stanley' cultivar which ranked first a total loss of 25,14 % for all storage conditions, almost without losses through spoilage 2.20%.

Conclusions on changes in chemical constituens

Initial content of the main chemical components registered important differences depending on cultivar. 'Anna Spath' presents a higher initial content in dry substance and total sugar comparative with 'Stanley' cultivar.

During storage the level of some chemical components increased or decreased, depending on variety and storing conditions.
The fluctuations have lower values comparative with other studied species.
During cold storage, tendency was to increase dry substance and total sugar content and to decrease titratable acidity content.

Conclusions on fruit size

The studied varieties differs in fruit size and form index. The average weight of the fruit ranges from 30.8 g and 34.1 g. Highest weight was registered at 'Anna Spath' cultivar, 34.1 g but the kernel weight (which was bigger than 'Stanley's) decrease the fruit weight.
The form index was between 1.04 and 1.30, depending on cultivar; the biggest value was registered at 'Stanley'.

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CONTRIBUTIONS REGARDING THE APPLE TREES GENETIC VARIABILITY INCREASE IN THE PROCESS OF OBTAINING IMPROVING BIOLOGICAL MATERIAL

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Abstract

The researches performed at the Fruit Growing Research and Development Station Voineşti in the period 2004–2012 point out the creation of some new apple trees selection bases from the biological material, obtained by sexuate hibridation. The genetic variability increase was remarked especially at the hybrid combinations, at which we used as genitors the recently created apple trees elites with genetic resistance against diseases, which included different resistance genes. The scab and mildew resistance feature was transmited to a greater percentage in the case of hybrid combinations between the resistant breeds - and in a more reduced percentage at the hybrid combinations: resistant breed x sensitive breed. The material undergoing selection and grafted in the nursery constitutes the base of the evaluation in test culture of the apple tree elites, regarding the features of productivity, fruits quality and genetic resistance against diseases.

Key words*: hybrid combinations, biological material, apple trees genotypes , genetic resistance against diseases*

INTRODUCTION

Obtaining apple breeds represents a long term and highly complex activity, especially when one has in view to obtain genetic diseases resistant breeds, regardless of the used research method. In the creation program of genetic disease resistant apple tree breeds at the FGRDS Voineşti, we annually realized the most diverse hybrid combinations, using matern and patern genitors, which had to respond to the proposed objectives. The researches performed at the FGRDS Voineşti in the period 2004 – 2012 point out the creation of some new apple trees selection bases, simultaneously with the increase of the genetic variability in the process of obtaining the biological improvement material. The increase of the genetic variability is realized especially at the hybrid combinations, at which we use - as apple tree genitors - recently created breeds and elitels with genetic resistance against diseases, which have included complex resistant genes.

MATERIALS AND METHODS

The researches, deployed in the period 2004 – 2012 in the experimental fields of the Fruit Growing Research and Development Station Voineşti, had as objective to obain some new apple tree hybrid generations, in view of the creation of new genetic disease resistant breeds.

We used matern and patern genitors with valuable productivity, fruits quality features and both partners - or at least one of them - shall possess the genetic diseases resistant gene, increasing the genetic variability - and a greater probability to obtain genetic diseases resistant breeds.

In the period 2004 – 2012 we performed 25 hybrid combinatons, at which we added 6 genitors, from which we used seeds obtained by natural polenation.

The used technology was specific to the creation of apple tree breds, mentioning that we applied no phyto – sanitary treatments with fungicides - only 4-5 treatments with insecticides.

The used working method was the selection of the apple tree hybridation, retaining those which presented resistance to the scab and mildew attack.

RESULTS AND DISCUSSIONS

The improvment process is continuos, so that this supposes the annual creation of new selection bases, composed of hybrid descendenes, which shall possess a complex variability, being implied as matern and patern genitors, breeds or genitors – so that the realization term of new valuable forms shall decrease.

The improvement succes is conditioned to a large extent by the clarity of the proposed objectives, but simultanously it depends on the existence and on the knowledge of the genertic resourses. The creation of a great genetic variability and diversity offers real sources to the searched for selection.

A newly created breed, besides the features of productivity, superior fruits quality, genetic diseases resistance - depending on the culture zone, has to meet also other features, which have to be added to the essential conditions, meaning:

- the adaptabiliy degree to the climate conditions;
- the destination of the a production;
- the knowledge degree of the breed;
- the market requirements of the obtained production;
- the security of the source of production and delivery of the tree growing seedling material;
- the economicity of the culture technology.

The use of some genitors, which possess the resistance and productivity gene, imprints into the descendency a greater transmission rate of the valuable characters, easing in a certain way the improver's work. The other characteristics, added to the essential conditions, are pointed out only based on further researches.

At the TGRDS Voineşti there is a rich selection base for future improvement works, obtained in the period 2004-2012, the evaluation of the apple tree hybridation program being presented in the table 1.

The multitude of hybrid apple tree seedlings has been obtaiined by sexuate hibridation, using as genitors:

- sensitive breeds of the present assortment: 'Idared', 'Goldspur';
- diseases resistant breeds: 'Florina', 'Goldrush', 'Topaz', 'Golden Lasa', 'Ariwa' of foreign origin, 'Rebra' obtained at the TGRDI Mărăcineni, 'Iris', 'Inedit', 'Remar' created at the TGRDS Voineşti, all having the resistance gene Vf; the 'Generos' breed, obtained at the TGRDS Voineşti, is scab tolerant - the Poly resistance gene;
- genetic dieseases resistant elites, selected at the TGRDS Voineşti: H 3/5; H 1/53; H 2/8; H 1/11; H 1/46; H 1/78; H 8/86; H 1/27; H 1/13; H 4/37; H 5/20; H 1/7, all have the Vf resistance gene.

From the data presented in the Table 1 it results, that in the period 2004 – 2012 we performed 25 hybrid combinations, to which we added 6 genitors, from which we used the seeds, obtained by natural pollination. From 6,013 pollinated flowers we obtained 2,187 hybrid fruits, from which we extracted 11,099 seeds; from these we sowed 9,811 hybrid apple tree seeds (7,955 obtained from hybrid combinatiom and 1,856 resulted by natural pollination). In total, 6,289 hybrid apple tree seedlings resulted, which were planted in the seedling nursery for fortification and then were transferred into the selection orchard.

In these 9 years of experimentation (2004-2012) resulted an initial improvement material of great genetic diversitaty, a fact that permitted and will permit to obtain some selection with perspective, with characteristics being superior to the genitors used in the improvement – and even to the apple tree breeds existing in culture. We annualy selected only apple tree hybrids presenting genetic diseases resistance, with superior quality fruits; those that corresponded to the previously established objectives were grafted in the nursery.

We realized the increase of the genetic variability especially at the hybrid combinations, where we use as genitors the recently created appple tree breeds and elites with genetic diseases resistance, with complex resistance genes.

Already from the first hybrid series, we remarked some selections with genetic diseases resistance and with superior quality fruits; these we grafted in the nursery and they were the object of some competition micro - cultures.

The material subjected to the selection and grafted in the nursery constitutes the evaluation base in the testing cultures of the apple tree elites, regarding to the features of productivity, fruits quality and genetic diseases resistance.

Table 1. Evaluation of the apple tree hybridization program in the period 2004 – 2012 (FGRDS Voineşti)

No.	Combination	Pollinated flowers	Obtained hybrid fruits	Sowed hybrid seeds	Resulted seedlings	
					nr.	%
	Year 2004					
1	Florina x Idared	450	80	341	216	63.3
2	Florina x H 3/5-90	60	9	49	16	32.1
3	Goldspur x Florina	422	117	854	280	32.7
	Year 2005					
4	Generos x H 1/53	380	87	604	525	86.9
5	Godspur x H 2/44	390	280	1520	1220	80.3
6	Goldspur x Florina	650	274	1287	928	72.1
	Year 2006					
7	Iris n.p.		188	818	365	44.6
8	Florina n.p.		66	389	273	70.0
	Year 2007					
9	Florina x H 2/8	112	38	109	59	54.1
10	Goldspur x H 1/11	281	76	268	152	56.7
11	Goldspur x H 1/46	248	96	202	121	59.9
	Year 2008					
12	Florina x H 1/78	215	48	256	146	57.1
13	Florina x Nicol	61	5	23	17	73.9
14	Florina x Iris	63	12	62	43	69.3
15	Remar x Golsrush	635	51	277	89	32.1
16	Remar x Iris	181	36	173	125	72.2
17	Inedit x H 8/86	214	44	225	146	64.8
18	Inedit x Remar	200	56	290	132	45.5
	Year 2009					
19	Iris n.p.		51	212	140	66.0
20	Goldrush n.p.		38	194	133	67.1
	Year 2011					
21	Ariwa n.p.		21	112	41	36.6
22	Rebra n.p. .		29	131	77	58.7
	Year 2012					
23	Topaz x H 1/27	128	7	38	29	76.3
24	Inedit x Ariwa	106	10	55	32	58.1
25	Goldrush x Golden Lasa	268	85	162	121	74.7
26	Goldrush x H 1/13	243	86	190	132	69.4
27	Goldrush x H 4/37	173	90	280	202	72.1
28	Goldrush x Inedit	256	78	275	208	75.6
29	Goldrush x Iris	102	23	90	60	66.6

No.	Combination	Pollinated flowers	Obtained hybrid fruits	Sowed hybrid seeds	Resulted seedlings	
30	Goldrush x H 5 / 20	93	49	160	128	80.0
31	Goldrush x H 1/7	82	57	165	133	80.6
	TOTAL	6.013	2.187	9.811	6.289	64,1

- n.p. = natural pollination

CONCLUSIONS

In the experimental fields of the FGRDS Voineşti, in the period 2004 -2012 we obtained a valuable biological material at the apple tree with a great genetic variability, which will permit the selection of some elites, which will become breeds or genitors for the future improvement works.

For the increase of the genetic variability at the apple tree, we performed t 25 hybrid combinations, to which we added 6 genitors, from which we used the seeds, obtained by natural polliniation.

From the hybrid series realized in the period 2004–2012, we obtained an initial improvement material, with a great genetic diversity, composed of 6,289 hybrid apple tree seedlings, of which we selected elites with perspective, with characteristics superior to the genitors used in the improvement – and even to the apple tree breeds existing în culture.

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PECULIARITIES OF GROWTH AND FRUITFULNESS OF APPLE CULTIVARS WITH GENETIC RESISTANCE TO DISEASES GROWN UNDER HIGH DENSITY SYSTEM

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Abstract

The researches performed at the Research Station for Fruit Growing Voineşti, in the period 2007-2012, had as object of study 13 disease-resistant apple tree cultivars in a high density system, grafted on M 9 rootstock, respectively: 'Ariwa', 'Golden Lasa', 'Goldrush', 'Enterprise', 'Inedit', 'Iris', 'Luca', 'Real', 'Rebra', 'Redix', 'Remar', 'Saturn', 'Voinicel', comparative with the 'Jonathan' cultivar, considered as control. The trees were planted at a distance of 4 x 1m (2.500 trees/Ha), spindle crown shape. With high production potential remarked the disease-resistant apple cultivars 'Ariwa', 'Remar', 'Inedit' and 'Saturn', which in the years 4 – 6 after planting realized over 29 t/ha. In the same conditions, the apple tree cultivar 'Real', 'Voinicel', 'Luca' and 'Iris' realized between 23,2 t/ha and 25,5 t/ha. The promotion of the high density apple tree system, in which disease-resistant apple tree cultivars are previewed, represents a modality for periodical and rapid replacement of the assortments, leading to the identification of new modern technologies in obtaining productions adapted to the requirements of the European quality standards.

Key words: *high density system, disease-resistant cultivars, productivity, fruits quality*

INTRODUCTION

The modern apple tree culture systems, with rapid fruit bearing start and short exploitation duration, represent a modality for the periodical and rapid replacement of the assortments, through this being encouraged the introduction of modern technics and ideas in obtaining of productions adapted to the exigences of the European quality standards (Comanescu, 2012).

On the European level, they generalized the use of the reduced vigour graft bearers (M9), with tree sustaining trellis and irrigation system, covering the orcharsds with an anti-hail net (Eremia, 2007). In the high density fruit trees exploitaion in France, Italy, Germany, Spain, Switzerland etc., with densities of 2,500 – 3,000 trees/ha, they obtain remarkable performances, concretized by productions of 40 – 60 t/ha.

The rersearches performed at the Research Station for Fruit Growing Voineşti Dambovita in the period 2007 – 2012, were aimed at the increase of the competity, corresponding to the principles of the enduring development and of the food security, concretized in the promotion of a high density apple tree culture system, in wich are previewed elements specific to the Romanian cultivars, comparative with the foreign ones, wich will lead in short time to the increase of the productive performances and economical efficiency, as well as immediate profitability, simultaneously with the implementation at private producers (Petre et al, 2010).

MATERIALS AND METHODS

The researches, conducted in the 2007 – 2012 period, at the Research Station for Fruit Growing Voineşti, had in view the establishing of an apple tree assortement, destined to the biological production, cultivated in the ecopedoclimatical conditions of the Voineşti area, being studied 13 genetic disease-resistant apple tree cultivars of local and foreign origin, respectively: 'Ariwa', 'Golden Lasa', 'Goldrush', 'Enterprise', 'Inedit', 'Iris', 'Luca', 'Real', 'Rebra', 'Redix', 'Remar', 'Saturn', 'Voinicel', comparative to the cultivar Ionathan, chosen as control. All cultivars were grafted on the rootstock M9.

The trees were planted at the distance of de 4 x 1 m (2500 trees/ha), spindle crown shape.

The soil of the experimental plot was fallow on the interval and maintained clean of weeds on the tree row. It is a brown eumezobazic, weakly preudogleizat, with clayish texture, with a weakly acid pH (5,7–5,9). The content in humus is medium at the surface (2,0–2,9%), medium supplied with nitrogen and weakly supplied with phosphorus and potassium.

Only 6–8 treatments were applied (insecticides).

The other works were performed according to the technology specific to the high density apple tree orchards.

At the apple tree assortment used at setting up the orchard, we followed up the vegetative tree growth, the fruit bearing precocity, the production levels, the fruits quality and other culture aspects, which represent factors to be taken into account at the promotion in culture of the high density system apple tree orchards.

RESULTS AND DISCUSSIONS

The growing vigour in the 6th leaf of the trees, cultivated in the high density system, when the growth potential is well differentiated, shows us that between the apple tree cultivars appear significant differences, regardig the trunk thickness growth, the height and the thickness of the fructiferous fence.

The trunk is one of the elements, which characterizes the tree vigour and it is always analyzed and correlated with a series of other biometrical processes and indices.

The values regarding the trunk thickness, registered in the year 5 from planting, are presented in Table 1.

Table 1. Trunk thickness growth in the year 6 from planting, at the genetic disease-resistant apple tree varieties, cultivated in the high density system (year 2012)

No.	Cultivar/rootstock	Diameter in the 6th leaf, 2012 (mm)	Medium growth inrease (mm)	Trunk section surface in the 6th leaf, 2012 (cm^2)	Differences ± to control (cm^2)	Significance
1	Ionathan/ M9 (C)	45,69	6,12	16,39	-	-
2	Ariwa/ M9	44,26	4,95	15,38	- 1,01	N
3	Golden Lasa/ M9	56,17	6,10	24,78	+ 8,39	***
4	Goldrush/ M9	38,52	3,95	11,65	- 4,74	000
5	Enterprise/ M9	59,28	6,75	27,60	+ 11,21	***
6	Inedit/ M9	47,70	5,35	17,87	+ 1,48	N
7	Iris/ M9	48,95	5,95	18,82	+ 2,43	**
8	Luca/ M9	58,03	7,35	26,45	+ 10,06	***
9	Real/ M9	44,50	5,30	15,55	- 0,84	N
10	Rebra/ M9	53,33	6,45	22,34	+ 5,95	***
11	Redix/ M9	55,83	7,65	24,48	+ 8,09	***
12	Remar/ M9	56,55	7,80	25,12	+ 8,73	***
13	Saturn/ M9	43,00	5,30	14,52	- 1,87	0
14	Voinicel/ M9	50,00	5,10	20,43	+ 4,04	***

DL 5% = 1,651 cm^2; DL 1% = 2,22 cm^2; DL 0,1% = 2,97 cm^2

Grafted on M9 rootstock, the most vigorous, resistant apple tree cultivars, cultivated in the high density sistem were: 'Enterprise' (58,28 mm), 'Luca' (58,03 mm), 'Remar' (56,55 mm), 'Golden Lasa' (56,17 mm), 'Redix' (55,83 mm) and 'Rebra' (53,33 mm).

Values of the trunk thickness, comprised between 40 and 50 mm, registered the apple tree cultivars: 'Saturn' (43,00 mm), 'Ariwa' (44,26 mm), 'Real' (44,50 mm), 'Inedit' (47,70 mm), 'Iris' (48,95 mm) and 'Voinicel' (50,00 mm).

Values less than 40 mm registered the cultivar Goldrush (38,52 mm).

At the Ionathan cultivar (control), the trunk diameter in the 6th leaf had an increase of 45,69 mm.

The medium growth increase shows values comprised between 3,95 mm at the 'Goldrush' cultivar and 7,88 mm at the 'Remar' cultivar, both cultivars grafted on M9.

The trunk vigour in the 6th leaf, represented by the trunk section surface, registers extreme values, comprised between 11,65 cm^2 at the 'Goldrush' cultivar and 27,60 cm^2 at 'Enterprise'.

The data, statistically analyzed as compared to the Ionathan cultivar, taken as control, point out very significant positive differences at the cultivars 'Golden Lasa', 'Enterprise', 'Luca', 'Rebra', 'Redix', 'Remar' and 'Voinicel'. Distinctive significant positive differences were assured by the 'Iris' cultivar – and very significant negative differences by the 'Goldrush' cultivar.

Vigour with unsignificant differences, as compared to the level of the Ionathan cultivar, have the cultivars 'Ariwa', 'Real' and 'Inedit'.

The tree dimensions and the crown volume registered in the 6th leaf are presents in Table 2.

The trees height registers the values comprised between 195 cm at the 'Goldrush' cultivar and 280 cm 'Luca' cultivar. The greatest trees height values are registered at the 'Real', 'Enterprise', 'Rebra', 'Redix', 'Golden Lasa', over 290 cm in height. The Ionathan cultivar riched 210 cm.

The fructiferous fence thickness was comprised between 110 and 165 cm.

The crown volume per tree, in the 6th leaf, oscillated between 1,82 cm/tree at the 'Goldrush' cultivar and 4,12 cm/tree at 'Luca', comparative to Ionathan, where 2,52 cm/tree were registred.

Calculated on the surface unit, the crown volume registers values from 4450 mc/ha at the 'Goldrush' cultivar – to 10300 mc/ha at the 'Luca' cultivar.

Lower values of the crown volume were registered at the cultivars: 'Goldrush' (4450 mc/ha), 'Inedit' (6300 mc/ha) and 'Saturn' (6500 mc/ha). At the other cultivars, the crown volume, calculated on one hectar, approaches the value of 6300 mc, registred at the Ionathan cultivar, taken as control, with unsignificant differences at the majority of cultivars. The statistical calculation registers very significant positive differences only at the 'Luca' cultivar, distinctive significant positive difference at the 'Enterprise' cultivar and significant positive differences at 'Golden Lasa', 'Real' and 'Rebra' cultivar, grafted on M9 rootstock.

Table 2. Tree crown dimensions and volume at the studied apple tree cultivars in the 6th leaf (2012)

No.	Cultivar/rootstock	Tree Dimensions (cm)		Crown Volume (cm)			
		Height	Fruits fence thickness	Per tree	Differences ± to control	Signifi-cance	Per Ha
1	Ionathan/ M9 (Mt)	210	140	2,52	6.300	-	
2	Ariwa/ M9	240	140	2,94	7.350	+ 1.050	N
3	Golden Lasa/ M9	260	150	3,45	8.625	+ 2.325	*
4	Goldrush/ M9	195	110	1,82	4.450	- 1.850	0
5	Enterprise/ M9	265	160	3,76	9.400	+ 3.100	**
6	Inedit/ M9	210	140	2,52	6.300	0	-
7	Iris/ M9	230	140	2,80	7.000	+ 700	N
8	Luca/ M9	280	165	4,12	10.300	+ 4.00	***
9	Real/ M9	270	140	3,36	8.400	+ 2.100	*
10	Rebra/ M9	265	140	3,29	8.225	+ 1.925	*
11	Redix/ M9	260	136	3,10	7.750	+ 1.450	N
12	Remar/ M9	245	145	3,12	7.800	+ 1.500	N
13	Saturn/ M9	230	130	2,60	6.500	+ 200	N
14	Voinicel/ M9	210	140	2,52	6.300	0	-

DL 5% = 1.729 mc; DL 1% = 2.336 mc; DL 0,1% = 3.117 mc

The productivity of the genetic disease-resistant apple trees, was pointed out by annual registering the apple production at cultivar level.

Among the appple tree cultivars in the apple tree high density system, the 'Iris' cultivar, grafted on M9 rootstock, has the tendency to bear fruits already from the 2nd leaf.

From the year 3 after planting, the 13 apple tree cultivars with genetic resistance to diseases and Ionathan/ M9 realized satisfactory productions, having in view that we used at planting seedling material from the field II of the nursery, without anticipations, as support of the fruit bearing buds differentiation – already from the planting year.

Table 3. Fruits production realized at the apple tree cultivars with genetic rezistance to diseases, cultivated in the high density system (2500 trees/ha)

No.	Cultivar/ rootstock	Production obtained in the year (t/ha)				Average of the years 4 – 6 (t/ha)	Differences ± to control	Significance
		3 2009	4 2010	5 2011	6 2012			
1	Ionathan/M9 (C)	3,5	15,8	24,0	21,5	21,4	-	
2	Ariwa/M9	5,5	21,9	42,0	39,0	34,3	+ 12,9	***
3	Golden Lasa/M9	5,5	19,4	29,8	19,5	22,9	+ 1,5	N
4	Goldrush/M9	5,0	20,8	29,5	17,0	22,4	+ 1,0	N
5	Enterprise/M9	1,3	12,5	28,5	31,5	24,2	+ 2,8	**
6	Inedit/M9	6,5	18,5	42,2	26,2	29,0	+ 7,6	***
7	Iris/M9	6,3	20,4	33,0	23,0	25,5	+ 4,1	***
8	Luca/M9	2,8	16,4	35,3	20,0	23,9	+ 2,5	**
9	Real/M9	7,5	18,8	29,3	27,0	25,0	+ 3,6	***
10	Rebra/M9	3,0	10,7	31,0	25,3	22,3	0 0,9	N
11	Redix/M9	2,8	16,6	23,0	21,0	20,2	- 1,2	N
12	Remar/M9	3,8	19,8	32,0	38,0	29,9	+ 8,5	***
13	Saturn/M9	5,8	21,6	42,5	31,5	31,9	+ 10,5	***
14	Voinicel/M9	4,3	18,6	29,8	21,3	23,2	+ 1,8	*

DL 5% = 1,67 t/ha; DL 1% = 2,25 t/ha; DL 0,1% = 3,01 t/ha

From the data presented in Table 3, rezults that the studied apple tree assortement, the Romanian cultivars, early and productive ones were: 'Real' (7.5 t/ha), 'Inedit' (6.5 t/ha), 'Iris' (6.3 t/ha), 'Remar' (3.8 t/ha).

From the foreign cultivars, we point out, with their productions in the year 3 after planting: 'Saturn' (5.8 t/ha), 'Ariwa' (5.5 t/ha), 'Golden Lasa' (5.5 t/ha), 'Goldrush' (5.0 t/ha).

At Ionathan cultivar, we obtained in the 3rd leaf, 3.5 t/ha.

Analyzing the medium production of the years 4 - 6 from planting, we observe that from the 13 apple tree cultivars with genetic resistance to diseases, cultivated in the high density system, the most productive are the cultivars: 'Ariwa', 'Remar', 'Inedit' and 'Saturn', at which we obtained over 29 t/ha. Appreciated with high potential are also the apple tree cultivars, which registred medium productions of over 23 t/ha, as follows: 'Real' (25,0 t/ha), 'Voinicel' (23,2 t/ha), 'Luca' (23,9 t/ha), 'Iris' (25,5 t/ha). The Ionathan cultivar, registered as a 3 years average a production of 21,4 t/ha.

The statistically calculated data confirm very significant positive differences, as compared to Ionathan, at the majority of the studied genetic disease-resistant apple tree cultivars.

The studied apple tree cultivars with genetic disease-resistance, cultivated in the high density system, manifested a very good resistance against scab (*Venturia inaequalis)* and an increased resistance degree against mildew (*Podosphaera leucotricha*), with unsignificant values, comprised between 0 and 6.5%.

The medium value of the fruits weight at cultivar level, in the perioad 2009–2011, shows that the 'Golden Lasa', 'Enterprise', 'Luca', 'Real', 'Rebra', 'Redix', 'Remar' and 'Saturn' cultivars have the potential to assure the suitable fruits size, which shall compete on the market, the fruits framing in the big fruits class – and the other cultivars frame in the medium fruits group.

The tree assortments are in a permanent change, the place of the cultivars, presenting inferior commercial qualities, being taken by the new breeded cultivars, which correspond to a higher degree to the consumers' continuously increasing requirements.

The experimented apple tree cultivars, can cover a great part of the consumption season, besides some of the genetic disease-resistant cultivars, muliplied in culture, already known and apreciated on the market by the consumers.

CONCLUSIONS

The growing in the 6th leaf, cultivated in the high density, sistem, represented by the trunk section surface, registers values comprised between, 11,65 cm^2 at the 'Goldrush' cultivar and 27,60 cm^2 at 'Enterprise'.

The crown volume, calculated on the surface unit, oscillated between 4450 cm^2/ha, at the 'Goldrush' cultivar and 10300 cm/ha at the Luca cultivar.
The highest production potential on the surface unit was realized in the years 4 - 6 from planting at 'Ariwa', 'Inedit' and 'Saturn', with over 29 t/ha, but also at the 'Real', 'Voinicel', 'Luca' and 'Iris' apple tree cultivars, at which we realize between 23,2 t/ha and 25,5 t/ha.
The genetic desease-reistant apple tree cultivars studied in the high density system, manifested a very good resistance to scab (*Venturia inaequalis*) and an increased rezistance degree to mildew (*Podosphaera leucotricha*), with unsignifiacnt values, comprised between 0 and 6.5%.
The apple tree cultivars, recently breeded at R.S.F.G. Voineşti, and also other studied foreign cultivars, cover a great part of the consumption season, besides some genetic disease-resistant cultivars already known and appreciated on the market by the consumers – these framing in differently in the conveyer recommended for the Dâmboviţa tree growing region.

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BIOTECHNOLOGICAL PRODUCING OF NATURAL FERTILIZERS THROUGH MICROBIAL COMPOSTING OF FRUIT WASTES

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Abstract

In Romania, to protect the natural environment and save its biodiversity there is an urgent need to solve a lot of serious damages caused by the pollution effects induced through discharging of fruit wastes into natural aquatic ecosystems (streams, rivers, lakes), in huge amounts, after their fermentation and alcohol extraction made in private horticultural farms. The efficient valorising of such organic compounds, represented by many derived wastes from fruit processing, through biotechnological means was establish to be the main aim of certain research experiments whose results are presented in this paper. There were carried out laboratory works to test the optimal needs of bacterial and fungal pure cultures to grow inside different marc made of apple, cherry and plum wastes (chemical composition, temperature, pH, oxygen/carbon dioxide concentration). In this respect, there were used pure bacterial cultures of Bacillus genus as well as the fungal ones belonging to species of Pleurotus for microbial transformation of different fruit wastes. The biotechnology of microbial composting was applied by using a laboratory-scale bioreactor of 15 L working volume. The submerged fermentations of different fruit wastes were set up for the following parameters: constant temperature, 23°C; agitation speed, 80-100 rev. min^{-1}; pH level, 5.7–6.0 units; dissolved oxygen tension within the range of 30-70%. After a period of submerged fermentation lasting between 140- 230 h, there was produced fermented composts containing the microbial biomass that was developed through biochemical transforming of marc into natural fertilizers. By using pure cultures of bacterial and fungal species, in strict hygienic conditions and permanent control of microbial processes induced for fruit waste composting it was ensured the optimum performance of tested biotechnological proceedings to increase the biochemical transformations of marc as compounds with a high content in proteins and other organic compounds that are very useful as natural fertilizers of agricultural crops.

Key words: *bacteria, composting, fertilizers, fruit wastes, mushrooms, submerged fermentation*

INTRODUCTION

It is well known that, annually, huge amounts of wastes and residues are produced in orchards during agro-industrial activities of fruit processing. The reuse and recycling of all fruit wastes could allow self-sustainable processes through the natural fertilizers that are produced by microbial conversion of cellulose into protein-rich biomass with beneficial effect on soil as well as efficient environmental protection tool (Bae *et al.*, 2000; Verstraeate and Top, 1992; Chahal, 1994; Moser, 1994).
The present work reports on the preliminary results carried out to study the bioconversion of cellulose wastes from fruit processing agro-industry into protein biomass by using pure cultures of bacteria and edible mushrooms (Carlile and Watkinson, 1996; Wainwright, 1992). The main aim of this study was to establish the optimal submerged culture conditions for controlled microbial co-fermentation in order to improve the bioconversion of cellulose from fruit wastes like apple and plum wastes into organic biomass with high protein content to be used as natural fertilizers in horticulture.

MATERIALS AND METHODS

Microbial strains and culture media

During the experiments there were tested two microbial strains of cellulolytic bacterial species, namely *Bacillus subtilis* as well as mushroom species *Pleurotus ostreatus*. These

two microbial species were used as pure cultures from the culture collection of microorganisms belonging to the University of Pitesti (Petre and Petre, 2008).
The stock cultures of bacterial species *Bacillus subtilis* were kept in viable shape on 0.5 LB agar plate (1% tryptone, 0.5% yeast extract, 0.5% NaCl and agar-agar 1.5%) and the mushroom species of *Pleurotus ostreatus* were maintained on malt-extract agar (MEA) slants. The culture medium composition for microbial conversion and protein synthesis was made of apple wastes 50%, previously treated by mixing with wheat bran 10%, barley bran 5%, d-glucose 5% and hydrated with pure water 30%. This was the first variant of culture substrate for mushroom growing (substrate 1). The second variant composition of culture substrate was prepared from plum wastes 50% improved by adding barley bran 10%, wheat bran 5%, d-glucose 5% and tap water 30% (substrate 2). The two variants of culture substrates were used in experiments for growing both monocultures and co-cultures of *B. subtilis* and *P. ostreatus*. In this respect, the optimal temperatures during the growth of bacterial and mycelial co-cultures were registered between 23–25 °C corresponding to initial pH levels of 4.5–6.0. The agitation speed was tested in the range of 30-90 rpm (Beguin and Aubert, 1994).

Methods used in experiments

The microbial strains of *B. subtilis* and *P. ostreatus* were used in pairs as well as separately to compare the efficiency of their biological potential in bioconversion of fruit wastes into protein biomass (Petre and Petre, 2013a).
These strains were tested both in monocultures and co-cultures for growing on two variants of culture substrates made of apple and plum wastes mixed with cereal wastes. The medium composition, pH levels, incubation temperature, agitation rate, inoculum age as well as inoculum volume during the submerged co-fermentation were registered as significant physical and chemical factors that could influence the bioconversion of fruit wastes used as growth substrates into protein biomass as well as microbial biomass formation (Ropars *et al.*, 1992; Chahal, 1994; Lamar *et al.*, 1992).

The experiments concerning the testing of microbial strains with the best potential of bioconversion of cellulosic wastes from fruit processing were carried out by using a laboratory scale bioreactor (Figure 1).

Figure 1. General view of the laboratory-scale bioreactor

The design of this bioreactor incorporates a device to keep the constant temperature, inoculum reservoir, sterile air supply in aerobic processes, culture vessel as well as an automation panel for bioprocess monitoring and management (Petre and Petre, 2013b).

RESULTS AND DISCUSSION

Bioconversion of apple and plum wastes requires a suitable environment for growth of pure bacterial and fungal cultures, in order to increase efficiency of submerged fermentation made by mono- and co-cultures of *B. subtilis* and *P. ostreatus* (Petre *et al.*, 2012; Smith, 1998; Stamets, 1993; Leahy and Colwell, 1990). The content of reducing sugars was determined by Kubicek technique and the total nitrogen content was analyzed by Kjeldahl method (Chahal and Hachey, 1990; Glazebrook *et al.*, 1992). The experimental data determined as total reducing sugars contents (Kubicek *et al.*, 1981) were correlated by complementary investigations with those values of dry weight loss measurements of fruit wastes bioconversion, for both mono- and co-cultures of *B. subtilis* and *P. ostreatus*.
All registered data regarding the evolution of total reducing sugars as well as total nitrogen contents during bioconversion of apple and

plum wastes into protein biomass by using monocultures and co-cultures of *B. subtilis* and *P. ostreatus* are presented in Tables 1 and 2. The evolution of dry weight loss of the same fruit wastes used as substrates for microbial cultures is shown in Table 3.

Table 1. Total reducing sugars evolution during bioconversion of apple and plum wastes into protein biomass by using monocultures and co-cultures of *Bacillus subtilis* and *Pleurotus ostreatus*

Culture time (h)	Total reducing sugars (mg g^{-1})					
	Bacillus subtilis (Monoculture)		*Pleurotus ostreatus* (Monoculture)		*B. subtilis – P. ostreatus* (Co-cultures)	
	Substrate 1	Substrate 2	Substrate 1	Substrate 2	Substrate 1	Substrate 2
72	2.10	2.80	4.50	6.90	9.30	12.80
144	4.10	4.90	5.80	8.10	11.10	15.50
216	5.70	6.80	7.90	10.40	14.90	18.30
288	7.80	8.10	10.70	12.80	18.30	21.80
360	9.50	10.90	14.10	15.50	21.90	25.30
432	10.70	12.50	16.30	18.20	24.50	27.50
504	11.45	15.30	19.70	21.50	26.30	30.10
576	12.50	17.70	21.80	23.30	28.80	32.50
648	14.80	19.30	23.50	25.80	30.10	33.90
720	15.10	20.50	25.10	28.30	30.50	35.10

All data are representative of three replicated determinations.

Table 2. Total nitrogen content evolution during bioconversion of apple and plum wastes into protein biomass by using monocultures and co-cultures of *Bacillus subtilis* and *Pleurotus ostreatus*

Culture time (h)	Total nitrogen content of fungal protein biomass (g % dry weight)					
	Bacillus subtilis (Monoculture)		*Pleurotus ostreatus* (Monoculture)		*B. subtilis – P. ostreatus* (Co-cultures)	
	Substrate 1	Substrate 2	Substrate 1	Substrate 2	Substrate 1	Substrate 2
72	3.50	3.90	4.50	5.10	7.90	9.50
144	4.10	4.75	5.80	6.40	9.30	12.10
216	5.70	6.55	7.70	8.50	14.10	15.80
288	7.80	7.90	9.80	10.10	15.80	18.10
360	9.50	9.80	12.10	12.50	18.30	21.90
432	10.70	11.10	14.00	14.40	21.50	23.30
504	11.45	12.70	16.70	17.30	23.60	25.70
576	12.10	13.50	18.50	20.10	25.90	27.10
648	12.80	14.30	20.80	21.80	27.20	28.90
720	12.90	14.50	21.30	23.20	28.10	30.30

All data are representative of three replicated determinations.

Table 3. The evolution of dry weight loss of apple and plum wastes used as substrates for *Bacillus subtilis* and *Pleurotus ostreatus* growing as monocultures and co-cultures

Culture time (h)	Dry weight loss (g %)					
	Bacillus subtilis (Monoculture)		*Pleurotus ostreatus* (Monoculture)		*B. subtilis – P. ostreatus* (Co-cultures)	
	Substrate 1	Substrate 2	Substrate 1	Substrate 2	Substrate 1	Substrate 2
72	2.50	4.30	5.10	6.40	7.30	10.40
144	3.10	5.50	5.90	7.00	8.50	12.80
216	3.90	6.70	6.70	8.50	9.70	14.10
288	4.80	7.90	7.90	9.40	10.80	15.30
360	5.50	8.80	8.80	10.50	12.50	16.50
432	6.40	9.50	10.90	12.80	14.80	18.30
504	7.30	10.90	12.70	14.30	16.30	20.10
576	8.50	12.10	13.50	15.90	17.70	21.80
648	9.30	13.70	14.90	17.40	18.50	23.70
720	10.10	14.30	15.80	18.50	20.80	25.50

All data are representative of three replicated determinations.

Finally, after 720 h of submerged fermentation the protein biomass obtained through bioconversion of apple and plum wastes by using the co-cultures of *B. subtilis* and *P. ostreatus* was collected from the culture vessel of laboratory scale bioreactor as it is shown in Figure 2 and Figure 3.

Figure 2. Protein biomass obtained through the bioconversion of apple wastes by using the co-cultures of *B. subtilis* and *P. ostreatus*

Figure 3. Protein biomass obtained through the bioconversion of plum wastes by using the co-cultures of *B. subtilis* and *P. ostreatus*

The amounts of protein biomass obtained through bioconversion of apple and plum wastes by using co-cultures of *B. subtilis* and *P. ostreatus* contain between 28.1 and 30.3 g % dry weight after 720 h of submerged fermentation in the culture vessel of the laboratory-scale bioreactor.

CONCLUSIONS

The microbial strains of *B. subtilis* and *P. ostreatus* were used in pairs as well as separately to compare the efficiency of their biological potential in bioconversion of fruit wastes into protein biomass.

These strains were tested both in monocultures and co-cultures for growing on two variants of culture substrates made of apple and plum wastes mixed with cereal wastes.

The experiments concerning the testing of microbial strains with the best potential of bioconversion of cellulosic wastes from fruit processing were carried out by using a laboratory scale bioreactor as controlled biotechnological system.

The optimal temperatures for both bacteria and mycelia cultures to produce microbial biomass through controlled submerged fermentation as mono- and co-cultures, were registered between 23–25 °C, corresponding to initial pH levels of 4.5–6.0 and the agitation speed was tested in the range of 30-90 rpm.

The registered results revealed an increasing of reducing sugars correlated with an increasing of protein content analysed as total nitrogen for the microbial biomass of co-cultures, in comparison with the control samples represented by the monocultures of the same bacterial and fungal species used in experiments

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IMPACT OF FOLIAR FERTILIZER ON PHYSIOLOGICAL COMPOUNDS IN DIFFERENT APRICOT VARIETIES

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Abstract

In the last decade applying of nutritive elements by foliar spraying became a usual practice for many crops, with benefit part in balancing of plant nutrition. Also, foliar fertilization have a practical and economical importance and have a low risk for environmental pollution. Implication in physiological processes, and in general in the plant metabolism of nutritive substances adsorbed by the leaves have an important role in increasing the adaptative capacity of trees to the stress conditions and therefore for growth production. The influence of foliar fertilizer on physiological and biochemical parameters of different apricot varieties was studied during two seasons at experimental orchard of Research Station Baneasa. Results indicated the increase of photosynthetic pigments content proved by a better function of photosynthetic apparatus. It also was found that polyphenolic compounds increased, which denotes the activation of tree defence mechanism for a better stress resistance.

Key words: *assimilation pigments, polyphenolic compounds, apricot leaves, enzymes*

INTRODUCTION

In the last decade, biological agriculture has the goal to use natural products with benefit part in balancing of horticultural crop nutrition for a better adaptation at different environmental conditions with increasing of economical efficiency by obtaining a significant increase of the crops quality.
From all this products, foliar fertilizers have a low risk for medium pollution and have a distinguished action in adjustment of the nutrient circuit among the agro ecosystem components.
In the world, foliar fertilization is an usually practice used in fruit growing for obtaining fruits of superior quality, high quantitative effects over fruits production and for providing a high resistance of trees to pest and diseases (Chamel, 1990; Bertschinger et al., 1999).
In Romania, it were tested a large range of foliar fertilizers for cultivated plants and among fruit trees species only for apple, plum, sour cherry (Borlan, 1995; Platon and Soare, 2002; Rusu et al., 2002; Dinu et al., 2009; Bochis and Ropan, 2011) with special regarding on tree growing and increasing of fruit yield.
Concerning a distinct interest for using foliar fertilization at fruit trees, our study was conducted to determine physiological and biochemical parameters at different apricot cultivars with Cropmax treatment, an ecological product.

MATERIALS AND METHODS

We take in study 33 apricot hybrids and three standard cultivars ('Dacia', 'Excelsior', 'Litoral') with the different ripening period. We determined in the leaves assimilating pigments, polyphenolic compounds, proteins and peroxidase. Also for the same hybrids it was evaluated the fruits quality through analyses of soluble solids content and titratable acidity and ratio among these parameters. In that sense it had been used specific methods for each parameters. To extract pigments, leaves of hybrids studied were ground with a mortar and pestle in acetone, with calcium carbonate added to prevent pheophytinization. Homogenates were centrifugate for 3-4 minutes at 3000 g.

The resulting extracts were immediately assayed. Absorbance of the clear extract at 645nm, 663nm and at 450nm were recorded and concentration of chlorophylls 'a' and 'b', carotenoids, chlorophyll a/b ratio and chlorophylls a+b/carotene+xantophylls ratio were computed after Arnon (1949) equations.

Quantification of total polyphenols was realized using the Folin Ciocalteu colorimetric method (Singleton and Rossi, 1965) and the results were expressed in mg/g fresh weight.

Determination of total dry matter was conducted by drying at 105°C until constant mass.

Assessment of proteins was realized by Bradford's method using bovine serum albumin as standard.

Peroxidase activity was determined by Bergmeyer's method based on the quantification of tetraguaiacol formation, spectrophotometrically at 436 nm and at 25°C.

To derive the chemical components, fruits were cleaned from their skin and core, sliced and homogenized; the obtained mixture was centrifuged and the supernatant used for analyzes.

For the soluble solid content it was used Abbe refractometer with temperature correction derive. Total acidity was determined by titrating each sample with solution 0.1 N NaOH until pH 8.1. The ratio between the soluble solids and the total acidity reflects the fruit taste feature.

RESULTS AND DISSCUSION

Determination of physiological and biochemical compounds indicated a differences between the leaves of treated hybrids with foliar fertilizer and samples untreated. For all studied hybrids we can observe a little increase of dry matter content in leaves for treated hybrids .

Also, for polyphenolic compounds it was observed a significative increase of their amount in the leaves of fertilized trees, which means a better resistance at pathogens because of their antioxidative character.

Protein content is substantial rising at hybrids fertilized with Cropmax. Among selection with early maturation it was relevant 77.23.39 BIV (952 mg/g dry weight) and 77.4.73 BV (948mg/g d.w), for selection with medium maturation can be noticed 82.28.62 BIV (888mg/g d.w), 82.32.29 BIII (950 mg/g d.w), 85.1.96BIII (809 mg/g d.w.) and for hybrids with tardive maturation distinguish 82.4.41 BIV (811 mg /g d.w.) and 82.15.10 BIV (613 mg/g d.w.).

Table 1. Variation of physiological and biochemical parameters at apricot variants without foliar fertilizer

Sample	Dry matter g%	Chlorophyll a/b	Chlorophyll a+b / carotene+ xantophyll	Polyphenols mg/g f.w.	Proteins mg/g d. w.	Peroxidase µmol/mg proteins
DACIA(control)	40.14	4.34	4.37	1.89	359.97	10.18×10^{-3}
77.23.39 BIV	36.53	3.01	6.48	2.54	736.38	6.32×10^{-3}
77.3.60 BIV	44.14	3.17	5.65	2.01	583.37	6.98×10^{-3}
77.4.73 BV	43.78	4.8	5.01	2.28	211.76	12.07×10^{-3}
82.6.62 BIV	45.85	5.26	3.97	2.21	460.06	0.67×10^{-3}
85.4.104 BIII	38.11	3.24	7.29	2.88	530.2	1.08×10^{-3}
EXCELSIOR (control)	45.11	3.9	6.94	2.55	582.57	0.45×10^{-3}
82.12.91 BIV	34.56	5.78	3.91	2.38	281.88	0.41×10^{-3}
85.5.100 BIII	39.08	3.18	4.13	2.11	108.67	1.88×10^{-3}
82.28.62 BIV	35.57	3.09	3.02	2.35	770.94	1.89×10^{-3}
85.18.5 BIII	36.59	5.19	4.60	2.6	552.22	0.198×10^{-3}
85.11.85 BIII	33.35	2.51	10.66	2.46	834.63	2.41×10^{-3}
85.11.95 BIII	37.58	2.44	8.66	2.67	589.78	0.40×10^{-3}
85.1.96 BIII	38.21	3.07	7.86	2.50	294.46	0.60×10^{-3}
82.32.29 BIII	41.43	4.02	5.54	1.70	517.55	0.68×10^{-3}
82.8.26 BIV	36.08	3.77	4.77	3.64	362.8	0.3×10^{-3}
85.4.95 BIII	44.09	5.02	5.26	2.09	408.09	2.7×10^{-3}
LITORAL (control)	45.01	4.16	5.42	1.56	174.11	2.42×10^{-3}
82.15.10 BIV	42.64	4.51	4.08	2.27	352.97	2.19×10^{-3}
82.12.7 BIV	37.93	4.20	5.32	2.20	264.55	3.86×10^{-3}
82.19.3 BIV	41.67	3.86	5.42	2.76	278.3	0.6×10^{-3}
82.15.48 BIV	47.77	3.32	6.41	2.89	238.45	1.58×10^{-3}
82.4.41 BIV	38.04	3.9	6.09	3.04	271.00	1.45×10^{-3}

The increasing of proteins quantity could be explained by the free amino acids content of foliar fertilizer which had a positive influence for protein synthesis at all apricot cultivars studied.

In the case of chlorophyll pigments we can observe at all hybrids analyzed a little decrease of chlorophyll content, this can be observed from ratio chlorophyll a/b. But clf. a+b/carotina+xantophyll ratio vary randomly at all hybrids.

We can concluded that in both cases (chlorophylls and carotenoids) the foliar fertilizer determine a different response reaction at almost all apricot hybrids studied but it was registered an exception in the positive sense: from early maturation selections were remarked 77.23.39BIV with a ratio of 3.09 and 85.4.104 with the ratio of 3.9; from the elites with medium maturation 82.28.62 BIV with the ratio clfa/clf b 3.31, 85.11.85 BIII (3.17) and 85.11.85 BIII (3.17) and 85.11.95BIII (3.15) are distinguished. Among hybrids with tardive maturation were remarked 82.15.10 BIV with ratio 4.83 and 82.15.48 BIV with ratio 3.43.

Peroxidase activity is, in general, higher at foliar fertilized hybrids with early and medium maturation, which means a better resistance of them at exogenous factors.

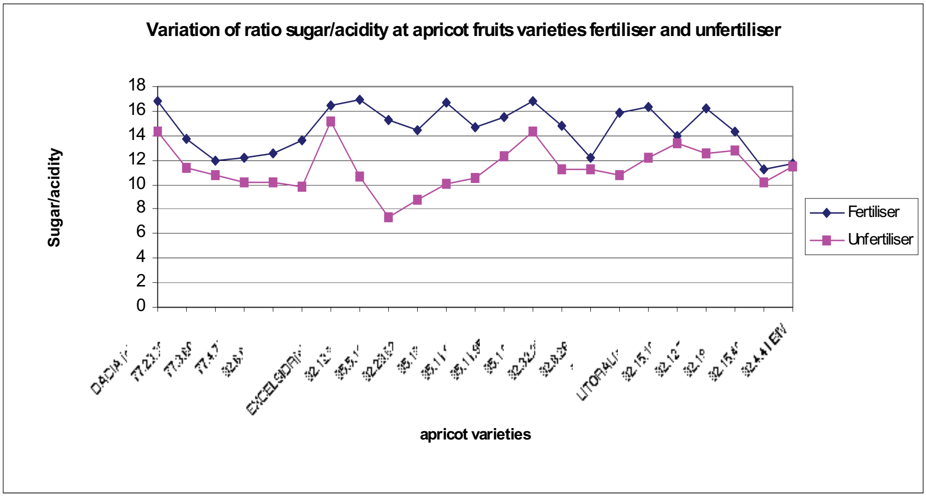
For fruit quality all hybrids have a positive response at foliar treatment by increase of soluble solids content and a little decrease of total acidity.

Table 2. Variation of physiological and biochemical parameters at apricot variants with foliar fertilizer

Sample	Dry matter g%	Chlorophyll a/b	Chlorophyll a+b / carotene+ xantophyll	Polyphenols mg/g f.w.	Proteins mg/g d. w.	Peroxidase µmol/mg proteins
DACIA(control)	43.48	2.29	4.71	3.44	929	9.67×10^{-3}
77.23.39 BIV	44.01	3.09	5.12	4.6	952	0.13×10^{-3}
77.3.60 BIV	41.23	2.47	5.54	2.83	770	0.16×10^{-3}
77.4.73 BV	42.87	2.30	4.13	3.52	948	0.27×10^{-3}
82.6.62 BIV	48.01	2.83	6.17	3.9	894	2.43×10^{-3}
85.4.104 BIII	38	3.90	4.77	3.71	756	6.06×10^{-3}
EXCELSIOR (control)	44.86	3.61	5.39	3.65	802	2.24×10^{-3}
82.12.91 BIV	32.47	2.35	6.39	2.65	410	15.9×10^{-3}
85.5.100 BIII	37.1	2.61	3.98	3.89	305	0.33×10^{-3}
82.28.62 BIV	46.18	3.31	4.67	3.23	888	0.15×10^{-3}
85.18.5 BIII	37.48	2.99	6.88	3.72	630	4.96×10^{-3}
85.11.85 BIII	43.25	3.17	6.22	3.67	840	9.29×10^{-3}
85.11.95 BIII	46.36	3.15	5.57	3.83	726	2.86×10^{-3}
85.1.96 BIII	39.4	3.09	5.99	3.31	809	5.39×10^{-3}
82.32.29 BIII	42.98	2.39	7.46	2.67	950	1.2×10^{-3}
82.8.26 BIV	47.13	2.36	4.4	4.03	781	0.62×10^{-3}
85.4.95 BIII	44.15	2.21	5.03	2.89	402	10.56×10^{-3}
LITORAL (control)	47.2	2.61	7.40	2.71	856	3.45×10^{-3}
82.15.10 BIV	46.88	4.83	7.44	2.63	613	9.99×10^{-3}
82.12.7 BIV	47.3	3.74	7.61	3.47	500	0.06×10^{-3}
82.19.3 BIV	37.4	3.94	4.09	3.58	453	0.99×10^{-3}
82.15.48 BIV	46.75	3.43	5.97	3.71	581	2.32×10^{-3}
82.4.41 BIV	49.6	2.86	10.56	3.9	811	2.27×10^{-3}

The ratio soluble solids/acidity indicated the ripening level of fruits. A value of this equal or higher than 8 for apricot fruit reflect the optimum maturity time.

From results obtaining at treated hybrids we can observe that ratio soluble solids/acidity varied between 11.25 at hybrid 82.15.48 BIV and 16.97 at 82.12.91 BIII which demand an equilibrate taste of them. As exception is the case of 82.12.4 BIV (16.22), 85.4.95 BIII (15.89), 85.11.95BIII (15.52), 85.18.5 BIII (16.73) and 82.12.91 that have a very sweet taste.



CONCLUSIONS

The foliar fertilizer tested have a significant effects in the mean of reaction response of apricot variants by studied parameters.

We can observe that all apricot hybrids studied had a positive response at foliar treatment with Cropmax from pathogen agents resistance point of view by increasing of polyphenolic compounds content and peroxidase activity from leaves.

Both polyphenolic compounds and peroxidase enzyme have an important role as part of defence mechanism of plant after microbial attack.

Foliar treatment determine a different reaction of apricot hybrids concerning chlorophyll and carotenoid pigments with a low decrease of them which is reflected by the ratio chlorophyll a /chlorophyll b.

Foliar fertiliser have a positive effects concerning apricot fruit quality emphasized by an increased ratio soluble solids/titratable acidity and a fresh fruit well-balanced taste.

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STUDY ON THE ACCUMULATION OF NUTRITIOUS SUBSTANCES IN GOOSEBERRY FRUITS

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Abstract

In the article it were presented the results of our investigations during 2011-2013 about the substances accumulation and fruit quality appreciation of the 10 gooseberry varieties introduced in Republic of Moldova on the new soil and climatic conditions.

Key words: *gooseberry, varieties, substances, fruits*

INTRODUCTION

Fruits and berries are particularly important in human nutrition as the richest source of biologically active substances. New cultivars must comply not only to commercial quality, taste and technology, but also with the increased content of precious substances including biologically active substances (Jdanova, 2002).
Presence of pectin substances in fruits of gooseberry (1.06 to 2.35%) attributes to them anti radiant qualities. However, the accumulation of pectin substances occurs until the technical maturity, and later on the extent of ripening of fruits, this process start reducing (Strelinikova, 1971).
Assessment criteria maturity apart from external qualities as: size, color, density and taste of fruits should be the content of soluble dry substances. The dry substances reach the maximum amount in a certain amount of time. The sudden rise of the dry substances further may be related to overripe and wilting berries as a result of reducing the amount of water in them (Franciuk; Strelinikova, 1983).

MATERIAL AND METHODS

Research conducted during the years 2011 - 2013 on the gooseberry cultivars studied in the Republic of Moldova allowed the appreciation for the quality and fruit weight, the amount of accumulated nutrients, according to the methods established for trees shrubs. Cultivars studied: 'Colobok', 'Sadko', 'Severny capitan', 'Smena', 'Slivovy', 'Captivator', 'Resistent de Cluj', 'Zenit', 'Somesh', 'Grushenka'.

RESULTS AND DISCUSSIONS

Studying the formation of fruit, evaluating the quality and accumulation of organic matter in their growth and maturation process determines the optimal terms of harvest and provides the ability to compare varieties by their qualities and chemical composition of the fruit.
Harvesting of gooseberry during biological maturity, allows increasing the yield and improving the quality of fruit from the account accumulation of substances soluble sugars and acidity decrease. Extension of harvesting period and transportation over long distances requires harvesting the fruit in the close biological maturity period when the accumulation of substances in fruits stabilizes.
Fruit mass increase occurs until reaching the biological maturity and therefore their harvest even in technical adulthood is lost about 30-40% of the harvest, which is 6.0 to 8.0 t/ha at harvest 20t/ha (Franciuk, Strelinikova 1983).
The content of vitamin C in gooseberry fruits is greater in a rainy summer than in one dry and over the years varies from 16.25 to 35.2 mg /%.
Biochemical characteristics of gooseberry fruit are of great importance in assessing and comparing cultivars (Sava, 2000). Knowledge of the chemical composition of gooseberries

allow correct assessment of cropping period output destination when substances accumulated reaches a high level. Chemical composition of gooseberry fruit for the studied varieties emphasizes their quality. Biochemical characteristics of gooseberry fruit are of great importance in assessing the quality of the berries and to the comparison of varieties. The results obtained on the nutrient content of the fruits of gooseberry are shown in Table 1.

Table 1. The amount of accumulated nutrients and weight of gooseberry fruits, years 2011-2013

Nr. Variety name	Fruit weight, g	Dry substances, %	The content of sugars, %	Titratable acidity, %	Tanning and coloring substances, mg %	Vitamin C, mg %	Coefficient sugar / acid
Colobok	2.57	13.29	10.57	2.42	90.07	30.87	4.67
Sadko	2.67	16.13	10.58	2.58	88.72	36.07	4.50
Severny capitan	1.33	16.22	10.61	338	120.56	25.08	3.13
Smena	2.63	14.80	9.39	2.68	69.28	27.13	3.53
Slivovy	1.70	16.49	10.61	3.02	93.54	28.49	4.22
Captivator	275	17.49	10.18	3.43	48.50	30.07	3.20
Resistent of Cluj	2.03	21.74	11.02	2.55	43.06	47.01	4.99
Zenit	1.47	19.89	12.52	2.50	27.81	49.79	4.98
Somesh	1.73	22.45	11.57	2.08	33.26	43.18	5.81
Grushenka	1.77	13.55	9.82	4.05	39.49	37.29	3.45
Average	2.07	17.21	10.69	2.87	65.43	35.50	4.25
Limit of variation	1.33-2.75	13.29-22.45	9.39-12.52	2.08-4.05	27.81-120.56	25.08-49.79	3.13-5.81

Fruit weight is an important feature in assessing the quality of the fruit. The larger fruits are and look more attractive, their quality is higher.

According to data obtained and presented in Table 1 average gooseberry fruit weight ranged from 1.33 g of cultivar 'Severny kapitan' and 2.75 g of cultivar 'Captivator'. Large fruits are good for use in fresh and smaller and sour fruits are good for processing, obtaining various products such as sauce, jam, juice, jelly, marinades, dried, frozen, etc. Evolution of gooseberry fruit weight for the varieties studied is shown in Figure 1. Cultivars 'Captivator', 'Sadko', 'Smena', 'Colobok' had the largest fruit and lowest for 'Severni capitan' and 'Zenit'.

High dry substance content, sugars, acidity, vitamin C contributes to improving the gooseberry berries. The amount of accumulated dry substances reached values between 13.29%, of cultivar 'Colobok', and 22.45% of cultivar 'Somesh', the average being 17.21%.

Gooseberry fruits are prized for high sugar content (Zotova; Inozemtsev, 1987), and according to data presented in Table 1 the accumulated amount varied between 9.39% at 'Smena' and 12.52% at 'Zenit', the average being 10.69%.

The amount of titratable acidity, gooseberry fruit is influenced by climatic conditions. If the annual average temperature is low, the acidity increases (Gherghi, A., Burzo, I., Bibicu, M. et al., 2001). Gooseberry fruit acidity accumulated during the period of investigation ranged between 2.08%, of cultivar 'Somesh' and 4.05%, at 'Grushenka', the average being 2.87%.

A valuable feature of gooseberry is sustainable conservation of vitamin P and ascorbic acid in fruit overripe (Zotova, Inozemtsev, 1987), and according to the obtained data the amount of vitamin C varies from 25.08 to 49.79 mg%, the average being 35 50 mg%.

The content of tanning and coloring substances accumulated in gooseberry fruits for the varieties studied ranged between 27.81 mg%, cultivar 'Zenit', and 120.56 mg% at cultivar 'Severny capitan', the average being 65.43 mg%.

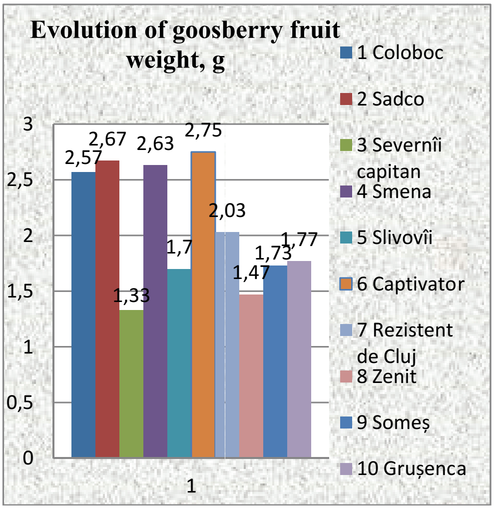

Figure 1. Evolution of gooseberry fruit weight

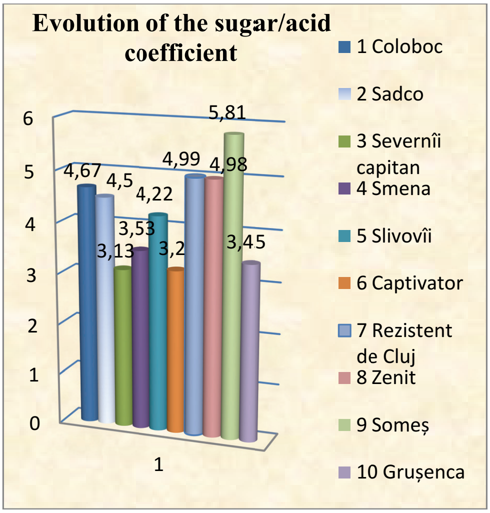

Figure 2. Evolution of the sugar / acid coefficient

Fruit quality is determined by the ratio of the accumulated contents of sugars and acid. The higher the ratio of these indices is, the higher is their quality. Evolution of the coefficient sugar/acid certifying the quality of gooseberry fruits for the varieties studied is shown in Figure 2. The highest values of the coefficient sugar / acid of 5.81, 4.99, and 4.98 have been accordingly registered for the cultivars 'Somesh', 'Resistent de Cluj' and 'Zenit' and the lowest value of 3.13 was established for 'Severni capitan', with an average value of 4.25.

CONCLUSIONS

Accumulation of nutrients in fruits depends largely on the cultivar and climatic conditions of the year of the formation of the harvest.

As a result of the conducted research on the capabilities that were introduced varieties of gooseberries reveal the amount of nutrients accumulated, based on which certifies the high quality of fruit was determined that:

- The average weight of the fruit reached maximum values of 2.75 g for the 'Captivator' cultivar.
- Highest amount of dry substances accumulated highlighted the 'Somesh' cultivar with 22.45%.
- Sugar content was accumulated in maximum amount of 12.52% at 'Zenit' cultivar.
- The highest acidity of the fruit of gooseberries in the amount of 4.05% was established for 'Grushenka'.
- The content of tanning and coloring substances accumulated - 120.56 mg% were recorded for 'Severny capitan'.
- The content of vitamin C in the largest amount of 49.79 mg% was accumulated in fruits of 'Zenit'.
- The value of the coefficient sugar/acid of 5.8 and high taste qualities of the fruit was recorded in cultivar 'Somesh'.

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AGRO-MORPHO-PRODUCTIVITY POTENTIAL OF SOME APRICOT CULTIVARS IN THE SOUTH-EAST ROMANIAN PLAIN

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Abstract

Making a modern fruit grow cannot be conceived without ensuring adequate hydric needs for fruit species alongside fertilization. Researching every attribute and specific character for complex systems which are biological known and new wants obtained through the process of apricot breeding required specific methods of collecting, processing and interpreting of data. Technological variants were studied using localized irrigation (b2) and irrigation associated with foliar fertilization (b2c2) on the behaviour of three cultivars of apricot ′Dacia′(a1), ′Comandor′(a2) and ′Tudor′ (a3) (Prunus armeniaca L.), grafted on Mirobolan, with different periods of maturation, in terms of the South-East of Romania, where optimal conditions are encountered. From this paper, studies found that a version of localized irrigation technology is associated with foliar fertilization (b2c2) and had influence on the characteristics of tree vigour, i.e. crown volume and trunk section area (TSA) on the production of fruit but also on the quality of the fruit items (average weight of the fruit, soluble dry matter, acidity and firmness). The crowns shape was not influenced by the application the experimental factors, these being the genetic attribute of each cultivar (′Dacia′ - spherical shape, ′Comandor′ - spherical and slightly alongeted shape and ′Tudor′ - pyramidal shape).

Key words: *apricot cultivar, drip irrigation, experimental variant, foliar fertilizer, quality*

INTRODUCTION

Modern conception of apricot culture in an high density system required a cultivar with an architecture which can allow small distance planting without diminishing complex mechanized possibilities, unhindered lightning of the crown with application upon the normal process of photorespiration and other physiological and biochemical processes which reflects production quality and uniformity.

Making a modern fruit grow cannot be conceived without ensuring adequate hydric needs for fruit species alongside fertilization (Amiri et al.,2008). Even in areas where 600-700 mm annual rainfall is recorded, there is a need to cover water deficiency during July-September, or sometimes in March and April, before and during flowering and fall in October, during the intense growth of roots. Irrigation with a drip system uses less water than sprinkler irrigation (Proebsting, 1994).

Foliar fertilization is necessary to create a favourable nutrient medium in order to obtain high yields of profitable indicators of quality (Bertschinger et al., 1997; Gradinariu et al., 2004). The method of localized irrigation achieved a fruit production increase of 11.06 kg in comparison with the method of micro irrigation with sprinkler which ensured a growth of 5.18 kg, both methods using 1 m^3 of water (Iancu; Septar, 2009).

Researching every attribute and specific character for complex systems which are biological known and new wants obtained through the process of apricot breeding required specific methods of collecting, processing and interpreting of data.

Considering this, the present work intend to highlight the technological options using localized irrigation and irrigation associated with foliar fertilization on the behaviour of three cultivars of apricot: ′Dacia′, ′Comandor′ and ′Tudor′ grafted on Mirobolan, with different maturation stages, in terms of the South-East of Romania, where optimal culture conditions are found. The three cultivars will be studied under the aspect of certain morpho-productive characteristics and the quality of fruits under the influence of irrigation and irrigation associated with fertilization.

MATERIALS AND METHODS

Obtaining an increase in the production of apricots can be attained through cultivar choice and ensuring adequate hydric needs. The experiment was carried out at the Moara Domneasca – Ilfov research station in 2011-2012 cropping year.

The soil that was founded typical reddish preluvosoil where the experiments began (according SRTS-2012 Chrome Luvisol according to WRB-ST-1998). In table 1 chemical analysis of soil (according Methodology ICPA-1987) are presented, which shows a weak acidic reaction of soil with values ranging from 5.82 to 6.19 (pH units).

Humus content is low (from 1.20 to 2.10%) at the top of the soil (0-72 cm, corresponding to the sequence of horizons Ap-AB), and very low (0.36 to 0.60%) based on the profile (72-150 cm).

Table 1. The main chemical properties of the soil - Moara Domnească

Level	Depth	pH_{H2O}	Humus (Cx1,72)	SB	Ah	T = SB + Ah	V_{Ah}
	Cm	units pH	%	me/100 g soil	%		
Ap	0-16	6.12	2.10	13.61	6.57	20.18	67
Apt	16-29	5.82	1.92	13.39	6.33	19.72	68
Am	29-40	6.19	1.80	15.98	4.30	20.28	79
AB	40-72	6.00	1.20	20.09	2.60	22.69	89
Bt_1	72-93	6.02	0.60	21.09	2.53	23.62	89
Bt_2	93-130	6.04	0.36	22.03	1.70	23.73	93
Bt_3	130-150	6.18	0.36	22.03	1.70	23.73	93

The plantation was established in 2004 with the planting distances of 5 x 4 m.

The following experimental scheme was organized: A factor – cultivar, with graduations: a1=′Dacia′, a2=′Comandor′ and a3=′Tudor′; B factor – norme of irrigation with the following graduations: b1=non-irrigated (control), b2=drip irrigation using a Nestos dripper type with dripping of 4l/hour; C factor - fertilization with Cropmax 0,1% with the following graduations: c1=nonfertilization and c2=fertilization with Cropmax 0,1%.

Drip irrigation was initiated since March 20, administering it daily for 4 hours. During the periods in which rainfall was recorded, irrigation was discontinued.

It has been established as the optimal time periods in which trees are most in need of water, these being: fruit sett, physiological fall, strengthening kernel, intensive growth of shoots and fruit, bud differentiation. The amount of water in watering is about 350 - 400 m^3/ha. The fertilizers were applied 3 times: immediately after blossom and every 2 weeks after. Soil samples were collected at two depths (0-20 and 20-40 cm), variants b1 (unirrigated) and b2 (irrigated) in the 3 cultivars a1, a2 and a3 and analytical data was interpreted according to the methodology ICPA (1986, 1997).

At the end of the vegetation period these measurements where recorded: tree height (m), crown height (m), crown projection through the rows direction (m), crown projection opposite to the rows direction (m), the trunk diameter on the rows direction (cm), the trunk diameter opposite to the rows direction (cm). Based on the crowns dimension these calculations were performed: the crowns volume (m^3) and shape (index) and based on the trunks dimension the trunk sectional area was calculated using specific breeding methods for fruit growing (Cociu, Oprea, 1989).

The crowns form and dimension depend on the cultivars vigour, on the angle of branch insertion, on the type of ramification, as well the length and the position of branches upon the axial branch. The more the angle of ramification is higher the more the diameter of the crown is higher as well.

After the obtained values the crowns shape can be:

- the value is around 1 or below 1 the shape of the crown can be spherical or even flat (<1,0)

-the more the values are higher than 1, higher crown shapes are resulted, reaching all to pyramidal shapes or fusiforme.

The crowns volume can be a particularity of cultivar, which can be influenced by rootstock and agrotechnics applied. Elements of fruit quality were determined by specific methods. The average weight of the fruit (g) was determined by weighing 25 fruits from each experimental variant of composite sample using an electronic balance. The content of soluble dry matter (%) was determined by a Zeiss refractometer using a sample juice resulting from 10 fresh fruits. Titratable acidity (malic acid g%) was determined by direct titration of a diluted extract with an alkaline solution of 0.1N NaOH in the presence of phenolphthalein solution (1% alcohol). Pulp firmness was made by portable penetrometer from a sample size of 20 fruits.

RESULTS AND DISCUSSIONS

Crown volume

Recorded measurements point out that in the VIII year the limits to volume values of the crown was between 5.65 – 9.35 m^3, which can show o high variability regarding the experiment. The cultivar with the lowest volume of crown was ´Dacia´ with differences between experimental studied variables of 5.65 m^3 and 7.10 m^3. The cultivar with the highest volume of crown from the 3 studied cultivars was b2c2 with differences from b1c1Mt cultivar between 1.45 m^3 for ´Dacia´ cultivar, 0.45 m^3 for ´Comandor´ and 0.80 m^3 for ´Tudor´ (Table 2).

Trunk section area (TSA)

Between the experimental studied variants, differences of trunk section was registred of 33.72 cm^2 for ´Dacia´ cultivar, 37.38 cm^2 for ´Comandor´ and 41.35 cm^2 for ´Tudor´ cultivar. For the b1c1Mt variants, the cultivar with the smallest trunk section area was ´Comandor´ (216.67 cm^2), followed by ´Dacia´ (220.48 cm^2) and ´Tudor´ (250.85 cm^2). The experimental variant with the highest growth spur of the trunk section area was b2c2 (Table 2).

Crown shape

The variability attribute given by the crowns shape is present in the index of form and through the valoric limits of these attributes, therefore the crown of ´Dacia´ cultivar is spherical (1.00-1.14), regardless of the experimental factor applied. As well, the ´Comandor´ cultivar through valoric limits of 1.13-1.21 express a spherical form, slightly alongeted, compared with ´Tudor´ cultivar which show a piramidal crown (1.50-1.58) (Table 2).

Table 2. Effect of experimental factors (b1, b2, c1, c2) on the vigor characteristics of apricot cultivars studied

Cultivar	Experimental variant	Crown volume (m^3)	Deference	TSA	Difference	Crown shape (index)
´Dacia´ (a1)	b1c1Mt	5.65		220.48		1.02
	b1c2	6.00	0.35	226.65	6.17	1.03
	b2c1	6.60	0.95	239.63	19.15	1.14
	b2c2	7.10	1.45	254.20	33.72	1.00
´Comandor´ (a2)	b1c1Mt	6.55		216.67		1.20
	b1c2	6.75	0.20	222.78	6.11	1.21
	b2c1	6.85	0.30	249.81	33.14	1.14
	b2c2	7.00	0.45	254.25	37.58	1.13
´Tudor´ (a3)	b1c1Mt	8.55		250.85		1.57
	b1c2	8.80	0.25	269.88	19.03	1.53
	b2c1	9.00	0.45	282.55	31.70	1.58
	b2c2	9.35	0.75	292.20	41.35	1.50

Regarding the characteristic of productivity, a significant difference has resulted between the experimental variants studied, b2c1 from b1c1, for the 3 studied cultivars with differences between the control variants of 6.25 (´Comandor´), 6.50 (´Tudor´) and 6.75 (´Dacia´). The b2c2 variant expressed a significant difference from b1c2 variant with values between 7.15 (´Tudor´), 8.25 (´Comandor´) and 8.50 (´Dacia´) (Table 3).

Table 3. The influence of drip irrigation levels on the production (t/ha) of apricot for the same cultivar and the same level of fertilization, 2011-2012

Cultivar (a)	´Dacia´ (a1)		´Comandor´ (a2)		´Tudor´ (a3)	
	Production (t/ha)	Dif	Production (t/ha)	Dif	Production (t/ha)	Dif
c1 (unfertilized)						
b1 (unirrigated)	18.75	Mt	17.5	Mt	16	Mt
b2 (irrigated)	25.5	6.75**	23.75	6.25**	22.5	6.5**
c2 (fertilized)						
b1(unirrigated)	21.50	Mt	18.25	Mt	18.75	Mt
b2 (irrigated)	30	8.5***	26.25	8.25***	25.90	7,15***
$DL_{5\%}$=3.499534 $DL_{1\%}$ = 4.924643 $DL_{0,1\%}$ = 7.014573						

Characterization of soil under the aspect of apparent density values (AD) which show the state of alignment (loosening or compaction) can be small and medium on the 0-20 cm layer and once with depth the indicator goes higher.
The lowest values of apparent density resulted from the 0-20 cm layer for the ´Comandor´ cultivar (1.20 g/cm^3) and ´Tudor´ (1.25 g/cm^3) at the experimental variant b1 and the experimental variant b2 the ´Tudor´ cultivar (1.28 g/cm^3) at the same depth. Very high values was observed at the 20-40 cm depth for both experimental variants (b1 and b2) for all 3 studied cultivars (Figure 1).
Regarding soil characterization in terms of total porosity (TP), we emphasize that the parameter values are inversely proportional to the apparent density (AD).
Note that the version b1 of 0-20 cm depth porosity is medium, recording higher values (44.5 to 54.5 % v/v) than version b2 (46 to 51 % v/v) where it is medium.
The same general trend is preserved and the depth of 20-40 cm between versions b1 and b2, with values between 36.5-40 % v/v (b1) and 37.5 to 38.5 % v/v (b2) (Figure 1).

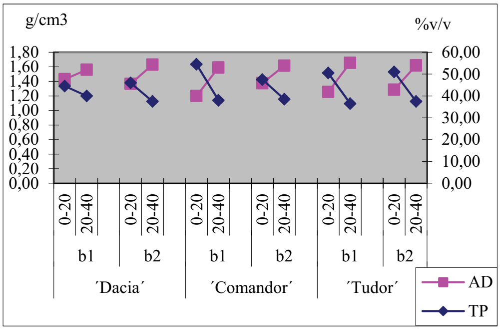

Figure 1. Effect of experimental factors (b1, b2) on the apparent density (AD) and total porosity (TP) of apricot cultivars studied

Available phosphorus values (mobile) shows small assurance for the 2 depths for both experimental variants, only ´Tudor´ cultivar at the 0-20 depth for both experimental variants the level of assurance is medium with 22.84 ppm (b1) and 20.07 ppm (b2) (Figure 2). Appreciation of ensuring soil with K^+ accessible (mobile) was made based on the values obtained for the layer 0-40 cm, values that are within the 25-40 ppm showing a lowered insurance with K (Figure 2).

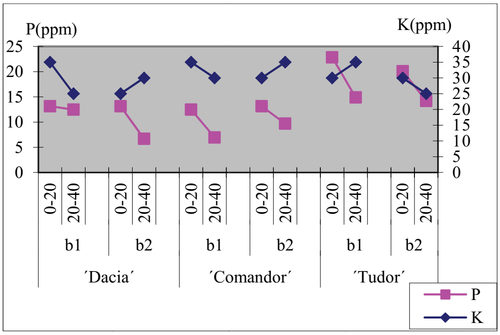

Figure 2. Effect of experimental factors (b1, b2) on the in phosphorus (P) and potassium (K) contents of apricot cultivars studied

The average weight of the fruits grew progressively from application of experimental factors in the following order: fertilization, irrigation and irrigation + fertilization.

´Dacia´ cultivar of b1c1Mt variant had fruits with a weight of 76.5 g and variant b2c2 fruits weighing 90 g ´Comandor´ and ´Tudor´ cultivars had fruits with low weight at b1c1Mt from 59 g to 75-76 g (Figure 3).

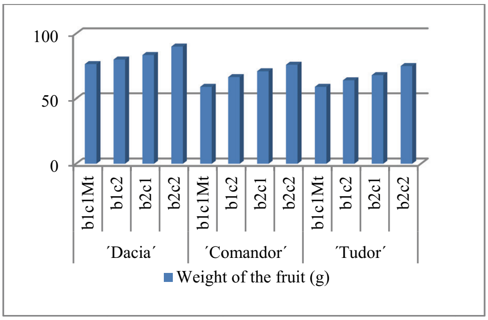

Figure 3. Effect of experimental factors (b1, b2, c1, c2) on average weight of the fruit (g) of apricot cultivars studied

Figure 4. Apricot cultivar ´Dacia´

The dry matter content presented stagnations for b1c2 (fertilized) variant and b2c1 (irrigated) and for b2c2 variant slight increases was registered, the most significant being ´Tudor´ cultivar of 1.6%. The ´Dacia´ cultivar, the difference between b2c2 and b2c1 was of 0.5% and for the ´Comandor´ cultivar just 0.8% (Figure 5).

Titratable acidity (malic acid/g%) presented progressive increase from the b1c1Mt variant to b2c2, the most significant being ´Tudor´ cultivar (0.59) (Figure 6).

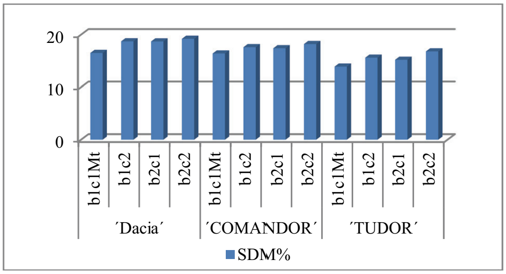

Figure 5. Effect of experimental factors (b1, b2, c1, c2) on soluble dry matter content (%) of apricot cultivars studied

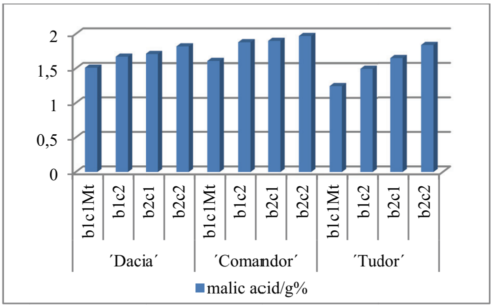

Figure 6. Effect of experimental factors (b1, b2, c1, c2) on titratable acidity content (malic acid/g%) of apricot cultivars studied

Under the aspect of fruit firmness, show in figure, shows that in the control variant that the ´Dacia´ cultivar has fruits with the best firmness (1.82 kgf/cm^2), followed by ´Comandor´ (1.7 kgf/cm^2) and ´Tudor´ with 1.3 kgf/cm^2. Under the aspect of irrigation and fertilization effect (b2c2), a significant increase was recorded for the cultivar ´Tudor´ reaching 2.2 kgf/cm^2 (Figure 7).

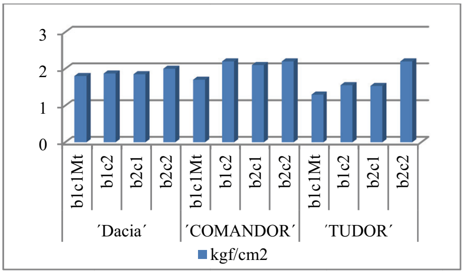

Figure 7. Effect of experimental factors (b1, b2, c1, c2) on fruit firmness (kgf/cm^2) of apricot cultivars studied

Figure 8. Apricot cultivar ´Comandor´


Figure 9. Apricot cultivar ´Tudor´

CONCLUSIONS

• The cultivar with the lowest crown volume was ´Dacia´ with differences between experimental variants studied of 5.65 m^3 and 7.10 m^3.
• Variant b1c1Mt the ´Comandor´ cultivar had the lowest trunk section area with 216.67 cm^2, followed by ´Dacia´ with 220.48 cm^2 and ´Tudor´ with 250.85 cm^2. The experimental variant with the highest growth spur of the trunk section area is b2c2.
• Regardless of the experimental factor applied, the crowns shape show the characheristic of the cultivar ´Dacia´ having a spherical crown (1.00-1.14), ´Comandor´ showing a spherical and slightly alongeted shape (1.13-1.21) and ´Tudor´ which presents a piramidal crown (1.50-1.58).
• Regarding the productivity characteristic, a significant difference resulted between the experimental variants studied, b2c1 for b1c1, for the 3 studied cultivars, with differences from the control between 6.25** (´Comandor´), 6.50** (´Tudor´) and 6.75** (´Dacia´). The b2c2 variant expressed a very significant difference from the b1c2 variant with values between 7.15** (´Tudor´), 8.25** (´Comandor´) and 8.50** (´Dacia´).
• Indicators of soil physical attributes, namely apparent density (ad) which shows the depth values 0-20, growing in depth alongside the percentage of clay in the soil. Regarding soil characterization in terms of total porosity (TP), we emphasize that the parameter values are inversely proportional to the apparent density (AD). Note that the version b1 of 0-20 cm depth, porosity is medium recording higher values (44.5 to 54.5 % v/v) than the version b2 (46 to 51% v/v) where it is medium. The same general trend is preserved and the depth of 20-40 cm between versions b1 and b2, with values between 36.5-40% v/v (b1) and 37.5 to 38.5 % v/v (b2). Soil potassium supply is low which implies the use of fertilizers with K.
• Regarding the elements of quality of the fruits (average weight of the fruit, dry matter content, acidity and firmness) a progressive increase has shown once the application of experimental factors in the following order: fertilization (c2), irrigation (b2) and irrigation + fertilization (b2c2).

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FIRST RESULTS OF SOME DAY-NEUTRAL STRAWBERRY CULTIVARS BEHAVIOR IN THE BUCHAREST AREA CONDITIONS

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Abstract

The main objective of the experiment consist in testing three cultivars of strawberries: two day-neutral ('Albion' and 'San Andreas') and one with one year harvest ('Benicia'). The goal was to monitor and evaluate their behaviour in the first year of vegetation in a plasticulture system considering the pedo-climatic conditions of Bucharest. We focused mainly on the following characteristics and traits: flowering and fruit maturation, the amounts of the accumulated temperatures during the bearing period, fruit weight, fruit shape, solid dry matter content, ascorbic acid content and total sugar content (glucose, fructose and sucrose). The earliest ripening date ranged between 18th and 25th of June and the amounts of temperatures accumulated up to this phenophase ranged between 1380 and 1515.5 ^{0}C. The strawberries reached the harvest maturity between June, 27th and July 1st for a period of 6-9 days. The day-neutral strawberry cultivars develop fruits who have been ripening during the entire summer and autumn season, the last significant harvest was remarked toward the end of October, when they accumulated 2317 ^{0}C each. The plants had continued to produce even in November and December, till the first frost. In the first months after planting, fruit weight was lower (8.33 g - 11.52 g) than achievable weight of each cultivar. All the studied cultivars have excellent organoleptic characteristics, a good shelf life potential and a high efficiency of fructification, taking into account the cultivation system and the overall technological measures. A high sugar content of 6.49% (glucose, fructose and sucrose) and solid dry matter content of 6.8% has been recorded by 'San Andreas' and the ascorbic acid content was of 71.92 mg/100g. A lower content in sugar was registered by 'Albion' with 4.81% and 4.28% by 'Benicia'. Regarding ascorbic acid content, 'Albion' accumulate the highest content in fruits (84.89 mg/100g) next by 'Benicia' with 74.6 mg/100g. The day-neutral cultivars remarked also by a notable number of runners at the end of the year.

Key words: *day-neutral, strawberry, traits, cultivar, 'Albion', 'San Andreas', 'Benicia'*

INTRODUCTION

The strawberries (*Fragaria* x *ananassa* Duch.) are along with cherries, early fruits which are ripening in May or June (Asanica A. and Hoza D., 2013).
Cultivating day-neutral strawberry cultivars (produce flowers regardless of the photoperiod and allow multiple harvests within one year) it is possible therefore to have fresh strawberries from spring until the end of the fall.
With a small stature (15 to 40 cm), as more or less compact/rare plants, strawberries could be grown successfully in pots, small kitchen garden, green houses or in large outdoors areas (Chira, 2000). Strawberries, also could be cultivated interleaved in plantations or between different other cultures (Hoza, 2000). Success depends on the location of culture, quality of soil, cultivar potential, plant age and moment of harvest (Montero et al.,1996).
The sugar, acid and vitamin C content of the strawberries is considered a quality factor both by consumers and food industry (Kim et al., 2013). The relationship between these components and sensory traits such as flavour or colour have been studied (Wrolstad et al.,1970) also in the postharvest technologies. Anyway, correlated with the time on the market, the strawberry fruit appearance including size, colour, shape as well as the flesh sensorial properties represent many times the consumer criteria for choosing one or another strawberry cultivar.

MATERIALS AND METHODS

The experimental field of the Fruit Growing Department is located in the geomorphological unit Romanian Plain, subdivision Vlasiei to 44^{0}29´50˝N and 26^{0}15´26˝E. The climate is temperate - continental with warm, sometimes hot and frequent droughts and cold winters, with large amounts of snow. The springs are short, with big jumps in temperature from

month to month and with significant variations between day and night amplitude. Autumns are distinguished by moderate thermal and slow transition to winter.
The annual rainfall volume is between 500 and 600 mm, the maximum occurring in the period from May to July.
In April 2013, within the Department of Fruit Growing field, it was established a demonstration plot where it were planted three new strawberry cultivars: two day-neutral ('Albion' and 'San Andreas') and one with a single major crop/year ('Benicia') in plasticulture system.
The soil was covered with Agrotextile mulch, three strips of strawberry rows have been performed for each cultivar (Figure 1). The planting distances were of 35 cm between strips and 20 cm between plants in the row. Each of the strawberry row benefits of irrigation through a 16 mm drip irrigation pipe with pre settled drip nozzles.

Figure 1. Experimental plot of strawberry cultivars in the Faculty of Horticulture field

For studying these strawberry cultivars, specific methods were applied for the following traits and plant/fruit characteristics: flowering period and fruit maturation, the amounts of temperatures accumulated during the fruiting period, fruit weight, fruit shape, dry soluble content, ascorbic acid content and total sugar content (glucose, fructose and sucrose).
The flowering period and fruit maturation were determined by distinct stages of each phenophase.
The temperatures accumulated during the fruiting period were calculated by summing, for each cultivar, based on daily temperature, corresponding trigger data and fruiting performance of each phase separately. The average fresh weight of the fruit (g) was determined by weighing 25 fruits from each type of composite sample using an electronic balance.
The shape index (SI) of the fruit was carried out by measuring with callipers the height (cm) and the diameter (cm) of 10 sample fruits. Thus, shape index was calculated as the ratio between these two dimensions.
The content of soluble dry matter (SDM%) was determined by a Zeiss refractometer using a sample juice resulting from 10 fresh fruits. The content of ascorbic acid (mg/100g) and the total glucidic content (%) (glucose, fructose and sucrose) was measured by means of a modular assembly HPLC detector with tuneable optical absorption in the spectral range of 190- 900 nm. For the ascorbic acid determination, the juice was diluted with 1% oxalic acid, filtered and then 2 ml analysed by HPLC. To determine the total sugar content, it was used a 2 ml filtered juice, obtained by boiling 50 g of pulp in distilled water until disintegration, afterward analysed with HLPC device.

RESULTS AND DISCUSSIONS

The April - October 2013 period was characterized by average monthly temperatures slightly higher than the climatologically average (1960-2004) (Figure 2), providing sufficient thermal resources for the strawberry crop growth and development of fruits.
In terms of water supply, however, rainfall was deficient in the first months after planting, compared to the multiannual average (1960-1990) (Figure 3), which is common in the South part of the country so that's why the irrigation system we consider it indispensable for the strawberry culture.

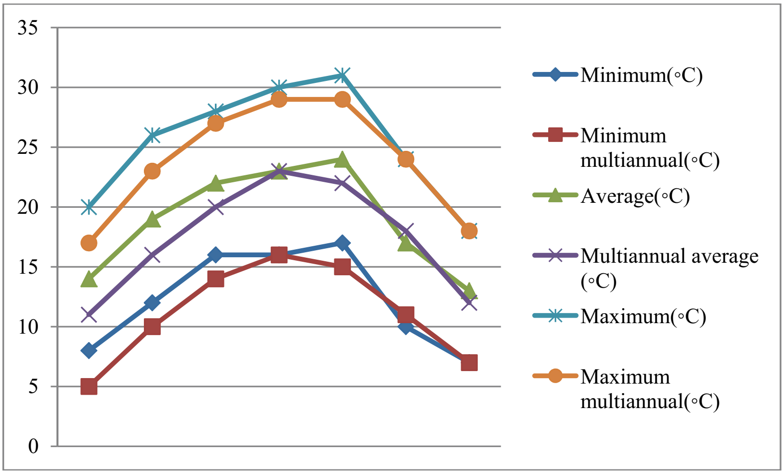

Figure 2. Variation of minimum, maximum and average temperatures compared to the multiannual averages (1960-2004), the first year of vegetation conditions of strawberry cultivars, 2013

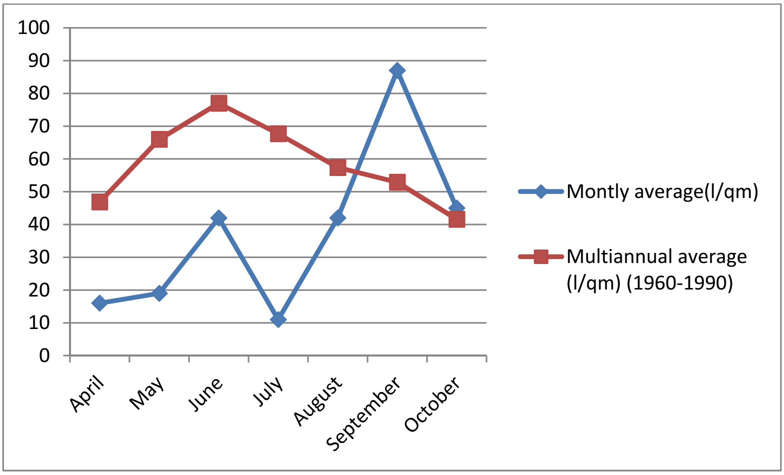

Figure 3.Variation in rainfall compared to multiannual averages (1960-1990), the first year of vegetation conditions of strawberry cultivars, 2013

In the newly established strawberry experimental crop, the day-neutral cultivars had ripening very early as follows: on 18.06.2013 'San Andreas' respectively 20.06.2013 for 'Albion'. From planting to the first ripened fruits were accumulated 1380^0C in the San Andreas case and 1407.5^0C at 'Albion' (Table 1). They had the ripening period over 6 and 9 days, around the date of June, 27^{th} when the temperature accumulated was of 181.5^0C ('Albion') and 205^0C ('San Andreas'). 'Benicia' had a quick development of foliage after planting and the first red fruits have been picked up around the 25^{th} of June, when it were accumulated 1515.5^0C. The fruits ripening in around six days and pass in the first days of July accumulating other 158.5^0C. The fructification continue during entire month and slows down in late of July.

The day-neutral cultivars have been blossom and fruiting continuously over the summer and autumn, the last harvest was towards the end of October (Table 1), when they accumulated 2317^0C each. Both cultivars continuing to produce some fruits even in late November, but more of them remains smaller, green or white with pale pink blush on the sunny part (Figure 4).

Table 1. Phenological data on the first year fruiting strawberries cultivars, 2013

Cultivar	Early ripening (date)	Temperatures until early ripening (^{0}C)	First fruit riped (date)	Fruit maturation period (days)	Temperatures from early ripening to first ripening fruits (^{0}C)	Last harvest (date)	Temperatures of the entire fruiting period (end of July to October) (◦C)
'Albion'	20.06	1407.5	27.06	6	181.5	20.10	2317
'San Andreas'	18.06	1380	27.06	9	205	20.10	2317
'Benicia'	25.06	1515.5	01.07	6	158.5	-	-

Figure 4. Day-neutral cultivars: 'Albion' (left) and 'San Andreas' (right), 28.11.2013

In terms of the fruits size, it seems that first fruits obtained had encounter modest weight. 'Albion' realised for the first picked fruits only 8.33 g/fruit as an average value, 'Benicia' 9.50 g/fruit and 'San Andreas' 11.52 g/fruit (Table 2).

But the biological potential of the cultivars former is far better. At UC Davis (University of California), fruit weight performance reported was of 32,1 g for 'Albion', 32,4 g for 'San Andreas' and 33,5 g for 'Benicia' (Watsonville Research Facility). In West Central Research and Outreach Center of University of Minnesota, 'Albion' in the same cultural conditions as we experimented, the day-neutral cultivars reach the average weight of the fruit of 14,32 g and 'San Andreas' 16,52 g/fruit.

In this regard, our results must be interpreted as the field potential of the strawberry cultivars, taking into account the fact that the land and culture was not fertilised at all and the fruits harvested comes from the runners planted few months ago.

In our technological and land conditions, the cultivars description have been made in order to compare them also by commercial aspect. 'Albion' cultivar develop long conical fruits with a shape index of 1.26. The colour of fruit is bright red (Figure 5) with red-whitey flesh.

'San Andreas's fruit is conical, with a shape index of 1.21, bright red, shiny (Figure 6), sweet and with a great fragrance.

'Benicia' cultivar has long shaped fruit conical with shape index of 1.32 (Figure 7), medium consistency, bright red outside colour, with a nice and attractive red pulp inside.

The three studied cultivars have an excellent taste (all organoleptic characteristics are superior), good shelf life potential and high fructification efficiency.

'San Andreas' had accumulated the highest content of sugars (glucose, fructose and sucrose) respectively 6.49%, with about 35% more than the next cultivar in range ('Albion'). Also, the dry soluble matter content of the 'San Andreas' fruits (6.8%) was higher, but the values between the studied cultivars were very closed to each other (Table 2).

Lower sugar content was recorded by 'Benicia' (4.28%) and 'Albion' (4.81), but this decreased content of total sugar in fruits, did not

disqualified the organoleptic feature of the cultivars.
Regarding the ascorbic acid content, 'Albion' recorded the highest value of 84.89 mg/100g next by 'Benicia' cultivar with 74.6 mg/100g and 'San Andreas' (71.92 mg/100g).

Table 2. The main characteristics of strawberry cultivars fruits harvested in the first year of culture, 2013

Cultivar	Weight (g)	Length/ width (cm)	Shape index (SI)	DSM %	Ascorbic acid (mg/100g)	Sugar content (%)			
						Fructose	Glucose	Sucrose	Total
'Albion'	8.33	2.94/2.32	1.26	6.7	84.89	1.97	1.86	0.98	4.81
'San Andreas'	11.52	3.39/2.78	1.21	6.8	71.92	2.7	2.47	1.32	6.49
'Benicia'	9.50	3.35/2.52	1.32	6.6	74.6	2.06	1.82	0.4	4.28

Figure 5. Albion

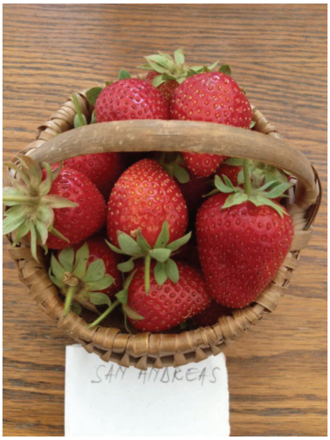

Figure 6. San Andreas

Figure 7. Benicia

Plants of each cultivar had shown different genetic finger print vigour. 'San Andreas' behave as a low vigorous cultivar and develop a dark green foliage. 'Albion' presented a medium vigour and 'Benicia' the highest height with big leaflets and thick petioles.
The data presented in the Table 3, reflect the average number of runners/plant at the end of the vegetation period. In comparison with 'Benicia', the day-neutral strawberry cultivars spread more runners/plant. 'Albion' has develop the highest number of runners/plant (4) and 'San Andreas' 3 runners/plant.
This trait is technologically important both for producers and traders, the total number of runners/plant as a genetic feature could also be used as a providing biological material capacity.

Table 3. Number of runners/plant after the first year of vegetation

Cultivar	'Albion'	'San Andreas'	'Benicia'
Average number of runners/plant	4	3	1

CONCLUSIONS

After the first year of plasticulture system in the Bucharest area conditions, the studied three strawberry cultivars proved a good agrobiologic potential.
Plants presented a good resistance to the main diseases and start fruiting in 57-60 days after planting. First fruits picked in these conditions recorded a moderate weight but with good organoleptic features, close to the cultivar potential.

The last fruits of both day-neutral cultivars ('Albion' and 'San Andreas') were harvested from the snow when the first frost occurred proving to be a very good cultivars especially for home garden use.

Day-neutral cultivars 'San Andreas' and 'Albion' develop more runners than 'Benicia'.

Research will continue in the next year for a better evaluation of these cultivars.

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THINNING AND FOLIAR FERTILIZATION INFLUENCE ON THE YIELD OF IDARED APPLE CULTIVAR

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Abstract

The research was conducted during 2010 - 2013 in an apple orchard planted in 2003 with 'Idared' cultivar grafted on M26 rootstock, at distance of 4x2 m. The trees are ruled by thin spindle-shaped crown. We studied the interaction between foliar fertilization and fruit thinning as the main determinants of the quantity and quality of apple fruit. The highest production (49.1 t/ha) was realized at application of 46% urea N in a concentration of 0.6% - when the 75% of the flowers while they fall, 0.9% - when the central fruit blossom has 10 to 12 mm, 1.2% - when the fruits are 25-30 mm in diameter and thinning of fruits by chemical preparation Bioprzerzedzacz SL 060, at a concentration of 0.075% when the diameter of the central fruit blossom is 10 - 12 mm.

Key words: *foliar fertilization, chemical and manual fruit thinning*

INTRODUCTION

Thinning species of fruit trees that tends to overload with fruits it becomes a common technological measure. Using chemicals for thinning of fruits are doing in order to obtain quality fruits and to increase the overall yield (Richard, 1998). The effect of thinning depends by climate and growing conditions of the species tree. (Sally, 1991; Black, 1995; Stopar, 1999, 2001).

The foliar fertilization, chemical and manual fruit thinning, all represent significant contribution to maintain the physiological balance between growth and fruiting and increasing the quantity and quality of fruit.

Obtaining high quality productions in terms of size, color, etc. increases the fruit quality, and the price, increases labor productivity in collecting, sorting and packing, because of the smaller fruits number, prevents the breakage and the split of the branches, keeping production volume of the crown for the coming years and prevents alternation of fruitfulness, increases the resistance to disease and frost trees due to store a sufficient amount of reserve substances, ensuring the formation of annual shoots that will form bearing formations for years (Stopar, 2001; Balan, 2009). The argumentation and refine the use of chemicals to obtain quantitative and qualitative fruits production represent a problem of great value to modern orchards (Babuc, 2012; Cimpoieş, 2012).

A prerequisite for a harvest of high quality is an adequate number of flowers and fruit trees so that their chemical thinning is a common measure in commercial apple orchards (Wertheim, 2000; Greene, 2002).

MATERIALS AND METHODS

We studied the influence of foliar fertilization and fruit load on growth processes and standardization of fruit during the years 2010-2013 in the apple orchard "Zubresti" Străşeni. It was studied 'Idared' apple cultivar grafted on rootstock M 26, planted in 2003, at a distance of 4 x 2 m. The trees are ruled by thin spindle-shaped crown, treatments applied with Urea 46% N concentration of 0.4% 1.2%. Each variant consists of four replications of three trees, each representative arranged by randomized block system. The chemical fruit thinning was performed when central fruit is 10-12 mm in diameter (Table 2) the 060SL Bioprzerzedzacz preparation in a concentration of 0.075%, and 1000 liters of solution per hectare. The fruit thinning is performed manually by physiological fall in June. The manually thinning is done when fruits are at a distance less than 10-15 cm. The small fruits, distorted, attacked by diseases are removed first and then the normal ones.

Table 1. Scheme applying foliar fertilizers for 'Idared'

No.	The period of foliar fertilization	Foliage fertilizer concentration			
		V1f	V2f	V3f	V4f
Urea 46 % active substance					
1	After bloom (when the 75% where in bloom)	water	0.4	0.5	0.6
2	When the fruit is size one nuts(fruit have 10-12 mm in diameter)	water	0.7	0.8	0.9
3	When the fruit are in size one walnuts (fruit have 25-30mm in diameter)	water	1.0	1.1	1.2
Polyfeed (N19:P19:K19)					
4	When fruits are in the ripen stages(20-30 July)	water	0.1	0.1	0.1
Calcium chloride ($CaCl2$)					
5	With 20-30 days before harvest	water	0.5	0,6	0,7

The harvest moment for each variant was chosen individually by weighing the fruits from 12 trees. The average weight of the fruit is determined by weighing electronically of 100 fruits.

Table 2. Metod of fruit thinning

Variant	Metod of thinning
V1r	Control
V 2r (Chemical thinning)	Management of chemicals when the central fruit diameter of 10-12 mm is blossoms Bioprzerzedzacz 060 SL preparation in a concentration of 0.075%.
V 3r (Chemical thinning + manual)	Administration of chemicals when the central fruit diameter of 10-12 mm is blossoms Bioprzerzedzacz 060 SL preparation in a concentration of 0.075% + manual fruit thinning.
V 4r (Manual thinning)	Manual thinning is carried out after the fall of physiological fruit when the fruit reaches 16-18 mm in diameter.

The experiment was installed in accordance with the method of organizing experiences factorial (foliar fertilizer, chemical and manually fruit thinning) and includes variants with the following scheme: V1 (V1f + V1r), V2 (V1f + V2r), V3 (V1f + V3r), V4 (V1f + V4r), V5 (V2f + V1r), V6 (V2f + V2r), V7 (V2f + V3r), V8 (V2f + V4r), V9 (V3f + V1r), V10 (V3f + V2r), V11 (V3f + V3r), rV12 (V3f + V4r), V13 (V4f + V1r), V14 (V4f + V2r), V15 (V4f + V3r), V16 (V4f + V4r).

RESULTS AND DISCUSSION

In 2010 the number of fruits per trees without foliar fertilization was from 164 at V2 to 180 per tree at V4 variant. The smallest number of fruits was observed in variant V11 (130 fruits/tree) and the highest in the variant V13 with 195 fruits/tree.

Thus leading to a high number of fruits, variants with chemical fruit thinning was as follows: 186 frutis/tree - variant V9: 178 fruits/tree in V10 and 184 fruits/tree at V14 variant.

In 2011, the number of fruits at 'Idared' cultivar increased in most variants but with a larger emphasis to the thinning variants without fertilization V2 (202 fruits) and 204 fruits in V3 trees. The fertilization variants without fruit thinning were remarked as an increase in the number of fruits but compared to the previous year 2010 growth was lower constituting up to 223 frutis/tree (V13).

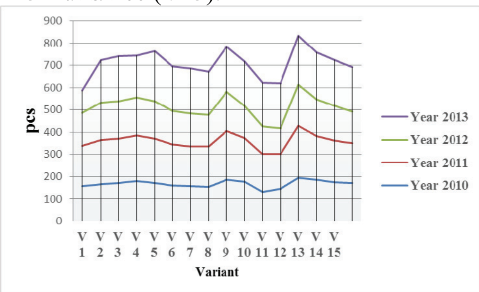

Figure 1. The number of fruits after fruit thinning and foliar fertilization application with mineral fertilizers of 'Idared' cultivar (fruits/tree) on M26 rootstock, planted at distances of 4x2 m in S.A. ‘Zubresti’ (2010-2013)

2012 was a critical year in terms of weather, the fruits number decreased. The smallest numbers of fruits were in the variant control V1 148 frutis/ tree and most fruits were gathered from V13 variant with 187 fruits. Among the variants with foliar fertilization and thinning and without thinning and fertilization variants we notice a slight increasing in the number of fruits depending on the concentration of urea

46% N applied to rich an average of 10 frutis/tree compared to the variants without fertilization.
2013 compared to previous years (2010-2012) is the year with biggest number of fruits, but in control V1 the number in 2010 was of 100 fruits/tree because of an insufficient number of fruit buds that had to be made in year 2012. In the variants with fertilization and thinning it was noted that the number of fruit is slightly smaller than the variants only fertilized (V5 204 frutis/tree) compared to V6 variants - 194 frutis/tree and V8 variant with 188 frutis/tree where was applied manual fruit thinning.
The fruit weight is an indicator that provides a good crop of fruit in 2010. The 'Idared' cultivar registered the smallest fruits in V1 with an average fruit weight of 99 g, and the largest fruits are remarked in the variant V16 with an average weight of 200 g.

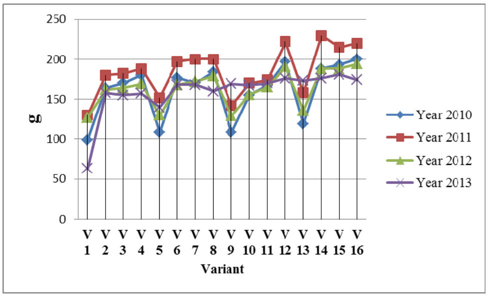

Figure 2. The fruit weight by fruit thinning application and foliar fertilization with mineral fertilizers - 'Idared' cultivar/M26 (2010-2013)

In the variants with different methods of thinning, the mean fruit weight varied from 164 g to 180 g (slow manual fruit thinning, V4). The application of different concentrations of foliar fertilization based on Urea 46% N increases the fruit weight. In the variant V13 fruit weight increased to 119 g with 46% N Urea application at a concentration of 0.6%, 0.9%, 1.2%. However, the biggest fruits weight where remarked in the variants with foliar fertilization applied and fruit thinning. Thus, the largest fruits were recorded in variants with chemical fruits thinning.
In 2011, the fruit weight increased in all cases, the highest average fruit weight was recorded in variant V14 with 230 g. Comparing with 2010, in 2011 the variants with foliar fertilizer recorded the highest growth. From 31% to 40 % variant V9 and V5.

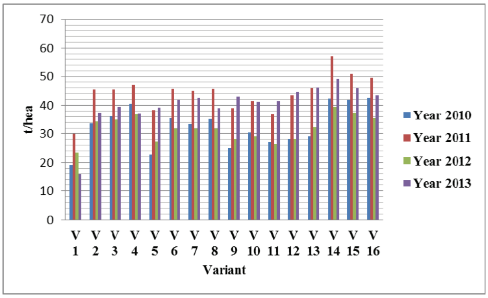

Figure 3. Yield obtained with fruit thinning and foliar fertilization application at 'Idared'/M26 (t/ha) (2010-2013)

In 2012 with some complicated climatic conditions, the fruit weight suffered. However, foliar fertilization variants only recorded difference of 16%.
In 2013 the lowest fruit weight was recorded in control (V1) with 63 g. In V2f where the concentration of Urea 46 % N was 0.4 %, 0.7 % and 1.0 % + thinning fruit weight according to the scheme experience was lower than or equal to 2011, from 140 g (V5) 168 g (V6). In the V3f foliar fertilization variants (Table 1) harvest was higher than in 2012. The chemical thinning variants (V10) and manual thinning (V11) weighing 170 g.
In 2010, the 'Idared' fruit production was of 19.2 t/ha at the control. In the V5 variant which concentration applied was of 0.4 % - 1.0 % yield was 22.9 t/ha and the V13 variant with Urea 46 % N concentration of 0.6 % - 1.2% yield was 29.0 t/ha .
The variants with fruit thinning and without fertilization, the fruits production riched to 33.6 t/ha; in V2 with chemical thinning to 40.5 t/ha; in the variant V4 with manual fruit thinning.
In variants with combined application of foliar fertilization of the crop and fruit thinning production varied between 27.1 t/ha in the variant V11, 42.5 t/ha with the V16 variant in the case of foliar fertilization application concentration of 0.6 to 1 2% 46 % Urea N and manual fruit thinning.
In 2011, fruit production reached to 50 tons per hectare with combined application of foliar fertilization and standardization load.
In 2012 fruits harvested per hectare recorded up to 23.4 t/ha in variant V14. Compared to

the 2010 -2012 harvest, the yield is with 30.3 % lower.

In 2013, production increased in all variants except the control variant (V1) which has been harvested from 15.8 t/ha. Decrease of the V1 harvest was due to insufficient deposition of fruit buds. The fertilization variants as in the 2010 - 2012 harvest was influenced by the concentration of Urea 46 % N applied. Thus, in the variant where the concentration of the fertilizer V5 was 0.4 %, 0.7 % and 1.9 % fruits yield was of 39.3 t/ha. But V14 solution where the concentration of the fertilizer applied was of 0.6 %, 0.9 %, 1.2 % fruit production was higher (49.1 t/ha).

CONCLUSIONS

The largest number of the fruits was recorded in the variant V13 (without thinning fruit and fertilization where the concentration of fertilizer applied was 0.6% - a 75% fall petals 0.9 % - when the central fruit blossom has a diameter of 10-12 mm, 1.2% - while the central fruit diameter is 25-30 cm).

The fruit weight in the studied period (2010 - 2013) recorded maximum value with foliar fertilization, concentration of 0.6%, 0.9%, 1.2% and chemical fruit thinning.

In the 2010 - 2013 production was influenced by the concentration of Urea 46% N. In the variant V5 the fertilizer was 0.4%, 0.7% and 1.0% fruit yield was of 39.3 t/ha. But in the V13 variant the concentration of fertilizer applied was 0.6% - at 75% fall petals, 0.9% - while the central fruit blossom has a diameter of 10-12 mm, 1.2% - when the central of fruit diameter 25-30 cm plus fruit thinning fruit harvest was 49.1 t / ha.

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EVALUATION OF THE QUALITY OF ORGANICALLY GROWN GRAPES IN THE VITICULTURAL CENTER OF MURFATLAR

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Abstract

The trend for organic grapevine cultivation has increased recently due to a higher demand for organic products in general, but also due to the fact that new legislation for organic wine has been passed in 2012. In order to produce quality and cost-effective wines the quality of the grapes is of utmost importance. The studies were performed during 2012 and 2013 on grapes for red wines of 'Feteasca neagra' and 'Cabernet Sauvignon' cultivars. The parameters followed were those specific for grape maturation (weight of 100 berries, sugar content, total acidity, anthocyanins accumulation and total polyphenolic index) along with the yield and sanitary quality of the grapes. These two years of organic cultivation proved to have been optimal for this type of technology, the grapes harvested being of the required quality for the production of wine with controlled denomination of origin.

Key words: *organic growing, resilience, pest and diseases resistance*

INTRODUCTION

As a result of health problems and environmental degradation effect due to the extended use of pesticides and synthetic chemical fertilizers applied to control diseases, pests and weeds, in many countries the concept of organic viticulture was promoted (Antoce et al., 2008).
In recent years the academic world and the staff involved in wine production showed increased interest in organic viticulture ideas, which are based on the concept of the harmonious combination of traditional culture methods and scientific research progress in such a way that the harmful impact on the environment to be minimized.
In the European Union, about 85 000 hectares of vineyards are organically grown (2,5%), the largest producers being Italy (5,5%), Greece (4,3%), Austria (3,5%) and France (2,2%). In Romania about 0,6% of the total of existing vineyards (180 000 ha) are grown organically. Although the percentage is very low compared to other countries with longer tradition, in Romania there is a steady increase in the number of wine farms practicing organic viticulture. At the Research Station for Viticulture and Oenology Murfatlar vines are grown organically since 2007. Currently, there are 45 hectares of vineyards registered for organic production, of which 15 ha are certified and 30 ha are in conversion to organic production. The varieties grown organically are 'Columna' and 'Chardonnay' - for white varieties and 'Fetească neagra', 'Cabernet Sauvignon' and 'Pinot noir' for red varieties (Ranca et l., 2013).

Organic farming systems rely on respecting a complex of rules contained in the Regulation (CCE) No 2092/91 of the Council, which Romania assumed once it jointed the EU on 27 June 2007. The new regulation enforced in 2007 (EEC 834/2007) and replaced the 1991 one was much clearer for both farmers and consumers, establishing a complete series of objectives, principles and basic rules for organic production (Ranca and Toncea, 2011).
It is well known that the quality of grapes and wine is influenced mostly by the varietal characteristics that imprint its own traits. Climate has significant influence too, on both the quality and the yield. Typically, the temperature influences the ripening period of various cultivars, allowing for a zone distribution of vine varieties in the regions of the country (Cotea, 1985).
Without any doubt, the ripeness of the grapes has a crucial importance in the production of quality wines. The maturation process has a great influence on the phenolic composition of grapes too, not only in the concentration of sugars and acids, which are usually the parameters mostly followed before harvesting.

MATERIALS AND METHODS

The research was conducted in Murfatlar wine center within the Research Station for Viticulture and Oenology Murfatlar, during two viticultural years, 2012 and 2013. The observations were performed on two organically cultivated vine varieties, 'Fetească neagră' and 'Cabernet Sauvignon', focusing on fruit quality and adaptability to organic cultivation in the conditions of Murfatlar area.
The total certified organic surface of the studied varieties is 12.39 ha, of which 5.25 ha is planted with 'Fetească neagra' and 7.14 ha with 'Cabernet Sauvignon'. The varieties under study were grafted on the rootstock Berlandieri X Riparia Teleki 4 - Oppenheim selection 4-4 and the training system used is Guyot with bilateral cordons, the planting distance being 2.2 m between rows and 1.1 m between vines. The row orientation is N-S.
The evaluation of resistance to pathogens was performed twice during the active growing season (June and July), using the descriptors of IPGRI (International Plant Genetic Resources Institute).
The descriptors 9.2.3. and 9.2.4. were used to establish the susceptibility to vine mildew (*Plasmopara viticola*) for grapes and leaves, respectively. For downy mildew evaluation (*Uncinula necator*) descriptors 9.2.5. (leaves) and 9.2.6. (grapes) were used and for the evaluation of gray mold (*Botrytis cinerea*) on grapes the descriptor 9.2.2. was used. The numerical evaluation scale used has values between 1 (resistant) to 9 (high sensitivity).
Due to the fact that in Murfatlar vineyard the most important pest is the grape moth (*Lobesia botrana*), for the population surveillance and control AtraBot pheromone traps were installed.
For the follow-up of the ripening dynamics weekly determinations of technological maturation parameters were performed. We measured the weight of 100 berries (g), the glucoacidimetric index (the ratio of sugar content and total acidity expressed as H_2SO_4) and for the phenolic maturation the anthocyanins content (mg/l) and total polyphenol index. The evolution of these parametes was easilly followed in the subsequently drawn maturation graphs.
The sugar content was determined using the electonic Smart refractometer produced by Atago, Japan; the total acidity - by titration with 0.1 N NaOH; the weight of 100 berries by weighing with the laboratory balance. The content of total anthocyans and polyphenol index were determined using the method ITV developed by the Institut Français de la Vigne et du Vin. This method is based on the extraction of phenolic compounds in an acid medium (15 ml of 95% ethanol, 85 ml HCl 0.1 % v/v) during 2 h, followed by the measurement of the absorbance at 520 nm of the extract previously diluted 1:20 with 1% HCl solution, for the anthocyanin content determination, and by the measurement of the absorbance at 280 nm in a 1 cm quartz cuvette of the same extract previously diluted 1:100 with distilled water for the total polyphenols index (IPT) estimation (Cayla et al., 2002).

Climatic data were provided by the weather station Weather Master 2000 produced by Environdata, Australia and includes daily observations for maximum and minimum temperature, insolation and precipitations.

RESULTS AND DISCUSSION

The climatic conditions of the viticultural years 2012 and 2013 can be judged as favorable for the organic cultivation of winegrapes, the vegetation period of the varieties studied being characterized by average temperatures with 2.0 – 2.7°C higher than the average calculated for the past 20 years. The viticultural year 2012 was a dry one, with higher values of air temperature during the growing season and also with an insolation that exceeded by 275 hours the multiannual average used as reference.

The amount of precipitation was significant in 2013, almost double as compared to the previous year, totaling 554.4 mm as compared to only 296.3 mm in 2012. In the same time, the sunshine hours in 2013 were with 626.9 hours less than in 2012 .

A summary of the climatic parameters can be found in Table 1.

Table 1. The annual average air temperature, the amount af precipitations and the insolation during the viticultural years 2012 and 2013

Climatic parameter	Viticultural year	2012	2013	Multiannual average 1991-2010
The air temperature (^{o}C)	The average temperature, ºC	13.5	14.6	12.6
	The average temperature during the growing season, ºC	21	21.7	19
Precipitation (mm)	∑ annual precipitations ,(mm)	450.8	727.1	513.6
	∑ precipitation during the growing season, (mm)	296.3	554.4	330
Insolation (hours)	∑ real annual insolation, (hours)	2791.2	2164.3	2203
	∑ real insolation during the growing season, (hours)	1973.1	1710.9	1698

The climatic conditions of 2012 and 2013 required an adaptation of the used methods to control and combat pathogens and pests in order to ensure a homogeneous profitable production. Pest control success was ensured by applying specific treatment schemes, considering the microclimate conditions, biological reserve of pathogens, the varietal susceptibility to attacks and last but not least, choosing eficient plant protection products form the list of the aproved ones for organic culture.

In 2012, five treatments were necessary for vine protection, while in 2013 six treatments were neccesary. Plant protection products used in the organic vineyards from the viticultural center Murfatlar are Kocide 2000 (copper hydroxide 53,8%) and Kumulus DF (sulfur 80%) obtained from DuPont International and BASF. The amount of copper/ha/year has not exceeded the 6 kg limit imposed by the Regulation (EC) no. 889/2008 of the European Commission.

Table 2. The resistance evaluation of the studied varieties to the vine pathogens

Variety	Year	Pathogenic agent				
		Plasmopara viticola		*Uncinula necator*		*Botritis cinerea*
		Leaf (9.2.3)	Grape (9.2.4)	Leaf (9.2.5)	Grape (9.2.6)	Grape (9.2.2)
Feteasca neagra	2012	5	3	3	1	3
	2013	5	5	3	1	7
Cabernet Sauvignon	2012	3	1	3	1	3
	2013	5	3	1	1	5

The evaluation of the two varieties resistance to pathogen attack showed that the downy mildew (*Plasmopara viticola*) and gray mold (*Botrytis cinerea*) occurred with greater intensity in 2013 as compared to the previous year (Table 2). Of the two varieties studied 'Feteasca neagra' showed greater sensitivity to these diseases, the control plan including a complex of cultural and environmental measures preventively applied during the incubation period of pathogens.

The attack of powdery mildew (*Uncinula necator*) recorded a moderate intensity in the studied years and it was manifested only at the foliar level, not affecting the grape yield. Manual works as bounding and tipping of shoots, nipping and partial defoliation were used to create optimal conditions for a good ventilation of the vine stock, which also improved the efficiency of the applied treatments .

Preventive control of the grape moth (*Lobesia botrana*) was achieved by performing correct canopy control and by using pheromone traps. With the help of pheromone traps the population density was determined. During the ongoing of the study the economic damage threshold of 100 butterflies/trap/week was not achieved in any of the two parcels.

The qualitative indices of production as the weight of 100 berries and glucoacidimetric index, on one hand, and the content of extractable anthocyanins and polyphenols index, on the other hand, reveals different levels of accumulation depending on variety and year.

The year of 2013 was the viticultural year in which both studied varieties expressed a higher acumulation in sugars, thus the glucoacidimetric index at harvest had values of 52.2 and 62.8 as compared to the previous year when the values were lower 33.4 and 38.3, respectively (Figure 1).

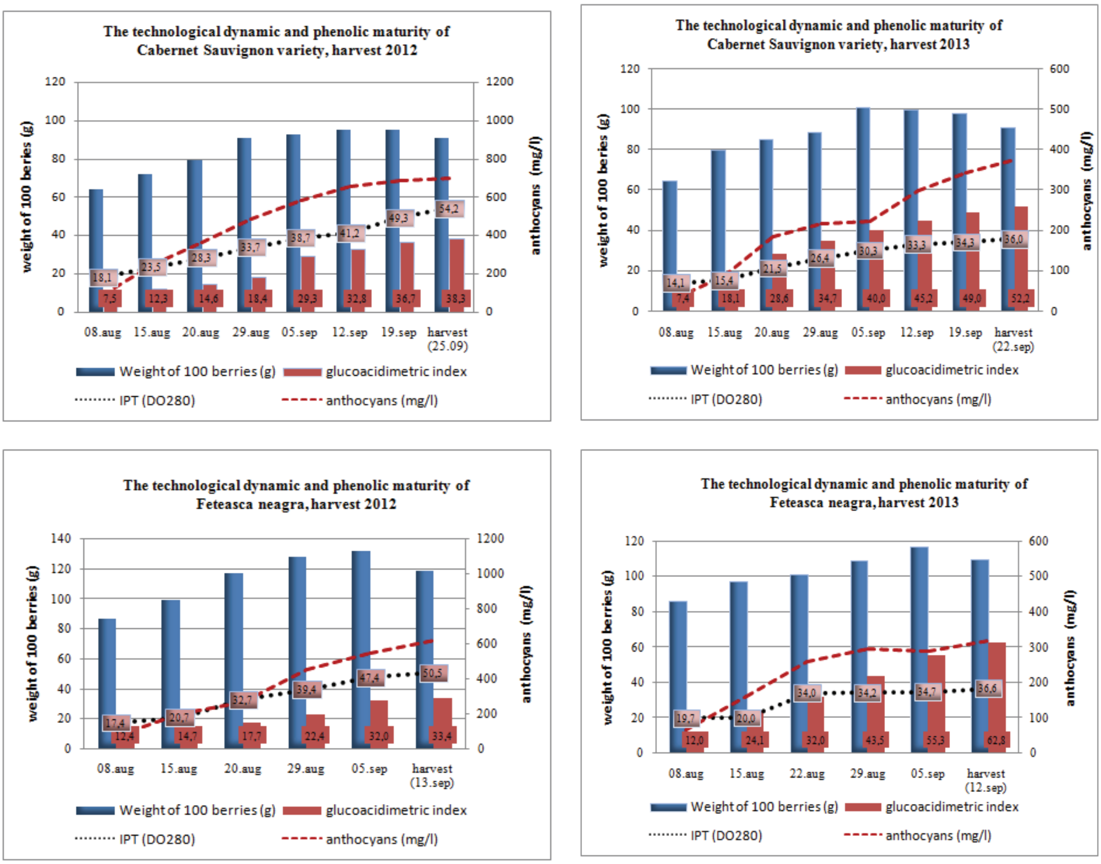

Figure 1. The evolution of qualitative parameters of the studied varieties, 2012-2013

In 2012, due to weather conditions with slightly lower temperatures during the growing season and with a quantity of precipitation similar to the average of the last 20 years, the accumulation of color compounds was superior, thus the total anthocyanins recorded a value of 621.11 mg/l for 'Feteasca neagra' and 699.26 mg/l for 'Cabernet Sauvignon', as compared to 2013, when the content of anthocyanins was only 317.44 mg/l for 'Fetească neagra' and 374.16 mg/l for 'Cabernet Sauvignon'. Regarding the polyphenols, the situation was similar, but with a less variation. The total polyphenol index in 2012 was 36.00 for 'Feteasca neagra' and 36.6 for 'Cabernet Sauvignon', increasing in 2013 to 50.4 and 54.2, respectively.

This indicates a slower phenolic ripening in 2013, coupled with the higher level of precipitations. However, in 2013 the harvest could not be delayed until a better phenolic maturity was reached because the glucoacidimetric index at harvest has already exceeded the previous year values. The option was to attempt the correction of polyphenols during winemaking processes. The evaluation of phenolic grapes ripening makes it possible to forecast the red wines quality and to model the technologies towards improving the wines phenolic structure. During the studied period, the yield was influenced by the action of the climatic factors in connection with the specific characteristics of each variety.

Table 3. The paramters and indices determined for the studied varieties organically grown during 2012 and 2013

Year	Variety	The weight of a bunch (g)	The volume of a bunch (ml)	100 berries weight (g)	No. of berries	Rachis Weight (g)	Yield (t/ha)	Maximum yield allowed for DOC wine (t/ha)
2012	Cabernet Sauvignon	140.86	116.67	135.2	144	5.66	9.0	12.9
	Feteasca neagra	234.44	203.33	229.32	200	5.12	8.8	15.0
2013	Cabernet Sauvignon	184.23	163.33	179.75	186	4.63	10.0	12.9
	Feteasca neagra	236.38	211.67	227.78	166	8.60	9.5	15.0

Under the same ecosystem conditions and technology, grape yield was variable, the most productive year proving to be 2013, when 'Cabernet Sauvignon' had an yield of 10 t/ha and 'Feteasca Neagra' 9.5 t/ha. Although close to the upper legal limit, the obtained values did not exceed the maximum allowed for production of wines with denomination of origin in Murfatlar vineyard and the quality of the raw material was not negativelly affected in any way.

CONCLUSIONS

The climatic conditions of winegrowing region Murfatlar in the years 2012-2013 proved to be favorable for organic growing of 'Cabernet Sauvignon' and 'Feteasca neagra'.

By adapting the pest control methods, especially by applying specific preventive and efficient schemes, the obtainment of a homogeneous and cost effective production was ensured. The selection of plant protection products depends on the microclimate and on the biological reserves of pathogens, but also on the crop sensitivity their attack.

Although the accumulation of glucides and anthocyanins in grapes depends on genetic traits of each variety, the decisive influence of the climatic conditions in specific production years was also observed. By monitoring the phenolic maturity each year it is possible to harvest at an optimal moment, to achieve a better phenolic structure in wines.

The grape harvest quantitatively evaluated by the average weight of the cluster and technological indices prove the good potential of these varieties and their adaptability in the Murfatlar climate. As regard to the quality, sugar content of both varieties showed values between 208 g/l and 245 g/l, allowing the classification of the resulted wines in the category of wines with denomination of controlled origin DOC.

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EVALUATION OF THE SUITABILITY OF SEVERAL CULTIVARS FOR ORGANIC GROWING IN THE VITICULTURAL CENTER OF MURFATLAR

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Abstract

The adaptability of the grape cultivars to the biotic and abiotic environment is one of the decisive factors for their suitability to be grown in organic culture conditions. The correct identification of highly suitable cultivars reduces the risks of important yield losses in difficult climatic years. The studies performed took into account the climatic factors evolution in the period of 2011-2013 as compared to the average values recorded for the 10-year period 1991-2010. The purpose was to identify some grape cultivars with suitable yields and genetic resistance to these changes to be grown in this region with organic viticulture technologies. Our studies show that the eco-pedo-climatic factors recorded during the last years were more favourable for the organic vine growing than in the past. Among the cultivars that proved to be suitable for the organic viticulture in Murfatlar, displaying high or moderate resistance to pests, drought and frost, are 'Cristina', 'Columna', 'Mamaia', 'Cabernet Sauvignon', 'Feteasca neagra' and 'Riesling italian'.

Key words: *organic vine growing, stress tolerance to biotic and abiotic factors, red and white cultivars*

INTRODUCTION

The industrialized agriculture with its accompanying shortcomings tends to be replaced by "organic agriculture" which, since the last decade, is starting to gain a more clear outline in our country too (Puia and Soran, 1981).
Organic viticulture has developed as a part of organic agriculture, whether you call it organic viticulture, biological or organic. The principles of organic agriculture are based on detailed know-how of production systems that maximize the local and economical resources, by integrating traditional knowledge with scientific progress. By organic agriculture as the accepted definition by the European it is understood that the culture system which aims at making and keeping productive biological systems without having to rely on products derived from chemical synthesis (Antoce et al., 2008).
Growing the most suitable cultivars with high productivity and quality traits with increased adaptability to the specific conditions of the main growing areas of the country, tolerant to biotic and abiotic stress remains the decisive factor in obtaining economic performance in the wine sector.
In general, all plant physiological processes are influenced by a number of abiotic factors: temperature, light, insolation and precipitation. The climatic potential suitability for optimal development of different plant parts can be appreciated monthly and annual by some dynamic values and strating from the growing season by various climate indicators.
Research undertaken in the recent years have allowed for a global evaluation of the oenoclimatic potential, evaluating also its suitability for organic viticulture in a specific region (Antoce et al., 2007; Carbonneau, 1994; Irimia and Patrichi, 2009; Irimia and Rotaru, 2009).

MATERIALS AND METHODS

The research took place at the Research Station for Viticulture and Oenology Murfatlar on the most popular varieties of white and red wines, recommended for the Murfatlar vineyard according to the Ministry Order 255 of 31.03.2006, evaluating their adaptability to the current climatic conditions and to the attack of various pathogens.
All works performed in the vineyard respected the technological steps specified for the organic culture system in the Regulation (EC) No 834/2007 of the Council concerning the labeling and organic production.
The growing of white wine varieties represents the main direction of production in Murfatlar wine center, but black varieties are cultivated here as well. Among the white varieties were studied: 'Chardonnay' (CH), 'Pinot gris' (PG), 'Italian Riesling' (RI), 'Sauvignon blanc' (SB) and 'Columna' (C). Among the studied varieties for the red wine 'Feteteasca neagra' (FN), 'Cabernet Sauvignon' (CS), 'Pinot noir' (PN), 'Mamaia' (M) and 'Cristina' (CR) were included. With the climate change, the black varieties started recently to enjoy better heliothermal conditions, which allowed the obtainment of quality red wines with denomination of controlled origin.
In biocenotic complexes within Murfatlar vineyard ecosystem, the damaging flora and fauna is relatively diverse and numerous, but only a few species of phytopathogens and pests can cause major economic damage, among them being found: downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*) and the gray rot of grapes (*Botrytis cinerea*).
The evaluation of biotic stress resistance was performed using a standard evaluation methodology (Genres project, 1999), which consists of visual estimation of the percentage of the infected leaf surface. For each variety a logarithmic scale was used with the following graduation: 1- without symptoms, 2-high resistance, 3-medium-high resistance, 4-moderate resistance, 5-low resistance, 6-moderate sensitiveness, 7-susceptibility, 8-high susceptibility, 9-high sensitiveness.
The evaluation of the cultivar resistance to abiotic factors (drought and frost) was performed in the field, where visual changes in the general appearance of the vine stock were observed and quantified. Each change was estimated using a logarithmic scale, graduated according to the degree of damage observed: 1 - very resistant, 2 - resistant, 3 - moderately resistant, 4 - slightly resistant, 5 - sensitive, 6 - very sensitive.
Climatic data were provided by the weather station Weather Master 2000 produced by Environdata, Australia. The colected data included daily observations regarding maximum and minimum temperature, sunshine and rainfall, based on which have been calculated a range of climate indicators commonly used in viticulture: the real heliothermal index (Branas et al., 1946), the bioclimatic vine index (Constantinescu et al., 1936), the oenoclimatic suitability index (Teodorescu, 1977), the aridity index (Martonne, 1926) and rain factor, Lang (1925).

RESULTS AND DISCUSSION

The climatic factors from the studied period corresponded to years with different conditions. A general analysis of the 2011-2013 period compared with the baseline period (average of 1991-2010), reveals the presence of mild weather that did not cause damage by frost. The absolute minimum air temperature values within the range of endurance for winter buds were -12ºC in 2011 and -14ºC in 2013, while the lowest temperature of -22ºC was recorded in 2012.
Monthly average temperature during the growing season ranged from 19.8°C (2011) to 21.7°C (2013), the average of the three studied years was 20.8°C, showing an increase over the reference period by 1.8ºC. The amount of rainfall during the active growing season varied from one year to another, being more abundant in 2013 (554.4 mm) and deficient in 2011 (238.9 mm).
The number of hours of insolation has been at an average of 2349.3 hours, the lowest values being recorded both in 2011 and 2013, while the highest ones were recorded in 2012 reaching a total of 2791.2 hours (Table 1).
In accordance to the measured climatic indicators it was found that the *heliothermal index* increased to 4.03 as compared to the average of 3.50 which indicates a raise of light

and temperature resources, important for the quality of the late maturing varieties. It is considered that conditions are optimal for grapevines when the value of IH is greater than 2.6 (Sandoiu, 2001).

The bioclimatic vine index (Ibcv) expresses the interaction of temperature, light and humidity; by analyzing these resources dring the period of our study, we observed a decrease in the average of 2011-2013 as compared to the reference period, that is a decrease from 13.6 to 12.2. The specific values of Ibcv for our country vineyards ranges from 5 to 15, the lower values of the range indicating rich water resources, while the higher end values and above indicating vineyards with rich heliothermic resource or years with deficit in rainfall. The values of the oenoclimatic suitability index (IAOe) assess the climatic suitability for a region to obtain red wines, that is the favorability to synthesize anthocyanins in grapes. Its values are between 3700 and 5200. Areas with lower values than 4300 are considered suitable only for white wines, those for which the oenoclimatic suitability index is between 4300 and 4600, have a medium favorability for red wines and the area where values are over 4600, as it is also in our case, are recommended for red wines. This index places Murfatlar vineyard in an area favorable for the production of red wines.

The hydrothermal coefficient (CH) had an average value of 0.85 in the period 1991 to 2010, identical to the average of the studied years. In our country this ratio ranges between 0.7 and 1.8, aside of this range the vine cultivation being not recommended.

Martonne aridity index value recorded over time in Murfatlar wine center falls on the borderline between semi-humid and semiarid climate. This index allows to determine the degree of aridity of a region for characteristic periods (a year or a month), as an expression of a restrictive character which climatic conditions impose to certain crops.

The rain factor proposed by *Lang* is used to illustrate the sequence of rainy or arid months, taking into account the precipitation /temperature ratio, as an expression of the water inputs and outputs from the system influenced by the temperature, which is one of the main factors in evapo-transpiration processes. Its values ranges between 20 and 160, from a desert climate to a wet one. Last years trend places Murfatlar vineyard into a semi-arid climate.

Table 1. Climatic indicators calculated for Murfatlar wine center

	Multiannual average 1991-2010	2011	2012	2013	Average 2011-2013
The average monthly temperature, ºC	12.6	13.5	13.5	14.6	13.8
The average monthly temperature during the growing season, ºC	19.0	19.8	21.0	21.7	20.8
Minimum temperature, ºC	-14.6	-12.2	-22.0	-14.0	-16.1
Maximum temperature, ºC	34.6	37.0	39.8	39.5	38.8
∑ Annual real insolation, (hours)	2203	2092.3	2791.2	2164.3	2349.3
∑ Annual real insolation during the growing season (hours)	1698	1708.2	1973.1	1710.9	1797.4
∑ Annual precipitations,(mm)	513,6	326,8	450,8	727,1	501,6
∑ precipitation during the growing season, (mm)	330	238.9	296.3	554.4	363.2
The hydrothermal coefficient, CH	0.85	0.6	0.6	1.2	0.8
The heliothermal index, Ihr	3.5	3.7	4.9	4.4	4.3
The bioclimatic vine index, Ibcv	13.6	15.0	14.3	7.2	12.2
The oenoclimatic suitability index IAOe	5093	5350.8	5840	5549.2	5580
Martonne aridity index $I_{ar\text{-}DM}$	22.9	13.9	19.2	29.6	20.9
The rain factor Lang	41	24	33	50	36

The ecopedoclimatic characterization and changes observed in recent years have highlighted the favorable conditions for organic cultivation of grapevine in Murfatlar ecosystem. The cultivar assortment in Murfatlar vineyard was selected in time to better sustain the interaction of eco-climatic, ecopedological and secondary biotope factors, but even so vine varieties behave differently to the attack of the main pathogens: downy mildew, powdery mildew and gray mold (Table 2).

Table 2. The relative resistance of the studied varieties to climatic factors and disease attack

Vineyard	Variety	DR	FR	MA	FA	PC
Murfatlar	Chardonnay	4	2	7	7	7
	Pinot gris	5	5	7	7	7
	Riesling italian	4	5	6	3	6
	Sauvignon blanc	4	5	7	7	7
	Columna	3	2	6	7	6
	Cabernet Sauvignon	2	2	6	4	6
	Feteasca neagra	3	3	7	4	6
	Mamaia	3	2	6	7	6
	Cristina	3	3	4	7	4
	Pinot noir	3	2	7	4	7

DR = drought resistance şi FR = frost resistence: 1 - very resistant, 2 - resistant, 3 - moderately resistant, 4 - slightly resistant, 5 - sensitive, 6 - very sensitive; MA – vine mildew resistance (*Plasmopara viticola*); FA – powdery mildew resistance (*Uncinula necator*); PC – gray mold resistance (*Botrytis cinerea*): 1- without symptoms, 2-high resistance, 3-medium-high resistance, 4-moderate resistace, 5 low resistance, 6-moderate sensitiveness, 7-susceptibility, 8-high susceptibility, 9-high sensitiveness.

Analyzing the evolution of studied varieties to the two abiotic stress factors, a normal reaction was observed, according to their genetic traits. The varieties resistent to frost were the red varieties, of which 'Cabernet Sauvignon' distinguished itself. Regarding the resistance to drought, the most sensitive were the white varieties 'Riesling Italian' and 'Sauvignon blanc' (Figure 1).

The vine mildew (*Plasmopara viticola*), has a dynamic life cycle, variable and strongly influenced by climatic factors. The vine mildew manifested on leaves and berries by showing spots on the leaves with various aspects, evoluating from oily-yellow spots with a confused outline in spring, to brown-center spots with dry aspect later on. *Plasmopara viticola* showed a stronger attack in 2013 for all studied varieties, the average infestation ranging from 3 to 6, from resistant to moderately susceptible, depending on the variety. Treated with anti-fungal products allowed in organic viticulture, the following varieties have shown a better resistance to vine mildew: 'Pinot gris', 'Sauvignon blanc', 'Columna', 'Cabernet Sauvignon', 'Mamaia' and 'Cristina'.

Powdery mildew (*Uncinula necator*), conidial shape Oidium tuckeri, is the most common disease after the vine mildew. In the case of powdery mildew the attack was both on leaves and berries. On the leaves was observed a whitish mycelium, ectoparasite, fine, powdery appearance that stretched forming spots on both surfaces of the limb. Under the mycelium the tissues became brown, but the leaves have only fallen late in fall. The powdery mildew presented higher values of the attack rate in 2011 and 2013, the white wine varieties being more affected, the average infestation being on the evaluation scale from 3 to 6, that is from resistant to moderately susceptible. Among the varieties studied, resistant to attack were 'Sauvignon blanc', 'Italian Riesling', 'Cabernet Sauvignon' and 'Pinot noir', while an opposite behavior displayed 'Chardonnay', 'Columna' and 'Cristina'.

The grape gray mold (*Botrytis cinerea*) is a disease difficult to control, due to the unusual nature of the fungus and the complexity of its life cycle. During the growing season, the pathogen is present in plantations (dormant), so that measures to combat the fungus should be preventively made. The intensity of the attack of gray mold manifested mainly during the months of abundant precipitation. *Botrytis cinerea* showed a strong attack in 2013, due to the year's favorable climatic conditions for the pathogen development. Most susceptible to the attack by gray mold are the varieties 'Chardonnay', 'Pinot gris', 'Italian Riesling' and 'Pinot noir'.

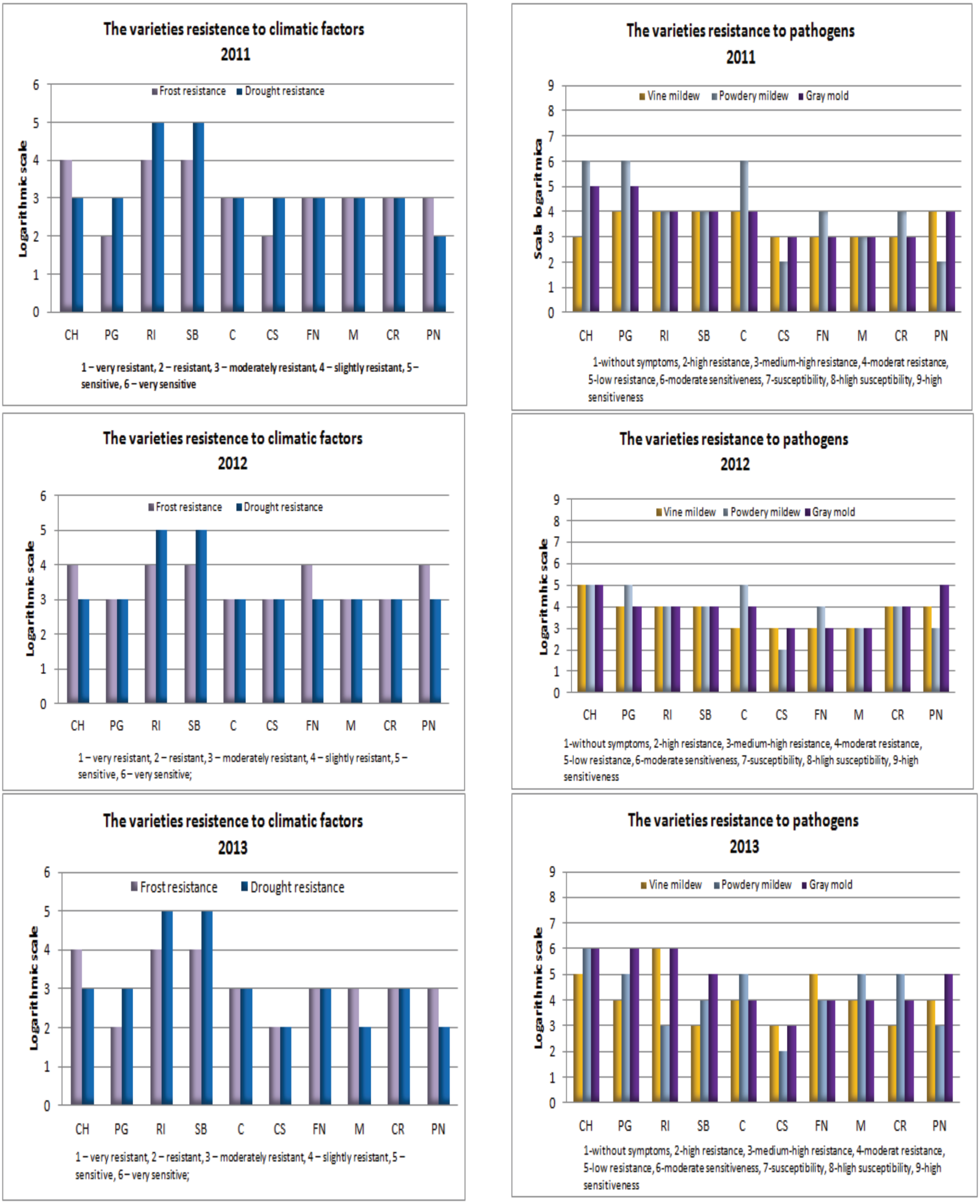

Figure 1. The evaluation of the resistance of the studied white and red varieties to biotic and abiotic factors in Murfatlar vineyard

In Murfatlar ecosystem vineyard the species of pathogens that cause the most significant economic damage and should be kept under control in organic viticulture are downy mildew and gray mold, and of the abiotic factor drought.

Table 3. The hierarchy of the studied varieties according to three factors:downy mildew, gray mold and drought

The suitability to the organic culture	Variety	Downy mildew	Gray mold	Drought resistance	Powdery mildew	Frost resistance
Recommended varieties	Cristina	4	4	3	7	3
	Columna	6	6	2	7	3
	Mamaia	6	6	2	7	3
	Cabernet Sauvignon	6	6	2	4	2
Recommended partially	Riesling italian	6	6	5	3	4
	Feteasca neagra	7	6	3	4	3
Not recommended	Chardonnay	7	7	2	7	4
	Pinot noir	7	7	2	4	3
	Pinot gris	7	7	5	7	5
	Sauvignon blanc	7	7	5	7	4

By taking into account the importance of these three main factors a classification of varieties was attempeted and included in Table 3, based on the resistance showed by the varieties to these factors. We classified the vaieties in there groups: the recommended varieties for organic viticulture the partially recommended and the variaties not recommended.

CONCLUSIONS

The adaptability of grape varieties to biotic and abiotic environmental conditions is one of the decisive factors for their suitability to the growing technologies, including the organic vine culture.

A general analysis of the 2011-2013 studied period compared with the reference period (average of 1991-2010), reveals an increase of the thermal regime and of insolation. The values of the synthetic ecological indicators from the Murfatlar center indicates a favorable area for growing vine with a very good suitability for both white and red wine varieties.

In the biocenotic complex of the Murfatlar vineyard only a few species of phytopathogens cause important economic damages, among them being: the downy mildew (*Plasmopara viticola*) and gray mold of grapes (*Botrytis cinerea*). Permanent supervision of vineyards in order to identify the first signs of pathogen attack during the vegetation period is a major objective to be followed in order to obtain quality grapes, especially in organic viticulture.

Our evaluation showed that the most recommended varieties to be grown organically in Murfatlar vineyard are: 'Cristina', 'Columna', 'Mamaia', 'Cabernet Sauvignon' and partially recommended are 'Feteasca neagra' and 'Italian Riesling'.

This fact is also supported by the ecopedoclimatic conditions, the calculated indexes showing that Murfatlar is a favorable ecosystem for the organic cultivation of vines.

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STUDIES REGARDING THE FOOD SAFETY MANAGEMENT SYSTEM IMPLEMENTATION IN PRODUCTION OF WINE GRAPES

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Abstract:

The wines quality and safety are depending of the raw materials quality and safety, respectively black and white grapes. These two requirements (quality and safety) were arise from the need to protect consumers and are found both in EU Directions and National Legislation. In the technology of wine grapes production it can be applied a HACCP system which allows for the identification of the key elements from this process which can affect the grapes quality and safety. HACCP is the abbreviation for the English expression "Hazard Analysis and Critical Control Points". Using the HACCP system, the microbiological, chemical and physical risks existing in the wine grapes production technology are identified, in order to find the CCPs (Critical Control Points). In order to keep under control the technology of wine grapes production a single CCP-1 was identified: Integrated plant protection, with two significant hazards: a) the attack of fungi, insects and mites; b) the pesticides and heavy metals residues. Our studies formulate the good hygiene requirements and work procedures that have to be fulfilled by each company specialized in the wine grapes production.

***Key words**: CCP, HACCP, hazard, residues*

INTRODUCTION

HACCP, is an acronym derived from English:"Hazard Analysis And Critical Control Points" and this is a systematic method to identify, assess and control significant hazards associated with plant and animal origin food (Pardo et al., 2005). This is designed to anticipate and control problems before they happen. It provides the most effective and sufficient way to ensure that food products are safe. The great calitologist J. Juran, 1999 said "We have to open eyes to ensure quality and food safety, if we want to live decently". Hygienic-sanitary quality is the essential condition for a food to be consumed by humans. Consumers always want the food at their disposal to be safe in terms of hygiene and sanitary quality so as to cause no illness (Mencinicopschi and Raba 2005). Hygienic quality is influenced by: - microorganisms and parasites; - pollution by: antibiotic residues in animal products, food additives, heavy metals, radioactivity, pesticides, organic substances (dioxins) and - other toxic substances: allergenic, cyanogen, antimetabolites etc.; - natural induced toxicity by: toxic plants, mycotoxins etc (Boboc, 2010). EC Regulation 852 transposed in Romania by HG 924/2004, article 5, paragraph 1, states: "Food business operators must implement and maintain a permanent procedure or procedures based on HACCP principles". In this case the white or black grapes can contain mycotoxins, pesticides and heavy metals residues above the permissible limits, being very dangerous for human health. During the technological flow of grapes producing, the hygiene rules should be respected, for does not lead to loss of product hygienic quality. Food safety management systems like ISO 22000:2005 and Hazard Analysis and Critical Control Point (HACCP) can assure the wine grapes safety by preventing potential hazard at the process source points. Using the HACCP system, we tried to identify the microbiological, chemical and physical hazards existing in the produced grapes technology, in order to indicate the CCPs (Critical Control Points) for the products hygienic quality.

MATERIAL AND METHOD

The studies were developed in a vine plantation according to the flow diagram described in Figure 1. For each process step was performed the risk analysis, in order to identify the biological, chemical and physical hazards correlated with the product and process and also the preventive actions and control measures which are necessary to keep under control these hazards (table 1). In order to establish the Critical Control Points in all steps of the technological flow of wine grapes production, where it's possible to implement specific control measures regarding food safety, it was applied the CCP decision tree (recommended by Codex). The control of each CCP, according HACCP principles are planned in a document named the HACCP plan (Table 3). The establishing and implementation of the control measures are shown by specific records. All researches and observations were made in a private company which owns vine plantations and vinification line. Each transfer of wine grapes from the farms to vinification line are accompanied by an analysis bulletin, issued by an approved laboratory.

RESULTS AND DISCUSSIONS

The technological flow of white and black grapes is shown in Figure 1. In researches it was checked each step from the technological flow of white and black wine grapes (Figure 1) in order to identify potential hazards such as: biological, chemical and physical hazards.

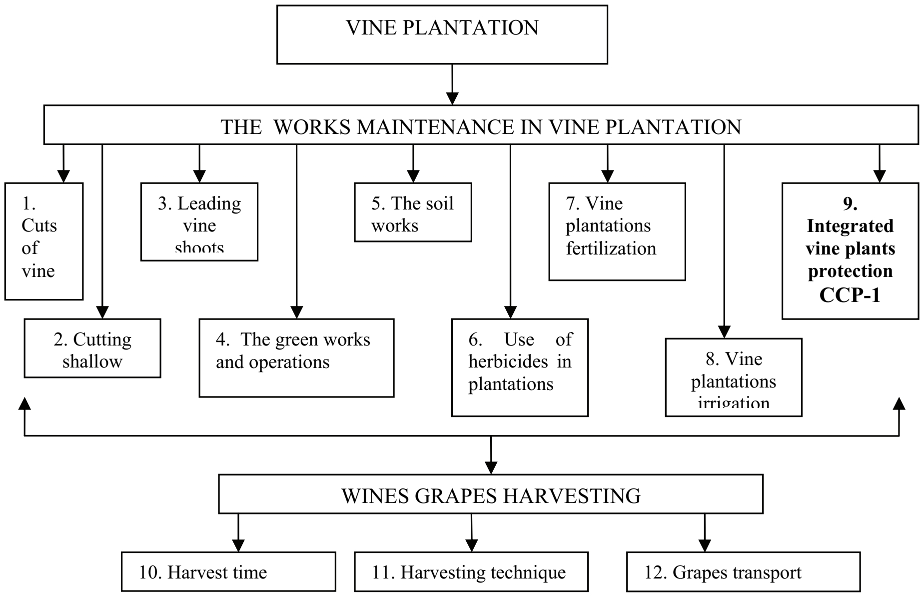

Figure 1. The technological flow of white and black wine grapes

All these hazards (Table 1) were identified by using the "decision tree" recommended by Codex Alimentarius. Each food safety hazard has been assessed according with the severity of possible adverse health effects and their appearing probability. By using the CCP decision tree (Table 2) only one CCP was identified in the technological flow of white and black wine grapes, which is focused to keep under the control the step 9 "Integrated plants protection". The critical Control Point is a point, step, or procedure where the hazard that's associated with the food can be prevented, eliminated or reduced to acceptable levels.

Table 1. Hazard analysis in the technological flow of white and black wine grapes

Process step	Hazard	Preventive actions/Control measures
1. Cuts of vine	*B and *C - unidentified	-
	*F- incorrect cuts	Visual inspection, cutters training; checking scissors
2. Cutting shallow roots	*B and *C - unidentified	-
	*F- Incorrect cutting shallow roots	Visual inspection, cutters training; checking instruments
3.Leading vine shoots	*B and *C - unidentified	-
	*F- Incorrect leading vine shoots	Avoid strangulation through a binding strings too tight; cutters training
4. The green works and operations	*B and *C - unidentified	-
	*F- Overdose fertilises	Making accurate and timely the works in green; checking scissors, knives, penknives; operator training; visual inspection
5. The soil works	*B and *C - unidentified	-
	*F- Incorrect soil works	Making accurate and timely ground work for: maintenance and keeping the humus in soil, nutrient accessibility, activation of chemical and biological processes in the soil and weeds destroying.
6. Use of herbicides in plantations	*B-– Affecting vines by incorrect herbicide usage	Setting recipes and time of herbicide; Uniform distribution of herbicides and avoiding contact with the leaves.
	*C-unidentified	-
	*F- Overdose of herbicides	Limiting doses to a minimum; respect the manufacturer's instructions for treatments; operator's training.
7. Vine plantations fertilization	*B and *C - unidentified	-
	*F- Overdose fertilises	Use of rational fertilization to avoid the dangers of polluting products and the environment; Knowing the soil level of nutrients supply and annual consumption; Extensive use of organic fertilizers and bio-fertilizers for the extention of the ecological concept.
8. Vine plantations irrigation	…………………	…………………
9. Integrated vine plants protection	*B- Attack by fungi, insects and spider mites	The fruit health maintaining; Combined treatments; Direct visual inspection of the field.
	*C- Ineffective chemical control	Treatments at the optimum time when is most effective; Choices based on efficacy, mode of action, side effects, the reshuffle. Active substance content; manufacturer's instructions checking; operator training; suppliers selection.
	*F- Overdosing or underdosing of pesticides	Professional advice to avoid danger to the user during application, fruit waste, environmental damage, etc..
10. Harvest timing	…………….	……………
11. Harvesting technique	*B- Microbial contamination in mechanical harvesting application	Equipment hygiene, harvested grape antimicrobial protection
12. Grapes transport	*B- Microbial contamination	Hygiene vehicles maintenance; Drivers training; Visual inspection
	*C-unidentified	-
	*F- – grapes crushing	Respect the limit of the load; Visual inspection. Training pickers

*B= biohazard; *C= chemical hazard; *F= physical hazard

The HACCP Plan (Table 3) is one of the most important document from food safety management system, which contains the main information necessary in order to implement the control measures and keep under control the identified CCPs.

Table 2. CCP determination during processing (in according with the decision tree)

Process step	Hazard	Decision tree questions				CCP no.
		Q1- there are preventive measures to prevent the risk of identified hazards?	**Q2-** stage is specially designed for eliminating / reducing the possibilities of developing a potential hazard	**Q3-** there is the Possibility of contamination due to a potential hazard till the acceptable level?	**Q4-** can a later stage to eliminate a potential hazard identified / to reduce possibility the occurrence of a potential hazard to an acceptable level?	
9. Integrated vine plants protection CCP-1	- Attack by fungi, insects and spider mites; - Overdosing or underdosing of pesticides	yes	yes	No	no	CCP-1

Table 3. HACCP Plan for wine grapes production

Stage	Significant hazard	CCP no.	Control measures	Critical limits	Monitoring				Corective actions
					Method	Respon-sable	Frequentcy	records	
Pest and disease control	a. Attack by fungi, insects and spider mites; b. Overdosing or underdosing of pesticides .	CCP -1	a. Specific laboratory tests for mycotoxins; b. Analysis of heavy metals residues and pesticides presence	a. Over 2µg/kg; b. Heavy metals presence (As<0.2, Cd<0.01, Cu<1, Pb<0.3 mg/l); organic pesticides: 2-10 ppm	a. ELISA test; HPLC b. gas chroma-tography or spectrophoto metry	head farm laboratory	Before harvest if the attack is high	Register for pest and disease control Analises register	Preventative health maintenance in plantations . Personal training

CONCLUSIONS

The HACCP system implemented in any company with wine grapes production activity, is a preventive self-control, whose principles can be applied to all food producing sectors.

Our studies formulate the good hygiene requirements and work procedures that have to be fulfilled by each company specialized in the wine grapes production.

In order to keep under control the wine grapes production technology one CCP was identified, which is focused on “Pest and disease control“.

The grapes should be sound without rotten parts, otherwise oxidative and microbial contamination can rapidly develop.

Harvesting should be conducted with greatest possible care and efficient disease management system should be applied.

Pesticides should be handled with care as they constitute chemical hazards.

At time of harvest, the grapes must have also reached correct maturity.

Pesticide and fungicide residues on surface of berries constitute chemical hazards.

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TAXONOMIC IDENTIFICATION OF TWO YEASTS STRAINS ISOLATED FROM CONCENTRATED MUST AND SUPRASULFITED MUST

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Abstract:

In 2012 year two yeasts strains were isolated from yeasts populations that were active in concentrated grape must and suprasulfited must. The aim of this work was the characterization and identification of two yeasts strains and established their affiliation at osmofile or sulfites species, or proof that this property was acquired as a result of their adaptation to unfavorable environmental conditions. Research shows that these two yeasts strains marked with an 1-MC and 2-MS belong to Saccharomyces oviformis and Saccharomyces cerevisiae var. ellipsoideus species. It was found that their growth and reproduction in unfavorable environmental conditions are a result of adaptation to these conditions. This adaptation is a warning to the fact that even if the equipment of work is technologically advanced (on the construction and operation of) can not prevent biological degradation of concentrated musts and of suprasulfited musts with these yeasts strains, if the hygiene measures are not taken for the technological lines, vessels and spaces for wines storage.

***Key words**: yeast strain, concentrated must, suprasulfited must, acetaldehyde*

INTRODUCTION

Loss of biological stability and degradation of wines quality are produced due to the presence of yeasts in the winemaking rooms that can be adapted to unfavorable conditions of life (Matei et al., 2012). Even in the modern and performant technological lines, the biological stability assuring of wines with sugar contents, concentrated musts and those suprasulfited is a major problem (Antoce et al., 2001). The literature in this field indicates that there are yeasts that can live in concentrated musts or protected by large amounts of SO_2, by changing musts composition (Cotea et al. 1985).

This paper aims is to identify and taxonomic classification of two yeasts strains isolated from concentrated must and suprasulfitated must (where these activities), establishing their membership to osmofile or sulfite species and prove that the property that they possess has been acquired as following a process of adaptation to unfavorable environmental conditions.

MATERIAL AND METHOD

Isolations were made, from concentrated must and suprasulfited must of SC VINEX MURFATLAR Ltd., situated in Murfatlar vineyard, in 2012. The yeast isolated from concentrated must were noted with 1-MC, and that from suprasulfited must with 2-MS. At the time of isolation concentrated must have had a sugar content of 670 g/l and the suprasulfited must have had the following composition: sugar - 200 g/l, total SO_2 - 910 mg/l and free SO_2 - 498 mg/l. Classical identification of these strains have been performed. Isolation was made from must in biological activity diluted with sterile water, and the cultivation was done on must-agar culture medium. Isolation was performed starting from a single cell using the method of successive dilutions (Domerq, 1956) and isolation in pure culture method (Barnett and Yarrow, 2001). To identify and characterize the isolated yeasts strains were used the classical methods of Lodder and Kreger van Rij (1984), Kurtzman and Fell (2011) and were followed: - cells shape and size (large and small diameters) after cultivation for three and six days at 25°C, in liquid medium (grapes musts and Wickerham medium) and on the solid (must grapes with agarose gel); - pseudohyfae-formation, after cultivation for 12 days, in medium potato agarose gel; - sporulation on synthetic medium-Gorodkova (comments after 30 days). Physiological characteristics were determined using the following tests: - different sugars fermentation into test-tubes with Durham tubes in yeast extract medium, with 2% of each sugar tested; - the sugars and nitrate assimilation by auxanographic methods using agar medium rich in mineral salts and vitamins - using the ethyl alcohol as unique carbon source; - split arbutin. To complete the taxonomic characterization were made also the following determinations: -total number of cells increased during alcoholic fermentation (Thoma blade counting); the fermentative processes evolution by gravimetric method and sugars content metabolized; preferential metabolizing of glucose and fructose by the yeasts during fermentation, was made by paper chromatography method; total and free SO_2, alcohol, acetaldehyde, volatile acidity contents, by OIV methods. In order to check the maximum temperature supported by these yeasts, they have been thermostated at different temperatures for 48 hours, after which the viability was tested by cultivation in must-agar medium to the optimal temperature. The concentrations in sugars and SO_2 to which yeasts strains can not activate were established by their cultivation on medium with high concentrations of these compounds (500 mg/l free SO_2 and 700 g/l sugars).

RESULTS AND DISCUSSIONS

The cells shape and size of the two yeasts strains lead to the conclusion that they belong to *Saccharomyces oviformis* (1-MC) and *Saccharomyces cerevisiae* var. *ellipsoideus* (2-MS) species.

1-MC yeast strain characterization:
- in liquid medium the cells are round, oval, isolated or grouped in pairs (Figure 1.a);
- budding type is polar; -cells sizes range from (4.0 to 6.0) x (9.0 to 12, 0) microns; -the strain isolated and tested does not form ring or film;
- the culture on solid medium is white to cream colored, smooth, shiny, with less lobes marked and fine ramifications at the edge of culture;
- on potato agar medium, the strain does not form pseudohyphae;
- sporulation test was performed on carrot-agar medium (Gorodkova medium);
- the yeast strain formed in asca two, rarely three spheroidal shape spores (Figure 1.b).

2-MS strain characterization:
- in liquid medium the cells are elliptical, oval, very rarely round isolated or grouped in pairs;

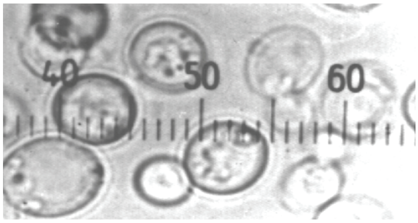

Figure 1.a. 1-MC strain – cells shape

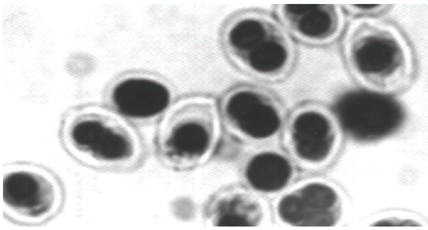

Figure 1.b. 1–MC strain – spores shape

- budding type is polar, there are cases of lateral budding (Figure 2a);
- cells sizes range from (5.0 to 8.9) x (4.0 to 8.1) microns, are similar to those in the specialized literature;
- the yeast strain isolated and tested does not form ring or film;
- culture on solid medium is white or creamy, smooth with matte or shiny aspect, straight edge;
- on potato agar medium, does not form pseudohyphae;
- in Gorodkova medium the yeast strain form 1-4 ascospores with round, spheroidal or elliptical shape (Figure 2b).

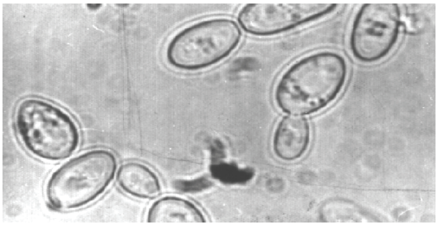

Figure 2.a. 2-MS strain-cells shape

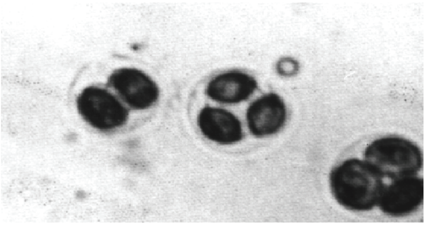

Figure 2.b. 2-MS strain: spores shape

Form cells incurred visible changes after their subjection to tests to establish maximum levels of sugar and SO_2 they support. At physiological characterization the two yeasts strains have responded as follows (Table 1, Table 2).

Table 1. Sugars fermentation at 1-MC şi 2-MS strains

Tested sugar	Fermentation	
	1-MC strain	2-MS strain
Glucose	+	+
Galactose	-	+
Zaharose	+	+
Maltose	+	+
Lactose	-	-
Rafinose	+1/3	+1/3

Table 2. Sugars asimilation at 1-MC şi 2-MS strains

Tested sugar	Asimilation	
	1-MC strain	2-MS strain
Glucose	+	+
Galactose	-	+
Zaharose	+	+
Maltose	+	+
Lactose	-	-
Rafinose	+	+

The table results shown us that the strain 1-MC, with the exception of galactose and lactose ferments and assimilates the rest of sugars, while 2- MS strain does not ferment, and do not assimilate lactose.

The tests of nitrate assimilation, using of ethyl alcohol as unique carbon source, split arbutin and the growth in the cycloheximide presence were negative for both strains and for 1-MC strain the growth in vitamin-free medium was absent.

The fermentation led in strictly aerobiosis conditions and monitored by the increase in the total cells number of yeast showed that the two yeasts strains also differ by the length of time during which the maximum cell density was achieved per unit volume. Strain 1-MC (*Saccharomyces oviformis*) recorded maximum $290x10^3$ cells/mm^3 in 5 days and 2-MS strain $245x10^3$ cells/mm^3 in 7 days (Figure 3).

The fermentation speed expressed by the release of CO_2 and daily sugars consumption has characteristic values for each strain.

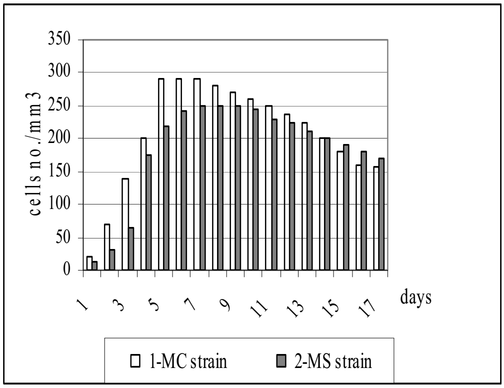

Figure 3. The total cells number increase under strict aerobiosis conditions

Regarding the selective sugars fermentation it was found that both species ferment glucose preferentially.

For both strains, in the time when 80% of the glucose has been fermented the proportion of fructose has not exceeded 50%, while the 1-MC strain fermented sugars at a higher speed and in a shorter time.

Alcoholic degree produced by 1-MC strain was higher with almost 2 vol% than 2-MS yeast strain and the capacity to produce volatile acids was higher for the 2-MS yeast strain (Table 3).

Table 3. The production of alcohol and volatile acids during alcoholic fermentation process

Yeasts strain	Alcohol vol. %	Volatile acids g/l CH_3COOH	Acetaldehyde mg/l
1-MC	12,0	0,90	113,1
2-MS	10,1	1,12	120,0

In the evolution of acetaldehyde content some differences were observed , in the sense that 1-MC yeast strain produced a larger amount by alcoholic fermentation than 2-MS yeast strain (Table 2 and Figure 4).

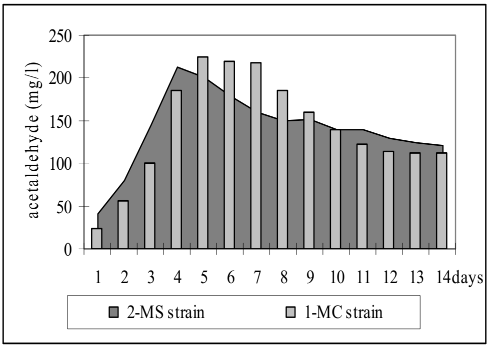

Figure 4. The evolution of the amount of acetaldehyde during the alcoholic fermentation

Keeping their viability at different temperatures was performed by thermostat for 2 days. It was observed that the lethal ranged temperature for both yeasts strains was around 47^0C. The MC-1 yeast strain was tested to establish the maximum quantity of sugars that it can support and the researches showed that at values about 650-700 g/l it activity is inhibited, while at the sugar level around 450 - 500 g/l, this yeast metabolizes sugars to 5-6 vol.% alcohol in about 52 days. The 2-MS yeast strain isolated from a suprasulfited must (498 mg/l SO_2), after isolation and purification was tested for the maximum level of SO_2 that can activate; it was found that it does not support large concentrations than 390-400 g/l free SO_2.

CONCLUSIONS

As a result of morphological and physiological measurements determined in laboratory conditions, the two strains belong to the species *Saccharomyces oviformis* (1-MC) and *Saccharomyces cerevisiae* var. *ellipsoideus* (2-MS).

The two yeasts strains were differentiated between them by cells shape and size, the aspect of giant colonies, by the sugars fermentation and assimilation tests and oenological characteristics (production of alcohol, volatile acids and acetaldehyde).

The property of 1-MC yeast strain (*Saccharomyces oviformis*) to activate in concentrated musts medium and to produce partial sugars fermentation in an inversely proportional quantity to the sugars concentration leads to the idea that the alcohol- their own product of metabolism- is an inhibitor of the yeasts activity that can be used in the preservation of concentrated grape must. In this case, the must can not be considered sterile because it contains yeasts which can become active.

The property of 2-MS yeast strain (*Saccharomyces cerevisiae* var. *ellipoideus*) to be active in suprasulfitated musts represents an adaptation that could be maintained if the yeast continues to be present in this medium. When the must is concentrated the microorganisms are concentrated too and thus the source of infection increased.

To ensure biological stability it is recommended to apply preventive must treatments and to ensure aseptic conditions as much as possible throughout the technological flow.

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INFLUENCE OF CLIMATE VARIABILITY ON GROWTH, YIELD AND QUALITY OF GRAPES IN THE SOUTH PART OF ROMANIA

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Abstract

The aim of this paper was to evaluate the trends of changes in climatic parameters and the effect of climate variability on vegetative growth, yield and quality of grapes in Bucharest (44°47'N; 26°07'E). There were examined the trends of changes in temperature, precipitation and the annual minimum temperature from 1961 to 2013 and from 1998 to 2013 - the production and quality of grapes of 'Feteasca regala' variety. Between 1961-2013 the annual temperature was higher than average with 1.0^{0}C, in the growing season (April-October) the average temperature was higher with 1.1^{0}C and in the summer time with 1.9^{0}C. There were established correlations between some climatic parameters and pruning weight, yield, sugar accumulation, titratable acidity etc. The temperature increase was associated with higher must sugar content, especially in the summer (from June to August). Also, more frequency of the minimum harmful temperatures for grapevine (under -20°C) was observed during the last decade. Grape production was correlated with the precipitations from the period March-May, and those of summer negatively influenced sugar accumulation in grape berries. In the last 16 years (1998-2013) there was a significant increase in the values of Winkler Index; maximum yield of grapes was obtained at values of this index comprised between 1600 and 1800; when the value was higher, the yield and must acidity decreased significantly. Maximum grape yields were obtained at values of Huglin Index comprised between 2300 and 2500; at higher values, there were registered significant decreases of the yield and of the must acidity as well as increased accumulation of sugars in berries.

Key words: *climate change; growth; quality; viticulture; yield*

INTRODUCTION

The grapevine is one of the cultivated plants most affected by climate change. Global warming, that is so obvious in the last two decades, has led to a number of positive effects on the physiology of plants (Burzo and Dobrescu 2011) on the production of grapes and on its quality (Cotea et al., 2008; Dejeu et al., 2008; Bucur et al., 2012), but also some negative effects, due to water stress (Bock et al., 2011; Carbonneau, 2011), obtaining less balanced wines, given the lack of acidity, the accumulation of a high concentration of sugar (and a higher alcohol content), decreased aging potential of wines, premature oxidation of white wines, increased variability vintages, flavoring changes, affecting the typicity of wines.
Given the predictions in the field, on the intensification of this phenomenon in the future, several scenarios were developed to adapt the viticulture to climate changes.
In viticulture, there are widely used a number of indicators for climatic zoning works, for studying phenophases development, for assessing the favourability of a specific area for quality viticulture: Winkler Index (1944); Huglin Index (1978) etc. (Winkler et al., 1974; Huglin, 1978). In the latest years, these indices have been widely used to study the effects of climate change on yield and quality in viticulture and winemaking (Neethling et al., 2012).
The study aims to note the evolution in time for the period 1961-2013(53 years), of the main elements of climate and bioclimatic indicators, in the context of climate change and their impact on yield and quality of grapes.
This study allows a better understanding of the current climate and a foresight of the consequences of climate changes on viticulture.

MATERIALS AND METHODS

The data recorded at Bucharest-Baneasa station (44°43'N; 26°10'E) were used for the present study. The present analysis relies on the observation data recorded during 53 years (1961-2013).

The climatic data analysed represents the average temperature, the minimum temperature and the maximum temperature, as well as the precipitations.

The climatic data allow the analysis of climate evolution over a long period of time, as well as the characterization and quantification of global warming in the area of Bucharest.

The temperature data were also used to calculate two bioclimatic indices, including the Growing degree-days (or Winkler Index, WI), Huglin heliothermal Index (Huglin Index - HI), (Winkler et al., 1974; Huglin, 1978). Winkler Index was calculated according to the equation: $\sum[(T_{max}+T_{min})/2-10^{o}C]$, for the period April – October (Winkler et al., 1974).

Huglin Index is calculated using the formula: $\sum[(Tavg-10^{o}C)+(Tmax - 10^{o}C)]/2 \cdot k$, for the period April-September (k = day length coefficient, varying from 1,02 to 1,06 between 40° and 50° latitude).

In the experimental plantation of the Horticulture Faculty within University of Agronomic Sciences and Veterinary Medicine of Bucharest, it was conducted an experiment during 1998 - 2013. The plantation was established in 1994, with 'Feteasca regala' variety, clone 21 Bl, grafted on Kober 5 BB, planted at distances of 2.2/1.2 m (3787 vines/ha), with spur pruning cordon and loading of 10 eyes/sqm.

Based on the data regarding temperature, precipitations, Winkler Index, Huglin Index and values of pruning wood, grape yield, accumulation of sugars, titratable acidity of must at 'Feteasca regala' variety in a long-term experience (1998-2013) there were determined the following correlations between: precipitations in spring (III-VI) and the annual wood eliminated at pruning; average temperature in the growing season (IV-X) and yield; average temperature in summer (VI-VIII) and sugar and titratable acidity; precipitations in winter (XII-II) and yield; precipitations in spring (III-V) and yield; Winkler Index and yield; WI and titratable acidity; Huglin Index and yield; HI and sugar accumulation in berries; HI and titratable acidity.

RESULTS AND DISCUSSIONS

Climate variability. As a consequence of annual average temperatures evolution during 1961-2013, there was a significant trend of increasing it by about 1°C (Figure 1). The average temperature increase was more obvious in the last two decades.

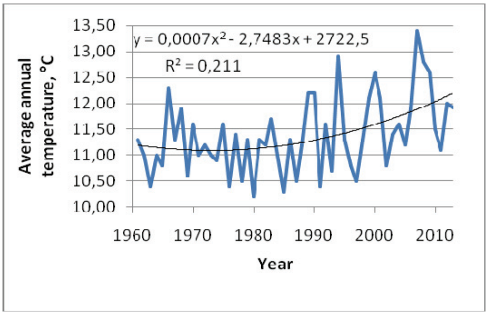

Figure 1. Evolution of the average annual temperature (°C) for the period 1961-2013

The evolution of average temperature during the growing season (IV-X) also highlights a significant increasing trend over the 53 years of observations (Figure 2). From 1961 to 2013, the average temperature in April-October increased by 1.1° C.

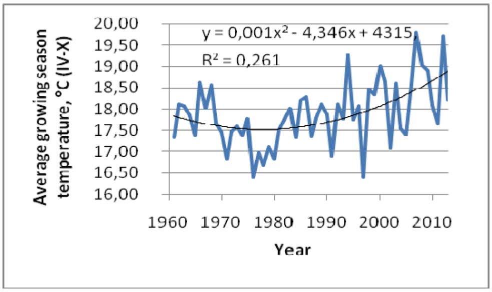

Figure 2. Evolution of the average temperature in the growing season (IV-X) for the period 1961-2013

The warming is even more accentuated during summer (VI-VIII), highlighted by a difference of 1.9ºC and the highest degree of significance (Figure 3).

The climate changes also act through an increased frequency of accentuated winter frosts. In our case we noticed an increased frequency during the last decade of minimum harmful temperatures for grapevine (< -20°C), but also by lower values (Figure 4).

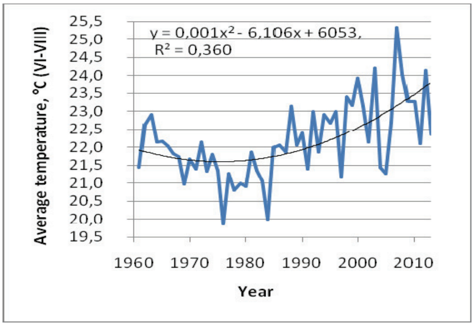

Figure 3. Evolution of the average temperature in summer, °C (VI-VIII, 1961-2013)

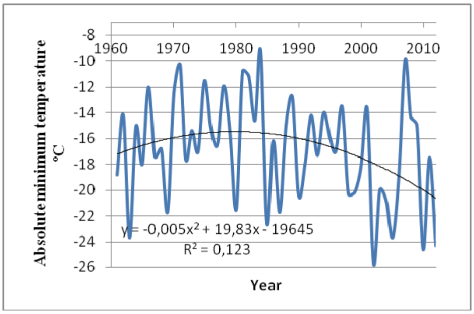

Figure 4. The absolute minimum temperature evolution, °C (1961-2013)

Regarding the evolution of the annual precipitation in the period 1961-2013 (Figure 5), of the precipitations during the growing season - IV-X (Figure 6) there are no significant changes, except for higher variations from one year to another.

The influence of climate changes on grapevine. Climate variability has influenced the growth, development of phenophases, grape production and quality.

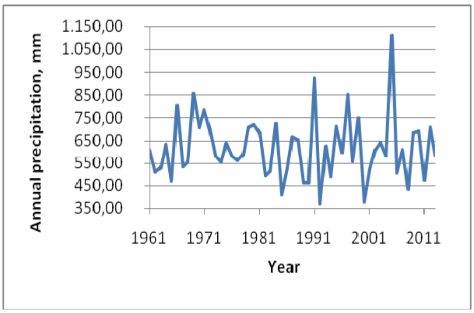

Figure 5. Evolution of the annual precipitations (mm) for the period 1961-2013

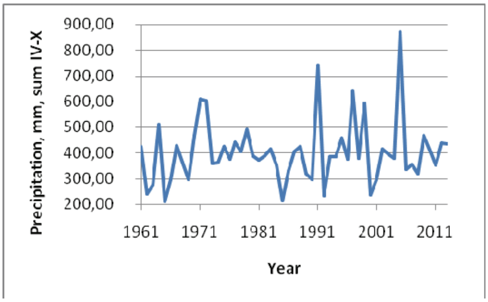

Figure 6. Evolution of the precipitations (mm) in the growing season (1961-2013)

Between the average temperature in the growing season (IV-X) and the grape yield, there was highlighted a significant parabolic correlation (Figure 7). The highest yields of grapes were obtained at average temperatures in the growing season between 17.5 - 19.0°C; above 19.0°C there has been a downward trend in the yield.

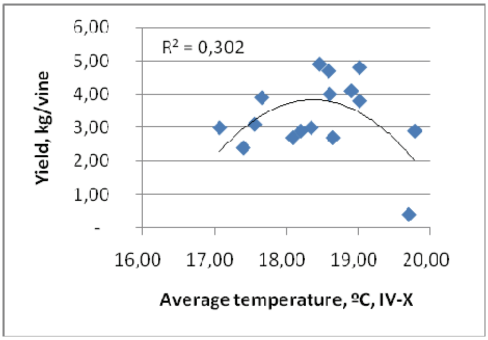

Figure 7. Correlation between average temperature in the growing season (IV-X) and the grape yield (kg/vine) (1998-2013)

The highest accumulation of sugar was obtained at an average temperature of more than 23°C in summer (Figure 8).

Extreme temperatures during maturation lead to an acceleration of the ripening process, at an increased sugar content, resulting in higher levels of alcohol or residual sugar content in wine, in conditions of a faster decomposition of acidity.

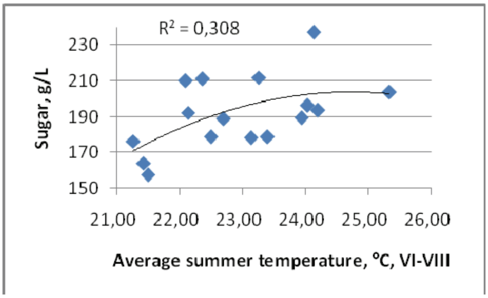

Figure 8. Correlation between average temperature in summer (VI-VIII) and sugar accumulation (1998-2013)

High temperatures in summer (VI-VIII) have a significant negative influence on acidity (Figure 9). At average temperatures of above 23.5°C in summer, titratable acidity falls below 4.0 g/L H_2SO_4, being necessary to correct the acidity of the must. Decreasing the acidity can have a negative impact on wine typicity and its capacity of aging.

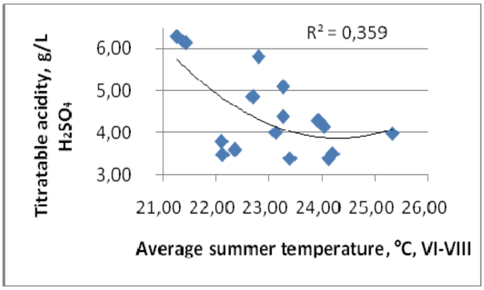

Figure 9. Correlation between average summer temperature (VI-VIII) and titratable acidity (1998-2013)

Between the amount of precipitations recorded in spring (III-V) and the pruning wood there was evidenced a significant correlation (Figure 10).

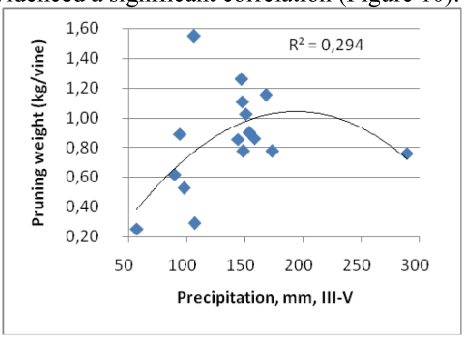

Figure 10. Correlation between the precipitations in spring (mm, III-V) and pruning weight (kg/vine) (1998-2013)

Between the precipitations in winter (XII-II) and the grape yield, there was highlighted a distinctly significant correlation (Figure 11). The highest yields of grapes were obtained at precipitations values situated, in winter, between 80 to 160 mm.

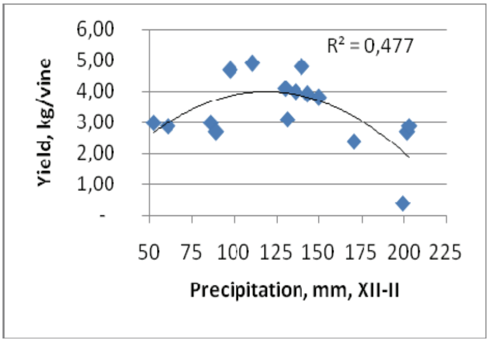

Figure 11. Correlation between the precipitations in winter (mm, XII-II) and yield of grapes (kg/vine) (1998-2013)

A distinctly significant parabolic correlation was also determined between the amount of precipitations in spring (III-V) and grape yield (Figure 12).

Winkler Index (WI) values increased significantly in the last 16 years (1998-2013), from values of 1700 to 1830 (Figure 13).

The highest yields of grapes were obtained at Winkler Index values between 1600 and 1800 (Figure 14).

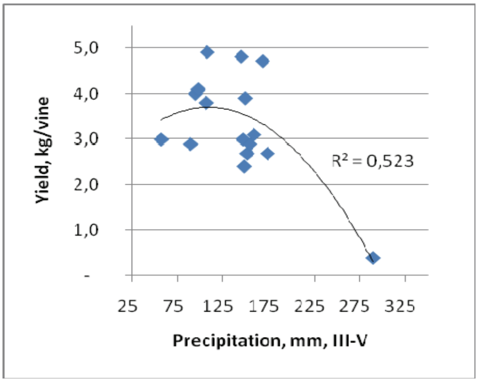

Figure 12. Correlation between the precipitations in spring (mm, III-V) and yield of grapes (kg/vine) (1998-2013)

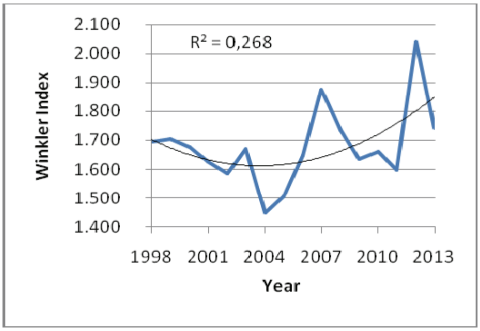

Figure 13. Evolution of the Winkler Index in the period 1998-2013

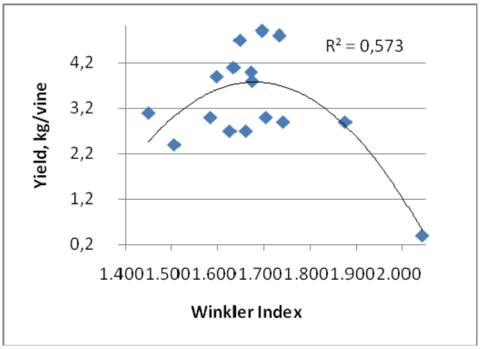

Figure 14. Correlation between the Winkler Index values and yield of grapes (1998-2013)

As Winkler Index values increase from 1400 to 2000, the titratable acidity of the must decreases significantly (Figure 15). At values of WI higher then 1800, titratable acidity of the must decreases significantly.

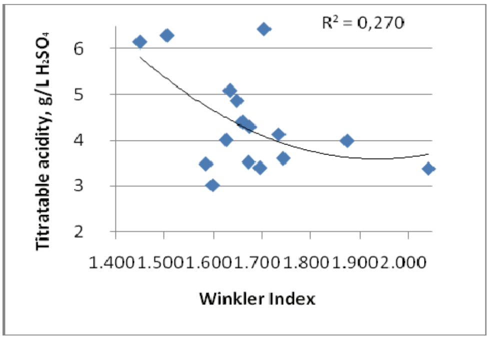

Figure 15. Correlation between the Winkler Index values and titratable acidity (1998-2013)

Between Huglin Index (HI) values and grape yield there has been established a distinctly significant correlation (Figure 16). At HI values of over 2600, specific to warm climate, grape production decreases significantly.

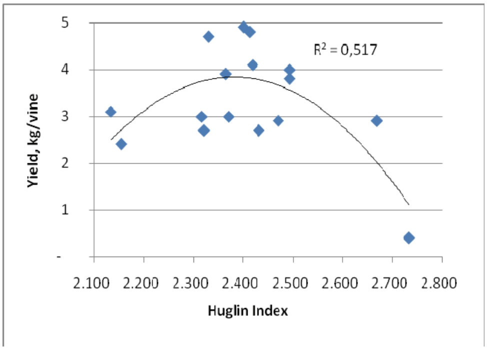

Figure 16. Correlation between the Huglin Index values and yield of grapes (1998-2013)

It was also found a significant increase in the accumulation of sugar in berries, while the index values increase (Figure 17).

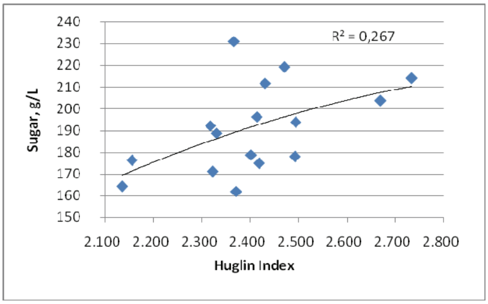

Figure 17. Correlation between the Huglin Index values and sugar content (1998-2013)

Between Huglin Index values and titratable acidity of the must there has been determined a distinctly significant negative correlation (Figure 18). At values of HI higher than 2600, titratable acidity of the must decreases below 4.0 g/L, requiring its correction.

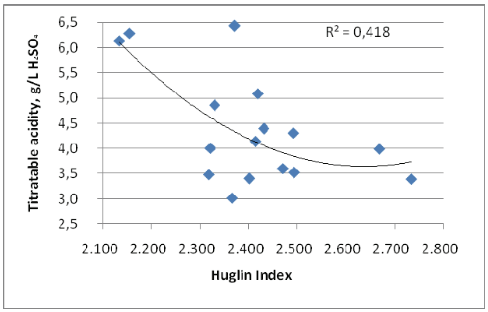

Figure 18. Correlation between the Huglin Index values and titratable acidity (1998-2013)

CONCLUSIONS

Between 1961-2013, it was registered an increase of the average annual temperature with 1.0°C, of the average temperature in the growing season (April-October) with 1.1°C and of the summer temperature with 1.9°C.

The temperature increase was associated with higher must sugar content, especially in summer (from June to August).

Also, a higher frequency was observed during the last decade of the minimum harmful temperatures for grapevine (under -20°C).

Winkler and Huglin indices have increased in recent years to values specific for warm climate.

The temperature rising during the ripening of the grapes results in an acceleration of the maturation, a quicker decomposition of the acids, an increase in sugar content, and it can also result in a loss of wine typicity.

Since it is expected that this trend of temperature rising continues in the future and that the ripening period of the grapes takes place in warmer conditions, there are necessary adjustments of the viticulture development strategies under the new conditions.

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ABUNDANCE AND POPULATION DYNAMICS OF FLAVESCENCE DORÉE PHYTOPLASMA VECTOR *SCAPHOIDEUS TITANUS* BALL ON ABANDONED GRAPEVINE IN SOUTHERN ROMANIA

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Abstract

The abundance and population dynamics of Scaphoideus titanus, the natural vector of the quarantine grapevine yellow phytoplasma, Flavescence dorée, (FD) were evaluated with yellow sticky traps on abandoned grapevine of 1.5 ha in the Northern part of Bucharest city (Southern Romania) during 2009-2011. The results revealed that the vector has developed permanent and increased populations every year. The nymphs were recorded from June to July in the year 2009, until August in 2010 and even up to September in 2011.The activity of adults started at the end of June in the year 2009 and at the beginning of July in the years 2010 and 2011. The adult's flight usually lasted until the middle of October. The present data can be of great help in developing precise control measures against S. titanus. They are the first reported on the population dynamics of Flavescence dorée vector in Romania, and the first time when a case of an abandoned vine infested by this vector has been detected.

Key words: *abundance, population dynamics, Scaphoideus titanus, abandond grapevine, Romania*

INTRODUCTION

Scaphoideus titanus Ball (Hemiptera: Cicadelidae: Deltocephalinae) is an extremely important leafhopper pest of grapevine, causing serious damage by transmitting the pathogen '*Ca. Phytoplasma vitis*' (Elm Yellows group or 16SrV group) (Seemüller et all., 1998), a wall-less intracellular bacterium restricted to phloem sieve cells, associated with the most important grapevine yellow diseases, the Flavescence dorée (FD).

The FD phytoplasma is a quarantine organism in the EPPO region (Annex IIA2 EU2000/29) and in Romania (HG563/2007/AnnexII S2), while its vector *S. titanus* is not regulated.

S. titanus is a species of North American origin, and was reported in Europe for the first time in France, at the end of the 50s (Baggiolini et al., 1968; Arzone at al., 1987, Papura et al., 2012).

Presently, it is widely spread into vine growing regions in the Central and Southern Europe. In the countries bordering Romania, it was firstly reported in Serbia (Magud and Toševski, 2004), then in Hungary (Dér et al., 2007) and Bulgaria (Avramvov et al., 2011). In Romania, the *S. titanus* presence was recorded in 2009 (Chireceanu et al., 2011).

Also, the vector and the Flavescence dorée disease were detected in abandoned vineyards (Dér et al., 2007; Pavan et al., 2012).

The increased risk of *S. titanus* vector in grapevine economy changed its status from a regional one to one of international importance, hence it is regarded as a major threat to viticulture production throughout the world.

According to the studies on S. *titanus* biology published in literature, this is an univoltine species, monophagous on grapevine in Europe, overwintering as eggs into the bark of two years or older woody canes of plants (Gargani et al., 2013).

The eggs hatch at the beginning of May; the nymphs pass through five moults; the adults are active from July to September (Boudon-Padieu, 2003; Linder and Jermini, 2007; Rigamonti et al., 2010).

The control of vector *S. titanus* as well as the destruction of abandoned vines which are reservoirs of diseases and vector is considered among the most effective actions to mitigate

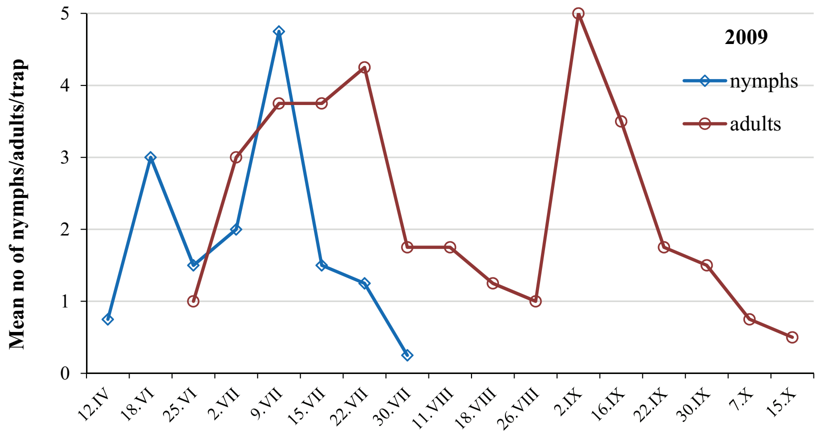
the Flavescence dorée disease spread in vineyards. For a successful control of the leafhopper a full knowledge on its biology along with other detailed studies on population dynamics and densities are needed.
The aim of this paper was to evaluate the abundance and seasonal development of the nymphs and adults of Flavescence dorée vector *S. titanus* on abandoned grapevine in the South Romanian weather conditions, in order to acquire basic knowledge for applying the control measures, according to the vector's development stages.

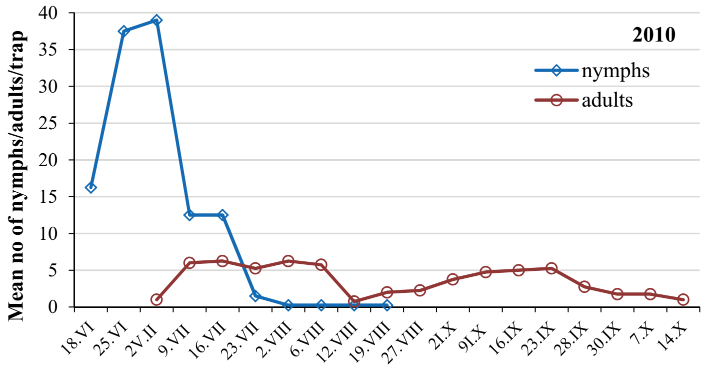
MATERIALS AND METHODS

The research was carried out during 2009-2011 in an abandoned grapevine of about 1.5 ha located in the Northern part of Bucharest city (Southern Romania), geographic position 260656508N, 445621388E, 89m, (WGS84/UTM zone35N).
The vine plot has shown the presence of symptomatic plants belonging to different cultivars, with symptoms of redness, yellowing and downward rolling of leaves. Shoots from rootstocks but also plant species others than of *Vitis* genera were observed (e.g. seedlings of *Acer negundo*, *Murus spp.*, herbaceous vegetation).
The sampling of *S. titanus*, nymphs and adults, was made using yellow sticky traps. Four traps were placed inside the grapevine canopy, approximately 30-50 cm above the ground.
The traps of *Atraceras* type (30×15cm) were produced at the "Raluca Ripan" Institute for Research in Chemistry from Cluj Napoca, Romania.
The traps were checked every week from June to October. All the captured *S. titanus* individuals were identified and counted under a laboratory stereomicroscope.
The populations' dynamics was designed on the basis of weekly nymphs and adults captures on the yellow sticky traps.

RESULTS AND DISCUSSIONS

In Figure 1 is presented the populations' dynamics of *S. titanus*, adults and nymphs (figure 2), performed in 2009, 2010 and 2011.
The results of this study showed that the captures of *S. titanus* nymphs were recorded from June to July in the year 2009, until August in the year 2010 and even up to September in the year 2011.
Data for the years 2010 and 2011 indicated that even though the presence of nymphs on traps was prolonged, they had a rate of 0.25-0.5/trap (1-2 nymphs/4 traps/week).
A maximum of nymphs was noticed early July in 2010 and 2011. In case of the captures of 2009, a maximum of nymphs in the middle of June and another early July were observed.
The maximum value of nymphs' density per trap was of 4.75, 39.0 and 73.75 in 2009, 2010 and 2011, respectively.





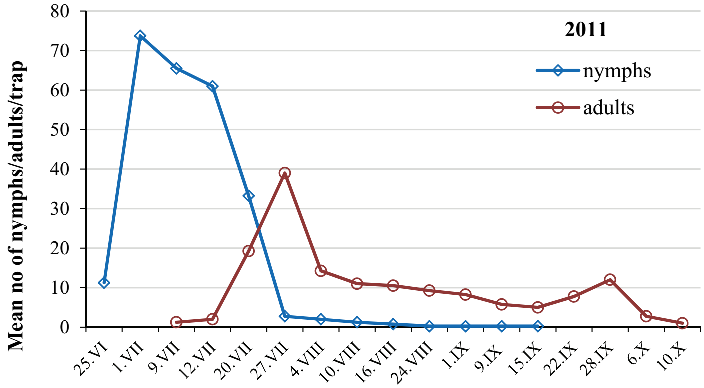


Figure 1. Seasonal population dynamics of *Scaphoideus titanus* Ball in Southern Romania (designed by means of captures per yellow sticky trap)

Figure 2. Adult and nymphs of *Scaphoideus titanus*

Considering the dynamics of captures in the three years of study (Figure 1), the *S. titanus* adult populations, the same as of nymphs, showed fluctuations in the rate of captures from one sampling date to the other and from year to year.

Data illustrated in this figure revealed that the activity of *S. titanus* adult started at the end of June in 2009 and at the beginning of July in 2010 and 2011. The earliest adults capture was recorded on June 25, in 2009. The adults' flight usually lasted until the middle of October. The entire flying time activity ranged from 93 to 112 days.

Captures of the year 2009 pointed out two periods of maximum in adult population dynamics, one in July and the other in September. But, taking into account the fact that this pest develops only one generation a year with one flight peak in its adult and nymph dynamics, this aspect could be considered the effect of rainfall fallen from spring to summer months in the study area and also of the changes in weather conditions that, together can produce modifications in the rate and dynamics of *S. titanus* local population.

The maximum value of adults' rate per trap was of 4.25, 6.25 and 39 in 2009, 2010 and 2011, respectively.

The cumulated populations' abundance of the vector *S. titanus*, nymphs and adults, on abandoned vine, is illustrated in Figure 3.

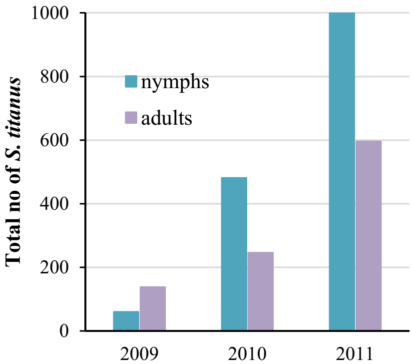


Figure 3. Total nymphs and adults of *S. titanus* population captured in the three years of trapping on abandoned grapevine in the South part of Romania

Comparison among the three sampling years revealed that the total captures of *S. titanus* were differed from one year to another, in the sense that the population has increased from the first to the third season. Both the nymphs and adults were much more numerous in the third season (2011) than in the first season (2009) and the second (2010) season of trapping.

In the year 2011, the total number of nymphs was 2.1 and 16.8 times more than in 2010 and 2009, respectively. The total number of adults in the year 2011 was 2.4 and 4.3 times more than in 2010 and 2009, respectively. The captures of nymphs were higher in number than those of adults, except for the year 2009, when more adults than nymphs were captured.

One of the factors contributing to the population densities of this pest could be the weather conditions of each year within study location. Throughout the monitoring period, local climatic conditions reached various values, such us the year 2011 that had less snowy winter and a hotter summer than 2009 and 2010. Lessio and Alma (2004) reported that the seasonal and daily activity of *S. titanus* is influenced by increased temperatures and humidity.

According to the captures data on the yellow sticky traps during the three year period of study, it was outlined that the leafhopper *S. titanus* developed permanent and stable populations every year in abandoned grapevine. Relative high number of *S. titanus*, collected in these conditions may confirm the fact that the abandoned vineyards offered optimum conditions for the vector development and can play a major role in epidemiology of FD phytoplasmas, as it has been reported in literature (Lessio et al., 2007; Beanland et al., 2006, Steffek et al., 2007, Pavan et al., 2010).

During visual observations, exuviae of *S. titanus* nymphs were commonly found on the lower side of maple trees (*Acer negundo*) leaves but also on *Cirsium arvense* leaves (Figure 4 and 5).

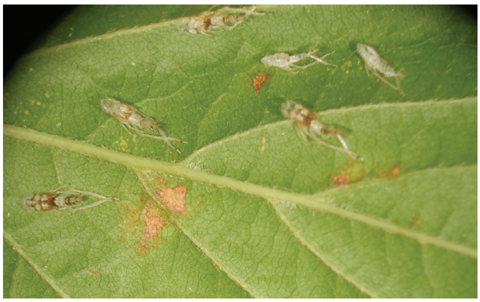


Figure 4. Nymphal exuviae of *S. titanus* on *A. negundo* leaf

Figure 5. Nymphal exuvia of *S. titanus* on *C. arvense* leaf

This observation is in line to others similar findings reported in literature and can be helpful in completing the range of plant species on which *S. titanus* was found in Europe, where it is considered to feed exclusively on *Vitis vinifera* species. In Switzerland, *Trifolium repens* and *Ranunculus repens* plants in vineyards were reported to host the *S. titanus* nymphs (Trivellone et al. 2009).

The presence of an increasing number of *S. titanus* on cultivated as well as on abandoned grapevine in Romania, as briefly shown in this paper, requests the need to apply obligatory chemical control against it. At this time, no insecticide has been approved for *S. titanus* control in our country. Its populations are covered through the insecticide treatments currently applied against main vine pests, such as *Lobesia botrana* and *Empoasca vitis*.

Therefore, the results of this work bring forward the first data on the biology and population dynamics of *S. titanus* in Romania, which can be used in warning the accurate time for insecticide applications in accordance with the development stages of the vector.

CONCLUSIONS

The results of this report regarding the abundance and population dynamics of *Scaphoideus titanus* - vector of Flavescence dorée phytoplasma - estimated on abandoned vine, showed that the vector developed high populations, increasing from one year to another, that this type of vine being a huge threat for the healthy vine of the vineyards.

We belief that this information can be of great help in developing precise control measures against *S. titanus*.

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A REVIEW OF THE CONVERSION RATE IN MONITORING THE MARKETING PERFORMANCE OF RETAIL WINE SHOPS

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Abstract

The success of a business can be evaluated by various performance indicators. Selecting the right key indicator for the marketing performance evaluation of a shop can often lead to the identification of potential improvements. For the evaluation of wine shops one of the most useful key performance indicators was identified as being the traffic conversion rate and the paper explains the possibilities to measure and calculate this parameter, its advantages and effectiveness. The objective of the paper is to integrate existing knowledge and findings about conversion rate, to highlights the implications and the advantages of this indicator in monitoring the marketing performance of physical (bricks-and-mortar) wine shops. The findings are drawn from literature review and is followed by analyse and discussion about the implications and the challenges of using the conversion rate indicator in practice. The paper is an overview of the conversion rate and findings reveal the importance of this indicator for assessing the marketing performance of the retail wine stores and the limits that generated lack of adoption on a large scale.

Key words: *traffic, conversion rate, purchasing behaviour, key performance indicators, wine shop*

INTRODUCTION

When analysing the shop performance, the most useful information collected are the ones that could not only show the outcome, but the ones that could help the managers find the cause and improve or prevent situations that generate loss in sales (Ryski, 2005).
The majority of retail analyse – wine shops included – relies on measuring sales and other indicators in relation with this values and the performance of the shop is monitored and evaluated based mostly on this indicators.
Faster and more effective availability of data is a competitive issue for most organizations. For example, businesses which have higher operational/credit risk (involving for example credit cards or wealth management) may want weekly or even daily availability of KPI (Key Performance Indicators) analysis, facilitated by appropriate IT systems and tools.
For retail, the most commonly used KPI's are: Total sales, Sales per hour, Average sale (per Customers/ Transaction), Sales per square meter, Customers per day, Items per customer/ transaction, Annualized sales per sales person, Sales compared to the previous year, Sales compared to target, Average Gross Margin, Revenue Market Share, Customer Profit, Gross Margin Return on Inventory Investment (GMROII) (Farris *et al*., 2006; PricewaterhouseCoopers LLP, 2011; Velimirovića et al., 2011).
The most relevant mean of understanding the success and the potential of the business is measuring the results comparative to the potential (opportunity), respectively how many customers purchased from the total that visited the shop. This can be accomplished using the conversion rate indicator, which reveals the performance in the context of the sale opportunities that existed (Underhill, 2009 and Ryski, 2011), as opposed to the sales only related indicators that offers no indication about the meaning of the values without considering them together with the circumstances.
The (traffic) conversion rate is as one of the 10 most important marketing key performance

indicators in e-commerce and for performance assessment in online industry in general (Perry, 2013).

For bricks-and-mortar retail wine shops, considering the high costs involved for measures taken for increasing the sales by generating more traffic or more prospects to enter the shop (advertising costs and the cost of the location), the traffic and conversion rate analyse offers a perspective on the existing potential – prospects already in the shop – allowing an incremental increase of the sales based on what is already there (intensive). Early identification and prevention of loss of sale, through conversion rate analyse, can work both as a *cost-cutting* and a revenue growth measure.

Therefore, in this work we assessed the options to better evaluate a shop performance by measuring and analyzing the traffic data, number of transactions and sale values.

MATERIALS AND METHODS

The study took into account the existent literature regarding the assessment of marketing performance of (physical/ bricks-and-mortar) shops in general, trying to select the most suitable key performance indicator to be used later on for the evaluation of the marketing performance of a wine shop.

The term marketing should be understood here as the broader area of all the actions undertaken in order to gain (paying) customers, while aiming the company goals (growth/ increasing the net income).

Generally, the marketing performance can be measured using metrics related to :

- Consumer attitudes and satisfaction: *Awareness, Perceived quality, Consumer satisfaction, Relevance to consumer, Image/personality, Perceived differentiation, Brand/product knowledge*, *Customer satisfaction, Number of complaints*;
- Comparison with competitors: *Relative consumer satisfaction, Perceived quality, Share of voice*;
- Consumer behaviour: *Total number of consumers, Number of new consumers, Loyalty/retention, Conversion, Number of consumer complaints*;
- Innovation: *Number of new products, Revenue of new products, Margin of new products*;
- Accounting/ Business performance: *Sales, Gross margins, Profitability* (Ambler *et al.*, 2004).

Metrics alone could only help the managers getting the information/ gain access to data, the correct selection of the metrics, trend and context analyse, could contour the insights, but most important when measuring performance is having clear goals and evaluating them with the right KPIs (Key Performance Indicators).

And one of the most important goals should be intensive growth based on the existing potential, hence using the most of what the company already has (prospective customers inside the shop).

RESULTS AND DISCUSSIONS

According to a benchmark survey by the Chartered Institute of Marketing in collaboration with Deloitte LLC, only 7% of organisations *always* set clear key performance indicators for marketing initiatives, and only 10% of businesses have core strategic meters that remain consistent to enable longer term reporting and over 2/3 of the companies feel that their KPIs are not optimal form measuring the impact of marketing (Brown and Turner, 2010).

Generally, retail companies use indicators related to capital expenditure, shop portfolio changes, expected return on new shops, customer satisfaction, same shop/like-for-like sales, sales per square metre (Pricewaterhouse Coopers LLP., 2011).

A performance indicator should be presented in form of numbers, should integrate well with present shop activity, should help to determine if the business is getting better and should be put into practice to produce a desired effect (Lake, 2013). In other words, the indicator should be Quantitative, Practical, Directional and Actionable. The most useful parameter found to obey these requirements and which can be used in assessing the marketing performance of wine shops is the conversion rate, as shown by the formula (1).

$$\text{Conversion rate (\%)} = \frac{\text{Number of transactions}}{\text{Traffic}} \quad (1)$$

This parameter can be used as such, monitored over time, used along with other indicators, analysing patterns and changes, or mapped together with other factors that influence the purchase occurrence.

As eloquently exemplified by Mark Ryski (2013) without conversion and traffic data it is impossible to understand if for example a 10 % sales improvement compared to the previous period is a success or a failure, because one needs to compared it with the values of traffic. If traffic increased with more than 10%, than the conversion rate is smaller and this means the performance of the shop, in the context of the sales opportunities it had, it is lower, in spite of the sales increase.

The key aspect of the conversion rate is actually the part that is missing (up to completing the 100%), as this represents the 'grey area' never reported, the unsuccessful shopping experiences from the customer's point of view or lost opportunity in sales from the business's perspective. All this missed customers are never captured in the sales results figures and neither in standard customer surveys (Ryski, 2011) and they can represent a large potential area of intensive expansionfor the business.

The conversion rate indicator, also referred as *customer conversion* (Conroy and Bearse, 2006), *buyers-to-shoppers ratio* (Chung and Hing 2010) or *close rate* (according to Ryski, 2011), illustrates the percentage of buying customers from the total potential customers entering the shop (traffic), as shown by the formula (1). For a higher accuracy it is recommended that instead of (total) traffic or gross traffic, only the total number of prospective customers (the number of individual customers that have entered the shop with intention of buying), or net traffic to be used (Ryski, 2011).

Regularly used in e-commerce and for performance assessment in online industry in general, conversion rate is not as frequent used in physical (bricks-and-mortar) shops.

According to Ambler, Kokkinaki and Puntoni (2004), accounting related metrics like Sales (Value and/or Volume), Gross margins and Profitability are still the dominant metrics used relative to consumer behaviour and according to their findings regarding the usage of marketing metrics in the UK in small, medium and large size businesses from various sectors, the conversion was not among the top 15 most used metrics by frequency (> 62 percent usage).

This could be probably be explained by the difficulty to measure the part of the traffic that is relevant as prospective customers. For a more accurate conversion rate, it is recommended that the value of the traffic used in calculating the conversion rate to be refined, in order to include only people entering the shop relating to buying/ with intention of buying (Ryski, 2011).

As follows, from the gross traffic or the total number of peoples entering the shop, a net traffic value can be extracted, by eliminated the staff's multiple entries, supply and maintenance personal entries, the people accompanying the prospect (often people shop in groups, with family or friends, but only one from the group is a prospect) and customers' multiple entries (especially for specific businesses, e.g. auto dealers) (Ryski, 2005). In order to obtain an adjusting factor that allows eliminating from the total traffic, the percentage of persons that are not entering the shop with intention of buying, a pre-study can be done, using the method of observation, and if possible re-tested periodically. The critical aspect is the consistency in usage of the adjustment factor to allow a correct conversion rate comparison on different time frames and factors.

A more useful formula for understanding the conversion rate is the one that reveals its impact on the growth potential of the business, as follows:

$$\text{Sales Revenue} = \text{Traffic x Conversion rate x Average Sale Value} \quad (2)$$

As the formula shows an increase in either of the factors involved – traffic, conversion rate or average sale value – will have a direct impact on the sales.

In comparison with the costs involved for increasing the traffic – high costs for advertising and for the location of the shop–

and the limits of the average sale increase techniques that, when used poorly, often leave a negative impression to the customer, the conversion rate can be more efficiently improved by monitoring the traffic and conversion rate, action upon the factors influencing the buying and measure the impact of this actions.

The conversion rate evolution reflects the success or the failure of all the factors involved in delivering customer experience – from marketing and sales to operational – in convincing them to becoming paying customers. The continuous monitoring and control of this indicator along with the factors involved allows shop managers to make better informed decisions.

The factors that influence the purchase decision and the conversion rate are:

- staff expertise and effectiveness (qualitative);
- staffing levels (quantitative)and waiting time/ till availability;
- the comfort of the environment (retail design, the dimension and the structure of the space/ shop, ambient, usability and ergonomic aspects);
- products availability, range and variety;
- price level (real and the customer perception);
- visibility and the ease of finding the products/ information about products (merchandising);
- the possibility to test (or touch/ interact with) the products;
- customer's group type;
- customer motivation, involvement and the occasion regarding the purchase;
- customer's preferences, expectations previous knowledge and understanding about the products (premium wines in this case);
- the factors with direct influence on the traffic: advertising and promotions, location (convenience), competition (competitors), weather conditions

(Underhill, 2009; Ryski, 2011; Ariely, 2008; Lindstrom and Kotler, 2011).

One of the most important factors influencing the conversion rate is the shop's personal, which has a positive impact on the conversion rate. Both the qualitative aspect – knowledge about the products, expertise and professionalism – and quantitative – enough personal to help, inform, answer inquiries and finalise the transactions with the customers – are important.

It was found that the existence of interaction with the personal (interception rate) has a significant impact on the conversion rate, this increased with 50% when the personal offered assistance to customers compared with the situation without any interaction (Underhill, 2009). Furthermore the type of interaction have direct influence on the conversion, personal testimony and offering suggestion generating higher conversion rate than simply informing about the product (Conroy and Bearse, 2006).

From quantitative perspective, long queues contribute at the failing of purchases, and not particularly solely in connection with the waiting time, solely the perception or the expectation of a long wait can discourage the customers.

Second, the discomfort created by a large number of customers present in a limited (small) space deter the purchase, particularly the repeated touching with the other customers (or the lack of possibility to avoid this) will determine more customers to leave without buying (Underhill, 2009) or will have a negative impact on the average sale value (Perdikaki, Kesavan, Swaminathan, 2011).

This effect can be visualised in Figure 1 (Ryski, 2011), reflected in the positive relation between the traffic and the number of transactions, up to a point of the traffic, after which the number of transaction will tend to level, with a negative impact on the conversion rate.

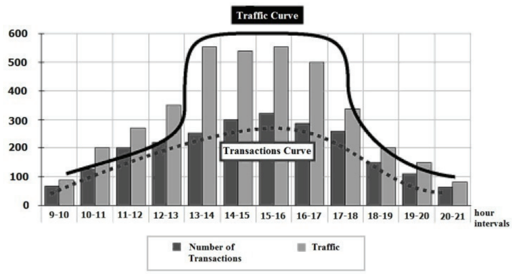

Figure 1. Typical evolution for traffic and number of transactions (Ryski, 2011)

Therefore, to assure comfort and deliver quality service for the consumer, in order to convert more of them into paying customers, the staff

scheduling should be optimise with the traffic level, not with the sales, not with the number of transactions.
Range, variety and availability of the products influence the conversion rate. According to Gruen, Corsten and Bharadwaj (2002) and illustrated in Figure 2, if a customer doesn't find the product she/ he is looking for, 55 % will not buy (do not purchase, buy from another shop or delay purchase) and for the remaining 45% to be satisfied an easy to substitute product should be available.
As recent finding from behavioural economics and neuromarketing shows, around 95% of our decision making is done at subconscious level (Halstead et. al., 2012) and the perception of the customer about the price of a product is mainly subjective and arbitrary.

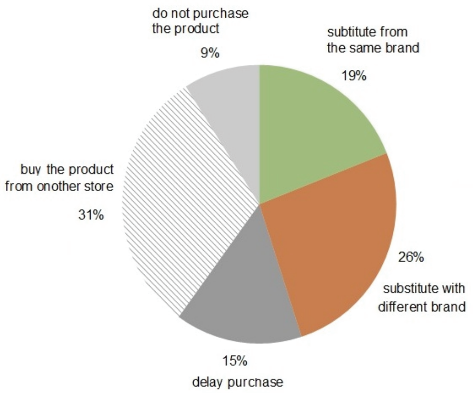

Figure 2. Consumer responses to out of stock (Gruen, Corsten and Bharadwaj, 2002)

Dan Ariely (2008), argues that the consumer behaviour is prone to a degree of irrationality, in regards to the perceived price of a product, mentioning the influence of the context (other prices or other options, not objectively sampled), by a so-called *herding* factor which implies that decisions are influenced by the comportment of other people and by *imprinting* or "*self-herding*" that means decisions based on our previous behaviour, in this context on the first or the previous price agreed to pay, that became an anchor and influences the price willing to pay today.
Furthermore, Lindstrom and Kotler (2011), in their book "Brand sense", argue that products which communicate through sensory channels – taste, touch and smell – create emotional connection/ engagement with customers that add a "hugely persuasive dimension" generating impulse purchasing, higher sales and high rate of loyal customers.
As shown in Figure 3, the present data available show a large distribution of the averages for this indicator, for different businesses, types of products and different contexts, which can be explained by the multitude and complexity of the factors involved, therefore only general guideline averages for conversion rate per type of business can be mentioned.
Also, when considering a conversion rate average for a retail category, it is important to accurately define the category along with the format, size, location of the shop, the level of price and quality of the products available, and so on. Without this delimitation, large variations can occur, as it can be observed from the data of Conroy and Bearse (2006) of Deloitte LLC and Atlanta Retail Consulting (2007) collected for Speciality Apparels and Department Store and included in Figure 3.
As Paco Underhill (2009) emphasises, the higher the margin of the business, a lower conversion rate tends to have, and the smaller the margins higher conversion the businesses have. Also the conversion rate for premium and luxury goods is lower than for the mass market merchandise.
According to Mark Ryski (2011), regarding general guidelines for conversion rate empirical encountered, the conversion rate for speciality shop in USA is situated around 20-50%.
The costs for traffic and conversion rate analyse are lower that the costs of other measures that could be taken for increasing the sales, and demands a people counter system/ people counter device(s), resources for correctly collect and interpret the data and training of all the staff involved (regarding the purpose of the indicator) for better results through their engagement.
Conversion rate analyse implies first collecting correct data about the traffic (the number of the visitors of the shop), analyse the number of transactions in rapport with the traffic to calculate the indicator, overlay the results with

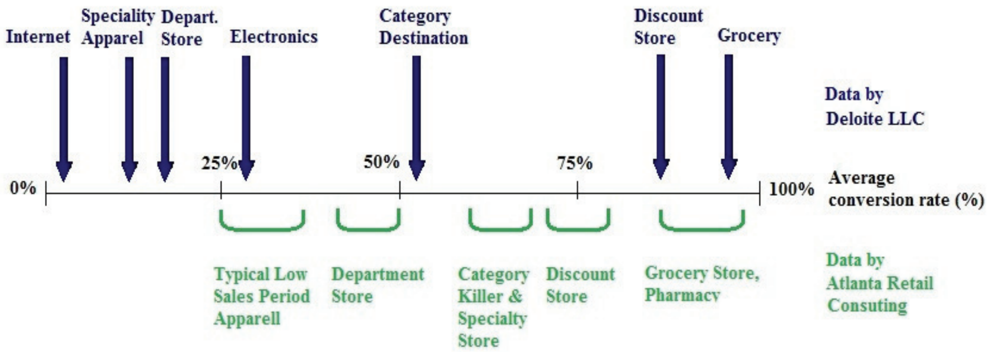

Figure 3. Conversion rate average guidelines for different retail segments and formats according to Deloitte LLC (Conroy and Bearse, 2006) and Atlanta Retail Consulting (2007)

Different factors of influence (staffing allocation, advertising and promotional programs and other factors identified through staff's observation) and interpret the results.
The further important step is taking measures/ action to improve the percentage of the customers (visitors) converted into buyers and then analysing the results of this actions with the same methods.
Usually the traffic data is collected using automated devices, but, in order to record additional aspects about purchasing behaviour and about the factors influencing the conversion rate, on a short term, some research using direct observation method is recommended.
As other authors highlighted, this method offers a better understanding of the actual actions of the customer, instead of their impressions about their actions or behaviour (as in surveys), which sometimes tend to be biased by the lack of correct recollection of the actual facts, improvisation when in lack of recollection or the "tendency to rationalize behaviour to make it appear in the best light" (Wells and Sciuto, 1966). From this point of view, Mark Ryski (2011) argues that traffic and conversion rate measurements are more accurate and objective, than the measurements obtained in other methods that involve the subject's opinion about its actions (surveys).
Regarding the limits of the method of observation, the aspects of validity should be taken into account, especially what Lynda Baker (2006) refers as the "researcher bias that may result from selective observation, selective recording of information, or the subjective interpretation of situations".
In order to define measurable goals for the study and to overcome the subjectivity of the observer, it is recommended to use structured observation. A form or a track sheet need to be designed with predefined aspects to be recorded and for each, predefined answers in tick boxes (for ease and faster recording), in scale, definite group intervals or categorical answers that cover all possibility. Also the aspects to be recorded and the answers should have clear meaning (not allow more than one interpretation) and allowing the observer to record objective aspects.

CONCLUSIONS

Of the many parameters reported in literature for assessing the performance of a commercial unit, the conversion rate allow for better informed decisions.
For retail shops, as it is the case of wine shops, by analysing together the traffic and the number of transactions (the constituents of the conversion rate), one can understand how, up to

a point, traffic has a positive impact on the conversion, as the number of transaction rise, up to a (critical) level, after which the large number of prospects and insufficient personal will generate conversion to decrease, which means losing customers and opportunity in sale.

By having permanent data about traffic and conversion rate, the impact of different factors on sales revenue and conversion can be monitored, acted upon and furthermore the impact of this actions can be measured.

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APPLICATION OF THE CONVERSION RATE AS AN USEFUL TOOL FOR ASSESSING AND MONITORING THE MARKETING PERFORMANCE OF A WINE SHOP

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Abstract

Starting more than a decade ago, there is an on-going shift towards customer-centric marketing, but the wine market has shown a slower turn into this direction. In order to confer to the costumer a superior overall purchasing experience and to increase the performance of the wine shop, an important issue should be finding the right tools for evaluating the purchase and consumption behaviour of these wine consumers. The aim of this study is to assess the indicators used by Wine Shops to evaluate the performance of their physical (bricks-and-mortar) retail shops, the consumers' satisfaction and the factors influencing the purchase decision. The data was collected during 2012 in a bricks-and-mortar wine shop using the method of observation and the conversion rate was analysed along with the involved factors. The results show that, when purchasing wine, a multitude of extrinsic factors have a significant impact on the buying decision and hence on conversion rate. Touching the products, tasting wine in the shop and the amount of time spent discussing with staff have shown a positive impact on the conversion rate. The loyal clients versus new prospective clients and the existence of interaction with staff versus no contact have shown significant differences in conversion rate. As the costs of increasing the traffic into the wine shops is high, a better use of the existing sales opportunity, measured through conversion rate, should be an essential part in analysing the performance of the wine shop.

Key words: *conversion rate, purchasing behaviour, wine preferences*

INTRODUCTION

Wine commercialization is a business which requires skilled staff and understanding of the particularities of wine consumers. Several surveys were conducted to better understand the preferences of the wine consumers (Gomez *et al*., 2004; Atkin and Sutanonpaiboon, 2007; Becker, 2013), including in Romania (Antoce and Paduraru 2012; Antoce *et al*., 2006, Antoce, 2003; Antoce *et al*., 2002).
A few studies pursued the purchasing behaviour of these consumers (Lockshin, 2003; Johnson and Bastian, 2007; Mueller et al., 2008; Chivu 2012) and revealed that consumers tend to be loyal to price categories, that wine expertise level found to be a factor which defines the model of purchasing behaviour and that the contextual factors inside the wine shop have influence on the purchase decision.
Furthermore there isn't a strong correlation between attitudes and behaviour or as Lockshin (2003) describe it "consumers will often say they do one thing and then actually do something else in the marketplace".
Therefore this study is based on the method of observation, monitoring the behaviour of the costumers in a retail wine shop, measuring the conversion rate and assessing the marketing performance of the shop based on this indicator.
Retail wine shops, such as the one in which this study was conducted, are facing great competition from the hypermarkets and internet wine shops, needing a special type of communication with their customers (Chis, 2009; Gómez and Morse, 2010). To stay in business, the selection of an effective strategy

is a must, so that, evaluation of the shop performance should be pursued regularly.

Retail shops use various key performance indicators (KPIs) to measure their activities (Bharath, 2013; Chivu and Antoce, 2014). There is no particular performance indicator which a retail shop should better use, but it is important to select an appropriate one in order to achieve their strategic goals. Upon an extensive literature research, the conversion rate was selected as the appropriate indicator of performance for wine shops (Chivu and Antoce, 2014) and it is used for several segments of the costumer population.

The data was collected using the method of observation. The average conversion rate was calculated and then compared with the conversion rates obtained in the presence and in the absence of different factors.

The paper aims to be the first to measure and analyse the conversion rate in a bricks-and-mortar wine shop in Romania, indicator considered to be a useful tool in assessing the performance of the shop and a measure of customer satisfaction for loyal clients.

MATERIALS AND METHODS

The study was conducted in a small wine shop in Bucharest and the data were collected via direct observation, using an observation sheet. The wine shop is situated in a premium real estate area in the neighbourhood of Floreasca Market, Bucharest with wines and spirits within the range of 5 to 200€.

Between the 21th of May and the 2nd of June 2012 a panel of 396 customers over 18 years old with interest in high quality wine, clients and potential customers (prospects) of the wine shop have been observed regarding their purchase behaviour.

All the customers who entered the wine shop during that period were included, but from the total gross traffic (403), the net traffic was extracted by excluding suppliers, family and friends of the staff. The sample population should accurately reflect the sub-group of persons with interest in wine, which visits wine shops, being potential customers and considered relevant for analysing the conversion rate in a wine shop and the factors involved.

A single observer was used, the dimension of the shop allowing observance of the customers at any moment. The observer posed as trainee employee in order to avoid changes in the behaviour of the subjects as a result of being aware that they were observed, also referred as *Hawthorne Effect* (Olson et al., 2004).

The observation sheet was structured to record the interaction of the customers with the products (seeing, touching, tasting), the interaction with staff (if occurred, the amount of time spent discussing), demographic characteristics of the customer and group type.

Measured Variables regarding demographics:

The customers were identified as male or female and classified into the following customer age categories: 18 to 28, 29 to 39, 40 to 50, 51 to 64, 65 and over. The classification of the customers into the age categories was made with approximation, based on the physical aspect, social context and conversation (when in group).

Customer group type was noted by classifying them as being either single, with a friend, a group of friends (of 3+ persons), male-female couple and family with children. For the categories *with a friend* and *group of friends*, the male-female couples were not included, as they were noted separately, due to the fact that the purpose of the classification was to investigate the influence of the group on the purchasing decision, hence on the conversion rate. For group of consumers, couple or family, only the age of the member which appeared to be with the most influence in the purchase decision was recorded. The same principle was used regarding the individual's characteristic recorded at gender.

Demographic characteristics of the sample are presented in (Table 1).

Each customer was observed during the whole time of presence in the shop and information was gathered in regards to: time spent in the shop, the percentage of time spent speaking with the staff, if they touched and held products, if they tasted wines, if they noticed the wines in promotion and if they purchased at the end. In addition, the sales values were obtained from the shop registry.

The amount of time each subject spent in the shop was calculated as difference between the

exact time at which the customers exited the wine shop and the time they have entered.
The final values obtained for the time spent in the wine shop for some of the customers were considered to be higher than normal, after observing that a few of them were spending one or more hours discussing with staff about wine and life in general. This was due to the fact that a number of the loyal clients developed a social relation with the staff and some of their visits and the time spent inside the wine shop were not specific to the purchasing context. As later to be discovered in our study, this type of clients proved to have a positive impact on conversion rate, but this situation was considered to generate bias regarding the average visit time of the customers, hence this was not analysed further as a factor.

Table 1. Demographic characteristics of the sample (n = 396)

Characteristic	Range	Complete sample (%)
Gender	male	71%
	female	29%
Age	18-28	7%
	29-39	48%
	40-50	36%
	51-64	8%
	over 65	1%
Customer group type	single	86%
	with a friend	4%
	group of friends (3+ persons)	2%
	male-female couple	5%
	family with children	3%

The percentage of the time spent for conversation between customers and personnel, from the total time of the visit, was recorded and analysed as a factor able to influence the purchasing decision and the conversion rate. This factor was approximated and recorded into four level groups, as presented in (Table 2). The values of 0 and 100% were not included, as zero means no conversation and the absolute value of 100% is difficult to define for this factor.

The factor *interaction with staff* (Table 2) is referring to the occurrence of any dialogue customers and staff, except greetings and formulas conventionally used in a purchasing contexts (e.g. I would like to buy this, I want to pay for this). Any polite conversations like small talk, amusing remarks, dialogue about the weather or other unimportant matters, where to be considered as presence of interaction and marked *yes*.

Table 2. Factors with levels and coding

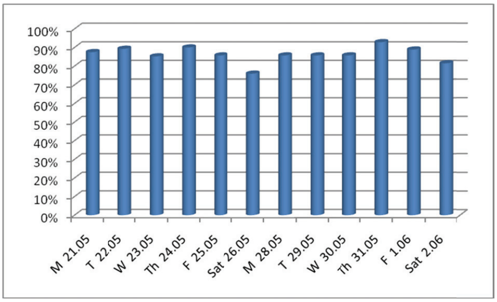
Factors	Level 0	Level 1	Level 2	Level 3
Interaction with staff	No (0)	Yes (1)		
Time spent discussing with staff*	<25%	<50%	<75%	<100%
Wine tasting	No (0)	Yes (1)		
Touching factor	No (0)	Yes (1)		

*in relation with the total amount of time spent inside the wine shop (percentage),to be read: < 25% = 0.01-24.99%; <50% = 25-49.99%;< 75% = 50-74.99% ; <100% = 75-99.99%.

The '*touching factor*' (Table 2) refers to the occurrence of direct contact of the prospective customers with the products, the layout of the shop allowing the majority of the products to be in the reach of customers. The *touching factor* was recorded *yes* when an item have been pulled from the shelf and held in hands by the customer.
Another factor recorded was identifying the customers as *loyal clients* (repetitive factor marked "*yes*") or *new prospective customers* (repetitive factor marked "*no*"). This classification was made interviewing the personal of the wine shop in regards to recollecting each customer as repetitive or not remembering at all seeing the particular customer. Answers under uncertainty were registered as *unknown information.* This can be regarded as biased samples, but the findings in this classification are considered useful for identification of general trends only.
The collected observations were introduced into an Excel database and the conversion rate was analysed along with the factors.
The average conversion rate was calculated as the total number of the customers that have bought, divided by the total number of customers that have entered the wine shop. This average was then compared with the conversion rate calculated for each level of the recorded factors.

RESULTS AND DISCUSSIONS

The evaluation of the collected data indicated a 84.60% conversion rate for the entire period analysed (Figure 1). This shows that from the total customers that entered the shop, approximate 85% have purchased (became paying customers).



Legend: Conversion rate marked with blue

Figure 1. Daily variation of the conversion rate (%) over the period of 2 weeks

Compared with the averages provided as general guidelines by Mark Ryski (2011), the conversion rate for this wine shop is positioned above the interval for the category, Speciality/ Gift Retail having a range of 20-50% conversion rate, and even above the level of the indicator for General Mass Merchandise, retail format with conversion rate in the range of 40-80%. In regards to the figures released by Atlanta Retail Consulting (2007) and Deloitte (Conroy and Bearse, 2006), the average conversion rate of the wine shop analysed is situated in the higher range, typical for Grocery shops and Pharmacies, which is a positive indicator, as usually retail offering necessity goods register the highest conversion rate.

In conclusion, when compared to the available ranges for conversion rate for different retail formats, the wine shop is high performing in regards to converting the prospective customers into paying customers and is using a very high part of the sales opportunity (considering that every customer who enters the shop is a sale opportunity).

However, these averages per industry are not absolute values; they represent guidelines, based on the figures recorded in USA by different retail consultants, and, as the above authors mention, the averages represent general guidelines, as insufficient data was recorded to have a clear image for different retail specific and for different formats.

The results of the present study could seem to indicate higher conversion rate compared with other figures available, but there aren't relevant data to compare with in the wine industry. There couldn't be found data regarding the consumer behavior in the wine shops in Romania which measure this indicator or about average conversion rate in bricks-and-mortar (physical) wine shops in general.

And as wine tend to be a more complex product from the purchase decision perspective (Lockshin 2003) and imply higher involvement than other products, most probably the conversion rate in wine shops will reveal different ranges than for other types of products. Furthermore, in this case, the dimension of the wine shop, the location and the staff facilitated a more personal relationship of the customers with the shop, which became more than a shopping destination, being a social hub. This situation could generate different results compared to a larger and more impersonal retail wine shop.

The principal utility of the conversion rate indicator is actually the opportunity to analyse the evolution of the wine shop in time, understanding the factors involved, the trends and identification of changes, in order to develop new strategies for increasing the performance of the business.

Investigating the factors influencing the conversion rate, a direct correlation can be identified between the traffic and the number of transactions, up to a peak of the traffic, above which, the number of transactions and the conversion rate, tend to stagnate (Figure 2). Correlating Figure 1 and Figure 2, it could be observed that the traffic has positive influence to the conversion rate up to a point, above which the conversion rate decreases. This indicates the insufficient number of personal at the peak of the traffic, which, along with the limitations of the space, generates customers' dissatisfaction and makes them leave without purchasing.

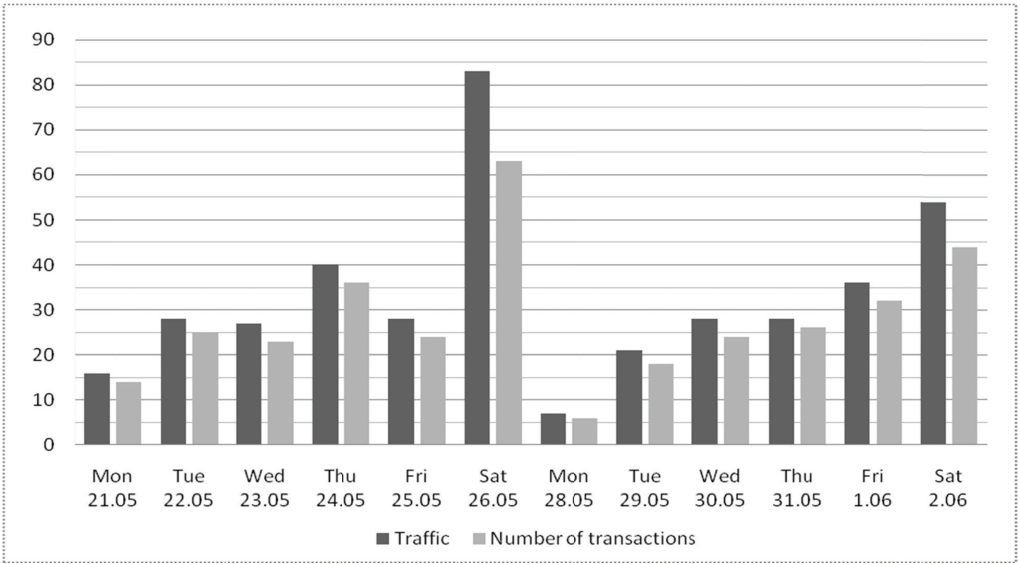


Figure 2. Traffic (the total numbers of customers who entered the wine shop) and number of transactions (or paying customers) per days

Analysing (Figure 1) and (Figure 2), one can understand that the most of the decrease in conversion rate is generated by higher values of traffic and the highest sale opportunity loss consequently occurs on Saturdays. This simple analyse would allow the management to identify the cause for the loss of sale and prevent it by supplementing the staff on Saturdays.

The conversion rate level was further analysed for the segment of *loyal (repetitive) clients* of the shop and compared with the conversion rate for the segment of *new customers*, as show in (Figure 3). The conversion rate in this case was calculated as the sum of the repetitive clients who purchased divided by all the customers who entered the shop and were identified as repetitive clients.

The evaluation showed a conversion rate within the new prospective customers (first time in the shop) of 67.42%, which is situated above the averages of the category (based on the guidelines of Mark Ryski, 2011), which could be a positive indicator of the performance of the shop in relation with the new customers if compared to empirical figures of the category, but situated below the average of the shop, which means that further improvement is needed in this area.

However, the results show a conversion rate for the loyal (repetitive) clients of 99.17%, situated far above the average of the wine shop, with less than 1% loss in sale opportunity among this category of customers (Figure 3).

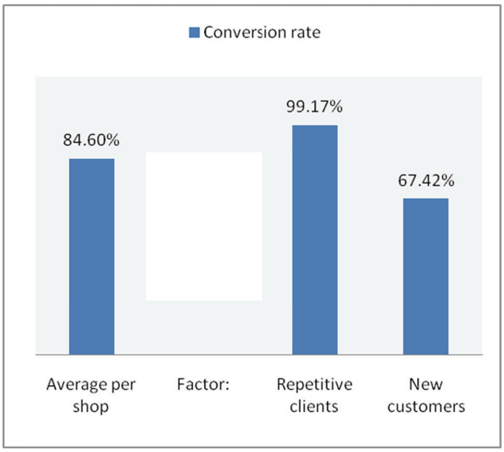


Figure 3. Comparative conversion rates for repetitive clients, new customers and the average shop conversion rate

The large segment of loyal (repetitive) clients, 61%, and the high level of conversion rate for this segment, above 99%, confers a solid base for the business and offers indication of high level of customer satisfaction.
By further investigating the customer group type (categories shown in Figure 4), a higher than average conversion rate can be identified in the category of family with children. Determined in their decision to purchase, and limited in time (and attention), this type of prospective customers tend to display higher predictability in regards to purchasing. In spite of representing a small segment, only 3% from total customers, this segment can be better served by prompt service and providing a more comfortable (and safer) environment for the whole family during the visit. One direction, considered to be instrumental in this matter, is making available online ordering, as the alternative of home delivery could prove helpful for these customers. Furthermore, as online shopping could decrease a part of the traffic by allowing some customers to order from home, small improvements in the comfort of the prospective customers inside the bricks-and-mortar wine shop will be accomplish.

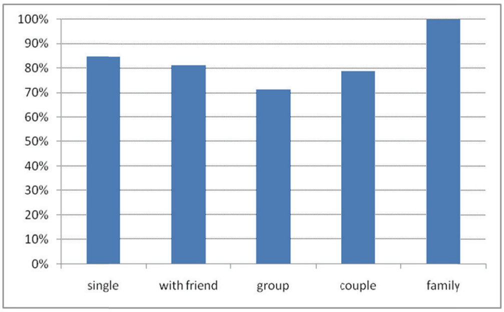


Figure 4. Conversion rate comparative for different customer group type

On the other side, smaller than average conversion rates can be observed for the other type of groups of customers, the ones accompanied by a friend, the ones accompanied by a male/female partner (couple), with the highest low identified for customers accompanied by group of friends.
The results also show that the presence or the absence of the factor of interaction with staff has a high impact on the conversion. As shown in (Figure 5), from the total number of the customers who entered the shop but did not have interaction with the staff, only 51.61% have purchased, compared with 87.40% from the ones who discussed with the personal.

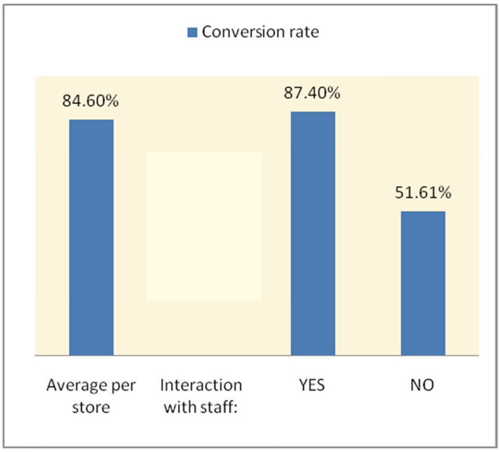


Figure 5. The impact of interaction with staff on conversion rate

As evidenced in the Table 3, the amount of time spent discussing with the staff has a smaller impact on the conversion rate.
A more distinctive low is noticed in the lower category, only 78.79 % from the customers who spent less than 25% talking with the staff have purchased.

Table 3. The impact of time spent discussing with staff on conversion rate

Time spent discussing with staff (from total time inside the wine shop)	Conversion rate
Between 75% and less than 100%	88.38%
Between 50% and less than 75%	88.46%
Between 25% and less than 50%	87.18%
Less than 25%	78.79%

The results support the important role of staff in helping customers decide, especially when they are confronted with large selection of merchandise, as previous findings of Conroy and Bearse (2006) suggest, the difficulty in choosing being a factor that frequent generates loss of sale. Furthermore a significant smaller amount of time spent discussing with the personal could mean that the problem of deciding wasn't sufficient or conclusive

addressed, hence generating a negative impact on conversion rate.
The possibility of tasting the product has been identified as a factor with a positive impact on the conversion rate. As shown in (Figure 6), from the total number of customers that have tasted wine in the shop, more the 93% have purchased, above the average conversion rate of the wine shop.
The results show that the possibility to experience (the characteristics of) the products will increase the willingness to purchase. The purchasing in this case was not made always from the product tasted.

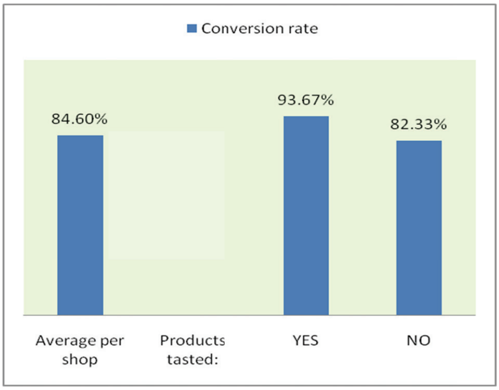


Figure 6. The impact of tasting products on conversion rate

Furthermore, our findings shows that the possibility of experiencing only the extrinsic characteristic of the product in the sense of touching and holding the bottle in hands also resulted in higher rates of purchases. From the total number of customers that have touched one or more bottled of wines in the shop, 100% have purchased (Figure 7). In comparison, from the total number of customers that have not touched the products a conversion rate of only 76% was registered. The findings above are consistent with Lindstrom and Kotler's (2011) theory that the simple touch of the product it brings a sensory input that adds a new dimension, emotion – creates an emotional connection with the product – that influences the customer decision. One can argue that the customer has been already decided when picking up the product, that they already intended to buy and it was just a matter of finding the detailed information they needed (reason for looking up closely to the product) but as findings about different other products showed (Conroy and Bearse, 2006; Lindstrom and Kotler, 2011; Underhill, 2008), the direct contact with the product (by touch) and merchandise trial make a difference in regards to conversion.

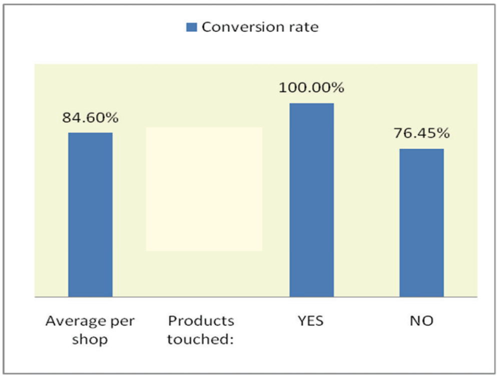


Figure 7. The impact of touching the products on conversion rate

The traffic and conversion rate analyse display important insights about the marketing performance of the wine shop:

1. The conversion rate of the wine shop, above the category average, indicates a high performance in converting prospective customers into paying customers, with only 1.5% of the sale opportunity lost;
2. The wine shop has a significant segment of loyal clients, with a high level of conversion rate, over 99%, which offers indication about high level of customer satisfaction for this category of customers and confers a solid base for the business;
3. Regarding the category of new prospective customers (first time in the shop) the figures shows low performance, compared with the average of the shop, with sale opportunity loss of approximate 33%, which indicate that further improvements to enhance first time customer experience in needed;
4. Further analyse shows that the wine shop is losing customers, due to insufficient staffing during the peak traffic, because the staff schedule is not adapted to total number of customers entering the shop (traffic); the most critical day being Saturday, with more traffic that the staff can service and in consequence with a level of 22% of the prospects lost;

By using the observation method, unobtrusive insights about customers' actions were obtained and have been avoided discrepancies generated by inaccurate recollection or dissonances in the subjects' perceptions of their own actions. While it is expected that the results of this study to have universal application for wine shops, some limitations in our study could relate to the subjectivity of the observer, aspect tried to overcome by using structured observation and other to variations that could occur in different wine retail formats and dimensions.

Considering the multitude of factors and the complex quest to determine the contribution of each on the conversion rate, further quantitative studies might reveal a better understanding of the impact of each of the factors identified in the present paper.

CONCLUSIONS

In a market with high competition it is essential to be able to understand the area of the lost customers – both as dimension and factors involved – and to develop actions to better serve them (next time), in order to capitalize on the existing sale opportunity which consists of the customers already in the shop.

One of the major errors that managers make is planning the staff allocation based on the sales figures, not on the actual needs of the store, which are represented by the total prospective customers inside the shop (net traffic). Therefore traffic and conversion rate analyse allows managers to take better informed decisions regarding optimising the staff level accordingly, in order to succeed in increasing the number of prospective customers converted into paying customers.

The conversion rate offers the opportunity to a better understanding of the factors influencing the purchasing and allows measuring the impact of the actions taken for delivering a better customer experience. The results revealed that personal assistance adds value on the customer experience, both, the occurring of interaction with staff and the higher degree of the time spent discussing with staff, having a positive impact on the conversion rate.

Tasting the product and the possibility of touching the product proved to have a positive influence on the conversion rate, the presence of the factor being associated with a higher than average level of the indicator. Also the loyal clients showed significant higher conversion rate then new prospective customers (first time in the shop).

The major utility of this indicator is to help managers analyse the evolution of the wine shop in time and, based on previous patterns and identification of changes, to forecast the future performance of the business and develop new strategies of enhancing customer experience and stimulate growth.

The limitation of the study consists in the inability to generalize the average of the conversion rate within the industry, taking into consideration that the dimension of the wine shop and the location allowed a more personal contact of the staff with the customers. This study needs to be replicated within different wine markets and in wine shop of different dimensions and formats, in order to have a relevant average for the industry.

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IMPLEMENTATION OF A DECISION SUPPORT SYSTEM FOR ACIDITY CORRECTIONS IN THE FRAMEWORK OF PRECISION OENOLOGY

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Abstract

Acidity corrections are currently performed in the industry in accordance to regulations and necessities. In the precision oenology for these corrections the dosage of the products used for the treatments can be calculated based on the values determined for the must and wine parameters, simplifying in this way the work of the winemaker. The performance and limits of the mathematical equations are evaluated in these paper, both for acidification and deacidification. The equation derived for acidification processes, irrespective of the acid used (tartaric, citric, malic) gives values which correlates well with the data experimentally obtained, making the application of this equation very useful. In the case of the deacidification the equation does not always give results correlated with the experimental data. For instance, the deacidification performed with alkaline salts containing potassium also react with other must/wine compounds than acids, so that the the exact dosage to be used cannot be calculated. However, for the deacidification with calcium carbonate, the most used salt for these type o treatments, the equation can be applied with good results.

Key words: *acidification, deacidification, decision, equation*

INTRODUCTION

Acidity corrections by acidification / deacidification are necessary to give the wine stability and flavor. Such corrections are usually made when grapes are crushed for a better stabilization of potassium hydrogen tartrate during fermentation, microbial stability and flavor. Musts and wines are very complex matrices, so that precise calculation of pH and titratable acidity changes during acidification and after potassium hydrogen tartrate precipitation is very difficult without laborious analysis. For this reason we propose an easier way to calculate these changes in red and white musts prior acidification / deacidification, although not as accurate as the laborious wine analysis of a the above prepared samples would be, but satisfactory for practical purposes. A methodology with even less accurate results was proposed by Moreno et al., 2012. For better results, we modified some equations on the methodology, by taking into account the solubility of potassium hydrogen tartrate with a correlation proposed by Ratsimba (1990) and previously used by Gerbaud, (1996) in his PhD thesis. Also, it is well known that the main complexing agents for potassium hydrogen tartrate precipitations are phenolic compounds and proteins, but potassium hydrogen tartrate can be inhibited by ionic reactions between potassium and sulphate. Potassium hydrogen tartrate precipitation changes the must titratable acidity and pH and is less affected by complexing agents in white musts than in reds, where to the presence of phenolic compounds is in greater quantities (Balakian, 1968). In white wines the titratable acidity after cold stabilization of musts was found to drop about twice as much than in red wines. The proposed methodology is less laborious, but still needs some routine analyses such the determination of pH, titratable acidity of musts and buffer capacity.

MATERIALS AND METHODS

Raw materials: White must of 'Feteasca regala' and red must of 'Dornfelder' from the experimental vineyard of USAMV Bucharest were used for the study.

Treatments. For *acidification* treatments tartaric, malic and citric acid were applied in doses of 6.86 meq/l for the white must and 5.78 meq/l for the red must. For *deacidification,* the salts used for treatments were alkaline salts of $CaCO_3$, $KHCO_3$ and K_2CO_3, in doses of 14.82 meq/l for the white must and 10.55 meq/l for the red must.

For a better representation of results three stages of experiment were considered:

Stage 0. Determination of musts characteristics before acidification / deacidification is performed. The principal physico-chemical characteristics that are analyzed are: titratable acidity (TA, meq/l), pH, buffer capacity (β_{HCl}, meq/l / β_{NaOH}, meq/l). From buffer capacity can be computed: the alkalinity of ash (AA, meq/l) and the value for the hypothetical monoprotic acid dissociation constant pKv, presented hereafter.

Stage 1. Determination and calculation of the acidification / deacidification effect on musts parameters (titratable acidity, pH, buffer capacity and alkalinity of ash) using the milliequivalent-for-milliequivalent basis. Two simulations are performed by computation of initial parameters and compared with the determined values obtained by physico-chemical analyses. The first one is a new model of simulation, while the second one is a model suggested by Moreno et al., 2012).

Stage 2. Determination and calculation of the potassium hydrogen tartrate precipitation effect on musts parameters (titratable acidity, pH, buffer capacity and alkalinity of ash) using the milliequivalent-for-milliequivalent basis and its solubility in musts in accordance with the temperature. For this stage, both musts were cold stabilized at 0°C for 2 weeks. Both simulations were performed on each must.

Methods of analyses and equipments: *pH* was determined with an Hanna pH 212 (OIV, 2009b). *Total titratable acidity* (TA) was determined with TitroLine easy Schott Instruments until the end point of titration at pH 7.0 was reached (OIV, 2009a), while the *buffer capacity* (β) was determined with the same equipment by titration with HCl 0.1 N or NaOH 0.1 N, using 20 ml of must until 1 pH unit was dropped or raised, respectively. The calculation of buffer capacity was done using the mathematical relations presented hereafter. The *alkalinity of the ash* (AA) was calculated based on titratable acidity and buffer capacity, in accordance to the mathematical relations presented hereafter.

Calculations: In order to achieve practical goals, it will be considered that a single monoprotic acid HV is present in the must. For this case acid dissociation constant can be easily calculated based on the laboratory determinations of pH, total titratable acidity (TA) (Moreno et al., 2012). Thus, the equilibrium reaction can be represented as: $HV \leftrightarrow H^+ + V^-$.

As shown in this chemical equilibrium, the acids present in musts are partly dissociated. Anions formed in this reaction are neutralized by cations [M^+] from the must leading to electrochemical neutrality. Thus, the following relations, can be established (Moreno *et al.*, 2012):

$[V^-] = [M^+] = AA$ and *[HV] = TA*, where:

$[V^-]$ - anions from musts; $[M^+]$ - cations from musts (alkali metals); AA - ash alkalinity; [HV] - undissociated acid from must; TA - total titratable acidity of the must;

In accordance to the Mass Action Law and Henderson-Hasselbalch equation (Țârdea, 2007; Usseglio Tomasset, 1992; Moreno et al., 2012), the value of acid dissociation constant (Kv) of the above equilibrium, can be calculated as:

$$Kv = \frac{[V^-] \times [H^+]}{[HV]}, \text{where:}$$

$$pKv = pH - \log_{10}\frac{[V^-]}{[HV]} = pH - \log_{10}\frac{[AA]}{[TA]}$$

Because the alkalinity of the ash (AA) determination is very laborious, it can be indirectly calculated by taking into account the buffer capacity and by applying the following equation (Țârdea, 2007; Usseglio Tomasset, 1992; Moreno et al., 2012):

$$\beta = \frac{L}{|\Delta pH|} = \ln(10) \times \frac{TA \times AA}{TA + AA}, \text{where:}$$

$$\beta, meq/l = \frac{V \times N \times F \times 1000}{|\Delta pH| \times S}, where:$$

$$AA = \frac{\beta \times TA}{\ln(10) \times TA - \beta}, \text{where:}$$

L – weight equivalent of a strong acid or base (HCl / NaOH) which changes the pH with one unit of one liter of must. $|\Delta pH| = pH_f - pH_i$; pH_i – initial value of pH, before titratation; pH_f – final value of pH after titratation; V- ml of acid or base (HCl 0.1 N/ NaOH 0.1 N) used for titration of musts; N – normality of solution NaOH / HCl (0.1 N); F – NaOH / HCl 0.1 N correction factor; 1000 – value that reports the result to a liter; S – quantity of the sample used in analysis, (20 ml); TA - total titratable acidity in meq/l, determined by physico-chemical analysis; AA – alkalinity of ash in meq/l, calculated indirectly from physico-chemical analysis of β;

According to Henderson-Hasselbalch equation, pKv value for hypothetical monoprotic acid in musts (equivalent to the combination of each acid present) and pH value can be calculated (Moreno et al., 2012):

$pKv = pH - \log_{10}\frac{[AA]}{[TA]}$, were:

$pH = pKv + \log_{10}\frac{[AA]}{[TA]}$

After acidity corrections are performed, precipitation phenomena of potassium hydrogen tartrate and / or calcium tartrate will occur, due to the abundance of potassium and / or calcium ions and low saturation point of these salts in musts, mostly at lower temperatures. Of greater interest for calculation of changes in titratable acidity, pH, alkalinity of ash and buffer capacity is the solubility of potassium hydrogen tartrate in hydro-alcoholic solutions at different temperatures. Although, there are tables which give the solubility of potassium hydrogen tartrate in hydro-alcoholic solutions (Berg et al., 1958; Ratsimba, 1990), Ratsimba (1990) proposes an equation which correlates the potassium hydrogen tartrate solubility in hydro-alcoholic solutions with temperature and alcoholic concentrations (Gerbaud, 1996):

$$C_{KHT}, g/l = a_0 + a_1 \times a_v + a_2 \times a_v^2$$

Standard deviation of equation is 0.01 g/l.

where:

$a_0 = 143.3747 - 1.14947 \times T + 2.319 \times 10^{-3} \times T^2$

$a_1 = -15.08854 + 0.114296 \times T - 2.182 \times 10^{-4} \times T^2$

$a_2 = 1.10444 - 8.01748 \times 10^{-3} \times T + 1.46 \times 10^{-5} \times T^2$

C_{KHT} – solubility of potassium hydrogen tartrate, g/l;

a_v – alcoholic concentration of liquid;

T – absolute temperature, °K, where:

$T^{\circ}K = (273.15 + T^{\circ}C)$;

$$C_{KHT}, meq/l = \frac{C_{KHT},\ g/l \times 1000}{188{,}177\ g/mol}$$

RESULTS AND DISCUSIONS

Effect of acidification:

Stage 1. Immediate effects of acidification on alkalinity of ash, titratable acidity and pH can be computed for both, red and white musts by following relations:

$AA_1 = AA_0$

$TA_1 = TA_0 + HA$

$pH_1 = pKv + \log_{10}\left(\frac{AA_1}{TA_1}\right)$

where:

HA – the quantity of acid added in meq/l;

Buffer capacity results of proposed mathematical model are very accurate, correlating well with the determined values of treated musts in the case of all acids used in the experiment (Figure 1.a.). The suggested model of Moreno *et al.*, 2012 is not so precise due to the overestimation of titratable acidity from figure 1.b. and 2.b. stage 1.

In this stage (1) alkalinity of ash remains the same as in the initial determination (Figure 1.c. and figure 2.c.). Both musts behaved similarly in stage 1.

The pH of both musts in stage 1 can be calculated using Moreno *et al.,* 2012 equation with accurate results (Fig. 1.d. – white must; Fig. 2.d. – red musts;). Slight differences in pH can be observed for both musts analyzed, due to the different dissociation constants of the three acids applied. At this stage, the mathematical models used does not take into account the pKa's of different acids.

Stage 2. This stage is more complex than the first one and hows that the restoration of ionic equilibrium occurs differently for each type of musts matrix. In the red musts the phenolic compounds of can inhibit potassium hydrogen tartrate precipitation by complexes formed with approximately 50%. Effects of hydrogen tartrate precipitation on alkalinity of ash (AA), titratable acidity (TA) and pH can be calculated depending on the type of must, white or red, using the equations presented further.

White musts equations:

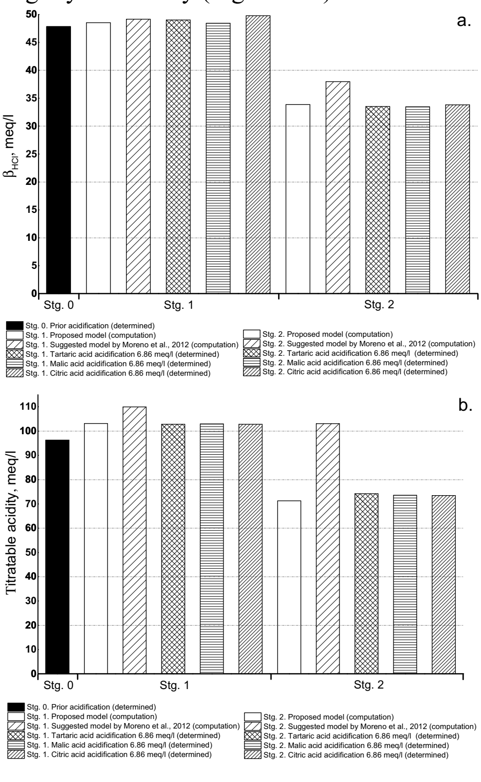
$$AA_2 = AA_1 - {}^1\!/_2 \times (AA_1 - C_{KHT})$$
$$TA_2 = TA_1 - 2 \times (AA_1 - C_{KHT})$$
$$pH_2 = pKv + \log_{10}\left(\frac{AA_2}{TA_2 + C_{KHT} + (AA_1 - C_{KHT})}\right)$$

In the case of white musts that are cold stabilized (stage 2.), the buffer capacity can be accurately calculated using the relation described by Usseglio Tomasset (1992), only if the values for TA and AA are accurately predicted.

We showed that the titratable acidity can be predicted with good results (Figure 1.b. and 2.b.), while the Moreno et al., 2012 model overestimates this parameter.

Alkalinity of ash is also important for buffer capacity prediction, as well as the titratable acidity and its prediction, which is well estimated by both models used for simulation.

The pH simulation on white must was predicted very close to the determined value for all samples, irrespective of the acid used for correction. Due to the different pKa's of acids, the pH of these samples behaved only slightly differently (Figure 1.d.).



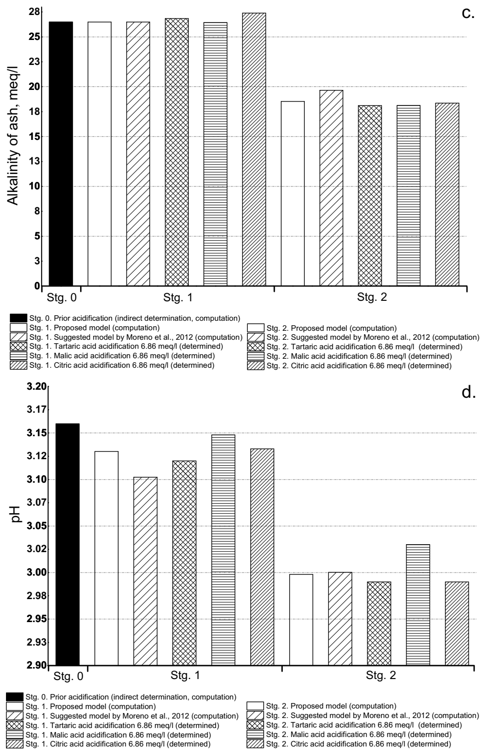


Figure 1. Effect of acidification with tartaric, malic and citric acid on white must buffer capacity, titratable acidity, ash alkalinity and pH (determined and computed values).

Red musts:

$$AA_2 = AA_1 - {}^1\!/_4 \times (AA_1 - C_{KHT})$$
$$TA_2 = TA_1 - (AA_1 - C_{KHT})$$
$$pH_2 = pKv + \log_{10}\left(\frac{AA_2}{TA_2 + C_{KHT} + {}^1\!/_2 \times (AA_1 - C_{KHT})}\right)$$

Red must behaved differently comparing to white must mainly due to the greater concentration of phenolic compounds.

Regarding buffer capacity of red acidified and cold stabilized musts (stage 2) the predicted values with both models are very close to the determined values (Figure 2.a.)

As in the case of white musts, in this case too, the titratable acidity is predicted accurately with proposed model and overestimated by the Moreno et al., 2012 model (Figure 2.b. and 1.b.).

Alkalinity of ash is slightly lower than determined values by physico-chemical analysis. Suggested model by Moreno et al., 2012 underestimates this parameter (Figure 2.c.).

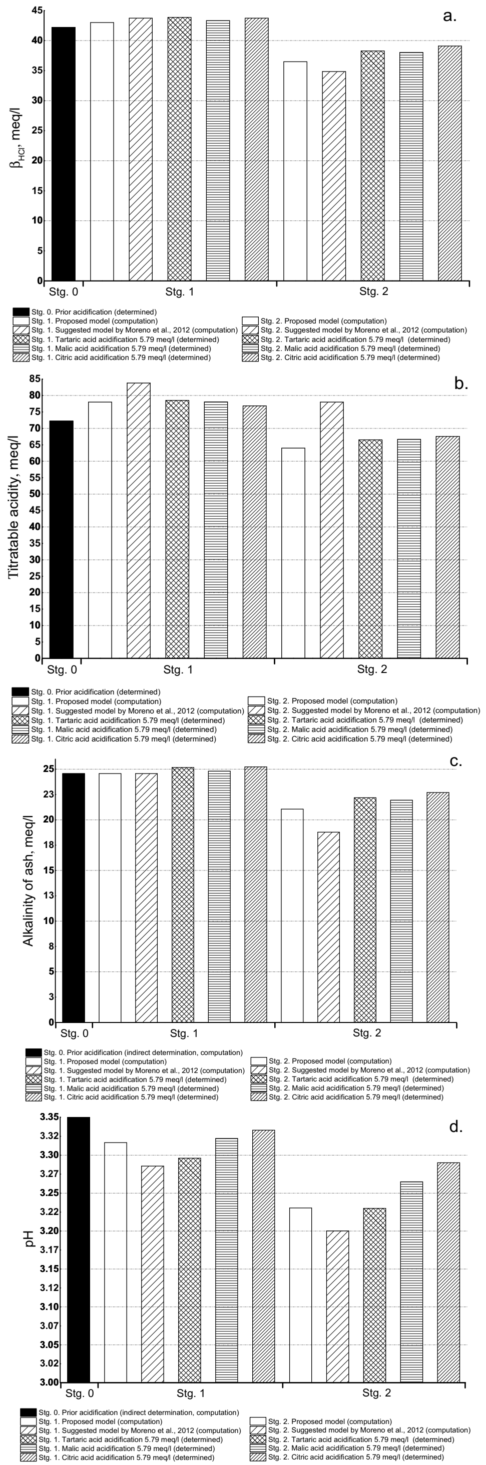


Figure 2. Effect of acidification with tartaric, malic and citric acid on red must buffer capacity, titratable acidity, ash alkalinity and pH (determined and computed values).

The pH of red wines can be predicted with good results using proposed model. Differences are higher than those obtained on white must (Figure 2.d. and Figure 1.d.) and all acids behaved slightly differently from one to another due to the pKa's and must matrix.

Effect of deacidification:

Stage 1. Immediate effects of deacidification on alkalinity of ash, titratable acidity and pH can be computed for both, red and white musts by following relations:

$$AA_1 = AA_0 + A^-$$
$$TA_1 = TA_0 - A^-$$
$$pH_1 = pKv + \log_{10}\left(\frac{AA_1}{TA_1}\right)$$

Buffer capacity can be satisfactorily calculated using the equation proposed by Usseglio Tomasset (1992), for both red and white musts. Its variation in the experiment is due to the titratable acidity and alkalinity of ash predictions. If the titratable acidity and alkalinity of ash are well predicted, the calculated buffer capacity is similar to the determined values (Figure 3.a. and 4.a).

In the stage 1, titratable acidity and alkalinity of ash are well correlated to the determined results for both of musts used (Figure 3.b., 3.c. and 4.b., 4.c.).

Stage 2. As in the case of acidification, phenolic compounds form complexes with potassium hydrogen tartrate and increase its solubility by approximate 50%. The effects of hydrogen tartrate precipitation after cold stabilization on buffer capacity, AA, TA and pH can be calculated depending on the type of must, white or red by following relations:

White musts:

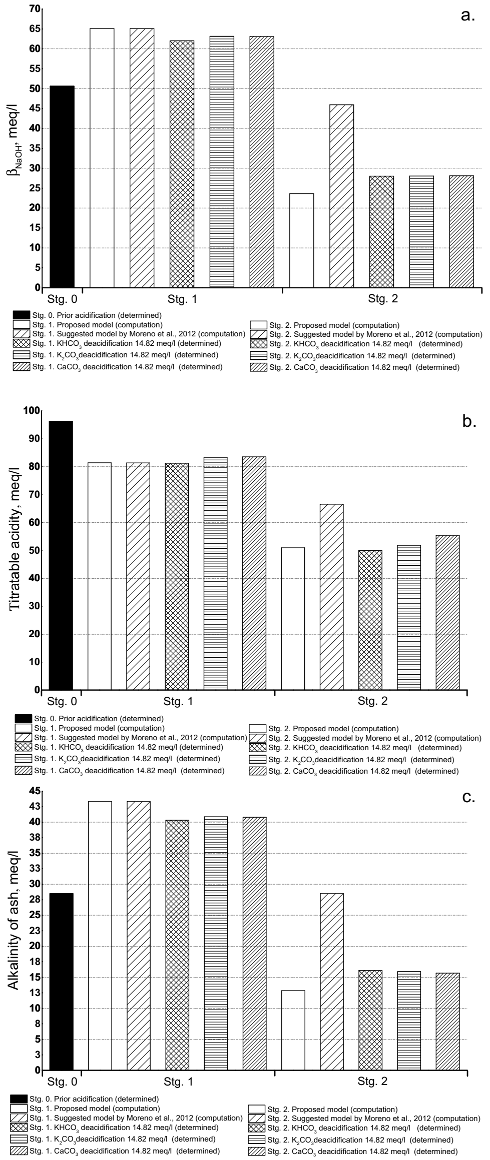
$$AA_2 = AA_1 - \Delta TA - {}^1\!/_2 \times (AA_1 - \Delta TA - C_{KHT})$$
$$TA_2 = TA_1 - (AA_1 - C_{KHT})$$
$$pH_2 = pKv + \log_{10}\left(\frac{AA_2 + {}^1\!/_2 \times (AA_1 - C_{KHT})}{TA_2 + {}^1\!/_2 \times (AA_1 - C_{KHT})}\right)$$

where:

$$\Delta TA = TA_1 - TA_2$$

Due to the overestimation of titratable acidity and alkalinity of ash in cold stabilized must (Figure 3.a, 3.b., 3.c.) the buffer capacity in stage 2 of white must is much greater using Moreno et al., 2012 model. As opposed, our proposed mathematical model gives for this parameter well correlated results in stage 2 (Figure 3.a, 3.b., 3.c.).

In white must, the pH calculated with both mathematical models in stage 2 give results with some differences, depending on the salt used for deacidification. The larger difference appears in the case of potassium alkaline salts, may be due to the reactions involved in must matrix (Figure 3.d.).



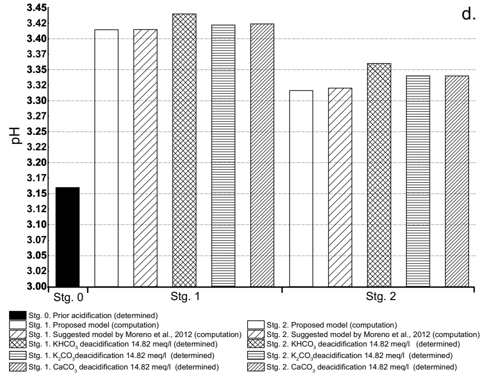


Figure 3. Effect of deacidification with potassium hydrogen carbonate, potassium carbonate and calcium carbonate on white must buffer capacity, titratable acidity, ash alkalinity and pH (determined and computed values).

Red musts:

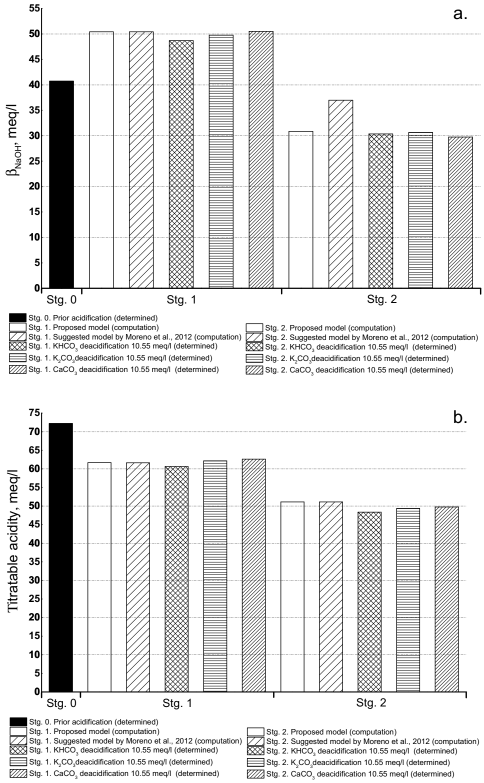
$$AA_2 = AA_1 - \Delta TA - {}^{1}\!/_{2} \times (AA_1 - \Delta TA - C_{KHT})$$

$$TA_2 = TA_1 - {}^{1}\!/_{2} \times (AA_1 - C_{KHT})$$

$$pH_2 = pKv + \log_{10}\left(\frac{AA_2 + {}^{1}\!/_{2} \times (AA_1 - C_{KHT})}{TA_2 + {}^{1}\!/_{2} \times (AA_1 - C_{KHT})}\right)$$

where:

$$\Delta TA = TA_1 - TA_2$$



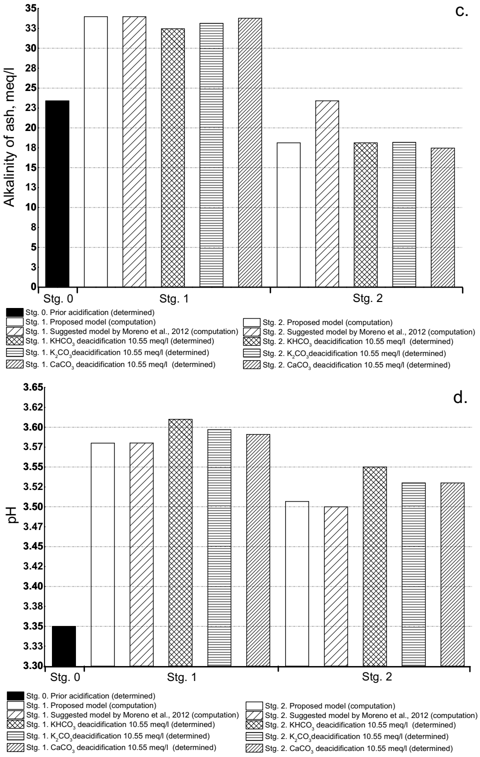


Figure 4. Effect of deacidification with potassium hydrogen carbonate, potassium carbonate and calcium carbonate on red must buffer capacity, titratable acidity, ash alkalinity and pH (determined and computed values).

Analyzing the buffer capacity in stage 2 of red must, a similar behavior of overestimation as in white musts can be observed in the case of Moreno et al., 2012 model due to the overestimation of alkalinity of ash (Figure 4.a., 4.c.).

Titratable acidity is well correlated in stage 2 irrespective of the mathematical models used (Figure 4.b.).

The pH of the red deacidified must is slightly higher in the analyzed samples than the results obtained by simulations (Figure 4.d.), probably due to the potassium reaction with another anions as the sulphate.

CONCLUSIONS

The presented results may be encouraging to be used as a decision support system in wine industry for acidity corrections and for its simplicity of computation.

The proposed model can gives oenologists a quick estimation of the dose of acid or alkaline salt to be used in some musts for acidification or deacidification, respectively.

Simulation models describe well the changes of must parameters after acid or alkaline salt addition.

To be on the safe side and avoid any dose overestimation or underestimation of the chemical used for the treatment, it advisable to prepare a laboratory sample first, check the final parameters of the corrected wine and only then proceed to the industrial scale treatment.

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OPTIMIZATION OF THE ALCOHOLIC FERMENTATION BY CORRELATING THE INITIAL SUGAR CONCENTRATION WITH THE INOCULUM SIZE OF YEASTS AND ASSIMILABLE NITROGEN REQUIREMENTS

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Abstract

It is known from the literature that the yeasts require assimilable nitrogen (YAN) in a certain dosage in order to ferment sugar and for the exact calculation of the nitrogen can be done by applying a certain equation derived from the Bisson and Butzke tables. This equation however does not take into account the yeast strain and its requirements for assimilable nitrogen, nor the possibilities of yeasts to ferment all the available sugar. Taking into account the high concentration of sugar which is more and more found in our grape musts in the later years the selection of yeast and the inoculum size is also important. We have used a 'Feteasca regala' must with various amounts of initial sugar and we have corrected the YAN in accordance with the Bisson and Butzke table for each sample. In order to optimize the fermentation, we tested 3 different yeast strains, used in quantities proportional to the initial sugar concentration. In this way the yeast quantity used is optimized in accordance to sugar fermentation requirements. We have observed that not all the yeasts are able to totally ferment the sugar in samples with 26-28° Brix, leaving in the same time unconsumed YAN. Therefore, the correction of nitrogen should not by applied only in accordance to the calculations, but should be also adapted and limited in the case of high sugar concentration musts. The moment of nitrogen correction, the yeast strain and the inoculum size are evaluated and discussed.

Key words: *fermentation, inoculum, optimization, YAN*

INTRODUCTION

Musts composition and the progress of alcoholic fermentation are essential for the quality of wine, therefore the amount of nutrients for yeast growth found in musts should be in good correlation with the yeast strain and sugar level. To help oenologists make the corrections, Bisson and Butzke (2000), proposed a table with the amount of YAN required for the yeast growth in musts with various sugar concentrations.

Underestimating the YAN requirement and the selection of an inappropriate yeast strain can lead to low rates of fermentation and, consequently, to stuck or sluggish fermentations possibly accompanied by hydrogen sulfide production. On the other hand, an overestimation of nutrient needs, can lead to high rates of fermentations and fast increase of medium temperature due to the rapid yeast multiplication, while the remaining amounts of YAN in wines can lead to microbial spoilage and again the possibility of hydrogen sulfide formation, due this time to a different mechanism than the one involved in the cases of low YAN content. Several authors shows that high concentrations of YAN do not necessarily protect against elevated H_2S formation (Butzke et al., 2011; Sea et al., 1997; Ugliano et al., 2009) and likewise a higher production of total H_2S was observed in musts with high initial concentration of ammonium ions, as compared to non-supplemented musts (Butzke et al., 2011).

Anywise, was confirmed that a typical must require a minimum 140-150 mg N/l YAN to successfully complete the alcoholic fermentation (Henschke et al., 1993; Bell et al., 2005).

Another important factor is the yeast rehydration process that should be done in accordance with producer's specifications. Moreover, the yeast dosage should be well correlated with the sugar concentration of the inoculated must.

This fact was also obverved by Kontkanen et al. (2004) during a research on icewine, that is a higher dosage of yeast forms a higher ethanol concentration and allows for a better rate of fermentation, rather than a sluggish fermentation that usually occurs in wines with high sugar concentrations. In these conditions, for an optimal alcoholic fermentation, oenologists should create the optimal equilibrium between sugar concentration, YAN, yeast inoculum, yeast strain and adequate temperature control.

MATHERIALS AND METHODS

Raw material. White must of 'Feteasca regala' from the experimental vineyard of UASVM Bucharest was used for the study. The physico-chemical parameters of the must are presented in Table 1.

Table 1. Must parameters of the must of 'Feteasca regala'

Harvesting date	09.09.2013
Brix, %	23.75
Titratable acidity, g/l acid tartric	4.58
pH	3.54
YAN, mg/l	68.05
Turbidity, NTU	620

Methods of analyses and equipments: *pH* was determined with a Hanna pH 112 (OIV, 2009b). *Total titratable acidity* (TA) was determined with TitroLine easy Schott Instruments until the end point of titration at pH 7.0 (OIV, 2009a), while the *YAN* was determined using the same titrator and the modified Sørensen method, in which titration with NaOH 0.1 N is performed until reaching the pH = 8.0, after the addition in the must of a solution of 38% formaldehyde with pH = 8.0, so that the amine basic function groups are blocked and the carboxylic acid functions are released (Filipe-Ribeiro et al., 2007; Gump et al., 2002; Shively et al., 2001). *Turbidity* of must was determined with a MRC, model TU-2016 portable turbidimeter by using an official method of OIV (OIV, 2009c). *Degrees brix* were measured with a digital probe refractometer Misco DFR123 by directly immersion of optical sensor in musts at 20°C. *Density* and *temperature* measurements were determined using physical methods. *Reducing sugar* was determined by Luff-Schoorl method (OIV, 2009d). *Alcoholic strength by volume* was determined by distillation and density measurement with a pycnometer (OIV, 2009e).

The growth of the yeasts in the musts was followed by recording the heat evolved in the medium by using an isothermal calorimeter working on the principle of the heat conduction (Antoce, 1998).

This calorimeter consists of 25 calorimetric units, in which 24 microbial cultures can be monitored in several inhibition conditions, while the last one is being used as a reference. A thermopile plate located on the bottom of each unit measures the amount of heat generated in the unit during the microbial growth, as it is transferred to the surrounding aluminum block, which is kept at a constant temperature by circulating water through copper pipes located around it. The heat flux established between the calorimetric unit and surroundings is detected by the thermopile plates (Melcore CF-70.1, New Jersey, SUA) and the difference between each sample and a reference cell is recorded as a voltage signal. The voltage signal is measured for each sample at a fixed time interval by using a Keithley digital voltmeter and a channel scanner. All 24 signals are thus digitalized and stored into a computer database. The specialized software for the data analysis works under Origin General Scientific 2.8v platform and is of in-house design (Antoce et al., 2011).

The recorded growth thermograms were used to calculate *growth rate constants* and times of *growth retardation* of yeast for each experimental culture.

Treatments. Preparation of the must consisted firstly in a pectolytic enzymatic treatment with 3 g/hl, followed by sedimentation of must at 15°C. The concentration of enzymatic product used was of 15200 U/g enzymatic activity including: 10000 U/g pectin lyase, 650 U/g pectin methyl esterase and 4550 U/g polygalacturonase.

After the sedimentation the must was racked from the lees and corrections of sugar, titratable acidity, pH, turbidity and YAN were performed. Sugar correction was done with inverted sucrose solution prepared, from 2 g/l tartaric acid to 1 kg of sucrose, boiled for 20 minutes.

In order to optimize and correlate the YAN corrections with respect to sugar concentration, experiment was conducted on musts with 18, 20, 22, 24, 26 and 28 Brix, obtained by correction from the initial raw must of 'Feteasca regala' with the parameters presented previously in Table 1.

Titratable acidity and pH were harshly corrected with tartaric acid to create a supplementary stress factor during alcoholic fermentation in order to make our evaluation for the worst case scenario winemakers can encounter in practice and subsequently correct. It is well known that fermentation of musts with very low pH and / or high sugar concentration leads to increased volatile acidity production by yeasts due to the passive ion influx stress and effect of osmotic pressure. Furthermore, the resulted acetic acid can inhibit the growth of yeasts and this situation can lead to a stuck or sluggish fermentation.

YAN adjustment / monitoring were made in correlation to the sugar concentration in the must samples, based on the correction table of Bisson et al. (2000), but using an equation devrived from it: $YAN\ (minimal), mg/l = 25 \times \%Brix - 350$.

For the YAN adjustment a commercial nutrient was used, consisting of a mixture of a 5 : 3 ammonium sulphate to diammonium hydrogen phosphate. The turbidity of musts was reduced to 100 NTU by using a cellulose filter aid. The composition of the musts resulted after these adjustments were re-analyzed and the physico-chemicals parameters included in Table 4.

Inoculum size. For the yeast inoculation we tried to obey the recommendations found in literature (table 2), which range from 10^5 to 10^7 cells/ml. In order to optimize the inoculum size and correlated it with sugar concentration, for this experiment we created a simple mathematical model, and devising the following equation: $i = v \times b \times 10^6$

where: i – correlated inoculum with % Brix, expressed in cells/ml; v - constant to correlate the inoculum size with the sugar content in must (v = 0.2); b – brix, % determined by refractometry;

Table 2. Recommended startup inoculum sizes

Recommended inoculum, cells/ml	**Reference**
10^5-10^6	Bisson, 2001
10^5-10^6	Jackson, 2008
1 x 10^6 - 3 x 10^6	Fugelsang, 2007
10^6-10^7	Jacobson, 2006
10^6	Ribéreau-Gayon *et al.*, 2006
10^6	Boulton *et al.*, 1996
*3,8 x 10^6 - 1 x 10^7	Kontkanen *et al.*, 2004
3 x 10^6 - 5 x 10^6	Monk, 1986; Monk, 1997
5 x 10^6	O'Brien *et al.*, 1990

*inoculums tested for icewine production;

Yeast strains. The selection of yeast strains for the experminent was based on availability and yeast oenological traits (Table 3).

Table 3. Oenological characteristics of yeasts

Strain	**Bayanus PC**	**Epernay 2**	**Premium Blanc 12V**
Species	*S. bayanus*	*S. cerevisiae*	*S. cerevisiae*
Origin	-	-	Alsazia region
Alcohol tolerance, % vol.	15	15	13
Alcohol yield (% vol./g of sugar)	0.057	0.058	0.058
Optimum temperature	11-30	12-30	10-35
SO_2 production	medium	low	medium
Action on malic acid (-%)	20-30	35-45	25-35
Glycerol production	medium	high	medium
Aromatic features	Crust bread	Fruity and fresh notes	Varietal expression

Experimental design. The experimental fermentations were conducted in 6 musts with 6 different sugar concentrations (18, 20, 22, 24, 26, 28%), each must being separately inoculated with one of the 3 yeast strains (Bayanus PC, Epernay 2 and Premium Blanc 12V).

Table 4. Quality parameters of 'Feteasca regala' musts with sugar level adjustments, inoculum sizes and yeast strains used in the experiment

Parameter	Sample with 18% sugar	Sample with 20% sugar	Sample with 22% sugar	Sample with 24% sugar	Sample with 26% sugar	Sample with 28% sugar
Brix, %	18.15	20.25	22.20	24.15	26.40	28.20
Titratable acidity, g/l tartaric acid	9.41	9.41	9.09	9.03	8.87	8.83
pH	2.96	3.06	3.11	3.13	3.16	3.15
[1]YAN_i, mg/l	50.84	57.06	63.42	67.39	65.07	62.80
[2]YAN_f, mg/l	120.29	154.18	194.28	254.71	303.28	348.46
[3]NTU_i	48.92	48.67	69.00	42.09	53.67	61.00
[4]NTU_f	100	102	101	102	100	102
Inoculum size, cells/ml	3.63 x 10^6	4.05 x 10^6	4.44 x 10^6	4.83 x 10^6	5.28 x 10^6	5.64 x 10^6
Yeast strains	Bayanus PC / Epernay 2 / Premium Blanc 12V;					

[1]YAN_i - yeast assimilabile nitrogen prior correction; [2]YAN_f - yeast assimilabile nitrogen after correction; [3]NTU_i – turbidity of musts prior correction; [4]NTU_f– turbidity of musts after correction;

The experiment was run in triplicate for each sugar concentration and yeast. In each must YAN was adjusted and yeast inoculated in accordance to the sugar level, as described in Table 4.

RESULTS AND DISCUSSIONS

After the completion of fermentation, the alcoholic concentration (Figure 1) and the residual reducing sugar in each sample (Figure 2) were determined.

It can be observed that all yeast strains used in our experimental conditions produced in the samples with high content of sugar more alcohol than the level the producer said they would normally tolerate (Table 3). This may be explained by the optimization of YAN and inoculum size with the the sugar concentration in each sample.

Kontkanen et al. (2004) found similar results regarding the alcoholic strength on icewines with high sugar concentrations when the yeast dosage was increased.

For reason of stability, in winemaking the interest is to produce wines with low sugar remaining after the completion of fermentation, of a maximum of 4 g/l (the limit between dry and semi-dry wines) or even less than 2 g/l, for the prevention of *Brettanomyces* infections (Antoce, 2005). This means that a better tolerance and a higher transformation yield of sugar into ethanol, will ensure the oenologists that most of the musts will ferment to dryness. To achieve this goal, a good correlation of YAN and yeast inoculum size would give satisfactory results in wine production.

From figure 2, it can be observed that only the musts with very high sugar concentrations cannot be fermented to dryness, especially in the case of using regular yeast strains, which are moderately resistant to alcohol. Even more, among the yeast we have used, the one more sensible to alcohol, Premium Blanc 12V, may be more prone to lead to sluggish or stuck fermentations. This behavior can be easily observed in figure 2, for the must samples containing sugar levels of 26 and 28% Brix and fermented with this yeast strain. Aside of these extreme cases, even using this less tolerant yeast strain would not generate any fementation inconveniences, provided the YAN and inoculum size is optimized in accordance with the sugar level.

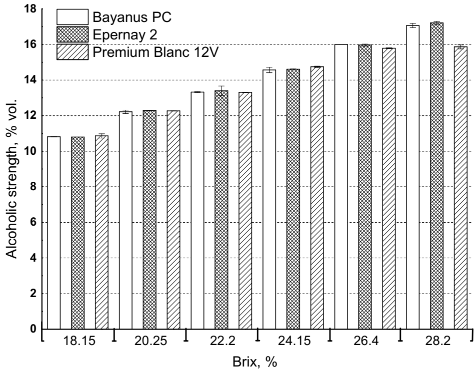


Figure 1. Alcoholic strength of the wines obtained form musts with various levels of sugar content and fermented with 3 different yeast strains

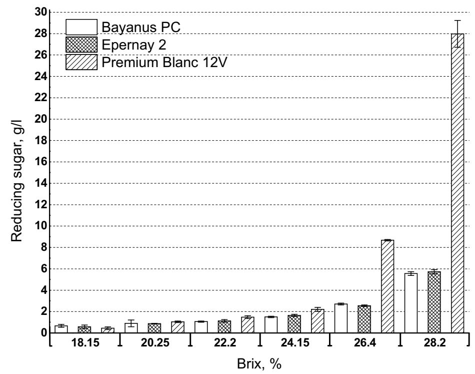


Figure 2. Residual sugar of wines obtained form musts with various levels of sugar content and fermented with 3 different yeast strains

Another crucial parameter for the wine quality is the volatile acidity, which was analyzed after fermentation in each sample, the values being presented in Fig. 3. In accordance to the knowledge in this field, we also observed a trend towards the increase in volatile acidity with the initial sugar concentration of must.

The values of volatile acidity are slightly higher in our experimental case, than would normally be in production conditions, due to the harsh changes of pHs which we artificially induced in the must samples, to create a supplementary stress for the yeasts and have, accordingly, a worst-case scenario. Even so, the legal EU limit of volatile acidity, which is 1.08 g/l acetic acid for white wines, was not exceeded.

By comparing the volatile acidity produced by each strain in all the must samples it can be observed that Premium Blanc 12V (Figure 4a) is the most productive, while Epernay 2 (Figure 4b) and Bayanus PC (Figure 4c) give similar results in our experimental conditions.

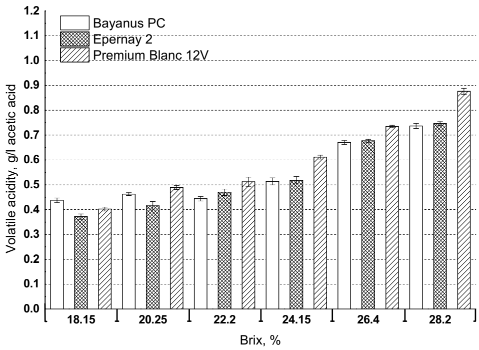
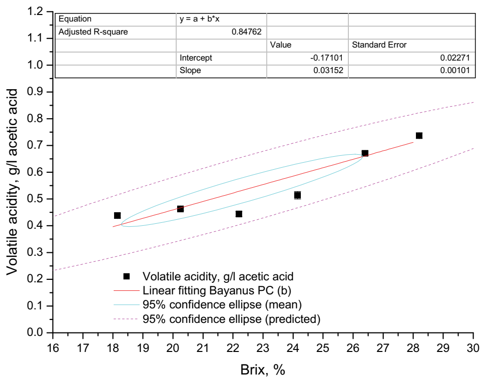
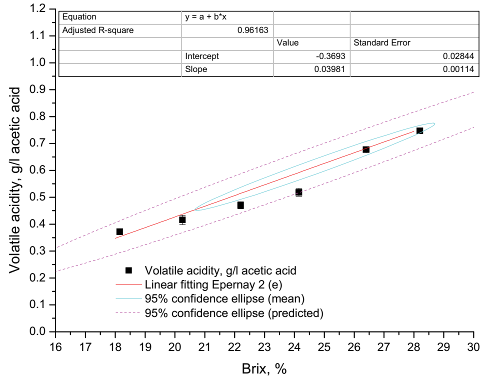


Figure 3. Volatile acidity of wines obtained form musts with various levels of sugar content and fermented with 3 different yeast strains





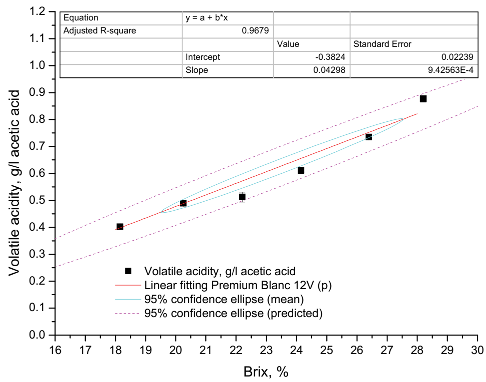


Figure 4. Volatile acidity production trend in wines obtained form musts with various levels of sugar content and fermented with Bayanus PC (a), Epernay 2 (b) and Premium Blanc 12V (c) yeast strains

The production of volatile acidity is very well linearly correlated (R^2 of 0.85-0.97) to the sugar concentration in the initial must, irrespective of the yeast strain used (Fig. 4a-c). This fact proves that the fermentation took place in optimal nutritional conditions even in the case of the samples with 26 and 28 % Brix, which usually lead to much higher volatile acidity (a surplus of 0.3-0.6 g/l), as the yeast struggles to survive in unfavourable conditions.

The growth thermograms obtained for each yeast culture introduced in the calorimeter showed that in our experimental conditions for each yeast strain the growth rates were similar for the samples with initial sugar concentration of 18, 24, 26, 28 % Brix, even though the inoculum size was different for each sugar level (Figure 5). This fact suggests a good correlation of the the inoculum size, sugar and YAN concentrations in the samples of these musts. It could be concluded that these growth rates with growth rate constants in the range of 0.1-0.2 min^{-1} are optimal and to slow down the growth in the samples of 20 and 22% Brix we should, for example, decrease the inoculum size. As it can be seen in Figure 5, for the Bayanus PC and Epernay 2 no further adjustments of inoculum size is needed, as the growth rate constant is between 0.1-0.2 min^{-1} irrespective of the initial sugar concentration. However, Premium Blanc 12V is a fastidious yeast strain, growing much faster in samples with medium sugar level (20-22% Brix) and high YAN concentrations. This fact is also confirmed by the yeast growth retardation chart (Figure 6), where we can see that Premium Blanc 12V yeast strain starts growing faster at 20-22% Brix, within 25-30 hours after inoculation, as compared to Epernay 2 and Bayanus PC, who need 33-53 hours for the same growth level. A faster growing can be an advantage in achieving the necessary number of yeast cells for the fermentation, but is not anymore if the rate of fermentation is also increased, because in a fast fermentation more wine aroma compounds are negatively affected or lost. Therefore, more studies are needed to decide if it is acceptable to apply the same equation for the adjustments in must composition for all the yeasts or the fast growing ones should be treated differently.

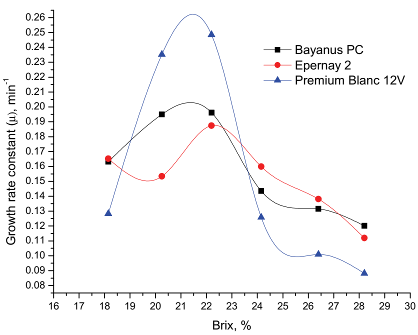


Figure 5. Growth rate constants of the 3 yeast strains in musts with different sugar concentrations

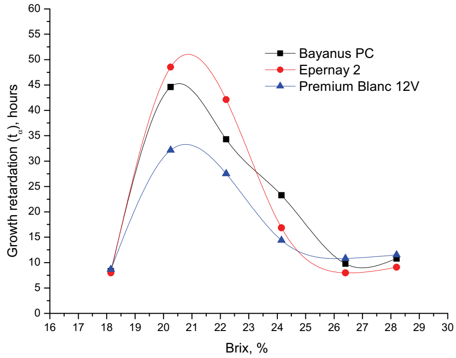


Figure 6. Growth retardation of the 3 yeast strains in musts with different sugar concentrations

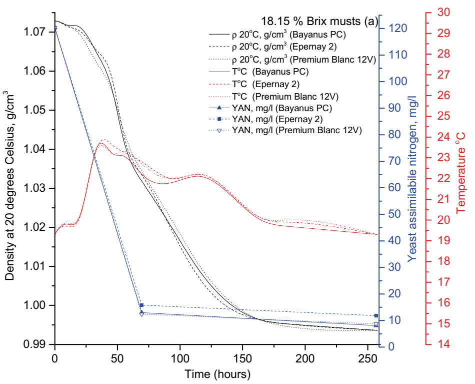
The growth retardation of yeasts (Fig. 6) determined by calorimetry in musts with various sugar concentrations and adjusted YAN levels is a good indication of the optimization achieved in the culture conditions. The growth retardation represents the time passed until a calorimetric signal of a certain level (in our case 10 mV) is reached on the growth thermograms mathematically processed (Antoce et al., 1996, 1997) to depict the real growth from the recorded calorimetric data.

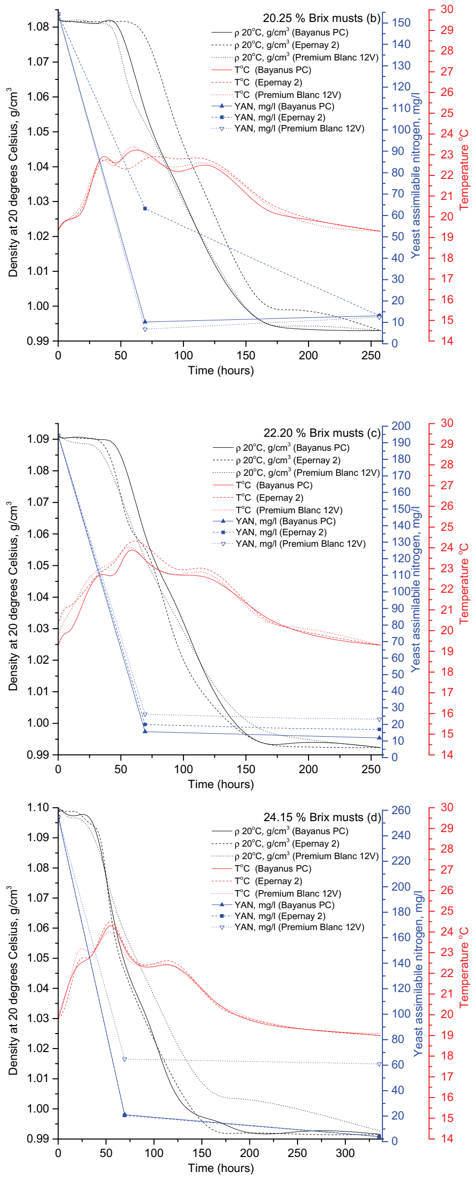
The fact that the lowest retardation (10-25 hours) is obtained in samples with 18 and 24-28 % Brix show that the inoculum size is best selected for these samples. The growth retardation of 35-53 hours observed in the samples with 22-24 % Brix suggests that for these musts the inoculum size was underestimated. However, because the growth rates for the musts with 22-24 % Brix are still in the normally expected ranges (Figure 5) and there is no risk for a sluggish fermentation, no need for further adjustment seems in order.

The proposed model for the adjustments in yeast dosage and YAN levels is particularly useful in the case of high sugar concentrations. This was proved by the rapid growth (10 hours) after the yeast inoculation in all samples with 26 and 28% Brix. Here, the high inoculum size and nutrient level, compensated the osmotic stress induced by high sugar concentrations. This type of approach is also supported by the wine yeast producing companies, which recommend in their technical sheets an increase in yeast dosage from the normal 10-20 g/hl to 30-40 g/hl in the case of high sugar content musts and a further yeast nutrient supplementation. In our work we were able to make more precise recommendations regarding the inoculum size and YAN levels required for a must with a certain initial sugar concentration. The fermentation process was monitored for each sample by following the evolution of density, temperature and YAN. Useful information was thus obtained regarding the consumption period of YAN, residual YAN and progress of alcohol accumulation.

Normally, irrespective of the sugar concentration, YAN concentration should not be excesive, so that yeasts should be able to consume it down to a level of 10 to 20 mg N/l. Higher residual YAN may lead to bacterial spoilage in wines. As it can be seen in Figure 7, the available YAN is consumed within the first 70 hours of fermentation, which corresponds mostly with the multiplication of yeast cells period and with the consumption of a 1/3 of the total sugar content. Another study shows similar results on YAN consumption period (Bely et al., 2003).

The administration of subsequent doses of YAN should be avoided, due to the risk of remaining unconsumed.





sugar concentration, where the sugar consumption is also retarded and only less than 1/3 of its quantity is used in the first 70 hours of fermentation.

To avoid bacterial spoilage, in the case of sweet wine production, the oenologists should choose low alcohol tolerant yeast strains with low nutrient requirements and limit the YAN level in must, so that it will not remain unconsumed in wines.

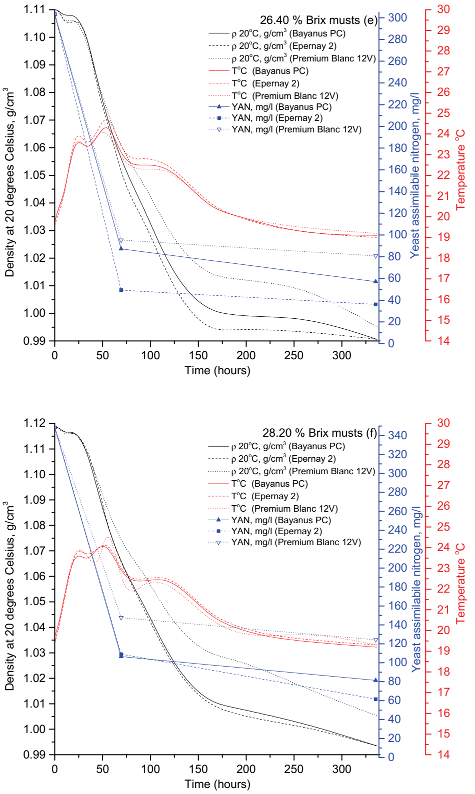


Figure 7. Evolution of density, temperature and YAN during alcoholic fermentation of musts with different level of sugar with yeasts with correlated inoculum sizes

Figure 7a-d, shows that for must with 18-24% Brix all the fermentations progressed normally, leading to dry wines (Figure 2), with residual YAN of no more than 20 mg/l. The musts with 26 and 28% Brix (Fig. 7e and 7f), for which the calculated and added YAN was in excess of 250 mg N/l, led to wines with high residual YAN, of 40-80 mg/l and 60-120 mg/l, respectively. The differences in the final YAN concentration for these high sugar samples were due to the yeast strain used for the fermentation, Premium blanc leaving the highest levels of nitrogen in the final wines.

In some cases, when the fermentation starts sluggishly (longer lag phase), YAN can be consumed even after the first 70 hours of fermentation. Such event happened for the fermentation of 20 % Brix musts with Premium Blanc 12V yeast strain (Figure 7b) and can frequently happen in musts with high

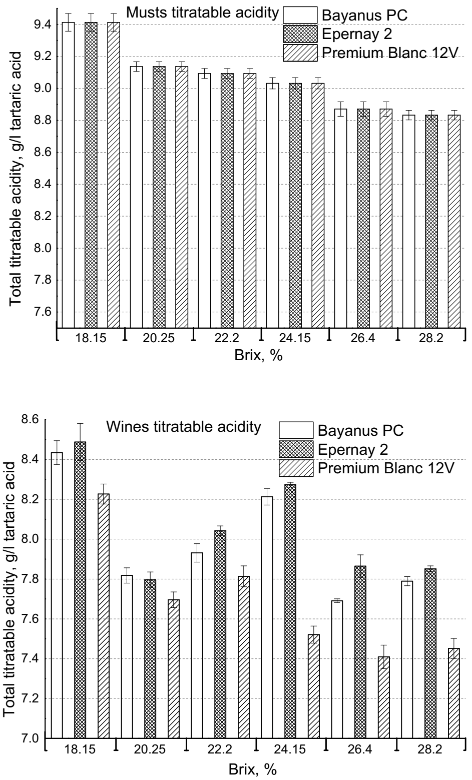


Figure 8. Titratable acidity of the musts (a) and the resulted wines (b) produced with 3 different yeast strains

The titratable acidity, another important parameter for the wine quality, generally dropped more in the wines obtained from musts with high sugar content (Figure 8b). This behaviour was not surprising, since the higher alcohol content produced in wines made from musts with high sugar concentration decreased the solubility of the potassium hydrogen tartrate, which precipitated in larger amounts. The small varations in the titratable acidity of the wines (Figure 8b) produced from the same must (Figure 8a) with various yeast strains can be accounted for the different metabolic mechanisms for some acid formation (eg. succinic acid) ore depletion (eg. malic acid). Generally, the wines resulted from musts inoculated with Premium Blanc 12V yeast strain had less titratable acids than the wines of the other strains (Figure 8b).

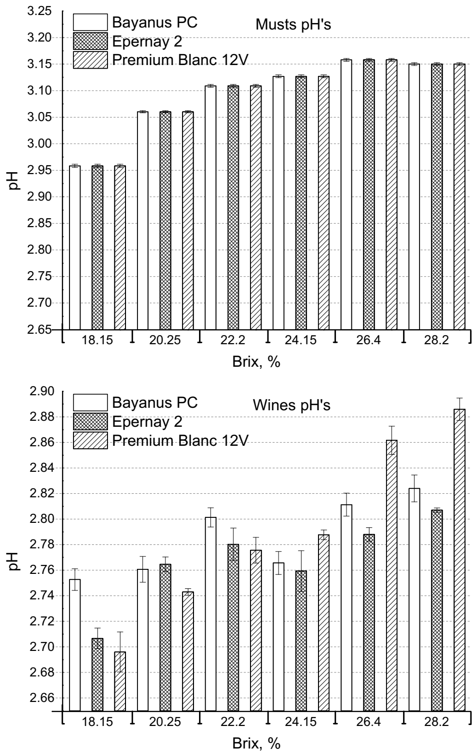


Figure 9. pH of the musts (a) and the resulted wines (b) produced with 3 different yeast strains

In Figure 9a and b the pH of the musts and the resulted wines produced with 3 different yeast strains are shown. As in the case of titratable acidity, pH was dependent on the metabolic pathways of yeasts and on the solubility of the tartaric acid salts and the precipitations of potassium hydrogen tartrate, but also on the added ammonium salts used as nutrients. As we can see, the adjustment of YAN in increasing amounts correlated to the sugar concentration, increased the pH of those musts accordingly (Figure 9a). A lower pH is good for the microbiological wine stability (Figure 9.b), but in must a pH below 2.9 is a stress factor for the yeast, especially when the alcohol also starts to accumulate. That is why, the YAN adjustments with ammonium salts are not only good for yeasts as nutrients, but are also good for the pH regulation in musts, increasing their fermentability.

CONCLUSIONS

Adjustments of YAN levels and inoculum sizes correlated with the sugar concentration of musts should be applied in wine technology to a certain extent, that is, to a maximum YAN concentration of 250 mg N/l. For a good management of fermentation YAN level should be at least 150 mg N/l, value reported in the literature by many authors as the minimal necessary to complete fermentation. This correction should be done prior to inoculation, while and a second correction should be applied only when necessary, 48 hours after the inoculation or, better, when sugar depletion is 1/3 of initial sugar concentration.

A recommended YAN level, correlated with the sugar concentration would be defined by the following equation: $YAN, mg/l = 10 \times \%Brix - 30$.

The second correction to be applied when 1/3 of initial sugar concentration is consumed may be calculated by substracting from the calculated YAN level the minimal recommended YAN level, that is 150 mg/l.

Aside of YAN corrections, the optimization of alcoholic fermentation implies also the adequate yeast strain selection and the inoculation of a optimum number of cell/ml. The formula we used for the inoculum size calculation in this experiment seems to provide good practical results and for this reason we recommend it to be applied in wine production sector.

If sweet wines are desired, a strain of yeast with low to medium tolerance to alcohol should be used and YAN should not be supplemented to more than 150 mg N/l.

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AGROTECHNICS APPLIED TO VARIETIES OF TABLE GRAPES GROWN IN VINEYARD ŞTEFĂNEŞTI-ARGES

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Abstract

Table grapes should be a pleasant and appealing. This condition is crucial because the consumer considers the first "eye" size, shape and color of the grapes. The production of grapes, in addition to climatic conditions and variety cultivated has a decisive influence on applied agrotechnics plantation. Obtaining high yields of high quality and at a low cost price agrotechnical requires application of differentiated varieties. Necessity to replace the old table grape varieties in plantations established many years ago, with new varieties suitable for large productions and qualitative study resulted in the collection ampelographic of new varieties such as 'Argessis' and 'Golden Stefanesti', compared with varieties known 'Muscat Hamburg', 'Augusta'. The research in the present paper, started from the assumption that pruning represents the most important agrotechnical link in the culture technology of grapevine. The execution of correct working, in accordance with the variety and the culture conditions of the grapevine ensures the achievement of large, constant, high quality production every year. At INCDBH Ştefăneşti-Argeş, located in the vineyard Ştefăneşti-Argeş, researches on the aspects regarding the level of the crop load for the table grapes varieties have not been carried on before. To this effect, we have considered useful to undertake a study on this agrotechnical intervention for a better promotion of these varieties in production.

Key words: *table grapes, consumer, quality, agrotechnics*

INTRODUCTION

The latest years have recorded an important rise in the consumption of fresh grapes, due to a general tendency towards a healthy nutrition, rich in vegetal resources (Dejeu L., 2010, 2011). Grapes represent a medicine-fruit as they have high therapeutic qualities as well as the grapevine and the wine (Necula C et al., 2010; Petrescu E., 2002).
The confrontation of Romanian viticulture with the international one and mainly with the European one, is a current issue which obliges us achieve high quality products (Messegue M., 1998). As the Romanian market contains only few varieties with early maturation and pleasant commercial aspect, new varieties and clones meeting these requirements must be promoted. Such new varieties and clones have been already cultivated in some plantations in the area Ştefăneşti-Argeş.
These are the newly homologated varieties: 'Argessis', 'Golden of Ştefăneşti', 'Muscat Adda 10 Şt'. The famous varieties cultivated in the area - 'Augusta' - can be added to the new cultivated varieties.

MATERIAL AND METHOD

The consumers' requirements, especially children and old people, for seedless grapes determine the obligatory extension of the seedless and other varieties in the viticulture of the countries having a warm or continental climate like Romania (Nicolaescu Gh et al., 2007).
The newly created varieties, homologated during the latest years, but known only in the units where they had been obtained, will be set

into culture in order to replace some older varieties which do not meet the continuously changing qualitative requirements of consumers (Popa C., Necula C., 2003).
Settlement experiences bifactorial type 4x3 for each experimental year (2011-2013) was identical, taking into study two factors, namely:
Factor A - variety, which included thee graduations: 'Argessis', 'Golden Ştefăneşti', 'Muscat Adda 22 St.', 'Augusta'.
Factor B - fruit load applying differentiated cuts bearing vines that covered graduations: b1 fruit load of 15 eyes per vine with pruning spigots; b2 fruit load of 20 eyes per vine, with cutting to the heart; b3 fruit load 25 eye hub with cutting the string.
Observations and determinations have been made on the experimental plot located in the viticultural area of Muntenia (Ştefăneşti Argeş), as regards the quality of the table grapes under the pedoclimatic conditions of the Argeş zone. The grapevine plantation has a distance of de 2,2/1 m, the applied cutting is the Guyot type on semistock.
The table grapes have been harvested when their maturation ensures a superior and efficient capitalization (Costescu A., 2013; Messegue M., 1998). This has been set in general, according to the variety, when the grapes reached full maturity.
The moment of full maturity for the table grapes has been established by approximation, through performing the following determinations: the weight of the berries, the sugar content of the must and the titratable acidity.
These determinations have been made periodically, every 3-5 days, beginning with the ripening of the grapes.
In the production of grapes, besides pedoclimatic conditions and the cultivated variety, a decisive role is played by agrotechnics applied in plantation.
The obtaining of large high quality production at a low cost price imposes the application of an agrotechnics differentiated on varieties.

RESULTS AND DISCUSSIONS

The shorten description of varieties:
'Argessis' (Figure 1) - variety homologated in 2002 at SCDVV Stefănesti.
The first table grape variety obtained under the pedoclimatic conditions specific to the vineyard Ştefăneşti-Argeş.
Pleasant commercial aspect, large berry (7,5-8,0 g), ovoidal, bluish-black colour.
Large, uniaxial grape (450-480g). Good tolerance to cryptogamic diseases (mildew, blight, rot).
High vigor vines, suitable for arbor culture. Average production reaches 15 t/ha (Popa C., Necula C., 2003; Petrescu E., 2002).

Figure 1. Variety 'Argessis'

'Auriu de Ştefăneşti' (Figure 2) - the first grapevine variety for white table grapes, having a very early maturation, obtained under the pedoclimatic conditions specific to the vineyard Ştefăneşti-Argeş, homologated in 2007 at INCDBH Stefăneşti.
Pleasant commercial aspect, large berry (5,5-6,8 g), globular, golden-yellow colour. Large grape (400-450 g), winged.
Good tolerance to cryptogamic diseases (mildew, blight, rot).
The maturation of grapes in phase I (28.07-05.08).
Suitable for ecological cultures.
Extension in culture of the variety ensures an average production of 14 t/ha (Popa C. et al., 2003).

Figure 2. Variety 'Auriu of Ştefăneşti'

'Muscat Adda 22 St.' (Figure 3) **-** has been obtained through clone selection from the variety 'Muscat d'Adda' at INCDBH Ştefăneşti and homologated in 2008; it is a variety destined for fresh consumption**;** the leaf is green; the grapes are medium sized, towards large (270-340 g); the berries are disposed equally on the cluster, being medium to large size; semicrisp pulp with specific taste and flavor; the skin is thick, black-aubergine coloured, highly bloomed; the vigor of the vines medium to large; it has good resistance to drought and diseases (mildew, blight and grey rot); the maturation of the grapes in September; a production of 4,9-5,3 kg/vine; it accumulates 187 g/l sugars and 3,9 g/l acidity; it has an increased resistance to handling, transport and storage (Necula C et al., 2010).

Figure 3. Variety 'Muscat d'Adda 22 Şt'.

'Augusta' (Figure 4) - it was obtained by controlled sexual hybridization Italy x Queen variety of vineyards, the Agronomic Institute in Bucharest. Variety approval was made in 1984. Required by earliness (II era of aging), size and look to the grapes.

At the rosette is starting in vegetation glabrous, green and brown shades, and young leaves are bronze. Adult leaf of medium size (16-18 cm long) and have fine grooves.

Autumn chords acquire a brown color - brown. Hermaphrodite flower normal guy May, variety is self-fertile.

Grapes are high (average 325g), conical and cylindrical-lacs. Berries large, oval, yellow-green, semicrisp flesh with pleasant flavor (Nicolaescu Gh et al., 2007).

The research in this paper have assumed that cutting is the most important link in the technology culture Agronomic grape-vine.

Correct execution of works in accordance with the variety and growing conditions, ensuring the achievement of high yields, consistent and quality from one year to another.

At INCDBH Ştefăneşti-Arges, located in the vineyard Ştefăneşti-Arges, research on issues related to establishing the level of load bearing table grape varieties created here, they have not been undertaken. In this respect it was considered useful to study the agro links to better promote further production of these varieties.

The production of grapes, in addition to climatic conditions and cultivated variety, has a decisive influence agricultural technique applied in the plantation. Achieve high yields of top quality and at a low cost price agrotechnical requires applying differentiated varieties.

Figure 4. Variety 'Augusta'

Shoot growth was recorded by variety, the load assigned to each block separately. The largest increases in shoots, regardless of year and fruit load were recorded 'Argessis' variety averaging 169 cm, followed by golden variety Stefanesti (165 cm) and 'Muscat Adda 22 Şt'. (162 cm) and the smallest increases were recorded in variety 'Augusta' 159 cm (Figure 5).

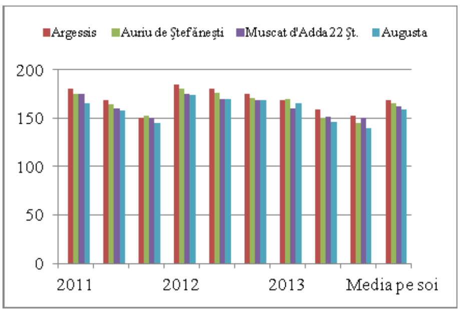

Figure 5. Shoot growth of the varieties (cm)

Analyzing the three variants and increases depending on the type of cut shoots was found that the highest increases were registered in the drill-cutting versions of fruits, to those cutting the string, so the variety 'Argessis' version 15 eyes / stump sprouts increases were 180 cm and Golden variety Ştefăneşti the variant with 25 buds / vine, shoots increases were only 152 cm.

In the three years of experimentation, 2011-2013, we found that leaf area per one cylinder varies within very wide limits between varieties because each variety is different vigor.

It was also found between study variation, climatic conditions of every individual year largely influencing the size of leaf area. Increased eye on the stump to dry pruning resulted thus increasing leaf area per vine, so that the highest values of leaf area were recorded in V3 (25 eyes/vine) for all varieties (table 1).

Growth vigor of the vine was assessed by the amount of wood removed from the cut. This amount of wood is made of wood and multi-year.

Data from the three years of experimentation (2011-2013) highlight the major differences between varieties, the number of strings left to cut.

Table 1. Pruning weight (g/block vine), depending on bud load, mean 2011-2013

Variety	Variant	The amount of wood removed (g / vine)		
		Total wood	**Anual wood**	**Multianual wood**
'Argessis'	V_1	1325	1150	275
	V_2	1375	1075	300
	V_3	1430	1050	380
	Average	**1410**	**1092**	**318**
'Golden Ştefăneşti'	V_1	1380	1200	180
	V_2	1250	1000	250
	V_3	1170	850	320
	Average	**1266**	**1016**	**250**
'Muscat Adda 22 Şt.'	V_1	1560	1210	350
	V_2	1550	1170	380
	V_3	1550	1150	400
	Average	**1552**	**1176**	**376**
'Augusta'	V_1	1120	820	300
	V_2	1150	800	350
	V_3	1150	760	390
	Average	**1139**	**793**	**346**

CONCLUSIONS

With vineyard wine Ştefăneşti-Argeş, table grape varieties grown obtained can compete in terms of quality with many other varieties produced worldwide ('Muscat d'Adda', 'Perllette').

'Golden Ştefăneşti' variety is appreciated as a very early variety for vineyard Ştefăneşti and successfully completes many varieties area (ripening in the first period).

By promoting the production of table varieties of high biological resistance to attack by pests and diseases will protect the environment in terms of the viticultural area.

Producing more grapes will reduce our country's imports of these products, which are sometimes straight from indigenous varieties grown in other European countries (e.g. 'Victoria').

'Argessis' and 'Golden Ştefăneşti' varieties successfully complete many varieties area as table varieties.

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FOUNDATION OF RED WINE MAKING STRATEGIES ON ASSESSMENT OF GRAPES MATURATION AND MATURITY

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Abstract

The maturation and maturity of the grapes are concepts which in recent decades have gained knowledge and approaches. In the classical system the maturation was considered as a process of accumulation of sugars in the grape followed by the modification of their appearance and weight, while in the present, grape maturation is clearly differentiated from grape maturity. The maturation of the grape represents a dynamic mathematical model of the compounds with enological value, while the maturity is a moment during the grapes evolution which is characterized by an enological potential. The researches were conducted during 2007–2013 period at the Research and Development Institute for Viticulture and Enology, Valea Calugareasca, located in the Dealu Mare viticultural area. The experimental device was organized in 4394 vineyard parcel representing a collection of varieties from Valea Calugareasca viticultural area wine assortment. The varieties which were taken into study were: 'Burgund mare', 'Cabernet Sauvignon', 'Feteasca neagra', 'Merlot' and 'Pinot noir'. The methodology concerning the maturation and maturity of the red grapes was developed during 2007 year by IC-DVV, Valea Calugareasca. The samples were taken during the ripening process, every 7 days and the following parameters were determined: sugars (g/l), titrable acidity (g/l sulfuric acid), weight of 100 grains (g), glucoacidimetric index, total phenolic potential and anthocyanins. Grape polyphenolic potential has been evaluated at harvest by means of standard Glories method. The information was organized into a database which has the following components: climate, maturation and oenological potential. The processing of information was done by mathematical modeling. The obtained results have allowed the identification and parametrization of the three types of grapes maturation and three levels of enological potential.

Key words: *Maturation, maturity, enological potential, mathematical modeling, winemaking strategies*

INTRODUCTION

In the classical way the maturity of grapes is evaluated by the dynamically analysis of the main compounds in grapes (Dubourdieu et al., 2004; Reynier A., 2007). In most cases these data are presented as such, and therefore the existing basic data remain unexploited. For maturation it is important to define its kinetics and to evaluate the potential of black grapes at harvest at the technological and polyphenolic level. In order to characterize the black grapes maturation kinetics, a parametric model of the grapes sugar content evolution was created by Severin et al., (2010). The model is a sigmoid described by the equation 1.

$$Ts = Tsver + \frac{Ts\max - Tsver}{1 + e\dfrac{t1/2 - japv}{\tau}} \text{ (equation 1)}$$

in which: Ts-sugar content of the grapes; Tsver-sugar content at ripening of the grapes; Tsmax-date on which Ts is Tsmax/2, inflection point of the curve, 1/τ-inflection point of the curve; japv-number of ripening days.

The assignation of the curve for each harvest in a period of time, gave the possibility to determine objectively the similarity between them. The phenolic potential of the black grapes was complexly defined by Glories (2004), which gave information about the content of the grapes in total polyphenols and anthocyannins, the anthocyannins extractibility and the tannins maturity in seeds. The above informations, to which the information related to the technological potential and the basic data of vinification are added, can be used in order to define the style of wine which could be obtained from the grapevine harvest. In the actual context, the maturation of the grapes is represened by the kinetics of the compounds with oenological value, mathematicaly modelated by the t1/2 indicator. The richness of the grapes in such compounds is a basic technological condition in vinification. The evaluation of the phenolic maturation of the grapes gave the possibility to design the style of the red wines and to modelate the vinification tehnologies of grapes.

The objectives of the study were the following:

- Identification the maturation types of the black grapes for wine;
- Determination the technological potential of the grapes at harvest;
- Analysis the phenolic potential of the grapes at harvest;
- Analysis the quantitative potential of the harvest.

MATERIALS AND METHODS

Five vinifera varieties for red Appellation of Origin Dealu Mare wines from the Valea Călugărească assortment were studied. These varieties are established in the National Collection of the Research and Development Institute for Viticulture and Enology, Valea Calugareasca (Table 1).

Table 1. Variety and origin of the tested wines

Code	Cultivar	Location
BM	Burgund mare	National Collection
CS	Cabernet Sauvignon	National Collection
FN	Fetească neagră	National Collection
ME	Merlot	National Collection
PN	Pinot noir	National Collection

The modeling of grapes maturation kinetics was based on the Tsmax. and $t_{1/2}$, parameters according to Dupin S. et al., (2010). Tsmax. represents the maximum content in sugar registered in the period of the grapevine maturation, while $t_{1/2}$ indicator is the day (Julian day) at which the sugar content reaches half from the maximum amount. Phenolic maturity is defined as a moment when seeds tannins and skin anthocyanins concentrations are minimum and maximum, respectively. It was evaluated according to Glories method (Anneraud C., Vinsonneau, 2009), by using the following parameters: Potential of the extractible antocyanins (ApH3,2); total antocyanins potential (ApH1); Total Polyphenol Index (TPI), Percentage of Anthocyanins Extractibility (PAE%), Contribution of grape seeds tanins (MP) and contribution of grape skins polyphenols (MS).

RESULTS AND DISCUSSION

The grapes maturation kinetics

In the 2007-2013 period, the parameter t1/2 varied between 194 and 217 with an average value of 206. The variation interval of this parameter was in good corelation with the grapes maturation kinetics: when its value was very big (210<t1/2<217), the period of maturation was long, when its value was medium (202<t1/2<209) the maturation was normal, and in case of a low value (194<t1/2<201), the period of maturation was short (Table 2).

Table 2. Defining of the grapes maturation kinetics function of t1/2 indicator

Code	Type of the maturation kinetics	Limit of variation for the t1/2 parameter
KM 1	short maturation	194-201
KM 2	normal maturation	202-209
KM 3	long maturation	210-217

The maturation kinetics is in relation with the harvest. Taking into account the 2007-2013 period the black grapes from the Appelation of Origin Dealu Mare Valea Calugareasca had a rapidly maturation in the 2008 and 2010 years, normal in 2007, 2011, 2013 years and slowly one during 2009 and 2012 years. The frequency of the years with normal grapes maturation kinetics was of 57% percent. The variety significantly influenced the t1/2 parameter of grapes maturation kinetics (Figure 1).

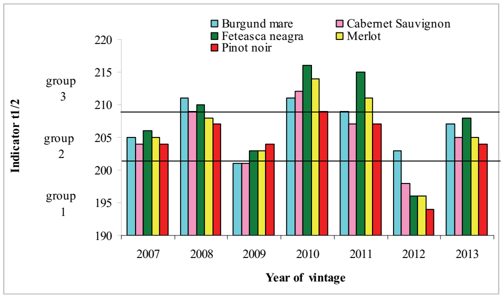

Figure 1. The variation of the t1/2 indicator depending on the variety

The analysis of the primary data conducted to the following: in case of Pinot noir, the t1/2 indicator had an weight of 86% in the group 2, represented by a normal maturation of the grapes at harvest, followed by 'Burgund mare', 'Cabernet Sauvignon' and 'Merlot' varieties with an weight of 57%; a late maturation given by variation of t1/2 parameter in the group 3 was observed with a weight of 43% at 'Feteasca neagra' variety, 29% at 'Burgund mare' and 'Merlot' and 14% at 'Cabernet Sauvignon'.

The technological potential of the grapes at harvest

At the variety level, a strong correlation between the maturation glucoacidimetric index (GI) and the type of the maturation kinetics was identified. In case of 'Merlot', 'Burgund mare' and 'Cabernet Sauvignon' varieties, the value of GI was lower than 45 when the periond of the grapes maturation was short, having values between 45 and 60 in a normal maturation condition and bigger than 60 when the maturation was long (Table 3). In normal maturation conditions, GI ranged between 45 and 55 in case of 'Feteasca neagra' variety and between 40 and 70 for 'Pinor noir' variety.

Table 3. Variation of the glucoacidimetric index function of the vinifera variety and the maturation kinetics

Vinifera variety	Maturation kinetics		
	KM 1	KM 2	KM 3
'Burgund mare'	<45	45-60	>60
'Cabernet Sauvignon'	<45	45-60	>60
'Fetească neagră'	<45	45-55	>55
'Merlot'	≤45	45-60	>60
'Pinot noir'	<40	40-70	>70

At the level of each variety, a strong correlation (R^2= 0,98-0,99) between GI and t1/2 was identifiend (Table 4).

The increase of the t1/2 indicator by one unit has determined the increase of the GI with values beteen 1,12 ('Merlot') and 2,00 ('Burgund mare').

Table 4. Modeling of the relation between the glucoacidimetric index and the maturation kinetics parameter t1/2 at the variety level

Code	Regression equation	Determination coefficient
BM	GI=-359,75+2,0016* t1/2	0,9860
CS	GI=-297,10+1,6962*t1/2	0,9808
FN	GI=-212,45+1,2896*t1/2	0,9900
ME	GI=-174,17+1,1204*t1/2	0,9840
PN	GI=-342,57+1,9649*t1/2	0,9926

The phenolic potential of the grapes maturation at harvest

The phenolic potential of the grapes maturation at harvest is in relationship with the grapes maturation kinetics.

The analysis regarding 'Feteasca neagra' variety showed that in case of a low value of the t1/2 indicator (short maturation) the phenolic potential was maxim registering the following values: the amount of the total polyphenols (TPI) 55 UA, the value of the total anthocyanins (ApH1) 1119 mg/l, the anthocyanins extractibility (PAE) 88,2% (987 mg/l) and the maturity of seeds (MS) 29,1%. The report between the amount of polyphenols in the skin and in the seeds was 2,06.

In 2013 year characterized by a normal grapes maturation kinetics, the phenolic potential had au averages value, the content in polyphenolys (TPI) being 42 UA and in total anthocyanins 835 mg/l

From the total amount of anthocyanins, only 55,7% were extractible anthocyanins (465 mg/l). The maturation registered 54,8%, percent in case of the seeds and 45,2% percent in case of the skins (Figure 2a. and Figure 2b.).

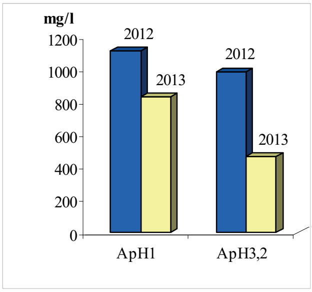

Figure 2a. Influence of the vintage upon the total and extractible anthocyanins content in the grapes, 'Feteasca neagra' variety

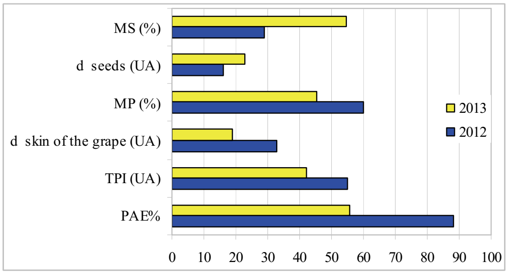

Figure 2b. Influence of the vintage upon the polyphenols and tannins in the grapes, 'Feteasca neagra' variety

Quantitative potential of the harvest

Quantitative potential of the variety is a typical characteristic of the harvest. It is in relation with the average weight of the grape and the number of clusters on the vine (Table 5)

Table 5. Influence of the harvest on the efficiency indicators in case of 'Feteasca negra' variety

Year of vintage	Average weight of the grape (g)	No. of grapes/ vine	Produc-tion (Kg/ vine)	Weight 100 grains (g)
2012	115	29	3,34	121
2013	181	26	4,71	138

The highest production (4,71 kg/vine) was registred at 'Feteasca neagra' variety in normal maturation conditions, typical for the 2013 year.

In these conditions, the cluster had an averege weight of 181g and the berry had an average weight of 1,38 g.

When the maturation was short, specific situation for 2012 year, the average weight of the cluster was lower with 66 g, which determined the decrease of the production (3,34 kg/vine).

The adaptation of the vinification technology to the harvest quality

For the enologist, the winemaking methods and multiple enological practices are available. Their theoretical and practical knowledge concerning the influence of each technological element allow to choose that combination which guarantees an optimal exploatation of the enologic potential of grapes.

In order to increase the enological potential of the wines, the vinification of the grapes which have a good enological maturity, can be achieved by using classical method or the method which involve a partial elimination of the grape free-run juice.

CONCLUSIONS

The maturation kinetics of the black grapes with the Appelation of Origin Dealu Mare Valea Calugareasca was evaluated based on the following parameters: the maximum content of sugar in the grapes (Tsmax), the minimum content the sugar in the grapes (Tsmin) and the date on which the sugar content was half from the maximum value (t1/2).

The black grapes maturation kinetics in relation with the harvest was the following: rapid maturation in 2008 and 2010 years, normal in 2007, 2011, 2013 and slowly in 2009 and 2012 years.

The phenolic potential of the grapes at harvest was influnced by the grapes maturation kinetics; the maximum value of the phenolic potential of the grapes at harvest was registered when the grapes had a rapid maturation.

The grapes production is specific to the harvest. In case of 'Feteasca neagra' variety, the grapes production was 4,71 kg/vine in 2013 year and 3,34 kg/vine in 2012.

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ASSESSMENT OF GENOTYPES AND ELITES OF GRAPE VINES *ON FARM* IDENTIFIED IN DRĂGĂŞANI VINEYARD

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Abstract

The assessment was carried out between the years 2010-2013, in the private plantations from Drăgăşani vineyard, for identification and conservation on farm of genotypes and elite of grape vines discovered. Have been identified, located, studied and evaluated of old grape vines varieties, autochthonous, local or cultivated by over time in the Drăgăşani vineyard. From this study were selected elites that can result valuable clones of varieties identified. The study also aimed at knowing and promoting of germplasm vines existing in Drăgăşani vineyard.

Key words*: elites, genotypes, germplasm, on farm, Vitis vinifera*

INTRODUCTION

On farm conservation is a very important measure for saving of local varieties from Romania vineyards, because comes to support the saving of local varieties in context in which they began to be the endangered and the default of viticultural germplasm.

In Romania, saving of viticultural germplasm *on farm* is very rare, the local varieties began to be very hard to fiind, because of rejuvenation vine plantations with new noble varieties appeared of market.

In situ conservation means the conservation of genetic diversity of traditional cultures in the environment where have developed their distinctive properties in the systems of traditional agricultural (Batîr, 2009). Conservation *in situ*, sometimes referred to as "*on farm* conservation", was defined as "the management and continuous cultivation by farmers of a diverse set of plants populations of a species in the agroecosystems as they have evolved " (Bellon et al., 1997).

Ex situ conservation is an action taken by man, to obtain of artificial biodiversity outside habitat that format. It emerged from the idea that genetic resources can disappear over time for various reasons. Therefore, conservation "*in situ*" must be supplemented by the "*ex situ*" for the safety and obtaining results beneficial with a view salvation of biodiversity (http://lori.academicdirect, 2012).

MATERIAL AND METHOD

The study was carried out during 2010 – 2013 in private plantations from Drăgăşani vineyard at old grape vines varieties and elites discovered, in order to assess the potential of agrobiological *on farm* of the vineyard. In the first stage were located and identified old genotypes of vines, which began to be increasingly less frequent in the Drăgăşani vineyard, with GPS to determine latitude, longitude and altitude.

Have been identified and studied a number of 7109 hubs of vines from these old varieties. Have been identified the region, plantation age, the number of varieties and elites discovered, synonyms and origin of variety, the countries where it is also cultivated, eco-pedological conditions, resistance to diseases and pests, color of the grapes, direction of production, after the technology of description *on farms* under vines.

The genotypes identified and localized as follows: 'Cârlogancă' ('Crâmpoşia'), 'Tămâioasă românească', 'Fetească regală', 'Fetească albă', 'Fetească neagră', 'Coarnă albă', 'Coarnă neagră', 'Coarnă roşie', 'Braghină roze', 'Braghină albă', 'Gordan', 'Gordin', 'Berbecel', 'Negru moale', 'Negru vârtos', 'Slaviţă', 'Moroştină', 'Românie', 'Ţâţa caprei albă', 'Sauvignon gros' 'Teişor', 'Băşicată' (Gorjan, 2013).

RESULTS AND DISCUSSION

In the period 2010 - 2013 were identified old varieties of vines in Drăgăşani vineyard, in many lands under vines in the vineyard, in the precise points at different farmers.
The study respected the technology of description *on farm*, in the one regards old vines varieties, by discussing with the farmers on this issue and conclusying about the importance of romanian germplasm viticole.
Have been identified and studied a number of 7109 hubs of vines from these varieties, resulting in a total of 261 genotypes of perspective for wine grapes and table grapes.
Parallel with the genotypes identified and studied, were studied 22 elites with very good potential agroproductive and technological in a view to selecting of new clones.
The altitudes were identified elites and genotypes of vines are between 145-321 m alt. We can observe that are meeting both in the area meadow as well as the hill, and did not suffered because frost over time, showing that they are genotypes and elites highly resistant.
Locations and the coordinates of elites and genotypes identified *in situ* in the vineyard are presented in tables 1 and 2.
Were studied grape color, age plantations in the *sites* identified. Plantations studied have aged between 40-90 years, hubs are viable and productive, this demonstrating their suitability to the ecological conditions of the vineyard. Those plantations must be conserved *in situ* and *ex situ* in order not to losing a valuable gene pool viticultural. Those genotypes and elites identified presents a yellowish green color, black-blue, dark red, red, rosé, yellowish green/ rosé.
Direction to the production is very good in commercial purpose, for obtaining white wines, red and rosé wines for current consumption, quality and high quality as well as for the production of table grapes with consumption in fresh condition and keeping over the winter (Table 3). (Gorjan, 2013).
The Drăgăşani Vineyard presents a benefic climate for the cultivation of vines namely the temperate continental with mediterranean influence, the soils are reddish brown, clays and regosoils. The viability of varieties and elites discovered it very good surpassing 90% between in the years 2010 and 2011 when not were recorded the negative temperatures, the absolute minimum temperature not exceeding threshold of –16º C. In the year 2012 was registered the frost over the vines with a the absolute minimum of –24 º C, registered in ianuary and february. The vines plantations existing on the valley and in the Olt meadow have suffered massive loss of eyes. The vineyards from the hill or the ones buried, mound from during autumn were mostly saved. So, during the three years studied we have medium viability of varieties and the elites discovered of 80-87%, which demonstrates that they are very resistant to frost.
The main characteristics agrobiological and physiological of genotypes and elites selected are very good, they are presented in table 4 (Gorjan, 2013).

Table 1. The distribution *on farm* of the main old varieties in the Drăgăşani vineyard

Lands under vines	**Variety**	**Point**	**Farmer**
Guşoieni-Spârleni Suteşti-Pietroasa Dobruşa	'Cârlogancă' ('Crâmpoşia')	point Carcadia point Bobocea point Victor	Carcadia Liviu Bobocea Ion Victor
Suteşti-Pietroasa Călina Zăvideni Orleşti Lunca Oltului –Drăgăşani	'Tămâioasă românească'	point Bobocea point Iulia point Roxana point Miu point Măcău	Bobocea Ion Mateescu Iulia Bădescu Roxana Miu Mircea Măcău Ilie
Guşoieni-Ţicleanu Lungeşti Creţeni Orleşti Dealul-Olt Drăgăşani	'Fetească regală'	point Iordache point Monastery point Nuţă point Ilie point Agricultural High School	Iordache Mihail Monastery Lungeşti Nuţă Gheorghe Gorjan Ilie Agricultural High School I.C. Brătianu Drăgăşani
Guşoieni-Ţicleanu Suteşti-Pietroasa	'Fetească albă'	point Iordache point Bobocea	Iordache Mihail Bobocea Ion
Dealul Olt-Drăgăşani Lungeşti	'Fetească neagră'	point Nedeluţ point Burugă	Nedeluţ Mircea Burugă Nicolae
Dealul Olt-Drăgăşani Guşoieni-Spârleni	'Coarnă albă'	point Iordache point Mitică	Iordache Mihail Mitică
Dealul Olt-Drăgăşani Mitrofani	'Coarnă neagră'	point Iordache point Ionela	Iordache Mihail Ionela
Lunca Oltului-Drăgăşani Guşoieni-Spârleni	'Coarnă roşie'	point Măcău punctul Niţu	Măcău Ilie Niţu
Suteşti-Pietroasa Călina	'Braghină roze'	point Bobocea point Mateescu	Bobocea Ion Mateescu Iulia
Suteşti-Pietroasa	'Braghină albă'	point Bobocea	Bobocea Ion
Dealul-Olt Drăgăşani Suteşti-Pietroasa Amărăşti	'Gordan'	point Costel point Bobocea point Ancuţa	Costel Bobocea Ion Ancuţa Maria
Suteşti-Pietroasa	'Gordin'	point Bobocea	Bobocea Ion
Drăgăşani-Momoteşti	'Berbecel'	point Lungu	Lungu Paul
Suteşti-Pietroasa Guşoieni-Ţicleanu	'Negru moale'	point Bobocea point Iordache	Bobocea Ion Iordache Mihail
Suteşti-Pietroasa Guşoieni-Ţicleanu	'Negru vârtos'	point Bobocea point Iordache	Bobocea Ion Iordache Mihail
Suteşti-Pietroasa	'Slaviţă'	point Bobocea	Bobocea Ion
Suteşti-Pietroasa	'Moroştină'	point Bobocea	Bobocea Ion
Suteşti-Pietroasa Drăgăşani-Bârsanu Călina	'Românie'	point Bobocea point Iulia point Mateescu	Bobocea Ion Mateescu Iulia Mateescu Ion
Mitrofani Guşoieni-Spârleni Lungeşti	'Ţâţa caprei albă'	point Ionela point Niţu point Monastery	Ionela Niţu Monastery Lungeşti
Dealul Olt-Drăgăşani	‘Sauvignon gros’	point Sandu	Sandu
Suteşti-Pietroasa	'Teişor'	point Bobocea	Bobocea Ion
Suteşti-Pietroasa	'Băşicată'	point Bobocea	Bobocea Ion

Table 2. Locations and coordinated identified from Drăgăşani vineyard

No.	Designation genotype	The elite code	Locations in the Drăgăşani vineyard	Coordinated
1.	'Cârlogancă' ('Crâmpoşia')	10-20-30	Guşoieni-Spârleni Suteşti-Pietroasa Dobruşa	(44º42'18 N, 24º07'56 E, 224 m alt.) (44º40'49 N, 24º12'09 E, 218 m alt.) (44º37'16 N, 24º12'41 E, 188 m alt.)
2.	'Tămâioasă românească'	1-2-3	Suteşti-Pietroasa Călina Zăvideni Orleşti Dealul Olt-Drăgăşani	(44º40'47 N, 24º12'06 E, 220 m alt.) (44º41'03 N, 24º15'09 E, 180 m alt.) (44º45'29 N, 24º13'31 E, 217m alt.) (44º47'59 N, 24º13'32 E, 190 m alt.) (44º39'48 N, 24º18'40 E, 145 m alt.)
3.	'Fetească regală'	4-1-4	Guşoieni-Ţicleanu Lungeşti Creţeni Orleşti Dealul-Olt Drăgăşani	(44º43'43 N, 24º07'53 E, 315 m alt.) (44º34'33 N, 24º12'19 E, 239 m alt.) (44º40'41 N, 24º11'24 E, 178 m alt.) (44º48'09 N, 24º14'00 E, 223 m alt.) (44º39'55 N, 24º14'55 E, 180 m alt.)
4.	'Fetească albă'	9-2-4	Guşoieni-Ţicleanu Suteşti-Pietroasa	(44º43'41 N 24º07'54 E 312 m alt.) (44º40'44 N 24º12'12 E 208 m alt.)
5.	'Fetească neagră'	4-3-4	Dealul Olt-Drăgăşani Lungeşti	(44º40'58 N 24º14'14 E 282 m alt.) (44º34'24 N 24º12'15 E 197 m alt.)
6.	'Coarnă albă'	30-40-50	Dealul Olt-Drăgăşani Guşoieni-Spârleni	(44º40'24 N 24º14'12 E 290 m alt.) (44º42'17 N 24º07'59 E 207 m alt.)
7.	'Coarnă neagră'	40-30-50	Dealul Olt-Drăgăşani Mitrofani	(44º40'26 N 24º14'13 E 292 m alt.) (44º44'12 N 24º12'17 E 231 m alt.)
8.	'Coarnă roşie'	1-40-50	Lunca Oltului-Drăgăşani Guşoieni-Spârleni	(44º39'49 N 24º18'57 E 146 m alt.) (44º43'46 N 24º07'55 E 321 m alt.)
9.	'Braghină roze'	11-12-13	Suteşti-Pietroasa Călina	(44º40'53 N, 24º12'10 E, 228 m alt.) (44º43'07 N, 24º13'38 E, 298 m alt.)
10.	'Braghină albă'	10-11-12	Suteşti-Pietroasa	(44º40'47 N, 24º12'06 E, 217 m alt.)
11.	'Gordan'	8-6-4	Dealul-Olt Drăgăşani Suteşti-Pietroasa Amărăşti	(44º40'05 N, 24º14'49 E, 208 m alt.) (44º40'49 N, 24º12'09 E, 218 m alt.) (44º46'35 N, 24º08'39 E, 223 m alt.)
12.	'Gordin'	7-5-3	Suteşti-Pietroasa	(44º40'49 N, 24º12'00 E, 195 m alt.)
13.	'Berbecel'	1-3-5	Drăgăşani-Momoteşti	(44º38'50 N, 24º15'50 E, 161 m alt.)
14.	'Negru moale'	3-2-1	Suteşti-Pietroasa Guşoieni-Ţicleanu	(44º40'48 N 24º12'05 E 206 m alt.) (44º43'09 N 24º07'48 E 310 m alt.)
15.	'Negru vârtos'	2-1-1	Suteşti-Pietroasa Guşoieni-Ţicleanu	(44º40'47 N 24º12'07 E 209 m alt.) (44º43'03 N 24º07'48 E 293 m alt.)
16.	'Slaviţă'	6-4-2	Suteşti-Pietroasa	(44º40'46 N 24º12'02 E 198 m alt.)
17.	'Moroştină'	4-3-2	Suteşti-Pietroasa	(44º40'47 N, 24º12'02 E, 196 m alt.)
18.	'Românie'	20-21-22	Suteşti-Pietroasa Drăgăşani-Bârsanu Călina	(44º40'46 N, 24º12'02 E, 197 m alt.) (44º41'03 N, 24º15'07 E, 185 m alt.) (44º43'06 N, 24º13'37 E, 296 m alt.)
19.	'Ţâţa caprei albă'	11-10-11	Mitrofani Guşoieni-Spârleni Lungeşti	(44º44'12 N 24º12'17 E 231 m alt.) (44º42'19 N 24º07'59 E 215 m alt.) (44º34'26 N 24º12'20 E 214 m alt.)
20.	‘Sauvignon gros’	60-70-80	Dealul Olt - Drăgăşani	(44º40'58 N, 24º14'18 E, 283 m alt.)
21.	'Teişor'	5-3-1	Suteşti-Pietroasa	(44º40'50 N 24º12'11 E 222 m alt.)
22.	'Băşicată'	26-27-28	Suteşti-Pietroasa	(44º40'46 N, 24º12'02 E, 196 m alt)

Table 3. The main characteristics of genotypes and elites identified

No.	Designation genotype	The elite code	Color of the grapes	The direction of production	Plantation age
1.	**'Cârlogancă' ('Crâmpoşia')**	10-20-30	yellowish green	- high quality white wines and table grapes for fresh consumption	approximately 90 years
2.	'Tămâioasă românească'	1-2-3	yellowish green	- aromatic wines of high quality	approximately 50-90 years
3.	'Fetească regală'	4-1-4	yellowish green	- high quality white wines	approximately 40 years
4.	'Fetească albă'	9-2-4	yellowish green	- high quality white wines	approximately 60-90 years
5.	'Fetească neagră'	4-3-4	yellowish green	- high quality red wines	approximately 40 years
6.	'Coarnă albă'	30-40-50	yellowish green	- table grapes with fresh consumption and keeping over winter	approximately 50 years
7.	'Coarnă neagră'	40-30-50	dark red	- table grapes with fresh consumption and keeping over winter	approximately 50 years
8.	'Coarnă roşie'	1-40-50	red	- table grapes with fresh consumption and keeping over winter	approximately 40 years
9.	'Braghină roze'	11-12-13	rosé	- white and rosé wines for current consumption	approximately 90 years
10.	'Braghină albă'	10-11-12	yellowish green	- whites wines for current consumption	approximately 90 years
11.	'Gordan'	8-6-4	yellowish green	- whites wines for current consumption	approximately 70-90 years
12.	'Gordin'	7-5-3	yellowish green	- whites wines for current consumption	approximately 90 years
13.	'Berbecel'	1-3-5	yellowish green	- whites wines for current consumption	approximately 90 years
14.	'Negru moale'	3-2-1	black-blue	- quality red wines	approximately 40 years
15.	'Negru vârtos'	2-1-1	black-blue	- quality red wines	approximately 40 years
16.	'Slaviţă'	6-4-2	yellowish green	- quality white wines	approximately 90 years
17.	'Moroştină'	4-3-2	yellowish green	- quality white wines	approximately 90 years
18.	'Românie'	20-21-22	yellowish green/ rosé	- whites wines for current consumption	approximately 50-90 years
19.	'Ţâţa caprei albă'	11-10-11	yellowish green	table grapes with fresh consumption and keeping over winter	approximately 50 years
20.	'Sauvignon gros'	60-70-80	yellowish green	- high quality white wines	approximately 40 years
21.	'Teişor'	5-3-1	yellowish green	- quality white wines	approximately 90 years
22.	'Băşicată'	26-27-28	yellowish green	- whites wines for current consumption	approximately 90 years

Table 4. The main traits agrobiological and physiological of genotypes and elites

No.	Designation genotype	The elite code	Time of maturation	Growth vigor	Resistance to disease, frost, drought *
1.	'Cârlogancă' ('Crâmpoşia')	10-20-30	medium	strong	P,O,B,F,D
2.	'Tămâioasă românească'	1-2-3	medium	strong	P,O,B,F,D
3.	'Fetească regală'	4-1-4	early	strong	P,O,B,F,D
4.	'Fetească albă'	9-2-4	early	strong	P,O,B,F,D
5.	'Fetească neagră'	4-3-4	medium	strong	P,O,B,F,D
6.	'Coarnă albă'	30-40-50	tardive	strong	P,O,B,F,D
7.	'Coarnă neagră'	40-30-50	tardive	strong	P,O,B,F,D
8.	'Coarnă roşie'	1-40-50	tardive	strong	P,O,B,F,D
9.	'Braghină roze'	11-12-13	tardive	medium	P,O,B,F,D
10.	'Braghină albă'	10-11-12	very tardive	medium	P,O,B,F,D
11.	'Gordan'	8-6-4	medium	strong	P,O,B,F,D
12.	'Gordin'	7-5-3	tardive	medium	P,B,F,D
13.	'Berbecel'	1-3-5	medium	medium	P,O,B,F,D
14.	'Negru moale'	3-2-1	medium	strong	P,O,B,F,D
15.	'Negru vârtos'	2-1-1	medium	strong	P,O,B,F,D
16.	'Slaviţă'	6-4-2	medium	strong	P,O,B,F,D
17.	'Moroştină'	4-3-2	medium	strong	P,B,F,D
18.	'Românie'	20-21-22	medium	strong	P,O,B,F,D
19.	'Ţâţa caprei albă'	11-10-11	medium	strong	P,O,B,F,D
20.	'Sauvignon gros'	60-70-80	early	strong	P,O,B,F,D
21.	'Teişor'	5-3-1	medium	medium	P,O,B,F,D
22.	'Băşicată'	26-27-28	tardive	strong	P,O,B,F,D

* P- *Plasmopara*; O-*Oidium*; B-*Botrytis*; F-Frost; D-Drought

CONCLUSIONS

For the first time in the Drăgăşani vineyard was carried out an assessment *on farm* of genetic resources existing in vineyard, purpose being salvation and promoting viticultural germplasm existing.

The conditions eco-pedological of Drăgăşani vineyard offers favorable conditions to cultivate vines, of romanian varieties and foreign through mineral-rich soils and through climate the temperatures not exceeding the critical threshold of -18 ºC, with the exception of year 2012 (-24 º C).

In Drăgăşani vineyard there are valuable genetic resources of vines that can contribute to the restoration and enhancement of the traditional assortment of vineyards.

The resources genetic from Drăgăşani vineyard were identified *in situ*, insisting especially on old varieties of vines, which are endangered, for conservation *on farm* and *ex situ* in collections ampelographic.

From the varieties and elites identified was collected of biological material with a view their multiplication, either to reintroduction *on farm* or to conservation *ex situ* in ampelographic collections from University Craiova and S.C.D.V.V. Drăgăşani.

These varieties and elites selected of vineyard Drăgăşani can modernize and develop fundamental viticulture of Oltenia and implicitly from Romania (Gorjan, 2013).

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THE IDENTIFICATION OF THE CLIMATE PROFILES IN THE VITICULTURAL AREA DOC DEALU MARE-VALEA CALUGAREASCA. THE DATABASE ASSOCIATED TO THE CLIMATIC PROFILE

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Abstract

The climatic profiles is a succint document, easy to understand, that contains the relevant information in a historical climatic context, the present and climate forecast and which specifies the viticultural potential effects of climate change, climatic variability and type of the climate. The study was achieved at ICDVV Valea Calugareasca, using the climatic parameters registered during the years 2004-2013. The objective of the study was to identify the climatic profile of the viticultural area DOC Dealu Mare Valea Calugareasca, which in terms of zoning is an area of the regional level. The viticultural climatic profile was designed with for basic components, one for climatic dormancy period, the second climate for the growing season, the third for the climate during the grapes ripening period (veraison-harvest) and the fourth for climatic risks in relation to the vines. The annual climate and the ripening period climate were defined by using the climatic indices recommended in the specialized literature. The differentiation between climatic types was done through the principal component analyses. The information obtained was conducted in an Excel database. By processing the information have been identified the following five types of climate. The climatic profile is an important viticultural technical document that can be used to predict the climatic changes, the climatic risks associated with the vine culture, establishing the possible adaptation of the viticultural technologies.

Key words: *climatic profile, climat of the growing season, climate of the ripening period, DOC Valea Calugareasca area, climate type*

INTRODUCTION

The annual behavior of vines and hence the quality of the grapes are in direct relationship with the climate vintage. In the last two decades significant progress in knowledge of the associated macro, meso and microclimate have recorded. A special importance had the realization of the Geoviticulture Multicriteria Climatic Classification System (MCC), (Carbonneau, Tonietto, 2000). It is a reference system of world viticulture, based on the using of three climatic indicators: Heliotermal Index, Dryness Index and Cold Night Index. This system also has the terms of comparison with the climate from any of the climate wine-growing regions.

Viticultural climate indicators are specific macroclimate, meso and microclimate. The defining of these indicators gave the opportunity to identify and characterize the types of climate in the main wine-growing regions (Dumas et al., 1997, Jacquet and Morlat, 1997, Tonietto, 1999, Tonietto and Carbonneau, 1998). In our country, were identified the climate types in the system MCC of the main wine-growing regions and the types of climate in the wine-growing region (Tudorache et al. 2013). The type of climate was multicriterial evaluated, by using the climatic indicators proposed by Joly et al. (2010).

Knowledge of the climatic vineyard has scientific and technical implications. The most important implications of climate are in the establishing of the vineyard management practices adapted to the climatic change and in the performing the strategies for making the best wine taking into account the enological potential of grapes. This work aimed to design a datasheet for the climatic of the vintage, to identify the climatic profiles based on a founded methodology for the climatic parameters registered in the 2004-2013 from Dealu Mare - Valea Calugareasca.

MATERIALS AND METHODS

Climate

Climate data used in this study were obtained from a meteorological station during the period 2004-2013 from the automatic station AddVANTAGE A720 only for 2013 and from the manual station for the period 2004-2012. The meteorological station is located in Valea Calugareasca with the following coordinates: 44°59’ N and 26° 1’E an elevation of 210 m. The data were manually collected by manually and consisted of 4 daily records for temperature, precipitation and hours of insolation. The automatic station records were made daily at an interval of 15 minutes. For each day were calculated the following statistical parameters: average, minimum, maximum and the amount.

Indicators of the bioclimate vineyards

These general climate parameters were used in order to derive other variables used in viticulture studies. The vegetation period (April 1-September 30) is characterized by Heliotermal Index (HI), Cold Night Index (FI), Winkler degree-days (GDD), Hours of insolation amount (INS) and the rainfall amount (Pp). For the determinations the rippening period was taken into consideration the conventional specification made by Carbonneau and Tonietto (2002), this can be considered during the maturation period of 30 days before the date of theoretical harvest. In this study it was used as a reference the time at which actuall harvest took place.. The grape ripening period was characterized by the specific indicators of the growing season, which included the following indicators: maximum temperature and thermal amplitude.

The statistical analysis

The statistical analysis of data was done with 2014 XLSTAT program. The fallowing modules were used: Data for the database, Descriptive statistique for the calculations, Principal component analysis (PCA), cluster analysis and Chart for graphic representation.

The climate risc indicators

The climate risc indicators were selected from the literature. They are: frost of winter (-17°C), spring and autumn (-3°C), the maximum temperature during the budburst to veraison and during the ripening period (35°C) and rainfall (>50 mm).

The database

Tha database was the list type and was created in Excel program. Its query was performed using two methods, the filter standard method and an advanced filter for the calculated criteria.

RESULTS AND DISCUSSIONS

The concept of climatic profile

The climatic profile of wine-growing vintage is designed as a technical data sheet, which contains data about the viticultural area (name, the type of weather station and its coordinates), the climate in the dormant period, the climate in the growing season, the climate in the ripenning period and the climatic accidents. Each period is described by monthly climatic parameters, bioclimatic indicators, climatic profile and through a synthetic text. The climatic accidents were evaluated based on the risk indicators.

The climate of the growing period

The climatic vegetative period is specific to the vintage. The integrative climate indicators were interpreted through the principal component analysis (Figure 1). The following climatic types were identified:

- type 1: 2012
- type 2: 2009, 2010, 2011, 2013
- type 3: 2008, 2004, 2006
- type 4: 2007
- type 5: 2005

The potential of the first climate type is maximum, all climatic indicators are in the range with maximum values.

Thermal resources the climate Type 2 are medium (HI between 2310 and 2540), insolation max level (INS>1560 hours), low rainfall and the FI had an average value.

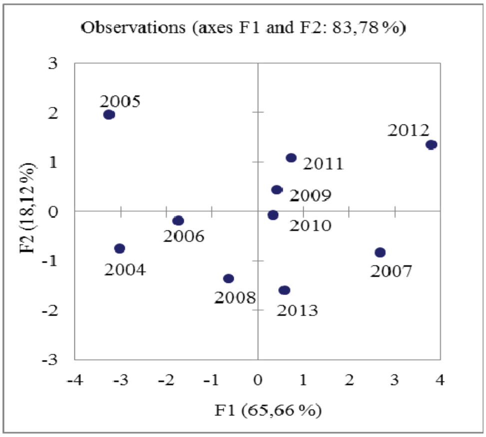


Figure 1. The score of the climate recorded during the growing season of the studied period (2004 to 2013), established by PCA

Climatic indicators of the 3rd type had the minimum level, except in the case of FI that had an average value. The 4th type to the wine climat differs from Type 2 by through insolation, which had a mean value (1410-1560 hours) and the cold night index, which had a low value (FI < 12.9°C). The climate Type 5 is characterized by minimum heliothermic resources and excess of rainfall (Table 1).

Table 1. The climate types during the growing period and their characteristics

The climatic indicator	Type 1	Type 2	Type 3	Type 4	Type 5
HI	>2540	2310-2540	<2310	2310-2540	<2310
Pp	500-660	<500	<500	<500	>660
INS	1410-1560	>1560	<1410	1410-1560	<1410
T° med	>20.6	19.8-20.6	<19.8	19.8-20.6	<19.8
FI	>14.3	12.9-14.3	12.9-14.3	<12.9	12.9-14.3

In terms of the frequency of the types of the climatic years Types (Figure 2), it is found that the frequency of the 2nd was the highest (40%), followed by Types 3 with 30%, while types 1, 4, and 5 showed a frequency of 10%.

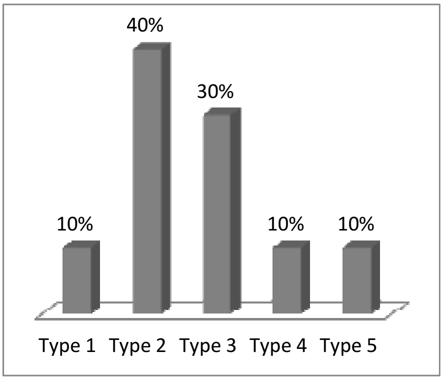


Figure 2. Frequent types of climate/vegetation period in the 2004-2013 period

The climatic profile of the growing season of the vine at the level of wine-growing vintage can be well represented in the form of a radial graph, in which each indicator has a nonparametric value. The transition to the values of climatic indicators to the nonparametric evaluation (score from 1-5) correspond to the specifications in Table 2.

Table 2. Correspondence between the score and the value of the climatic indicators

Score	T° med	HI	FI	Pp	INS
1	<19.48	<2214	12.34	<433 and >727	<1350
2	19.48-19.96	2214-2355	12.34-13.18	433-531 and 629-727	1350-1439
3	19.96-20.44	2355-2495	13.18-14.02	531-629	1439-1527
4	20.44-20.92	2495-2636	14.02-14.86		1527-1616
5	>20.92	>2636	>14.86		1616

The rainfall are evaluated with a score between 1 and 3, because the excess rainfall influence negatively the vegetative cycle of the vine. For example, the climatic profile/the growing period of the years 2008 and 2009 is presented in Figure 3.
The year 2009 had a normal climate. Specific indicators of year, characterized by a higher values than the average, were FI and INS.

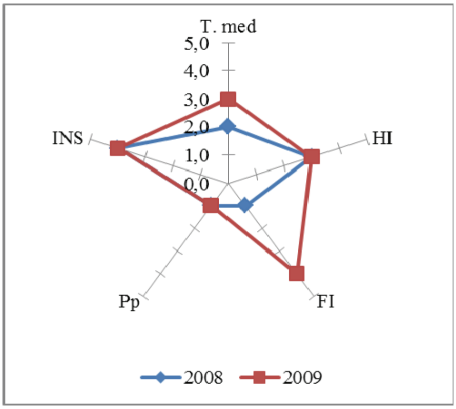


Figure 3. The climatic profil/the vegetation period of the years 2008 and 2009

In terms of hidrological year 2009 was a poor year in rainfall. As favorability, the climate of 2008 was below 2009. This was a year of poor rainfall and low favorability for the grapes maturation (FI = 1).

The climatic profile of the ripening period of the red grapes

The climate types of the grapes ripening period established by principal component analysis are as follows: (Figure 4):

- type 1: 2011, 2012
- type 2: 2009, 2010
- type 3: 2013, 2008, 2004, 2006
- type 4: 2007
- type 5: 2005

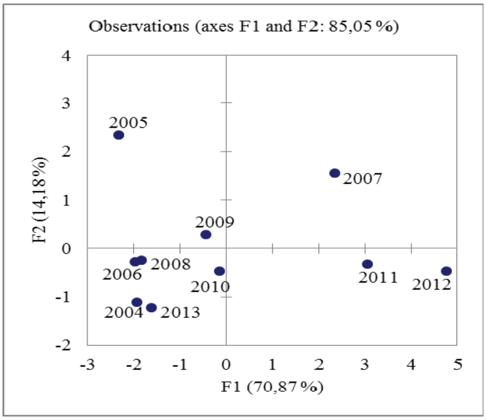


Figure 4. The vintage score by the principal component analysis

Each type of the climate is characterized by specific values of the parameters and climatic indicators. The type 1 represents an exceptional climate for the grape maturation. The thermal resources and cold night index are at the maximum level and the rainfall are below the risc value of the vine.

The type 2 is characterized by an average level of the thermal regime and the cold night index an average level and normal values for rainfall. The climatic characteristics of the type 3 presented values situated below their average values and rainfall is normal. The type 4th different from the first type by an excess of rainfall. The last type is an unfavorable climate for vine, all parameters being at the minimum level, with the exception of rainfall which is at the maximum level (Table 3).

Table 3. The climatic types is the grapes ripening period and their characteristics

The climatic indic.	Type 1	Type 2	Type 3	Type 4	Type 5
HI	>400	<400	<400	>400	<400
Pp	<65	<65	<65	>65	>65
INS	>250	>250	<250	>250	<200
T° med	>20	<20	<20	>20	<20
FI	>15	14-15	<14	>15	<14

The climatic profile in the grapes ripening period is similar with the climate of the growing period.

Comparing climate in the growing period with that in the ripening period

The climate in grape ripening period has similar characteristics with as the climate in the growing period in 2012, 2009, 2010, 2004, 2006, 2008 and 2005 (Figure 5). Thus, in the year 2011, although the climate in the growing season has been framed in type 2, the climate of the ripening period was exceptional of level (type 1). 2007 year was classified in type 2 as well as the climate in the growing season, but the rainfall has similar characteristics with that classified in the type 4. In 2013, the climate of the growing season was framed in level 4 and the ripening period in level 3.

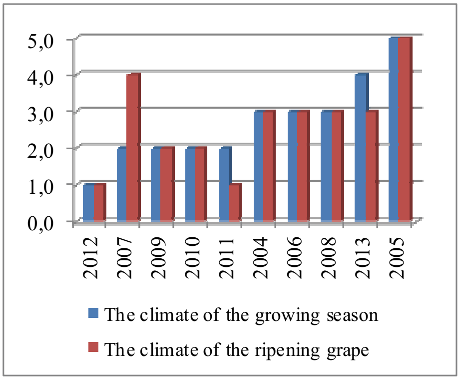


Figure 5. The specificity of the growing season climate type and the ripening in relation to vineyard milesima

The frequency of the years with the same type of climate during the growing season and the ripening period was of 70%.

The database associated to the climatic profile of wine-growing vintage

There had been two list for the database, one for the wine climate and another for the climatic risks. The daily climate list had the following fields: the year, the day and the month, hours of insolation, medium, minimum and maximum temperature and rainfall (Figure 6).

1	Anul	Ziua luna	Insolatia reala	Temp. med. aer °C	Temp. max. aer °C	Temp. min. aer °C	Precipitatii
2	2004	1-ian.	0,0	5,0	5,2	5,0	4,0
3	2004	2-ian.	0,0	4,1	4,2	4,0	5,7
4	2004	3-ian.	0,0	-1,3	0,2	-2,3	3,9
5	2004	4-ian.	0,0	-4,8	-4,3	-5,6	1,0
6	2004	5-ian.	0,0	-7,1	-6,5	-8,4	4,4
7	2004	6-ian.	0,0	-8,3	-6,5	-10,0	0,8
8	2004	7-ian.	0,4	-4,8	-3,6	-10,6	0,0
9	2004	8-ian.	2,2	-5,0	-3,2	-8,2	0,0
10	2004	9-ian.	6,8	-5,0	-2,0	-6,6	0,0
11	2004	10-ian.	7,5	-4,4	0,8	-9,0	0,0
12	2004	11-ian.	0,0	-2,5	1,0	-8,6	0,0
13	2004	12-ian.	4,2	-2,3	0,0	-3,8	0,0
14	2004	13-ian.	0,0	-1,0	0,5	-4,6	0,0
15	2004	14-ian.	1,8	2,1	3,6	-0,6	7,0
16	2004	15-ian.	0,0	2,1	4,2	0,5	11,9
17	2004	16-ian.	2,4	2,7	4,4	0,5	2,6
18	2004	17-ian.	6,1	1,3	5,7	-4,6	0,0
19	2004	18-ian.	4,5	4,1	9,4	-3,2	0,0
20	2004	19-ian.	0,0	2,1	4,2	0,6	0,0
21	2004	20-ian.	0,0	1,9	3,6	0,0	2,2

Figure 6. The structure of the daily climate list

Query parameters and the climatic indicators were achieved by using the data form, ilustrated for the grapes ripening climate (Figure 7).

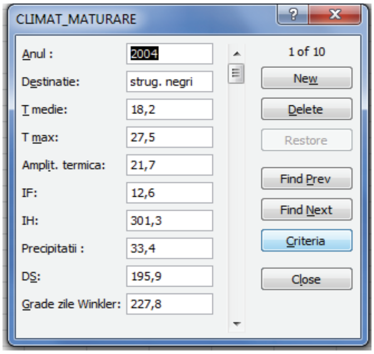
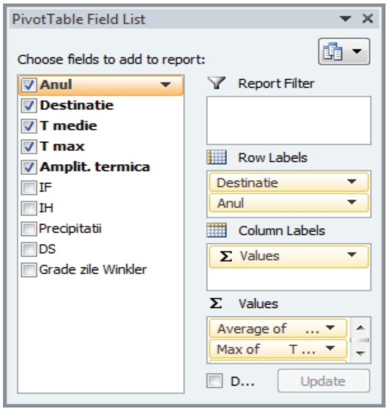


Figure 7. The query to view the ripening period climate

The analysis in detail and the comparison of the climatic data were achieved through the Pivot Table report which represent an interactive way to summarize the data. In the Pivot table (Figure 8), it is easily seen how to report the climatic parameters of medium temperature, maximum temperature and thermal amplitude during 2004-2013, according to the destination of the production.



Destinatie	Average of T medie	Max of T max	Average of Amplit. Termica
strug. negri	19,7	37,2	23,1
2004	18,2	27,5	21,7
2005	18,7	22,6	19,6
2006	18,7	22,4	18,3
2007	21,6	37,2	25,6
2008	18,6	22,2	26
2009	19,8	23,9	21,8
2010	19,8	23,4	25,2
2011	22,3	27	22,6
2012	21,9	29,4	28,7
2013	17,7	23,2	21,3
Grand Total	19,7	37,2	23,1

Figure 8. The query of the data through Pivot table

The reports were made by the model VBA Project. As an example was generated the climatic profile report of the ripening period of the year 2004 (Figure 9).

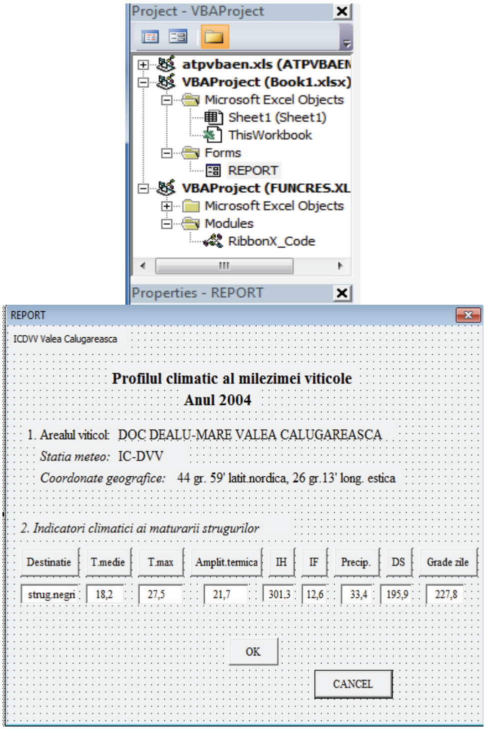


Figure 9. The climatic profile report of the ripening period of the year 2004

CONCLUSIONS

A file was conceived for the description and the identification of the vintage climatic profile, in which the summary information regarding the climate of the dormancy period, the growing season, the ripening period and the climatic risks are shown.

5 types of the viticultural climate, evaluated on numerical scales from 1 to 5, during the years 2004-2013 were identified

The frequency of the climatic types in the growing season was the following: 10% type 1, 40% type 2, 30% type 3, 10% type 4 and 10% type 5.

The climatic type in the ripening period was similar to the climate in the growing season for 7 years (2012, 2009, 2010, 2004, 2006, 2008 and 2005) and different for 3 years (2011, 2007, 2013).

The climatic profile is presented in the form of a radar type chart.

The information concerning wine climate of the period 2007-2013 was organized in a database of Excel type and was processed by queries and specific reports.

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INFLUENCE OF SOIL MAINTENANCE SYSTEMS AND FRUIT LOAD ON GRAPES QUALITY UNDER DROUGHT CONDITIONS

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Abstract

In order to diminish the disturbing effect of climatic changes in viticulture there were studied two agrotechnical factors which have an important impact on water regime in soil and on vine growth, respectively the soil maintenance system and fruit load. In comparison with black furrow (considered as control) there were experimented the total mulching with straws, the partial mulching (between the rows) with marc compost and minimum tillage. To reduce the negative impact of drought conditions in case of each soil maintenance systems a reduction of normal fruit load with 20 and 40% was also experimented. The experimental data obtained reveled that soil maintenance systems by mulching had a positive effect especially on the technological features of the grapes, assuring higher values for grain composition index and for efficiency index, that is a higher must content in grapes and a higher winemaking efficiency. The chemical properties of the grapes (sugar content, acidity, glucoacidimetric index) were not influenced in an obvious manner. Opposite, the reduction of fruit load determined in case of all soil maintenance systems an increase of sugar content in grapes, parallel with an evident decrease of must acidity, determining high values of the glucoacidimetric index, unfavorable for obtaining qualitative and typical wines. The changes induced in grapes quality by the two experimental factors have been reflected also in the wines quality (alchoolic degree, total acidity, unreducing extract). Ranking the wines obtained from all the experimental variants (combinations between the two experimental factors) by using the synthetic index of wines quality (Ntaj) one could notice that the soil maintenance systems by mulching and the fruit load of 15-18 buds/m^2 had a positive effect on wines quality under drought conditions.

Key words: *grapevine, climatic changes, technological solutions, grape quality*

INTRODUCTION

The climatic studies undertaken in the last two decades in our country have shown a tendency of climatic changes in most viticultural regions, put into evidence by the increase of thermal regime and by a deficient and generally unfavorable distribution of the rainfalls, leading to the appearance of severe soil drought conditions, especially during the growing season of the vine (Busuioc et al., 2004). Although the vine is considered a resistant plant to drought conditions, this phenomenon can affect in an obvious manner the vegetative development of the vine, the production potential and especially the qualitative potential of the grapes (Bindi et al., 2001; Tate, 2001; Jones et al., 2005; Dejeu et al., 2007). To diminish this disturbing effect of the climatic changes in the last years there were experimented new agrotechnical solutions in order to preserve the water in soil and to reduce the vine transpiration. These agrotechnical solutions were focused especially on the soil maintenance system and on the rationalization of fruit load (Şerdinescu et al., 2013). The aim of our study was to find in which manner the use of different soil maintenance systems and different fruit loads can influence the yield quality under drought conditions.

MATERIALS AND METHODS

The researches were conducted during two years (2012 and 2013) in Valea Călugărească viticultural center, in an experimental plot with Fetească regală/SO4 located on hills in the conditions of a molic reddish-brown soil. There were studied two agrotechnical factors which have an important impact on water regime in soil and on vine growth and productivity. One of them was the soil maintenance system, with the following variants: black furrow (considered as control), total mulching with straws (in a layer of 10 cm), partial mulching (interval between rows) with marc compost (in a layer of 10 cm) and minimum tillage. The second factor was the fruit load. For each soil maintenance system three different fruit loads were experimented, respectively: 18 buds/m^2 (considered as normal fruit load), 15 and 12 buds/m^2 (a reduction with 20 and 40% of the normal fruit load). The investigations were carried out using a bifactorial split plot experiment with 12 variants representing the combinations between the two experimental factors. All the variants received during the experimental period the same amounts of fertilizers and the same plant protection treatments. In order to determine the effect of the two experimental factors on grapes quality there were determined the following parameters: the weight and volume of 100 berries, sugar content, acidity, glucoacidimetric index (GAI) and the technological features (by using the data obtained from the mechanical analysis). It was also investigated the effect of the changes in grapes quality on wines quality, respectively on the alcohol degree, total acidity and unreducing extract. Based on these parameters it was calculated the synthetic index of wine quality (Ntaj), in order to rank the experimental variants (combinations between the two experimental factors).

RESULTS AND DISCUSSIONS

The climate during the experimental period was characterized by an excessive thermal regime and by a deficient and generally unfavorable distribution of precipitations, especially during the growing season of the vine (Table 1).

Table 1. Climatic conditions during the experimental period

Climatic parameters	Multiannual average value (1936-2012)	2012	2013
Sum of active temperatures (°C)	3645	4502	4063
Sum of useful temperatures (°C)	1738	2282	1883
Mean temperature of the air (°C)	11.2	12.3	11.9
Precipitations (mm)	612.5	665.9	661.3
Sunshine duration (hours)	2017	2520	2255

As compared with the multiannual average values, the mean temperature of the air, the sum of active temperatures and the sunshine duration presented higher values. The annual precipitations were in normal limits, but during the growing season of the vine there were some intervals (shorter or longer) with very low values of precipitations which induced severe soil drought conditions. Along with the climatic conditions, the experimented soil maintenance systems influenced in an obvious manner the evolution of soil moisture during the growing season of the vine, having a strong impact on grapevine productivity and on the quality of the grapes. Analysing the evolution of soil moisture on the depth of 0-60 cm during the two years of experimentation we can notice that at the beginning of the growing season (in April and May) the soil moisture was at a normal level, but after that period decreased constantly until the end of the growing season, reaching values under the value of Pmin (50% from active soil moisture interval), closed to the limit of wither coefficient (CO), especially in August and September (Figure 1).

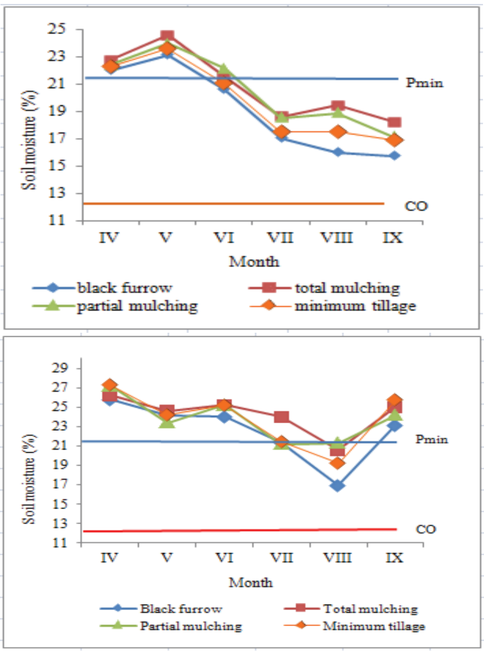

Figure 1. Influence of soil maintenance systems on soil moisture during the growing season of the vine (values for 2012 and 2013)

In comparison with the black furrow the soil maintenance systems by mulching ensured higher values of soil moisture, as a result of reducing water evaporation at the soil surface. The changes occurred in the soil water regime had an obvious effect on grapes quality.

Thus, we can observe that soil maintenance systems by mulching had a positive influence on the weight and volume of 100 berries and on must acidity, determining optimum values for the glucoacidimetric index (Table 2).

Table 2. Influence of soil maintenance systems on grapes quality (average values for the three fruit loads)

Soil maintenance system	Weight of 100 berries g	Volume of 100 berries cc	Sugars g/l	Acidity g/l H_2SO_4	GAI
Black furrow	194.0	177.0	188.7	3.43	55.0
Total mulching	177.9	161.7	178.3	3.96	45.0
Partial mulching	204.0	186.7	182.7	3.86	47.3
Minimum tillage	197.3	179.5	182.4	3.40	53.6

More evident was the effect of soil maintenance systems on the technological features of the grapes.

The experimental data obtained shown an obvious increase of the values of grain composition index and of the efficiency index in case of using the soil maintenance systems by mulching (total or partial), suggesting that in case of severe drought conditions these systems can ensure a higher must content in grapes and as a consequence a high winemaking efficiency (Table 3).

Table 3. Influence of soil maintenance systems on the technological features of the grapes (average values for the three fruit loads)

Soil maintenance system	Technological indices			
	Grape comp. index	Grain index	Grain comp. index	Efficiency index
Black furrow	41.69	67.13	7.94	5.22
Total mulching	35.79	63.18	10.85	6.41
Partial mulching	30.86	62.86	10.02	6.06
Minimum tillage	33.50	61.79	9.60	5.64

Concerning the influence of the reduction of fruit load (with 20 and 40%) on the grapes quality we can notice an increase of the weight and volume of 100 berries, of sugar content and a decrease of must acidity, parallel with the decrease of fruit load from 18 to 12 buds/m^2 (Table 4).

Table 4. Influence of fruit load on the grape quality (average values for the four soil maintenance systems)

Fruit load	Weight of 100 berries g	Volume of 100 berries cc	Sugars g/l	Acidity g/l H_2SO_4	GAI
18 buds/m^2	190.5	172.5	175.7	3.73	47.1
15 buds/m^2	191.5	174.3	184.6	3.57	51.7
12 buds/m^2	198.0	181.9	188.8	3.49	54.1

In case of the technological features of the grapes the experimental data didn't allow to formulate a pertinent conclusion.

However, we can notice that the reduction of fruit load led to a slow decrease of the values of the grain composition index and of the efficiency index (Table 5).

Table 5. Influence of fruit load on the technological features of the grapes (average values for the four soil maintenance systems)

Fruit load	Technological indices			
	Grape comp. index	Grain index	Grain comp. index	Efficiency index
18 buds/m^2	35.90	66.26	10.17	6.12
15 buds/m^2	35.22	66.46	9.90	5.93
12 buds/m^2	35.26	58.51	8.74	5.45

As a consequence of these changes in grapes quality the wines quality was also influenced.

Thus, high values for the alcoholic degree and for unreducing extract were obtained in case of using the partial mulching with marc compost and black furrow as soil maintenance systems (Table 6).

The reduction of fruit load determined, as a consequence of the increase of sugar content in grapes, an increase of the alcoholic degree and of unreducing extract of wines, but also a decrease of the total acidity, conducting to the obtaining of unequilibrated wines (Table 7).

Table 6. Influence of soil maintenance systems on wines quality (average values for the three fruit loads)

Soil maintenance system	Alchoolic degree % vol	Total acidity g/l	Unreducing extract g/l
Black furrow	12.04	6.52	19.44
Total mulching	11.69	6.11	19.41
Partial mulching	12.27	5.68	19.66
Minimum tillage	11.50	6.06	18.59

Table 7. Influence of fruit load on the wines quality (average values of the four soil maintenance systems)

Fruit load	Alchoolic degree % vol	Total acidity g/l	Unreducing extract g/l
18 buds/m^2	11.79	6.15	18.49
15 buds/m^2	11.83	6.05	19.21
12 buds/m^2	12.11	5.89	20.13

Using the synthetic index of wines quality (Ntaj), which allow an interpretation of the general composition of the wines, we could do a classification of the wines obtained in case of the 12 experimental variants (combinations between the two experimental factors). Depending on the values of this index the wines were grouped in three classes: high quality wines, medium quality wines and common wines.

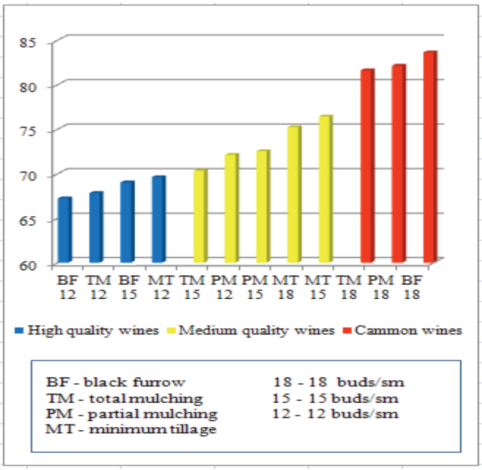

Figure 2. Classification of the experimental variants using the synthetic index of wine quality (Ntaj)

One can notice that in the first class, which include the high quality wines, are presented the wines obtained from the variants with mulching soil maintenance systems and a fruit load of 18 buds/m^2.

CONCLUSIONS

Under severe drought conditions the soil maintenance system applied and the amount of fruit load have an obvious influence on grapes quality and, as a consequence, also on the quality and typicality of the obtained wines.

The soil maintenance systems by mulching (total or partial) had a positive effect especially on the technological features of the grapes, suggesting that in case of a severe drought stress these systems can ensure a higher must in grapes and a high winemaking efficiency.

The reduction of fruit load (with 20 and 40%) determined in case of all soil maintenance systems an increase of sugar content in grapes, parallel with a decrease of must acidity, determining high values of glucoacidimetric index, unfavourable for obtaining typical and qualitative wines. For this reason, this agrotechnical operation is indicated to be used only in the conditions of an excessive soil drought, which can affect in an irreversible manner the vegetative development of the vine.

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EVALUATION OF QUALITATIVE PARAMETERS OF TABLE GRAPES VARIETIES IN ECOPEDOCLIMATIC CONDITIONS OF THE EXPERIMENTAL FIELD U.S.A.M.V. BUCHAREST

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Abstract

In the last years, there has been a higher and higher growth of consumers' interest towards the nutritive value of fruits and vegetables, in the context of a correct alimentation, which has a major impact on the health state of the human organism. Within the healthy alimentation framework, grapes represent genuine sources of micronutrients with antioxidant effect, due to their high content of whole polyphenols, flavonoids, and anthocians with a varying qualitative and quantitative distribution in the grape. The purpose of this study is to assess and compare the quantity of these beneficial substances, accumulated in the skins and the seeds, upon full maturation, for the most grown and known table grapes varieties in the varietals conveyor in Romania. From the achieved results, as expected, the total phenolic content of the red grape is higher when compared to white grape.

Key words: *flavonoids, grape table, phenolic compounds, maturity, varieties*

INTRODUCTION

The crop quality at the grape vine mainly depends on its metabolites. The diversity of grape vine varieties is greatly due to secondary metabolites. A large range of specific chemical compounds represents these and they form part of different groups, such as phenols, terpenes, antibiotics, volatile oils, resins, glycosides, sterols, alkaloids, saponins of which many proved to be very valuable for the pharmaceuticals, agrochemical, food and cosmetics (Zhang et al., 2001). Phenols are a large and complex group of secondary metabolites, which contributes especially to the features of the red grapes and vines. Phenolic compounds are known especially for their contribution to the pigmentation of different parts of the plant, but also due to the role, they play in the plant's resistance to biotic and antibiotic stress. In addition, they have an important role in determining the food quality of grapes. Phenolic composition of grapes depends not only on their degree of ripening but also on the growth parameters of the grape vine such as soil and weather conditions, together with the applied viticultural agrotechnics (pruning, fertilisation or irrigation). In the recent years, consumer's interest has grown more and more on the nutritive value of fruits and vegetable, in the context of the right feeding which has a major impact upon the health condition of the human body. Grapes represent real sources of nutrients with antioxidative effect, these compounds having a diverse spread in the different parts of its berries. Important representatives of phenols, flavonoids can be found, in the skin and seeds of the grapes berry, having a wide spectrum of pharmaceutical, antiallergenic, anticancerigenic, properties etc. Anthocyanins, as red pigments, can be found in the skin of berries (in few cases also in their pulp), as red-coloured heterosides (at low values of pH) or blue (at high values of pH) and they are represented mostly by malvidin, delphinidin, peonidin and petunidin. Anthocyanins gathering in grapes starts at the beginning of the ripening stage, but the content in anthocyanin pigments occasionally drops to the end of the ripening, especially in the areas with warm climate (Fournand et al., 2006). The regular consumption of fruits, vegetables, wine, jam, jellies with a high content of anthocyanins is associated with the decrease of risk of developing chronic diseases, such as cancer, cardiovascular diseases, Alzheimer's diseases (Kroon, 2005). Flavonoids, together with other substances assimilated from the daily diet, such as vitamin C, vitamin E and carotenoids, protect the tissues of the human body against "oxidative stresses" by their action upon free radicals often associated with cancer, cardio-vascular diseases and inflammations (Manach et al., 2004, Di Lorenzo et al., Milella et al., 2013). Their beneficial effects upon health depend greatly on the administrated quantity and on their bio-disponibility. It is thereof obvious how important is studies thoroughness on the identification, evaluation and quantification of these compounds with therapeutic role on one hand and energy suppliers on the other hand, in the main varieties of table grapes grown in Romania.

The phenolic composition of grapes becomes a useful parameter for the selection of the varieties, which should take into account their natural nutraceutical qualities., apart from the grapes appearance (size, colour, berry shape), related to marketing (sales). Generally, in the worldwide viticulture practice, to determine the ideal moment of the table grapes consumption, are used parameters as sugar concentration (g/l) and total acidity (g/l tartaric acid) as well as the implicitly gluco-acidometric index in which the evolution of the first two parameters, during grapes ripening, being in inverted correlation. Despite all that, these quality parameters seem to be insufficient, in approaching the quality term used in the case of varieties of table grapes, since their nutritional and therapeutic quality is also given by the concentration of phenolic compounds (over 500 compounds in *Vitis vinifera*). The phenolic content of the grape berry is distributed as follows: 1% in solid pressed pulp; 5% in juice; 50% in the skin of red grapes or 25% in the skin of white grapes; and the remaining 46 to 69% in seeds. As result, the determination of the content in phenolic compounds (polyphenols, flavonoids, anthocyanins) gets a significant importance and it is useful in the study of table grapes varieties, offering clues about reaching full ripeness as well as about their nutritional value.

MATERIALS AND METHODS

Plant material.
In the present paper, there have been analysed 6 genetically related table grape varieties, grown in Romania. 'Bicane' variety has been used as common maternal genitor for varieties in Italia, 'Xenia' and 'Tamina'; 'Muscat Hamburg' variety for the same varieties represented the paternal form; 'Muscat d'Adda' variety has been obtained by self-pollination of 'Muscat Hamburg' variety. The study has been approached starting from two reasons: for the compositional rating and evaluation of the above-mentioned compounds and for studying their conveyance to descendants. Grape vine varieties are located in the experimental field of the ampelographic collection from the University of Agronomic Sciences and Veterinary Medicine of Bucharest. They have been conducted on the semi-stalk; the type of pruning in the prior year was Guyot on semi-stem, with a load of 42 buds/vine. During the developing of the seasonal phenological stages, there were performed measurements and ratings to evaluate the fertility and productivity elements, and on the date of harvesting, on an average sample of 10 harvested grapes from 10 shoots. There were made physical-carpometric and chemical ratings: fertility indexes, the number of grapes per vine, the average weights of one grape, the average weight of 100 berries, production/vine, sugar (g/l), total acidity (g/l tartaric acid), gluco-acidometric index, as well as the content of total polyphenols, flavonoids, anthocyanins, present in the skin and in the seeds. The harvesting of the grapes *samples* were performed upon their technological maturity.

Preparation of grape skin and seed extracts. After harvest, the grapes samples were processed immediately, separating skin, pulp and seeds from 10 grapes berry / replicate and the phenolic compounds were obtained with an ethanol: water: hydrochloric acid as extractant (70: 29: 1, v/v/v), 20 ml per sample. The extracts were centrifuged for 20 min at 6000 rpm, using a centrifuge EBA 20, stored in a refrigerator (4°C) and analyzed in the short-term from the extraction.

Total phenolic assey

The determination of the total phenolic content was made using the Folin-Ciocâlteu method, (Singleton and Rossi, 1965). In brief, an aliquot (0.5 ml) of the appropriate diluted wine was added to a 10 ml volumetric flask, containing 2.5 ml of distilled water. Then, 0.5 ml of Folin-Ciocâlteu reagent was added and the contents mixed. After 3 min, was added 2 ml Na_2CO_3 solution of concentration 10 g/l and made up to a total volume of 10 ml distilled water. After keeping the samples at 50°C (water bath) for 16 min in sealed flasks and subsequent cooling, their absorbance were read at 700 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (0÷100 mg/l). The concentration of total phenolic is expressed as the gallic acid equivalent per litter of extract. All samples were prepared in triplicate.

Total flavonoids assay. Total flavonoids content was evaluated according to a colorimetric assay with aluminium chloride. For the determination of the total flavonoids, a colorimetric method using $AlCl_3$ was applied for the analysis of the fruit pulp and skin extracts (Zhishen et al., 1999). This method is based on the formation of stable complexes with the C-4 keto group and either the C-3 or the C-5 hydroxyl group of flavones and flavonols, which exhibit maximum absorbance at 510 nm. A 1 ml aliquot of wine (appropriately diluted) was added to a 10 ml volumetric flask containing 4 ml of distilled water, followed by the addition of 0.3 ml of solution of $NaNO_2$ (0.5 g/l). After 5 min, 0.3 ml of a 1 g/l solution of $AlCl_3$ was added and 6 min later, 2 ml of NaOH (1 mol/l) was added to the mixture. The total volume was made up to 10 ml with distilled water, the solution was mixed and the absorbance was measured at 510 nm against water blank. Catechin was used as the standard for the construction of a calibration curve and the concentrations are expressed as catechin equivalents (mg/l).

Total anthocyanins assay. The samples were diluted with a solution consisting of 70/29/1 (v/v/v) ethanol/water/HCl (concentrated) and the absorbance was measured at 540 nm. Due to the lack of a malvidin-3-glucoside standard, the total anthocyanins contents are expressed as malvidin-3-glucoside equivalents and calculated using the following equation purposed by Di Stefano and Cravero, 1991

TA (mg/L) = A540 nm x 16.7x d

Where A540 nm is the absorbance at 540 nm and d is the dilution.

Total acidity and ***sugar*** content were made according to the official methods of O.I.V. (1990).

RESULTS AND DISCUSSIONS

In Tables 1, 2 and 3 are presented obtained results with the most representative Romanian table grape varieties in the agro-climatic conditions of the 2013 year. Assigning the same load of buds per grapevine to all six varieties taken to study shows a differential behaviour of these, with regard to yield quality and quantity.

To facilitate the evaluation of the achieved results, the data interpretation was made taking as point of reference the 'Muscat Hamburg' variety (Fig. 1). From the presented data (Table 1), there can be noticed that the control variety 'Muscat Hamburg' achieved, for a load of 42 buds/vine, the highest values of the fertility indexes, this having as direct result the obtaining of a greater number of grapes per vine (32 bunch) as compared to the other varieties. In opposition, somewhat expected, carpometric parameters have had the lowest values (Table 2).

Figure 1. 'Muscat Hamburg' variety

Thus, the reference variety recorded more reduced sizes of berries and bunch as compared to the other varieties (2.75g – average weight of a berry, 220g – average weight of a bunch as compared to the 'Tamina' variety (Fig. 3). which recorded 6.2g – average weight of a berry and respectively, 460g – average weights of a bunch). These values are reflected in the yield per hectare, relatively low production for a table grape variety. At maturity, the reference variety 'Muscat Hamburg' gathers an impressive amount of sugar, together with an acidity which confers to it a balanced gluco-acidometric index, content which surpasses by far the normal limits of the variety group which it forms part of (Table 3). With regard to the analysed parameters, the other varieties behave differently, recording fertility indexes with low values.

A low number of grapes per grapevine, but an average weight of 100 berries and of a cluster obviously higher as compared to those recorded at the reference variety so that, these varieties have entailed better productions, even providing a surplus of 4 kg/grapevine (for instance, 'Tamina' variety).

With regard to the amount of gathered sugar, it was noticed that only the varieties 'Muscat d'Adda' with 214 g/kg (Fig. 2) and 'Xenia' (Fig. 5), 188 g/kg of grapes are closer to the performance reached by the control variety – 'Muscat Hamburg' (228g /kg of grapes).

The table grape varieties can be harvested before the full maturity, practically at the consumption maturity, based on the gluco-acidometric index. Usually, this index has values between 2.5÷4.5. For the tested varieties, it was between 2.88 and 6.88, the highest values being recorded for the varieties 'Muscat Hamburg' and 'Muscat d'Adda' (6.83; 6.88 respectively). Although the varieties 'Italia', 'Bicane', 'Xenia', 'Tamina' usually reach their full ripeness later than the reference variety, since they belong to the V^{th}-VI^{th} maturity ages, in the conditions of the 2013 year they have reached the optimum level of harvesting simultaneously with the variety 'Muscat Hamburg' (September 12^{th}, IV^{th} age of maturity).

The above-analysed qualitative parameters seem to be insufficient in approaching the quality term used in the case of table grape varieties, since their nutraceutical feature is due to the phenolic potential gathered both in the skin and in the grape berries. The level of gathering of phenols is different depending upon the genotypes, culture range, (soil, climatology), fertilisation, eye load left on the grapevine and, in general, upon the applied technology.

Figure 2. 'Muscat d'Adda' variety

The analysis of the recorded data (Table 3) has indicated a higher phenolic potential of the varieties with red grapes. 'Muscat Hamburg' (intense red to black), 'Muscat d'Adda' (very dark red) and 'Tamina' (red-violet) - as compared to the varieties of which berries are coloured in green-yellow ('Xenia'), golden yellow ('Bicane') and green yellow grapes – 'Italia', (Figure 4, 6).

Figure 3. 'Tamina' variety

Thus, the content of total polyphenols in the skin was framed between 2.074 g of gallic acid/kg of fresh weight-fw (the variety 'Muscat d'Adda') and 0.283 g of gallic acid/kg of fw, ('Bicane' variety).

It was noticed the fact that 'Xenia', a white grapes variety, gathered in the skin a considerable amount of polyphenols, higher to that recorded for the maternal variety, 'Bicane', and also, as compared to the reference variety, 'Muscat Hamburg' (paternal variety). The total content of flavonoid compounds in the skin recorded values between 0.698 g catechin/kg of fw ('Tamina') and 1.27 g catechin/kg of fw ('Muscat d'Adda') - varieties with the skin coloured in different intensities of red - and between 0.166 g catechin/kg of fw ('Xenia') and 0.585 g catechin/kg of fw ('Italia') - varieties with yellow grapes.

Table 1. Productivity parameters of the studied grapevine varieties over the vegetative 2012-2013 year

Experimental variants and specification	Absolute fertility coefficient	% Dif. compared to the control	Relative fertility coefficient	% Dif. compared to the control	No. of grapes/ vine	% Dif. compared to the control	Average weight of a grape (g)	% Dif. compared to the control	Production (kg /vine)	% Dif. compared to the control
Italia	0,92	-0.18	1,09	-0.19	23	-9	410	190	9,43	2.39
Bicane	0,81	-0.29	1,08	-0.2	27	-5	270	50	6,75	-0.29
Xenia	1,19	0.09	1,4	0.12	31	-1	265	45	8,21	1.17
Tamina	1,04	-0.06	1,26	-0.02	24	-8	460	240	11,04	4.00
Muscat Hamburg (control)	***1,10***	-	***1,28***	-	***32***	-	***220***	-	***7,04***	-
Muscat d'Adda	1,00	- 0.1	1,14	-0.14	31	-1	300	80	9,03	1.99

Table 2. Carpometric indexes of the studied table grapevine varieties

Experimental variants and specification	Weight of 100 berries (g)	% Dif. compared to the control	Weight of 10 berries (g)	% Dif. compared to the control	Weight of 10 skins (g)	% Dif. compared to the control	No. seeds in to 10 berries	% Dif. compared to the control	Weight of seeds (g)	% Dif. compared to the control
Italia	450	175	50	7	9,91	3.5	22	-1	1,038	0.485
Bicane	520	245	61	18	11,27	4.86	19	-4	1,272	0.719
Xenia	420	145	42	-1	6,96	0.55	16	-7	0,881	0.328
Tamina	620	345	62	19	8,12	1.71	17	-6	1,075	0.622
Muscat Hamburg (control)	***275***	-	***43***	-	***6,41***	-	***23***	-	***0,553***	-
Muscat d'Adda	345	70	42	-1	5,61	-0.8	19	-4	0,757	0.204

Table 3. Physical and chemical characteristics of the grapes belonging to the studied varieties

Experimental variants and specification	Sugar (g/kg)	% Dif. compared to the control	Total acidity (g tartaric acid/L	% Dif. compared to the control	Gluco-acidometric index	% Dif. compared to the control	Total polyphenols g gallic acid/ kg f.w. (skins)	% Dif. compared to the control	Total Flavonoids g catechin/ kg f.w. (skins)	% Dif. compared to the control	Total anthocyanins mg malvidin-3-O-glu/ kg f.w. (skins)	% Dif. compared to the control	Total polyphenols g ac. gallic/ kg f.w. (seeds)	% Dif. compared to the control	Total flavonoids seeds g catechin/ kg f.w. (seeds)	% Dif. compared to the control
Italia	167,6	-60.1	5,81	2.48	2.88	-3.95	0,720	-1.007	0,585	-0.403	0,4	-281.0	5,98	-2.79	2,57	-5.07
Bicane	162,8	-64.9	4,83	1.5	3.37	-3.46	0,283	-1.444	0,199	-0.789	0,3	-281.1	3,46	-5.31	1,97	-5.67
Xenia	188,1	-39.6	3,92	0.59	4.79	-2.04	1,864	0.113	0,166	-0.822	1,4	-280	3,84	-4.93	2,24	-5.4
Tamina	167,6	-60.1	4,70	1.37	3.56	-3.27	1,029	-0.698	0,698	-0.290	112,6	-168.8	5,98	-2.79	3,39	-4.25
Muscat Hamburg (control)	***227,7***	-	***3,33***	-	***6.83***	-	***1,727***	-	***0,988***	-	***281,4***	-	***8,77***	-	***7,64***	-
Muscat d'Adda	214,2	-13.6	3,11	-0.22	6.88	0.05	2,074	0.347	1,270	0.282	763,8	482.4	12,67	3.9	10,39	2.75

Figure 4. 'Italia' variety

Figure 5. 'Xenia' variety

Anthocyanins in the skin have a similar evolution to that of flavonoids, namely, the red varieties (especially, the variety 'Muscat d'Adda' with 764 mg malvidin-3-O glucosid/kg of fw) distinguished themselves by a high anthocyanin potential, as compared to the three varieties with green yellow grapes to which the gathering was low. Regardind to the content of the seeds of total polyphenols, it is mentioned that the highest concentration in these compounds was recorded for the 'Muscat d'Adda' variety (12.67g gallic acid/kg of fw and the lowest for the 'Bicane' and 'Xenia' varieties (3.46g gallic acid/kg of fw respectively, 3.84g gallic acid/kg of fw). 'Tamina' and 'Italia' varieties recorded almost 6 g gallic acid/kg of fw while the reference variety contented 8.77 gallic acid/kg of fw. The analytical tests revealed a high phenolic potential for the 'Muscat d'Adda' variety, both in skins and in seeds, the values of the polyphenols, flavonoids and anthocyanins surpassing those recorded for the reference variety, 'Muscat Hamburg'.

It could be noticed an exception in the group of studied varieties with green-yellow grapes and namely, 'Xenia' variety gathered an amount of total polyphenols significantly higher than the paternal variety 'Muscat Hamburg', surpassing it by a value of 9.93%.

In addition, the 'Muscat d'Adda' variety surpassed the reference variety by 20.09%, being followed by 'Xenia' variety. By analysing the flavonoids content in the berry skin, it could be noticed a significant difference of the 'Muscat d'Adda' variety which surpasses the reference variety by 28,54%.

The varieties 'Italia' and 'Tamina' reach a level a little bit beyond the half of the value of 'Muscat Hamburg' variety, (0.585 mg catechin/kg fw and respectively 0.698 mg catechin/kg fw).

Figure 6. 'Bicane' variety

From this point of view, 'Bicane' and 'Italia' varieties recorded values under the limit of the control variety. From the correlation of the recorded data, it could be noticed that on the consumption maturity, which coincided with the full maturity, in the agro-climatic conditions of the 2012-2013 vegetative year, the studied varieties also reached their phenolic maturity, conferring to them special nutritional value.

CONCLUSIONS

Climatic particularities of the vegetative year 2012-2013 caused for the varieties such as

'Italia', 'Bicane', 'Xenia' and 'Tamina' to reach the optimum level of harvesting simultaneously with the reference variety, although usually these ripen later, belonging to the ages of maturity Vth-VIth.

The varieties 'Muscat d'Adda', 'Xenia' and 'Tamina' distinguished them self by a high phenolic content in the skin recording 2.074 g gallic acid/kg of fw, 1.864 g gallic acid/kg of fw and respectively 1.029 g gallic acid/kg of fw, as compared to the reference variety 'Muscat Hamburg', 1.727 g gallic acid/kg of fw.

It can be appreciate that the rating of the phenolic compounds (polyphenols, flavonoids, anthocyanins) is also useful in the study of the table grapes varieties, not only of those for wine, the achieved data providing clues about the nutritional and therapeutic value of these varieties.

It is recommend the extension of culture of new varieties, 'Xenia' and 'Tamina', for their organoleptic and nutraceutical qualities which equal those of the genitor varieties, 'Bicane' and 'Muscat Hamburg'.

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USE OF PRIMARY AMPELOGRAPHIC DESCRIPTORS IN ESTABLISHING THE SIMILARITY-DIFFERENCE DEGREE BETWEEN VINE VARIETIES WITH DIFFERENT ORIGINS

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Abstract

Vine varieties, no matter which their production direction may be, are characterized by an emphasized morphologic variability, given by their genetics, but also by the fact that these are influenced in a higher or lower degree by climatic and agro-technical factors, thus manifesting differently, according to the crop area. Within this study, it was aimed to morphologically express the 'Pinot noir', 'Pinot blanc', 'Pinot gris', 'Chardonnay', 'Traminer rosé' varieties under the conditions of the experimental field of U.A.S.V.M. Bucharest. The studied varieties show a common lineage and they all show their stressed character of morphological variability. For the ampelographic description, there have been used 37 ampelographic descriptors according to the International Organisation of Vine and Wine (OIV) descriptors as modified by EU project Genres CT96 No81. The achieved results highlight different degrees of similarity of these varieties with the 'Pinot noir' variety, taken as standard, and the variety with the lowest degree of similarity is 'Chardonnay' - with a percentage of only 45.94% compared to the standard.

Key words: *ampelographic descriptors, biodiversity, dissimilarity, morphological, similarity*

INTRODUCTION

Approaching such a theme and that is, establishing the degree of similarity, dissimilarity respectively, between five varieties of grape using as reference variety the variety 'Pinot noir', it can appear as an approach of which end is greatly known. For sure, studies carried out so far (Bowers et al., 1999; Regner et al., 2000; Christensen et al., 2003; Bettiga, 2003; Stroe, 2012) established, over the course of time, which is the degree of similarity between several varieties of grape.

As a matter of fact, this has been a perpetual preoccupation of the scientific research, to find out the way in which the grape varieties appeared, the way in which they evolved and their degree of kinship.

Despite all this, grape in general can develop differently, depending upon the crop area, both from the phenotype and agrobiologic and technological perspective.

Practically, within this study there was evaluated the morphologic expression of varieties and the extent to which it is influenced by the crop area, within a comparative system, using as control the 'Pinot noir'.

In recent years, as result of advanced research, both in terms of establishing the genetic origin of varieties and their degree of relatedness, scheme description used to describe and identify varieties of grape-vines, was confined to a small number of characters analyzed, 147 of the total descriptors, restricted scheme comprises of 37 descriptors that highlight and demonstrate in the highest degree the similarities and differences between them.

This has been agreed upon within an agreement with O.I.V., amended by EU, by Genres CT96 No81 Program (Damian et al., 2011; Bergamini et al., 2012).

MATERIAL AND METHODS

Plant material

The research developed in the winegrowing year 2011-2012, in the experimental field of U.A.S.V.M. Bucharest.

The varieties which form the object of these researches are: 'Pinot noir', 'Pinot blanc', 'Pinot gris', 'Chardonnay', 'Traminer rosé', varieties

which are very appreciated by consumers with regard to wines quality and specificity which are obtained from these.
The selected type of pruning was Guyot on semi-stem, the planting distance of 2.0/1.2 m, with a load of 30 buds/vine.

Ampelographic characterization

It was performed comparing morphology in two consecutive years (2011-2012) by means of primary and secondary descriptors, as indicated in the frame of the 2nd edition of the OIV Descriptor List for grape varieties and *Vitis* species.

(http://www.oiv.int/oiv/files/5%20-%20Publications/5%20-%201%20Publications%20OIV/EN/5-1-9_Liste_descripteurs_2ed_EN.pdf).

37 characters were selected:
6 characters for the description of the shoot - 001, 003, 004, 007, 008, 016;
2 for the young leaf - 051, 053;
17 for the adult leaf - 065, 067, 068, 070, 072,073, 074, 075, 076, 078, 079, 080, 081-1, 081-2, 083-2, 084, 087;
5 for the bunch - 202, 203, 204, 206, 208
6 for the berry - 223, 225, 228, 231, 235, 236;
1 berry: formation of seeds- 241.

RESULTS AND DISCUSSIONS

Ampelographic characterisation of each variety was performed using 37 markers; as a reference (Table 1, Table 2) are reported data related to varieties that first reached fruit production.
As result of interpretation of data in which there can be found the synthesised scheme of the most representative ampelographic descriptors (37), specific for all the organs of the vine, this being the reduced variant of establishing the degree of similarity – dissimilarity between the varieties submitted to study, the following have resulted:
The variety with the highest degree of similarity is the 'Pinot blanc' (Figure 2) which has a number of 28 ampelographic characters common to the 'Pinot noir', (Figure1) having a degree of 75.67% similarity to it, as follows: in the case of the young shoot of the three studied

Figure 1. 'Pinot noir' variety

characters, (001 -opening of the shoot tip, 003 - intensity of anthocyanin coloration on prostrate hairs of the shoot tip, 004 - density of prostrate hairs on the shoot tip) 'Pinot blanc' is similar for two of these, that is 001 and 004.
In the case of the young leaf, the similarity is at the colour of upper side of blade (4th leaf) - 051, and in the case of adult shoot of the two studied characters the similarity is complete, that is at the colour of the dorsal side of internodes and colour of the ventral side of internodes (007, 008).

Figure 2. 'Pinot blanc' variety

When we talk about the adult leaf, the most representative body of ampelographic recognition, the degree of similarity is very high, respectively of the 17 studied characters; 'Pinot blanc' is similar for a number of 14 characters, which is in a proportion of 83%.

The similarity can be found in the case of size of blade descriptors (065), goffering of blade (072), and area of anthocyanin coloration of main veins on upper side of blade end profile of blade in cross section (074).
Also, shape of teeth (076), as well as degree of opening – overlapping of petiole sinus (079) and shape of base of petiole sinus (080) are similar.
At the grape-cluster, the similarity is a four of the five studied characteristics, respectively length (peduncle excluded) – (202), width (203) and their density (204), as well as length of peduncle of primary bunch (206).
The berry is similar in the case of four characters out of six, which is shape (223), intensity of flesh anthocyanin coloration (231) firmness of flesh (235) and particular flavour (236).
The seeds are present and fully developed (formation of seeds 241) this being valid for all the studied varieties.

Figure 3. 'Pinot gris' variety

The following variety as degree of similarity is the 'Pinot gris' (Figure 3) which with a number of 23 characters shows a degree of 62.16% similarity, as compared to the control variety, which in the case of the young shoot is similar for (001 – opening of the shoot tip end 004 – density of prostrate hairs on the shoot tip.
In the case of the adult leaf, 'Pinot gris', it is similar in the case of ten characters that is to shape of blade (067), profile of blade in cross section (074) shape of teeth (076), length of teeth compared with their width (078).
Degree of opening – overlapping of petiole sinus (079) as well as of shape of base of petiole sinus (080) and teeth in the upper lateral sinuses 083-2 end density of prostrate hairs between main veins on in the upper lateral sinuses 083-2 end density of prostrate hairs between main veins on lower side of blade (084) is similar.
At the grape cluster, the similarity is for three out of five characters, length (peduncle excluded) – (202), width (203) and their density (204).
In the case of the berries the similarity is higher than in the case of the 'Pinot blanc', the latter being similar for five out of the six characters. Thus, the shape of the berry, the skin thickness, the pulp colouring intensity, the degree of consistence of the pulp and the taste are similar, only the skin colour descriptor being different (225).

Figure 4. 'Traminer rosé' variety

The third is the 'Traminer rosé' (Figure 4), which with a number of 19 characters, showing a degree of 51.35% similarity, as compared to the control variety.
In the case of this variety, the similarity can be found at the young leaf, the adult shoot with only one character, and at the adult leaf there are seven similar characters, among which: shape of blade (067), area of anthocyanin coloration of main veins on upper side of blade (070), degree of opening – overlapping of petiole sinus (079) end shape of base of petiole sinus (080) and teeth in the upper lateral sinuses 083-2.
The grape cluster of the 'Traminer rosé' variety is similar to that of 'Pinot noir', for three out of five characters, 202, 203, 204 (length peduncle

excluded, width and density). For the berry, the number of characters defining the degree of similarity is three, these being shape - 223, intensity of flesh anthocyanin coloration - 231 and particular flavour - 236.
The highest degree of dissimilarity is represented by the 'Chardonnay' (Figure 5), by the 17 characters, leading us to a percentage of 45.94%, similarity as compared to the control. This is similar to the control for the young shoot, and in the case of the adult leaf, the number of similar characters is only eight. These are represented by undulation of blade between main or lateral veins (073), goffering of blade 072, profile of blade in cross section 074, shape of teeth end length of teeth compared with their width (076, 078).
Shape of base of petiole sinus (080) and teeth in the upper lateral sinuses (083-2) are identical.

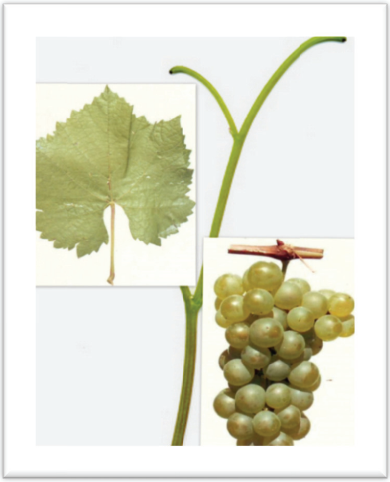

Figure 5. 'Chardonnay' variety

The grape cluster is similar in the case of width, compactness and shape.
The berry of the 'Chardonnay' grape cluster is identical to that of 'Pinot noir' regarding shape, colouring intensity of the pulp, the skin thickness and taste, the difference appearing at the code 225 (skin colour) and 235 (degree of consistence of the pulp).

CONCLUSIONS

The data obtained in the case of using the reduced scheme which contains only 37 ampelographic descriptors, out of the total of 147, the order regarding the degree of similarity – dissimilarity between the studied varieties is the following: the 'Pinot blanc' is the closest to the control variety with a percentage of 75.67% similarity.
The 'Pinot gris' is ranked the second with a degree of 62.16% similarity, as compared to the same variety taken as control.
The third in order of similarity is the 'Traminer rosé' with a percentage of 51.35% similarity, the last being the 'Chardonnay' variety, with a degree of similarity of 45.94% as compared to the control variety.
The results can provide us with a series of information with regard to the varieties origin, their degree of kinship, the way in which they develop within a specific area and at the same time, they demonstrate the necessity of closely knowing the grape varieties in general and their behaviour and development in a specific area, in particular.
This order is an order which appears almost natural, and which has its origin in the theory that there are numerous families and group sorts of varieties, very similar to one another and that approximately 75% of these are related, which indicates to us the fact that, genetics – by genome scanning, shall settle the potential uncertainties with regard to the origin and degree of kinship in the case of grape varieties.

Table 1. 18 ampelographic primary descriptors are reported in highlighted columns

Varieties / Cod OIV	001	003	004	007	008	016	051	053	065	067	068	070	072	073	074	075	076	078
Pinot noir (control)	**7**	**1**	**5**	**2**	**1**	**1**	**1**	**7**	**5**	**3**	**3**	**2**	**5**	**1**	**4**	**3**	**2**	**5**
Traminer rosé	7	5	7	3	1	1	3	7	3	3	1/2	2	7	0	4	5	3	3
Pinot gris	7	3	5	1	3	1	1	5	3	3	2/3	0/1	7	0	4	5	2	5
Chardonnay	7	5	5	3	3	1	3	1	3	4	1/2	0/1	5	1	5	3	2	5
Pinot blanc	7	7	5	2	1	1	1	5	5	4	2/3	2/3	5	1	4	3	2	5

Table 2. 19 ampelographic primary descriptors are reported in highlighted columns

Varieties / Cod OIV	079	080	081/1	081/2	083/2	084	087	202	203	204	206	208	223	225	228	231	235	236	241
Pinot noir (control)	**3**	**1**	**1**	**1**	**1**	**5**	**3**	**3**	**3**	**7**	**5**	**3**	**2**	**9**	**3**	**1**	**1**	**1**	**3**
Traminer rosé	3	1	0	1	1	5	5	3	3	7	3	2	2	5	5	1	3	1	3
Pinot gris	3	1	1	1	1	5	0	3	3	7	3	2	2	4	3	1	1	1	3
Chardonnay	2	2	0	3	1	1	5	3/5	3	7	3	3	2	2	3	1	3	1	3
Pinot blanc	3	1	0	1	1	5	3	3	3	7	5	2	2	2	5	1	1	1	3

(red)	**dissimilarity**
(green)	**similarity**

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THE VARIABILITY OF THE ANTIOXIDANT CAPACITY OF RED WINES IN RELATION WITH GRAPEVINE VARIETY

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Abstract

The red wine is one of the foods rich in antioxidants, compounds that protect the organisms against the destructive action of the free radicals on the health. In this context, for the promotion of wines on markets, an identification of the wines assortments with high antioxidant capacity should be achieved. The present study, performed during 2012– 2014 period, at Research and Development Institute for Viticulture and Enology Valea Calugareasca, aimed to define the antioxidant capacity of the wines made from 11 grapevine varieties belonging to the three groups of wines assortments and to make a correlation of the antioxidant capacity with the phenolic potential of the grapes and wines. Depending on the period when the grapevine variety was created, an old wine assortment, a current and a newly created one can be differentiated. The antioxidant capacity of the wine was performed by means the TEAC method, the phenolic potential of the grapes by Glories method and the phenolic composition of the wines by OIV spectrophotometric methods. The data processing was realized by using the Regression and correlation analysis. The obtained results showed that the varieties 'Negru aromat', 'Novac' and 'Olivia' are characterized by a high antioxidant capacity, the group formed by 'Feteasca neagra', 'Pinot noir' and 'Merlot' showed a medium antioxidant capacity. The lowest values of the antioxidant capacity were registered in the case of 'Negru moale', 'Negru vartos', 'Blauerzweigelt' and 'Burgund' mare varieties. The antioxidant capacity of the wines was closely correlated with the following parameters of the phenolic potential of the raw material: total polyphenols, extractable anthocyanins, tannins originated from seeds and the maturity of the seeds.

Key words: *antioxidant capacity, phenols, wine, grape, grapevine variety*

INTRODUCTION

Grape and wines are an important source of natural antioxidants (Kanner et al., 1994). Different wines have different quantities and spectra of native antioxidants and therefore different health benefits. Wine composition, including the contents of phenolic compounds, varies markedly depending on the grape cultivar, soil, nutrition, climatic conditions, conditions of grape maturation, winemaking procedure and conditions of wine maturation and storage.

Antioxidant activity of grapes and wine had been studied all over the world and varieties with high antioxidant capacity were identified: 'Pinot Noir', 'Merlot', 'Chardonnay', 'Cabernet Sauvignon' and 'Malbec' varieties (Landrault et al., 2001; Mitic et al, 2012; Kostadinovic et al, 2012 etc).

The relationship between the antioxidant activity and polyphenolic compounds in wines has been studied by many scientists (Cano Lario and Guerrero, 1999; Arnous and Makris, 2002).

The present study, performed during 2012–2014 period at Research and Development Institute for Viticulture and Enology Valea Calugareasca (IC-DVV), aimed to define the antioxidant capacity of the wines made from 11 grapevine varieties belonging to the three groups of wines assortments and to make a correlation of the antioxidant capacity with the phenolic potential of the grapes and wines.

MATERIALS AND METHODS

Materials

All varieties used were *V. vinifera s*pecies, harvest 2012. Eleven varieties were chosen from the collection of the (ICDVV). Five varieties, 'Cabernet Sauvignon', 'Merlot', 'Feteasca neagra', 'Pinot noir' and 'Burgund' mare represent the current assortment located in the appellation of controlled origin (AOC) Dealu Mare - Valea Calugareasca vineyard, two varieties ('Negru moale' and 'Negru virtos') belong to the old Romanian wine assortment and three varieties ('Negru aromat', 'Olivia' and 'Novac') are Romanian newly research creation. 'Blauerzweigelt' is a variety recently introduced in the viticultural area from the world's assortment. Details about the cultivars and their location are given in Table 1.

Table 1. Variety and origin of the wines tested

Codes	Cultivar	Location
BM	Burgund mare	National Collection
BL	Blauerzweigelt	National Collection
CS	Cabernet Sauvignon	National Collection
FN	Feteasca neagra	National Collection
ME	Merlot	National Collection
NA	Negru aromat	National Collection
NM	Negru moale	National Collection
NV	Negru virtos	National Collection
NO	Novac	National Collection
OL	Olivia	National Collection
PN	Pinot noir	National Collection

Sample preparation

The grapes used for the production of the experimental wines were harvested at optimum technological maturity, as judged by indices of ripening and indices of the phenolic maturity. All the tested wines were produced in the winery of the ICDVV under similar oenological practices and stored in the same conditions. Crushed grapes stayed in contact with the must for six days at 28–30°C.

Antioxidant capacity (CAO)

All samples were analyzed in April in the year following the vintage (2012). Antioxidant capacity was determined by scavenging of the radical 2,2,-azino-bis (3-ethylbenzothiazoline) - 6 sulfonic acid (ABTS+), as described by Re et al. (1998). Stock solution was prepared by stirring ABTS (7 mM) and potassium persulfate (2.45 M) aqueous solution and allowing the mixture to stand in the dark at room temperature for 18-24 hours before use. This solution was diluted in ethanol to obtain an absorbance of 0.7 at 734 nm. In the assay, 100 µl of wine, diluted 1:100, 2500 µl ABTS and 100 µl distilled water were mixed. The absorbance at 734 nm was determined after 3 min. For each extract, a blanc with 2500 µl ABTS and 500 µl distilled water, was included to correct for any sample absorbance at 734 nm. Trolox (6-hydroxy-2,5,7,8- tetramethyl - chroman-2-carboxylic acid) was used as a standard. The antioxidant capacity was expressed as µM trolox equivalents (TE) per 100 ml.

Phenolic composition

For the determination of the total content of polyphenolic compounds in wines the DO280 index was considered: wine was diluted with distilled water (1:100) and the absorbance was measured directly at 280 nm. The value of DO280 index for each sample was given as the absorbance multiplied by the proper dilution rate.

The determination of catechins (flavan 3-ols) is based on the reaction of the phloroglucinol ring with vanillin, that produces a red colour with a maximum absorption at 500 nm (Pompei and Peri, 1973).

The anthocyans were measured using the method of Ribereau-Gayon and Stonestreet (1965).

Phenolic maturity of the grapes at harvest

The phenolic maturity of the grapes is defined as a moment when the tannins in seeds and the concentration of anthocyanins in the grape skin are minimum and maximum values, respectively. It was evaluated according to the Glories method (Anneraud and Vinsonneau, 2009), by using the following parameters: Total Polyphenol Index (ITP), Anthocyanines extractibility (AE), Contribution of grape seeds tannins (MP) and Contribution of grape skins polyphenols (MS).

RESULTS AND DISCUSSIONS

The antioxidant level of the wines from the old and new red wine assortment

The wines from the studied assortment had a medium value of the antioxidant capacity of 1808 µM TE/100 ml wine, which varied between 1181 and 2600 µM TE/100 ml. The identification of the antioxidant level of wines, namely low, moderate or high, is very important in the evaluation of wines from pharmacodynamic point of view. For this purpose, based on the obtained information, three classes of variation of the antioxidant capacity of wines were defined, the class range being of 570 µM TE/100 ml. The antioxidant level was identified for each sample of wine. The richest in antioxidants have been 'Olivia', 'Negru aromat' and 'Novac' wines. The wines 'Negru moale', 'Negru virtos', 'Blauerzweigelt', 'Burgund mare', 'Cabernet Sauvignon' and 'Merlot' presented a low CAO value and Feteasca neagra' and 'Pinot noir' wines a moderate one (Figure 1).

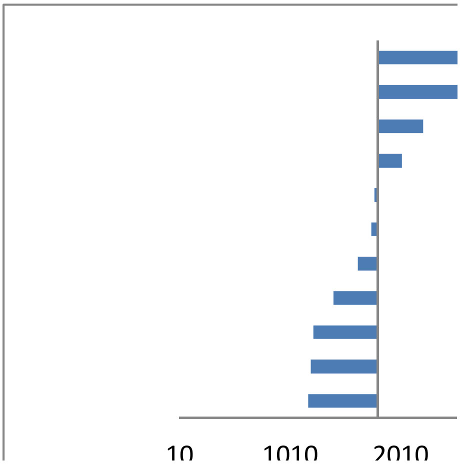

Figure 1. The ranking of red wines from the old and new assortment function of CAO

The correlation between the antioxidant capacity and the phenolic compounds

The phenolic compounds are involved in the antioxidant capacity of wines. The setting of the relationship between the antioxidant capacity of wines and their polyphenolic composition is important for the organization of the winemaking process. The analysis of linear regression CAO-total polyphenols (DO 280) showed a strong relation between the two parameters (R^2=0.77) (Figure 2).

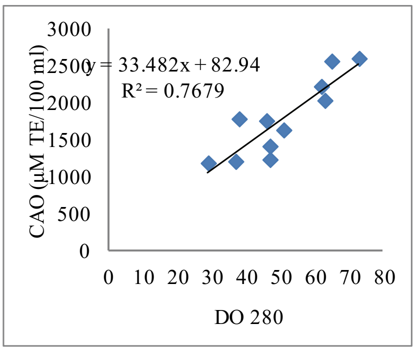

Figure 2. The correlation between CAO and DO 280

The increasing of the DO280 index by one unit determined the upwards changing of the antioxidant capacity with 33 µM TE/100 ml. The anthocyanins are in a weak relationship with the antioxidant capacity (R^2=0.29) (Figure 3).

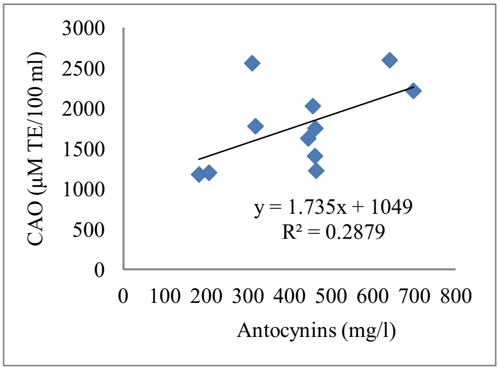

Figure 3. The correlation between CAO and anthocyanins

A strong correlation with the antioxidant capacity of wines was put into evidence at the catechins and tannins level (R^2=0.77) (Figure 4 and Figure 5).

When the amount of catechins increased with 100 mg/l, the antioxidant capacity was raised by 51 µM TE/100 ml. The increasing of tannins value by 1 g/l determined the upwards changing of the antioxidant capacity with 706 µM TE/100 ml.

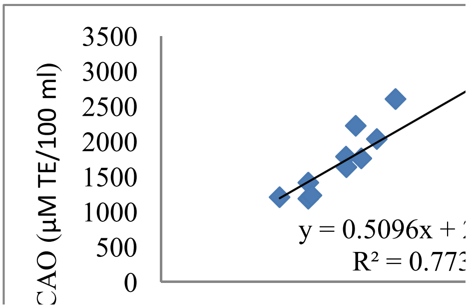

Figure 4. The correlation between CAO and catechins

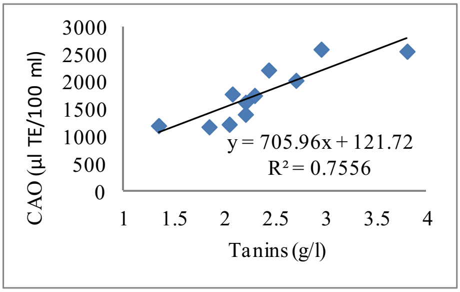

Figure 5. The correlation between CAO and tannins

The correlation between CAO and the phenolic maturity of grapes

The evaluation of the relationship between CAO and the phenolic maturity of grapes is essential to determine the optimal harvest time and for the establishing the correct vinification technique. The analysis of the multiple dependence between CAO and the parameters of the phenolic maturation of grapes (ITP, AE, MP and MS) put into evidence a strong correlation (R^2=0.89).

The regression equation (1) had the following parameters included in Table 2:

CAO=-8070+31ITP+0.10AE+8516MP+7618MS (1)

Table 2. Analysis of the multiple regression between CAO and the parameters of the phenolic maturation of grapes

	Coefficients	Standard Error	t Stat	P-value
Intercept	-8069.8	11539.68	-0.699	0.5105
X ITP	31.46	6.52	4.829	0.0029
X AE	0.1	0.25	0.435	0.6791
X MP	8515.7	11550.60	0.737	0.4888
X MS	7618.4	11691.29	0.652	0.5388

The equation 1 can be used to estimate the antioxidant capacity of wines based on the values of the parameters characterizing the phenolic maturation of grapes at harvest, provided the maceration during winemaking is kept at 6 days at 28-30°C, as in the experimental conditions .

CONCLUSIONS

The antioxidant capacity of the wines belonging to the AOC Dealu Mare Valea Calugareasca old wine assortment and to the newly created one varied between 1181 and 2600 µM TE/100 ml.

The highest values of the antioxidant potential were registered in case of the 'Olivia', 'Negru aromat' and 'Novac' wines; 'Negru moale', 'Negru virtos', Blauerzweigelt', 'Burgund mare', 'Cabernet Sauvignon' and 'Merlot' wines were characterized by a low antioxidant capacity. 'Feteasca neagra' and 'Pinot noir' wines showed a medium antioxidant capacity.

There was a strong correlation between the antioxidant capacity of the wines and total amount of polyphenols, catechins and tannins.

The antioxidant capacity of the wines can be estimated based on the phenolic maturity indicators using the relationship CAO=8070+31ITP+0.10AE+8516MP+7618MS.

This classification is valid only for the winemaking processes in which the maceration on skins is performed for only 6 days at 28-30°C. For longer maceration periods the antioxidant capacities displayed by high tannic cultivars, such as 'Cabernet Sauvignon', 'Merlot' or even 'Pinot noir' and 'Feteasca neagra', may be significantly increased.

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EFFECT OF A NEW COMPLEX OF TRACE ELEMENTS MICROCOM-V ON SOME METABOLIC PROCESSES AND PRODUCTIVITY OF *VITIS VINIFERA* L.

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Abstract

The first local complex compound of trace elements Microcom-VA dedicated for grape has been tested in field conditions on 4 cultivars in comparison with two iron containing compounds – $FeSO_4$ and Dissolvin. The results of foliar treatment of vine, which were obtained in the 4 - year experiments (modification of content of photosynthetic pigments, microelements in the tissue, phosphor components and carbohydrates compounds, optimization of shoots growth and maturation, grape quality and quantity, winter resistance), let to assume, that complex of trace elements Microcom-VA led to optimization of plant nutrition, improving grape and wine quality, fuller realization of genetically based potential of frost and winter resistance.

Key words: *amino acids, carbohydrates, plant nutrition, sugar, trace elements*

INTRODUCTION

Vine is cultivated, in generally, on the soils with low fertility due to its morpho-physiological particularities, high level of plasticity and high adaptive potential. But such soils require permanent amelioration of the nutritive regime. Provision of grape plants with macro and microelements, prevention and attenuation of currency and sub currency of nutritive elements on the period of vegetation, increase of the quantity and quality of grape is possible through the foliar treatment of plants. The problem of fertilization is especially important for Moldova, because of high degree of soil degradation, slopes, and semi-arid conditions (Kuzneţov and Dmitrieva, 2006; Toma and Veliksar, 1992). Moreover, trace element content plays a very important role in many metabolic processes of plants and is the basic limitative factor (Alloway, 2006; Burzo and Toma, 2000; Toma et al., 2003).

Foliar fertilization is an important factor as supplementary and compensatory fertilization due to the high degree of uptake and utilization of the applied nutrients. Elaboration and utilization of the complex compounds for fertilization predominate actually in the world. Taking into account the insufficient supply of soils in mobile forms of microelements Fe, B, Mn etc. in our region, and also their high necessity for perennial plants, a special complex of microelements *Microcom-V* was created in the Institute of Genetics and Plant Physiology Academy of Sciences of Moldova (Patent MD: 2654).

From the other hand vine is frequently injured by the critical negative temperatures during the winter period. The majority of the varieties cultivated in the country, possess an enhanced genetic potential of producing capacity and relative resistance to frost. An eloquent objective provided for both intensive and ecological technologies within the strategies of a durable agriculture development in view of production of stable and high quality yields is to ensure the most complete manifestation of this potential.

The multiple studies (Burzo et al., 2000) have demonstrated that plant responses to stress are accompanied by accumulation of N-containing compounds (proline, other amino acids, polyamine compounds) and hydroxyl compounds (soluble glucides, oligosaccharides, sorbitol, inositol etc.) The problem is to provide evidence demonstrating the impact of nutrients in this process. A specific microelement complex Microcom-V has been developed for this purpose. This study has been conducted to elucidate the impact of the Microcom-V microelement complex on some metabolic processes and productivity.

The main objective of this article is elucidation of some physiological aspects of the influence of specific complex of trace elements Microcom-V on grape metabolic processes and productivity.

MATERIALS AND METHODS

The researches have been performed in field conditions (central region of Moldova) on the technical varieties of grape ('Codrinschi', 'Aligote', 'Traminer', 'Chardonnay') in the years 2007 - 2011. Three trace element compounds were used for plants foliar fertilization: $FeSO_4$, Dissolvin and Microcom-V. Dissolvine - universal fertilizer for the prevention and remedying of micronutrient deficiencies in plants (manufactured in the Netherlands). The last compound - Microcom-V is created in Moldova; it contains 6 especially necessary for grape trace elements (Fe, Mn, B, Zn, Mo, Ni, Co) in optimal combination. The foliar treatment by the micro fertilizers was conducted two or three terms – before flowering and at the stage of intensive growth with an interval of 12 to 14 days. Water treated plants were used as control. Working solutions for spraying: $FeSO_4$ and Dissolvin – 0,3%, Microcom-V – 0,15%.

Leaves for analyses were sampled three and six days after the foliar treatment, thoroughly rinsed with water, allowed to dry and used for analysis. The berries were collected in two steps - at the beginning of their maturation and at full maturity, rinsed with water, allowed to dry and analyzed.

The following analytical methods were used: the content of free amino acids - using an AAA-300 analyzer, the carbohydrate content - according to Bertan; photosynthetic pigments determination - using ethanol extraction (Foy, 1987), trace elements content - using an atomic absorption spectrophotometer Perkin Elmer after dry digestion at 480°C. The results were analyzed statistically according to Statistica-7.

RESULTS AND DISCUSSIONS

The content of photosynthetic pigments in grape leaves. The content of photosynthetic pigments in leaves is one of the important indications of the plants status during the growing season. Determination of these indices in plant leaves revealed positive influence of plants foliar fertilization by micronutrients on plant photosynthetic activity. More effective were compounds containing several trace elements - Dissolvin and Microcom-V. The amount of chlorophylls *a* + *b* in leaves of those variants increases after foliar treatment by 9.47 and 11.0 % compared to the control (Table 1). Ratio of chlorophyll forms essentially did not change. It was observed the tendency to decrease of the carotenoids content after treatment with micronutrients. Fertilization of plants by Microcom-V maintains the content of chlorophylls at a higher level during the vegetation.

Table 1. The content of photosynthetic pigments in vine leaves after the treatment with Fe - containing compounds, v. 'Aligote', % f. w. (15.06.2009)

Variants	Chlor."a"	Chlor."b"	Sum a+b	Carotenoids
Control	0,516 ± 0,009	0,202 ± 0,005	0,718± 0,009	0,302±0,004
$FeSO_4$	0,530 ±0,008	0,202 ±0,005	0,736± 0,008	0,304±0,004
Dissolvin	0,573 ±0,005	0,213 ±0,005	0,786 ±0,011	0,286±0,005
Microcom -V	0,588 ±0,033	0,209 ±0,005	0,797 ±0,035	0,285±0,010

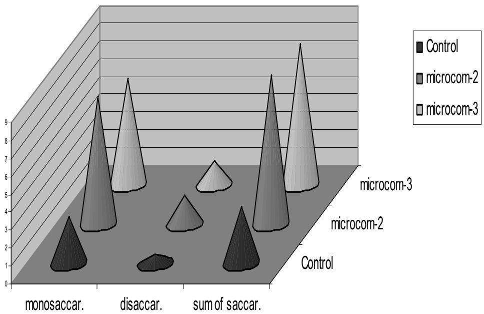
The content of sugars, free amino acids (FAA) and phosphoric compounds in grape leaves. The total sugar content in grape leaves changes quite significantly during the vegetation. The analyses performed three and six days after foliar fertilization of plants demonstrate that plant foliar treatment with Fe-containing solutions contributes to decrease of sugars in leaves immediately after treatment (after the first three-four days) and a subsequent increase in leaves and grape berries (after the sixth days). A dynamics evaluation of the sugars content in vine leaves after the foliar fertilization by trace elements has demonstrated that it increases in the course of vegetation (Table 2).

Table 2. The content of sugars in vine leaves and berries, mg/100mg d.w.

Treatments	leaves			berries
	6 days after I foliar treatment	6 days after II foliar treatment	6 days after III foliar treatment	before harvesting
Control	0,67	0,80	2,40	18,77
$FeSO_4$	0,93	1,13	3,06	17,77
Dissolvin	0,67	0,87	2,73	18,23
Microcom-V	1,07	1,33	3,46	19,67

The most favorable effect has been obtained after the treatment with the trace element complex Microcom-V. Comparison of two-and three-fold foliar fertilization of plants by micronutrient complex Microcom-V showed that more effective is a three-fold processing (Figure 1).

a)



b)

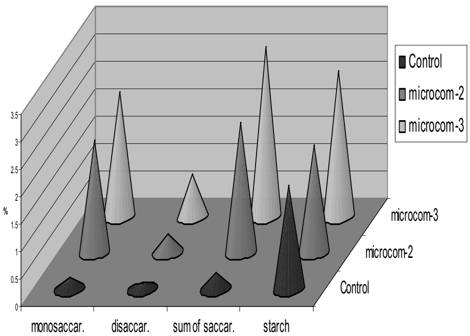


Figure 1. Carbohydrate content in grape leaves (a) and shoots (b) %, after 2 (Microcom 2) and 3 (Microcom 3) foliar treatments, v. Chardonnay

A concomitant increase of starch in shoots after fertilization of plants with Microcom-V indirectly denotes an intensification of synthetic processes in plants.

The total content of FAA in plant tissues is quite a mobile indicator and depends on many factors including the plant condition, vegetation stage, nutrition conditions etc. Determination of FAA in dynamic demonstrate that it decreases in leaves three days after foliar fertilization, especially that of non-essential ones, and increase after six days. Treatment of plants with trace element solutions maintains the total level of FAA at a higher level (Table 3).

Table 3. The content of free aminoacids (FAA) in vine leaves and berries, mg/100mg d.w.

Treatments	leaves			berries
	6 days after I foliar treatment	6 days after II foliar treatment	6 days after III foliar treatment	before harvesting
Control	0,244	0,022	0,05	0,020
$FeSO_4$	0,262	0,024	0,103	0,036
Dissolvin	0,266	0,024	0,133	0,041
Microcom-V	18,23	0,028	0,164	0,052

The quantitative and qualitative changes are more pronounced after the third treatment. Utilization of Microcom-V was more beneficial. The analysis of the qualitative content of FAA shows that the content of proline, valine, tyrosine, and phenylalanine increased in the treatments with trace elements. The content of glutamic acid + glutamine rises by 2-3 times after three days of treatment. It is a common knowledge, that accumulation of sugars and other compounds having a stress protective action is one of the mechanisms of plant resistance to the action of negative temperatures, as well as other stress factors (Bohnert et al., 1995; Mazzucotelli et al., 2006).

The content of FAA, particularly indispensable acids, and total sugars significantly increased in grapes, the highest value registered after the fertilization by the microelement complex Microcom-V. This evidences about improving the quality of berries under the influence of foliar fertilization of plants, in comparison with the control.

Importance of phosphorus for the synthesis processes activation, transport in plants, role of this element for the formation and manifestation of the level of frost resistance and wintering of perennial plants is known. The influence of foliar treatment by Microcom-V on the content of phosphoric compounds in grape leaves was studied in 2007-2008. The studies have revealed essential modifications in the content of some forms of these compounds in the vine organs after microelement treatment. A significant increase of the content of phosphorus lipids and nucleotides, acid soluble phosphorus and a significant reduction of etheric glucides has been established. The obvious modifications found in the content of the phosphorus compounds after the plants fertilization by Microcom-V attest a beneficial effect of the microelements on enhancement of plant resistance to frost and winter conditions.

Plant productivity and resistance to wintering. The results obtained in our long-term experiments on the different varieties of grapes in production conditions of Moldova shoved stimulating effect of foliar fertilization by Microcom-V on the metabolic processes in the grape leaves during the growing season. Improvement of metabolic processes contributed to increasing of plant productivity. A part of conducted records of grape yield under the influence of foliar fertilization by micronutrient complex Microcom-V are presented in table 4. A beneficial action of the specific microelement complex on the yield volume and grape fruit quality has been detected. Depending on the variety and year conditions, the number of clusters from one bush grows by 103-120% as compared to control bushes and the average yield per bush - by 108-140%. Some increase of bunches number took place as foliar treatment before flowering influenced the additional development of buds because of better mineral status of plants and improvement of leaf photosynthetic activity.

Table 4. Effect of Microcom-V on the grape quantity

Treatment	The quantity of bunches	The average weight of bunches, g.	Average yield, kg/ bush
2007, cv. Aligote			
Control	48,86±3,22	144,44±7,50	7,05
Microcom-V	56,40±3,63	156,33±7,64	8,82
2008, cv. Codrinschi			
Control	38,03±2,15	189,17±11,61	7,19
Microcom-V	42,10±1,97	240,54±11,04	10,13
2009, cv. Codrinschi			
Control	32,33±2,65	149,56±17,65	4,84
Microcom-V	33,05±2,67	160,58±15,51	5,31

The optimization of the metabolic processes in the course of plant vegetation through application of trace elements has influenced shoot growth and maturation. Table 5 summarizes the results of the estimation of the trace elements effect on the length and maturation degree in the shoots in 2009. The effect of complex of trace elements Microcom-V is much more pronounced than Fe-containing substances ($FeSO_4$ and Dissolvin). Best shoot maturation provides a higher resistance of plants to conditions of wintering.

Table 5. Growth and maturation of vine shoots depending on the foliar treatment, October 31, 2009

Treatment	Total mean shoot length, cm	Mean mature shoot length, cm	Shoot maturation degree	
			cm	± to control
Control	134,5±6,13	110,4±4,73	82,1	
FeSO4	167,1±7,64	151,1±5,92	90,4	8,3
Dissolvin	152,3±5,34	131,7±3,41	86,5	4,4
Microcom -V	173,4±9,53	159,2±7,86	91,8	9,7

The state of buds after wintering in the plants treated with trace elements during the preceding vegetation period has been assessed. The data obtained in the years 2008 and 2009 demonstrate that buds viability has increased significantly, the number of dead buds has decreased; the plants treated during the vegetation period of 2008 with Microcom-V or $FeSO_4$ saved the highest number of viable buds. Eloquent results have been obtained regarding the condition of vine buds after the action of critical negative temperatures in the winter of 2009-2010, which proves a significant increase of bud viability and reduction of the number of dead buds in the plants treated during the vegetation period with Microcom-V, Dissolvin, and $FeSO_4$ in comparison with the control. It has been found that the number of viable buds constituted only 38.39% in the control (Table 6). In the plants treated with Microcom-V, this index reached a value of 44.56%, a significant increase of 16.07% being recorded. It should be also mentioned that a high number of injured buds was detected in the treated plants in comparison with the control. The high number of injured buds, in which the central bud died as a result of the freezing action on January 26, but at least a lateral bud remained viable, developed in annual buds in the control treatment, while in the treated plants, the percentage made 23.59% of the number of the completely injured buds detected in January, 22.48% and 25.15% respectively. The action of the negative critical temperature of -27^o has been found to provoke a loss of 39,13% of buds in the control, while in the treated plants it made only 30.29%, which is by 22.59% lower in comparison with the control.

Table 6. The grape buds state following the action of the temperature of -27^o, January 26, 2010, %

Treatment	Viable buds	Injured buds	Dithered buds
Control	100.00	100.00	100.00
FeSO4	104.58	127.58	79.66
Dissolvin	105.34	134.12	75.16
Microcom-V	116.07	111.88	77.41
LSD 0.5	3.07	5.33	6.63
C I	2.03	3.53	4.4

Content of trace elements in leaves and grapes. The content of trace elements in the grape leaves is one of the indices that testify about the conditions of plants mineral nutrition and stipulate the realization of potential of plants productivity and ecological resistance. It was showed that foliar treatment influenced first of all the concentration of introduced element in leaf blades of treated plants - Fe, Mn and Zn. The content of Cu decreased in comparison with the control plants. It is well manifested the antagonism between Fe and Cu, that was mentioned in our previous investigations too. It is necessary to take into consideration in the elaboration of the technology of Microcom-V application on the vineyard.

As a rule the content of trace elements in generative parts of plants is lower, than in vegetative ones. We determined the amount of trace elements Fe, Mn, Zn, Ni and Cu in the berries of three grape varieties ('Traminer', 'Aligote' and 'Codrinschy'). Foliar fertilization of plants by micronutrient solution to our knowledge has not significant effect on the content of trace elements in grape berries. The weak tendency to the increase of Fe and Mn quantity in berries of treated bushes was marked, that is very important to improve the process of fermentation and wine quality. Our data showed relatively high level of Ni in grape. After the foliar treatment with trace elements clear tendency to the efflux of Ni, Cu and Zn from generative parts was evident.

CONCLUSIONS

Foliar treatment of grape during vegetation period with the complex of trace elements Microcom-V induces more significant modifications in same metabolic processes in plants in comparison with two iron containing compounds – FeSO4 and Dissolvin.

The modifications in the content of photosynthetic pigments, amino acids, carbohydrates, phosphorus compounds in vine leaves under the influence of micronutrients, especially the Microcom-V were revealed.

The obtained results let to assume, that complex of trace elements Microcom-VA led to optimization of plant nutrition, improving grape quality, fuller realization of genetically based potential of productivity and winter resistance.

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BEHAVIOR OF VINE VARIETIES WITH BIOLOGICAL RESISTANCE UNDER THE SOUTH ROMANIAN CONDITIONS

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Abstract

Main behavior of vine varierieties with biological resistance in the south romanian climatic conditions were analyzed during 3 years of study. Thus they was determined: phenology varieties, fertility and productivity for table and wine varieties, behavior of these varieties at the main attack of major diseases and pests attack, physico-mechanical characteristics, the technological attributes of varieties, the physico-chemical characteristics of wines. For content of grapes in volatile compounds was used the gas chromatography/mass spectrometry method. The wine varieties studied were: 'Moldova' and 'Andrevit' (table wine varieties), 'Admira', 'Radames' and 'Brumariu' (varieties of wine).In ecopedoclimatic terms from the southern Romania, varieties mature later, in maturation ages IV ('Andrevit', V ('Admira') and VI ('Moldova', 'Radames' and 'Brumariu'). From the point of view of fertility and productivity varieties, variety Admira had the best behavior, also the same variety with the table wine 'Moldova' had the lowest degree of the main attack of vine diseases (powdery mildew, downy mildew, grey mold) and phylloxera attack. Regarding the accumulation of sugars in the grape, sugar content varied between 164 and 190 g/L at the wine varieties and between 154 and 171 g/L of table wine varieties, distinguishing varieties are 'Admira' and 'Radames'. Regarding the analysis of the main volatile compounds shows that limonene, hexanol, 2-methyl propanol and 3-methyl butanol is found in highest concentration in all varieties; as regarding terpenes, at the expression of olfactory characteristics of grapes, in the highest proportion is limonene, in lower concentration being found terpens as: cymene, pinene, myrcene.

Key words: *gas chromatography method, downy mildew, powdery mildew,grey mold, phylloxera attack*

INTRODUCTION

Vine varieties with biological resistance are improved varieties, derived by crossing varieties of *vinifera* vines with American hybrid direct producers (Grecu V., 2010).
They are also called green varieties because they require a much smaller number of treatments against diseases and pests compared with *vinifera* varieties.
Although their quality doesn't equal the noble varieties, these varieties have their importance in winemaking, both in improving vine and obtaining green products (juice, alcoholic and non-alcoholic drinks, in different type of food industry).
Using organic varieties are suitable for industrialization in getting juices is a well-known practice in countries like Europe, USA etc.
These drinks, originating from a healthy raw material, the green environment have been the subject of study of many researchers and nutritionists as an outstanding source of antioxidants, vitamins, minerals etc. (Visan et al., 2007).
The behavior of these varieties, and here we refer to their biological resistance, productivity and applied technology depends on several factors including crop area, year of harvest, ecopedoclimatic conditions etc.

MATERIALS AND METHODS

A detailed study on some of vine varieties with biological resistance was conducted in ecopedoclimatic conditions in southern Romania.
The studied grapevine varieties were 'Andrevit' and 'Moldova' (table varieties), 'Admira', 'Radames' and 'Brumariu' (wine varieties).
The study, conducted over a period of three years referred to determining phenology variety, fertility, the agrobiological characterization of varieties (percentage of fertile shoots, the fertility coefficients, productivity indices, reaction to major diseases and pests of the vine) and technological

characterization of variety (total production grapes/ha, physical and mechanical characteristics of the grapes, technological indices).

Grape musts were analyzed under sugar and total acidity terms (after the standardized methods in effect) and characterized in terms of concentration in volatile compound by gas chromatography method coupled with mass spectrometry.

The extraction of compounds was achieved by *headspace* technique under the *vacuum dynamic* method (Serot T. et al., 2001) with Tekmar device and identification of volatile compound and their quantification were achieved using complex devices Varian Star 3400 gas chromatograph with FID detector CX/gas system Hewlett Packard 5890 Series II chromatograph/mass spectrometer Hewlett Packard 5971 Series II.

Identification of volatile compounds was realized based on retention index and standard database. In order to extract the aromatic compounds was chosen version of 10 mL sample that is mixed with 0,02 mg/mL of the internal standard *2-methyl-2-patenal*, the extraction temperature below 40°C (35°C) and the extraction time of 20 minutes. To identify the isolated aromatic compounds we use the GC/MS system (Guth, 1997). For each sample were made every two repeats.

RESULTS AND DISCUSSIONS

The 'Andrevit' table variety, genitors *SV 23-18* x *Queen vineyards*, with selection in F_1 (Oprea et al., 1994) breaking leaf buds around 30 April (Table 1) and has the shortest vegetation of 184 days (Fig.1) fits in IV maturation era.

'Moldova' table variety (genitors *Guzali kara* x *Villard blanc*) is a variety with a late breaking leaf buds, in early May (Table 1) has a vegetation period of 187 days and fits in the VI maturation era. Both varieties have large force, a percentage of 64-67% fertile shoots (Fig.2), weighing more than 250 g (Table 2) and productivity index with high values, grater in 'Andrevit' variety. The total production from the 2 grape varieties exceed 14-15 t/ha, the highest production from the studied varieties (Figure 3).

Physico-chimical analysis of grapes showed a higher sugar content at 'Andrevit' variety (171 g/L) towards 'Moldova' (154 g/L) and a glucoacidimetric index of 32.9 due to an optimum total acidity of 5.2 g/L sulphuric acid (Figure 4).

Behavior at diseases and Phylloxera is different at the two table variety, the 'Andrevit' being characterized by a higher biological resistance. The highest degree of infection was recorded in 'Moldova' variety in case of powdery mildew on grapes (Table 3).

'Admira' variety (genitors *Villard noir* x *Pearl Csaba*) breaking leaf buds on April 30, has a vegetation period of 189 days and fits in the V maturation era.

'Radames' variety, genitors *Traminer Pink* x descendant *Villard blanc* x *Queen vineyards* (Moldovan et al., 1994) breaking leaf buds earlier, on April 12 but has the longest vegetation period (209 days) thus sweeping later, in the VI maturation era.

'Brumariu' variety breaking leaf buds on April 27, has vegetation period of 194 days and fits in VI maturation era.

The last three vine varieties with technological traits shows the average force ('Admira' and 'Brumariu') and high force ('Radames'), a greater percentage of fertile shoots in 'Admira' and 'Brumariu' varieties and quite low at 'Radames' variety (60%).

The Grape weight had record optimum values for category in which the 3 varieties are (146-177 g). Among wine varieties the largest production was recorded for the 'Admira' variety (13.9 t/ha), 'Radames' records a smaller production under 10 t/ha.

Regarding the potential of the alcoholic variety, the highest concentration of sugars was recorded in 'Admira' variety with 190 g/L sugars, so with a 11 vol% alcohol potential, followed by 'Radames' with 175 g/L sugars. Wine analysis results confirmed evaluating the alcoholic potential, wine variety 'Admira' recorded a 11 vol% alcohol (Fig.5).

Wine obtained from 'Admira' variety presented the best characteristics of the varieties of wine; thus, on an alcoholic background concentration comparable with some *vinifera* wine varieties, total acidity was 5 g/L sulfuric acid and total extract of 21.55 g/L. Regarding the biological resistance of wine varieties the best behavior has 'Admira' variety, recording the lowest level of attack both hand, mildew and mold. Varieties 'Radames' and 'Brumariu' shows a lower resistance at mold. The GC/MS analysis of volatile compounds revealed a number of compounds that characterize the studied varieties, some of these compounds can be found in higher concentration in comparison with the other compound identified (Table 4).

Thus, regarding esters were identified: Ethyl acetate, Methyl hexanoate, Ethyl hexanoate, Methyl caprylate (Figure 6).

Esters are formed in grape fermentation process in large quantities by enzymatic esterification and in the process of maturation and aging of wine by chemical esterification (Chisholm et al., 1994). It distinguishes these varieties a very small number and a low concentration of esters compared with *vinifera* varieties (Baek et al., 1997). Among aldehyde were identified 2-Methylpropanal, in the highest concentration of all examined varieties, 2-Methyl butanal, 3-Methyl butanal, Pentanal, 2-hexenal, 2-Heptenal, Octanal, 2-Octen-1-al, Benzaldehyde, Nonanaldehyde etc. (Figure 7).

The higher alcohols were represented by: Propan-1-ol, 2-Methyl-1-propanol, 2-Methyl-1-butanol, 2-Ethyl-1-butanol, Ethyl propyl carbinol, 3-Hexanol; This alcohol that leave a taste of greenery is found in highest concentration in the studied varieties (Fig.8).

The terpenes identified were, in order of concentration traced at most of studied varieties: limonene, p-cymene, β-pinene, α-pinene, β-myrcene etc. We can observe a higher concentration of limonene, in all varieties of case, this terpene being majority, with exception of 'Andrevit' variety, that 2 terpenes characterized this variety: limonene and p-Cymene (Figure 9).

Table 1. Phenology of the biological resistant varieties

Variety	Average data of phenophases				
	breaking leaf buds	flowering	veraison	maturation	fall leaves
Andrevit	30.04	6.06	11. 08	12.09	31.10
Moldova	4.05	8.06	16.08	6.10	7.11
Admira	30.04	8.06	12.08	25.09	5.11
Radames	12.04	5.06	20.08	15.10	7.11
Brumăriu	27.04	4.06	15.08	10.10	7.11

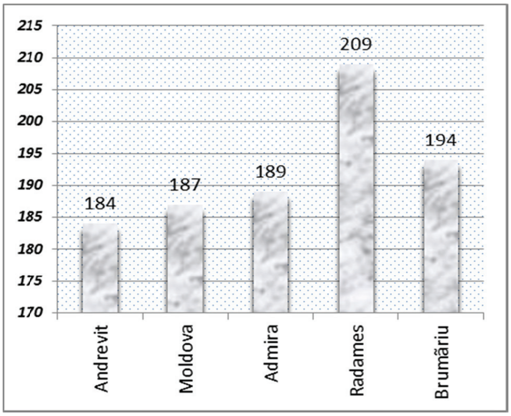

Figure 1. Vegetation period of the varieties (days)

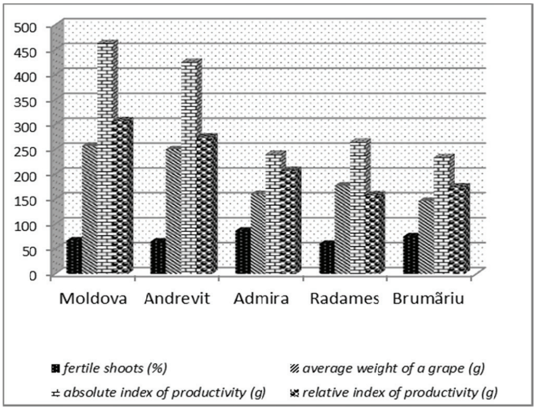

Figure 2. Fertility and productivity of organic varieties

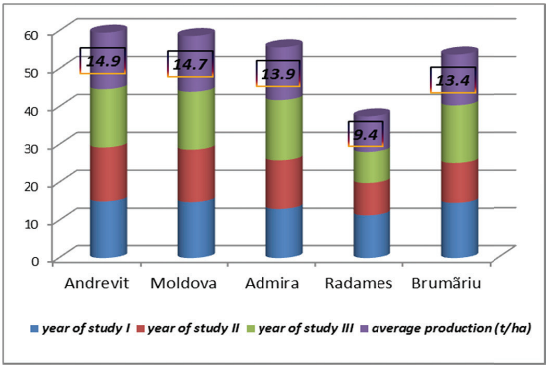

Figure 3. Production of varieties with biological resistance (t/ha)

Table 2. The physico-mechanical and technological indices of biological resistant varieties

Characteristics	Variety				
	Andrevit	Moldova	Admira	Radames	Brumariu
	Mechanical composition / kg grapes (g)				
Grain weight	981	975	967	962	967
Weight of must	721	725	720	629	635
Skin and pulp	241	218	208	288	310
Rahis	19	25	33	38	33
Seeds	19	32	39	45	22
Marc	279	275	280	371	265
The composition of 100 berries (g)					
grain weight	375	390	240	160	135
Skin weight	56	46	35	36	45
pulp weight	311	328	195	114	84
seed weight	8	16	10	10	6
Technological indices					
Index structure of the grape	51.6	39.0	29.3	25.3	29.3
Index berry	28.0	28.0	39.0	40.0	55.0
Index composition of berry	4.8	5.3	4.3	2.5	1.6
Yield index	2.6	2.6	2.6	1.7	1.7

Table 3. Behavior of vine varieties with biological resistance to major diseases and pests of the vine

Variety	behavior of vine diseases				phylloxera attack
	downy mildew	powdery mildew		grey mold	galicola
		on leaves	on grapes		
	(attack degree %)				
Andrevit	1	1	2	4	0
Moldova	2	2	10	0	0
Admira	0	1	1	2	0
Radames	2	2	2	4	0
Brumăriu	0.8	2	2	4	0

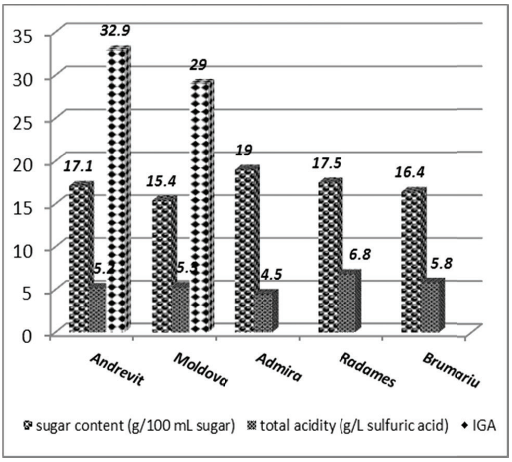

Figure 4. Composition characteristics of grapes

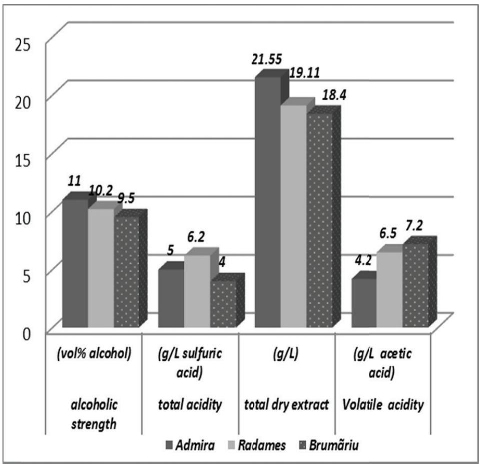

Figure 5. The Main Physico-Chemical Parameters Analyzed wines

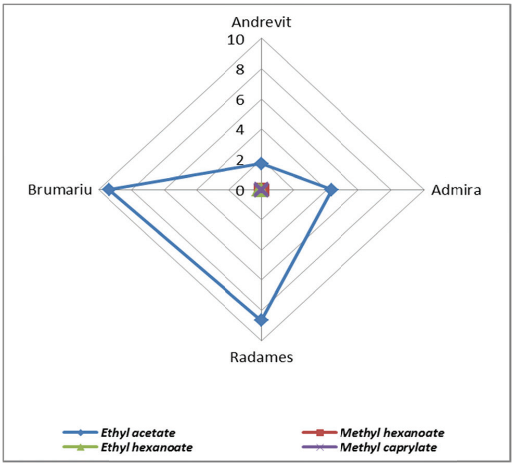

Figure 6. The main esters of a Analyzed musts

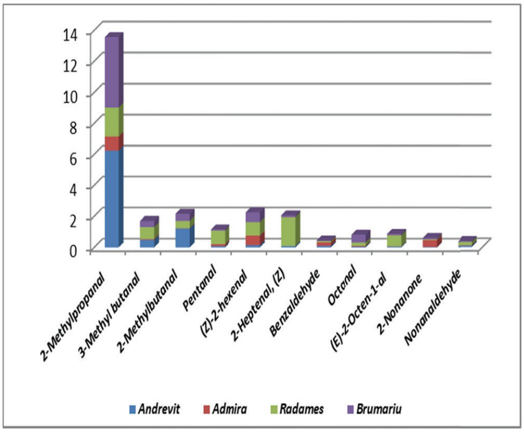

Figure 7. Aldehydes concentration in musts

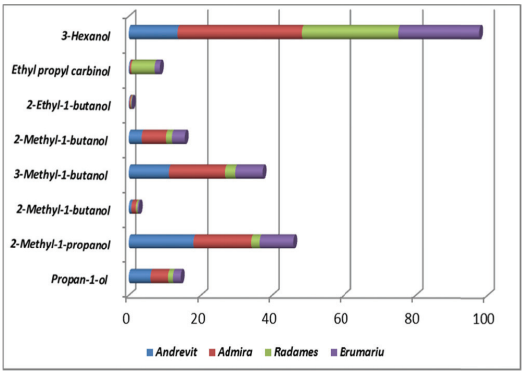

Figure 8. Concentration of higher alcohols

CONCLUSIONS

The longest period of vegetation was recorded by variety 'Radames'; Wine varieties have force average and large and a higher percentage of fertile shoots in wine varieties, especially the variety 'Admira'.

Table varieties, 'Andrevit' and 'Moldova' shows a weight of grapes beyond 250 g and an production index with high values, production of variety exceeds 14-15 t/ha.

At wine varieties the most production was recorded by 'Admira' variety with 13.9 t/ha, 'Radames' records the lowest production between studied varieties.

The concentration of grape sugars record the highest values at 'Admira' variety, resulting wine reaching a degree of alcohol above 11 vol% alcohol and a high value of extract.

Although with increased biological resistance to disease and *phylloxera*, the varieties had different degrees of attack, the best behaved variety was 'Admira'; 'Moldova' variety registered higher attack degree mildew on grapes. GC/MS analysis of volatile compounds revealed a number of compounds that characterize the varieties studied; so, identified esters are: Ethyl acetate, Methyl hexanoate, Ethyl hexanoate, Methyl caprylate. Is distinguished from these varieties a very small number and a low concentration of esters compared with *vinifera* varieties.

Among aldehyde were identified: 2-Methylpropanal, in the highest concentration examined in all varieties, 2 and 3-Methyl butanal, Pentanal, 2-hexenal, 2-Heptenal, Octanal, 2-Octen-1-al, Benzaldehyde, Nonanaldehyde etc. Higher alcohols found: Propan-1-ol, 2-Methyl-1-propanol, 2-Methyl-1-butanol, 2-Ethyl-1-butanol, Ethyl propyl carbinol, 3-Hexanol; this alcohol that leaves a taste of greenery is found in the highest concentration of studied varieties.

Identified terpenes were, in order of traced concentration at the majority of studied varieties: limonene, p-cymene, β-pinene, α-pinene, β-myrcene, etc. We can observe a higher concentration of limonene, in all varieties of case, this terpene being majority, with exception of 'Andrevit' variety, to that 2 terpenes characterized this variety: limonene and p-Cymene.

Table 4. Volatile compounds from musts analyzed

Volatile compounds	*Andrevit*	*Admira*	*Radames*	*Brumariu*
	µg equivalence I.S			
2-Methylpropanal	6.24	0.91	1.87	4.54
Propan-1-ol	6.04	4.86	1.35	2.17
Ethyl acetate	1.70	4.32	8.67	9.36
n-Butanoic acid	0.81	0.06	4.13	0.05
2-Methyl-1-propanol	17.93	16.27	2.32	9.35
3-Methyl butanal	0.49	0.05	0.77	0.39
2-Methylbutanal	1.22	0.02	0.47	0.45
2-Methyl-1-butanol	0.74	1.25	0.44	0.37
Pentanal	0.08	0.13	0.87	0.08
3-Methyl-1-butanol	11.13	15.57	3.07	7.60
2-Methyl-1-butanol	3.57	6.75	1.64	3.57
2-Ethyl-1-butanol	0.17	0.34	0.20	0.16
1-Hexanol	0.42	0.19	4.22	3.14
(Z)-2-hexenal	0.14	0.62	0.87	0.62
Ethyl propyl carbinol	0.24	0.45	6.47	1.47
3-Hexanol	13.52	34.74	26.90	22.59
3-Methyl-1-butanol acetate	0.09	0.09	0.04	0.04
Heptan-2-one	0.07	0.32	0.13	0.05
Methyl hexanoate	0.04	0.05	0.00	0.04
2-Heptenal (Z)	0.09	0.00	1.85	0.12
Benzaldehyde	0.10	0.22	0.08	0.05
2-carene	0.22	0.21	0.24	0.94
2-octanone	0.10	0.08	0.22	0.44
β-myrcen	0.6	0.26	0.13	0.13
Ethyl hexanoate	0.00	0.04	0.05	0.11
Octanal	0.06	0.04	0.21	0.51
delta-3-carene	0.01	0.20	0.42	0.10
β-pinene	0.32	0.72	1.53	2.33
p-Cymene	5.93	0.28	0.35	21.93
Limonene	5.17	22.94	27.91	62.42
(E)-2-Octen-1-al	0.06	0.01	0.71	0.09
α-pinene	0.28	0.61	1.10	1.38
2-Nonanone	0.02	0.46	0.05	0.08
Nonanaldehyde	0.08	0.04	0.21	0.06
Methyl caprylate	0.06	0.05	0.14	0.01
Naphthalene	0.04	0.04	0.02	0.10

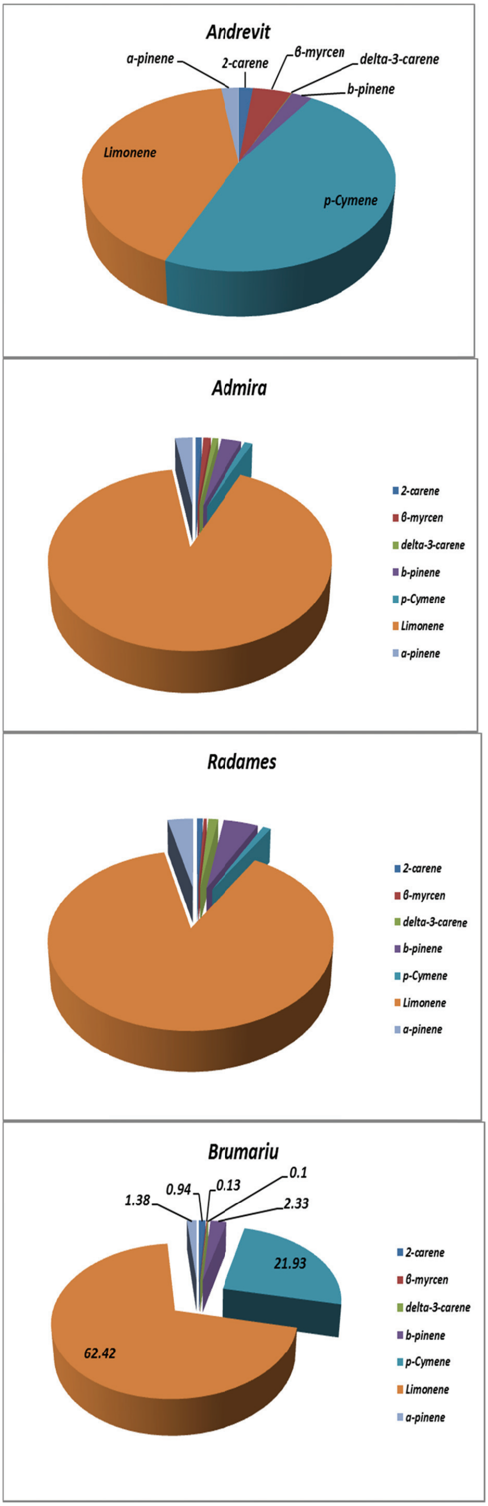

Figure 9. Concentration of terpenes

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EFFECT OF FOLIAR SPRAYING WITH ZINC AND/OR GAMMA RADIATION ON OIL CONTENT AND OIL COMPOSTION OF ANETHUM GRAVEOLENS DURING THREE DEVELOPING STAGES

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Abstract

This study was to investigate the influence of pre-sowing treatment of Anethum graveolens L. seeds, consisted in irradiation with gamma radiation (0, 2, 4, 8, 16, 32 and 64 k-rd doses) with or without zinc (150 ppm), on the oil content and oil composition of plants during vegetative, flowering and fruiting stages. Spraying dill plants with zinc increased the oil content. Generally increasing gamma rays doses increased essential oil. However interaction treatment between zinc and gamma radiation at 8 k-rad dose recorded the highest values of essential oil in vegetative and fruiting stages, while interaction between zinc and gamma radiation at 2 k-rad dose recorded the highest essential oil in flowering stage. The major compounds were found to be α-phellandrene, limonene, β-phellandrene and p-cymene in the vegetative stage, p-cymene, carvone, dillether and dillapiole in the flowering stage, whereas, carvone, dillapiole and limonene in the fruiting stage.

Key words: *dill (Anethum graveolens L.) essential oil, gamma radiation, zinc*

INTRODUCTION

Anethum graveolens L. "Dill" is an annual herb from *Apiaceae* family used as a spice and medicine, It originates from the Mediterranean and West Asia. Herb and seed oil have also been widely investigated in respect of their antiseptic and exhibits anticarcinogenic, antimicrobial and antioxidant activity (Weiss, 2002).

Zinc acts as a metal component of various enzymes or as a functional, structural or regulatory cofactor (Marschner, 1995). Irradiated seeds with gamma rays induced biochemical, physiological and cytological changes (Korosi and Pal, 1989).

Therefore, this study aims to study the effect of zinc and gamma radiation treatment on essential oils content and composition of dill plants at different developing stages.

MATERIALS AND METHODS

The experiment was carried out at the Farm Station of National Research Centre, Egypt during the seasons of 2000/2001 and 2001/2002. Dill seeds were sown in 15 of October in both seasons. Dry seeds of dill were irradiated with gamma rays doses at 2, 4, 8, 16, 32 and 64 k-rad before sowing. The source of irradiation is installed at the Middle Eastern Radioisotope Centre for the Arab Countries, Dokki, Giza, Egypt. Zinc EDTA (150 ppm) was applied as foliar spray at interval times of 30 and 60 days after sowing. The essential oil of fresh herb at vegetative stage (90 days after sowing), flowering stage (180 days after sowing) and air dried seeds (fruiting stage, 210 days after sowing) was subjected to hydro-distillation for 3 hours using a modified Clevenger apparatus according to Guenther (1961). The resulted oil was dehydrated over anhydrous sodium sulphate and stored at freezer till used for gas liquid chromatographic (GLC) analysis.

RESULTS AND DISCUSSIONS

1-Essential oil ratio

The results presented in the Table 1 show that, volatile oil ratio in dill herb during vegetative stage in both seasons was significantly increased by zinc application and gamma radiation doses in most cases. In this concern, the largest ratio of volatile oil in fresh herb was found by the irradiated seeds at 8 k-rad with zinc (0.0800 and 0.0866%) in the two seasons, respectively.

From the same table it can be noticed that all treatments caused a positive effect on the volatile oil ratio in herb during flowering stage in both seasons in comparison to control in most cases. Generally, volatile oil ratio in herb responded significantly to zinc application in the second season.

Gamma rays significantly increased volatile oil ratio of herb at flowering stage in both seasons. However, the highest volatile oil ratios was recorded by irradiated plants with 2 k-rad (1.1666 and 1.1183%) in the flowering stage during the two seasons.

The results given in Table 1 also show that, application of zinc insignificantly increased volatile oil ratio in dill fruits in the two seasons. In this regard, the irradiated seeds with gamma doses up to 32 k-rad led to significant increases in volatile oil percentages of fruits in the two seasons, excepting the dose of 64 k-rad with or without zinc which induced a reduction of volatile oil percent in the second season comparing to the control.

There was significant effect on volatile oil ratio for the interaction of zinc and gamma radiation in the two seasons. The largest ratio of volatile oil in dill fruits was resulted by the treatment of 8 k-rad (3.4166 and 3.2333%) in both seasons, respectively. This superiority might be the result of the cumulative effect of the zinc application on enzymes activity and metabolism improvements.

These results are in agreement with those found by Jeliazkova et al. (1997), Said-Al Ahl and Omer (2009) on *Coriandrum sativum* and Yadegari (2013) on *Borago officinalis.*

Table 1. Effect of foliar spray with zinc, gamma radiation and their interaction treatments on volatile oil content (%) of dill plants

Zinc (ppm) / Gamma radiation (k-rad)	First Season								
	Vegetative stage (90 days after sowing)			Flowering stage (180 days after sowing)			Fruiting stage (210 days after sowing)		
	Herb			Herb			Fruits		
	Zn (0 ppm)	Zn (150 ppm)	Mean	Zn (0 ppm)	Zn (150 ppm)	Mean	Zn (0 ppm)	Zn (150 ppm)	Mean
0	0.0600	0.0700	0.0650	0.7150	0.7666	0.7408	2.3666	2.7333	2.5499
2	0.0633	0.0733	0.0683	1.1666	0.9733	1.0699	2.9166	2.9500	2.9333
4	0.0633	0.0733	0.0683	0.9383	0.9650	0.9516	2.8333	2.8666	2.8499
8	0.0766	0.0800	0.0783	0.8550	0.8650	0.8600	3.4166	3.1500	3.2833
16	0.0733	0.0733	0.0733	0.8983	0.9316	0.9149	2.8833	2.9166	2.8999
32	0.0566	0.0600	0.0583	0.8350	0.8900	0.8625	2.8166	2.6833	2.7499
64	0.0600	0.0633	0.0616	0.8850	0.8916	0.8883	2.5833	2.5333	2.5583
Mean	0.0647	0.0704		0.8990	0.8975		2.8309	2.8333	
L.S.D. at 5%	**Zinc** = 0.0029 **Radiation** = 0.0055 **Interaction** = N.S			**Zinc** = N.S **Radiation** =0.0328 **Interaction** =0.0463			**Zinc** = N.S **Radiation** =0.0832 **Interaction** =0.1177		
	Second Season								
0	0.0633	0.0733	0.0683	0.7183	0.8400	0.7791	2.4833	2.5500	2.5166
2	0.0766	0.0833	0.0799	1.1183	1.0416	1.0799	2.8166	2.9500	2.8833
4	0.0766	0.0800	0.0783	0.8966	0.9033	0.8999	2.8500	2.9833	2.9166
8	0.0800	0.0866	0.0833	0.8866	0.8933	0.8899	3.2333	2.9333	3.0833
16	0.0733	0.0800	0.0766	0.8450	0.9100	0.8775	2.6500	2.6666	2.6583
32	0.0666	0.0700	0.0683	0.8150	0.8716	0.8433	2.9166	2.8000	2.8583
64	0.0733	0.0766	0.0749	0.8650	0.9150	0.8900	2.4666	2.4000	2.4333
Mean	0.0728	0.0785		0.8778	0.9106		2.7737	2.7547	
L.S.D. at 5%	**Zinc** = 0.0031 **Radiation** = 0.0059 **Interaction** = N.S			**Zinc** = 0.0147 **Radiation** = 0.0276 **Interaction** =0.0390			**Zinc** = N.S **Radiation** =0.0804 **Interaction** =0.1137		

2-Essential oil constituents

a- In the vegetative stage

The main constituents of the essential oils distilled from fresh herb during vegetative stage as affected by spraying zinc and gamma radiation doses are shown in Table 2; the results revealed that in all treatments, α-phellandrene ranged from 33.9058 to 54.5671%, limonene ranged from 10.8714 to 13.7269%, β-phellanrene ranged from 6.4833 to 13.7149% and p-cymene ranged from 11.6728 to 27.0142%. The results within hand indicated differences in the volatile oil compositions due to the action of different treatments. Thus, zinc treatment was distinguished with high content of α-phellandrene (54.5671%), followed by the treatment of 16 k-rad, 8 k-rad and then 2 k-rad giving the contents of 54.3210, 53.6559 and 49.5163%, respectively, on the expense of limonene, β-phellandrene and p-cymene that reduced. On the other hand, all treatments decreased the content of limonene while control plants recorded the highest content of limonene (13.7269%). β-phellandrene content increased with the treatment of 32 and 64 k-rad giving the highest contents (13.7149) and 11.9062%), respectively, compared to the other treatments. All treatments except of 2 k-rad + zinc and 64 k-rad + zinc decreased p-cymene content and these doses resulted in the highest p-cymene content (27.0142 and 24.5739%), respectively, compared to the other treatments. However, Nagiub et al. (1998) on *Anethum graveolens* found that foliar application with zinc at 50 and 75 ppm led to increase linalool content and decreased the contents of limonene and carvone of herb essential oil. El-Sawi and Mohamed (2002) found differences in the oil constituents of cumin herb by application of zinc spray which increased the main constituents such as cumin aldehyde and decreased β-pinene, p-cymene, α-terpineol and thymol.

Table 2. Effect of foliar spray with zinc, gamma radiation and their interaction treatments on the constituents of dill volatile oil "herb" (90 days after sowing) in the second season (2001/2002)

Treatments / Identified compounds	Control	2K-rad	4K-rad	8K-rad	16K-rad	32K-rad	64K-rad	Zn(150ppm)	2K-rad +Zn	64K-rad +Zn
α-pinene	1.6383	1.7974	1.8901	1.9763	1.9059	1.7525	1.9106	1.9403	trace	trace
β-pinene	0.2781	0.2673	0.2881	0.3030	0.2980	0.2602	0.3333	0.2879	trace	trace
α-phellandrene	46.3320	49.5163	41.7208	53.6559	54.3210	33.9058	47.1898	54.5671	trace	trace
Limonene	13.7269	12.8234	11.7102	12.9282	13.1126	10.8714	12.9978	13.1349	trace	trace
β-phellandrene	11.0111	9.1626	10.9918	9.6725	9.4752	13.7149	11.9062	8.7047	6.6631	6.4833
γ-terpinene	0.3637	trace	trace	0.2759	0.2590	0.3384	0.2690	trace	trace	trace
P-cymene	17.8812	13.4692	15.6183	12.8502	12.0912	16.1557	11.6728	14.5944	27.0142	24.5739
Dillether	0.4540	0.3238	0.4013	0.2772	0.2914	0.4138	0.2669	0.3519	trace	trace
Dihydrocarvone	0.2261	0.2549	0.2345	0.2649	0.1923	0.7562	0.3387	trace	0.3129	trace
Sabinol	1.0462	0.9648	1.3265	1.1019	1.2430	2.5898	0.8829	0.9599	2.2044	trace
Carvone	2.1161	1.9052	2.8169	1.5604	1.3942	4.0910	2.0420	1.3117	3.2173	1.9718
Piperitone	0.2391	0.2070	0.5379	0.3164	0.1971	0.5233	0.1876	trace	5.2361	4.9990
Carveol	0.6624	0.8569	1.4896	0.8318	0.6479	2.1876	0.9592	0.5573	0.9825	7.2734
Nerolidol	0.7168	1.5388	1.0765	0.6572	0.6761	2.3750	2.4901	0.4743	4.4874	6.5424
Eugenol	0.7961	1.2686	1.0898	0.6958	1.1251	1.6109	1.1639	0.9984	6.6546	8.4158
Thymol	0.5909	0.6691	1.0498	0.4810	0.8397	1.1915	1.7360	0.3305	1.9846	4.5253
Carvacrol	0.2442	0.2911	0.6435	0.2303	0.1913	0.6637	0.3205	trace	3.8849	6.9117
Myristicin	1.0782	0.2451	0.2560	1.3051	0.1304	0.6478	0.3162	1.1032	1.8029	2.0332
Dillapiole	0.5977	0.2148	0.4870	0.6150	0.1518	0.5421	0.2644	0.6829	2.5156	2.6660
Total	99.9991	95.7763	93.6286	99.9990	98.5432	94.5916	97.2479	99.9994	66.9605	76.3958

Table 3. Effect of foliar spray with zinc, gamma radiation and their interaction treatments on the constituents of dill volatile oil "herb" (180 days after sowing) in the second season (2001/2002)

Treatments / Identified compounds	Control	2K-rad	4K-rad	8K-rad	16K-rad	32K-rad	64K-rad	Zn(150ppm)	2K-rad +Zn	64K-rad +Zn
α-phellandrene	0.5948	0.4488	0.27375	0.1389	0.21505	0.1768	0.52785	1.13205	0.7287	5.7479
Limonene	0.4896	13.9558	13.09455	1.56535	0.2465	0.1850	0.65075	2.4146	1.2625	3.40155
β-Phellandrene	2.7002	3.84975	1.9140	1.6345	1.4729	0.99805	2.7177	7.67235	11.84175	3.51335
P-cymene	33.4236	30.13605	12.4088	15.6645	20.9028	10.93595	18.9239	14.1821	22.66095	19.80595
Linalool	0.3549	2.15815	3.19355	0.5088	0.08135	0.2745	0.2579	trace	1.8701	2.06605
Dillether	19.6369	7.4865	6.9993	23.4015	6.04855	25.32085	14.6603	26.53655	19.8128	13.2396
Dihydrocarvone	0.7368	0.7338	1.7367	1.23395	4.0528	1.7255	1.37045	1.2932	2.9191	2.9056
Sabinol	0.3132	1.09445	0.8439	trace	1.9289	0.41315	0.1487	1.4508	2.2814	0.23635
Carvone	13.1097	8.10495	14.98475	28.3321	16.2905	19.94225	15.42275	21.20685	9.0318	10.9535
Piperitone	4.60385	2.7639	2.9082	4.2495	4.01995	6.70485	4.5431	0.6625	2.85235	4.7520
Carveol	3.2440	4.28835	4.15145	1.6229	0.91125	3.0084	1.75465	0.7319	1.0365	6.8739
Nerolidol	1.48645	2.4969	3.8250	0.9874	0.63875	1.5302	1.18235	0.5108	0.95215	2.8895
Eugenol	1.55765	1.4469	1.3751	1.5779	1.24535	1.13135	1.4503	0.51765	0.75135	1.58405
Thymol	0.85815	1.33125	1.4006	0.4414	3.0775	0.8049	1.53185	2.96245	1.5438	1.11485
Carvacrol	1.6281	0.98895	1.1322	1.5123	1.79115	1.1125	1.3649	0.66955	1.12255	trace
Myristicin	0.6435	1.72055	1.44405	1.1224	2.06025	0.72205	1.00725	0.6748	0.81925	1.0386
Dillapiole	4.1622	8.40175	17.50245	11.7855	17.30025	19.1895	12.54755	10.34655	8.44505	13.88545
Total	89.5436	91.4068	89.18835	95.7789	82.2838	94.1758	80.06225	92.9647	89.9321	94.0082

Table 4. Effect of foliar spray with zinc, gamma radiation and their interaction treatments on the constituents of dill volatile oil "fruits" (210 days after sowing) in the second season (2001/2002)

Treatments / Identified compounds	Control	2K-rad	4K-rad	8K-rad	16K-rad	32K-rad	64K-rad	Zn(150ppm)	2K-rad +Zn	64K-rad +Zn
Limonene	14.6156	15.2200	21.1883	22.1267	21.7131	17.8006	7.2642	17.4400	9.2528	trace
P-cymene	0.3043	0.4831	0.2931	0.2633	0.3151	0.2167	trace	0.3474	trace	trace
Linalool	0.0173	0.0467	0.0191	0.0375	0.0168	0.0147	trace	0.0431	trace	trace
Dillether	1.6490	2.1049	1.6696	2.0552	2.3632	1.6495	1.8385	2.1059	20.3966	trace
Dihydrocarvone	0.0740	0.0668	0.0620	0.0656	0.0518	0.0488	trace	0.0636	trace	trace
Sabinol	0.0301	0.0496	0.0250	0.0225	0.0180	0.0033	trace	0.0321	1.9877	trace
Carvone	62.4883	57.2566	57.2408	51.5096	57.5308	59.5012	65.6520	60.1803	1.8758	26.6766
Piperitone	0.2106	0.1693	0.1688	0.1676	trace	0.0066	trace	0.2455	5.2112	2.3167
Carveol	0.0072	trace	0.0079	trace	0.0146	0.0107	trace	trace	trace	trace
Nerolidol	0.0165	0.0229	0.0042	0.0165	0.0049	0.0013	trace	trace	trace	5.4549
Eugenol	0.0053	0.0094	0.0042	0.0086	0.0084	0.0064	trace	trace	3.9766	3.8817
Thymol	0.0144	0.0194	0.0215	0.0323	0.0225	0.0196	trace	0.0202	0.7513	trace
Carvacrol	0.0299	0.0461	0.0329	0.0323	0.0246	0.0303	0.0322	0.0367	5.5313	trace
Myristicin	0.0555	0.0756	0.0722	0.0354	0.0339	0.0891	0.0812	0.0934	2.5200	0.8192
Dillapiole	19.5113	23.4544	18.3871	22.8572	17.1895	19.7647	22.4265	18.4222	5.1628	13.9000
Total	99.0293	99.0248	99.1967	99.2303	99.3072	99.1635	97.2946	99.0304	56.6661	53.0491

b- In the flowering stage

The influence of zinc and gamma radiation singly or collectively on the compounds of volatile oil distilled from fresh herb during flowering stage are recorded in Table 3. It is obvious that four main compounds were identified such as p-cymene ranging from 10.93595% (under 32 k-rad treatment) to 33.4236% (control plants), carvone ranging from 8.10495% (under 2 k-rad treatment) to 28.3321% (under 8 k-rad treatment), dillether ranging from 6.9993% (under 4 k-rad treatment) to 26.53655% (zinc treatment) and dillapiole ranging from 4.1622% (control plants) to 19.1895% (under 32 k-rad treatment). Zinc treatment increased dillether, dillapiole and β-phellandrene. All treatments caused an increase in dillapiole content. On the other hand, all treatments caused a decrease in p-cymene content. Gamma ray doses from 4 to 64 k-rad increased carvone content. Gamma ray at 32 k-rad resulted in the highest piperitone content (6.70485%), whereas, 2 k-rad+zinc and 64 k-rad+zinc gave the highest compound of β-phellandrene (11.84175%) and carveol (6.8739%), respectively, when compared with the other treatments.

c- In the fruiting stage

Data presented in Table 4 pointed out that pre-sowing gamma radiation and spraying zinc application alone or together caused variable changes in the ratios of different ingredients which represented the constituents of volatile oil distilled from dill seeds under investigation. It can be remarked that 15 compounds were identified. All treatments showed as main compounds carvone, dillapiole and limonene. Carvone ranged from 1.8758% (under 2 k-rad + zinc treatment) to 65.6520% (under 64 k-rad treatment), dillapiole ranged from 5.1628% (under 2 k-rad + zinc treatment) to 23.4544%% (under 2 k-rad treatment) and limonene ranged from 7.2642% (under 64 k-rad treatment) to 22.1267 (under 8 k-rad treatment). As general trend, limonene percent was considerably increased by spraying zinc alone and pre-sowing seeds with gamma radiation from 4 to 32 k-rad. Gamma rays at 2, 8, 32 and 64 k-rad increased dillapiole, whereas 64 k-rad treatments increased carvone and 2 k-rad + zinc treatment gave the highest content of dillether (20.3966%) at the expense of carvone (1.8758%).

CONCLUSIONS

Our two-year experiment involving several treatments showed the great differentiation in dill volatile oil content extracted from fresh herb at vegetative stage, fresh herb at flowering stage and seeds at fruiting stage as well as major components of essential oils during vegetative, flowering and fruiting stages.

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FLOATING HYDROPONIC CROPS PROPOSAL: TONLE SAP LAKE, CAMBODIA

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Abstract

The paper presents an innovation project elaborated by the authors as an entry for the Empowering People Award, an international competition launched by Siemens Company in 2013. The aim of the competition was to develop sustainable solutions in order to improve the quality of life in local communities from emerging countries. An important condition was the use of eco-friendly technologies, in order to solve supply problems considering financial constraints. The proposed solution represents a floating vegetables garden based on a simple hydroponics technology system which is powered by solar energy and is mostly made of recyclable and local materials. The location of the project is the area of Tonle Sap Lake (Kingdom of Cambodia), the largest freshwater lake in South-East Asia, a rural region with more than 1 million inhabitants. The aim of the design was to improve and to diversify food supply for the residents of the floating villages, considering the primary food resources are fish and rice. Thus, the main objectives of the solution were to enhance the life quality of the local communities in a sustainable manner, both at social, economic and ecological level. The results of the study include: target groups, technical feasibility and functionality, technical plans and illustrations of the proposed solution.

Key words: *Cambodia, Floating villages, Hydroponics, Sustainability, Vegetables growing*

INTRODUCTION

The proposed solution represents a floating vegetables garden based on a simple hydroponics technology system, using mostly recyclable and local materials. The location of the project is the area of Tonle Sap Lake (Kingdom of Cambodia), the largest freshwater lake in South-East Asia.
The aim of the design is to improve and to diversify food supply for the inhabitants of the floating villages, considering the primary food resources are fish and rice. Thus, the main objectives of the solution are to enhance the life quality of the local communities in a sustainable manner, both at social, economic and ecological level.

MATERIALS AND METHODS

Following the competition launched by Siemens a study has been done for the implementation of a floating hydroponic garden system for Tonle Sap Lake. The first phase included a theoretical research regarding local conditions. The results led us to analyze to what extent a sustainable solution to improve food security in the local communities can be implemented. The second phase of the study included the evaluation of the premises for making a product based on sustainable principles: renewable energy, using recycled materials, using local resources, minimal environmental impact and landscape integration. The costs to obtain a minimum budget were also taken into consideration. Following the two research stages a floating hydroponic culture system resulted, responding to the identified issues in the Tonle Sap Lake.

RESULTS

Tonle Sap is the largest freshwater lake in Southeast Asia, a generator of biodiversity and source of living for most of the population of Cambodia. The food is provided by fishing and rice cultivation, while the shelters in the form of floating settlements are made of local materials. Intensive exploitation and lack of conservation strategies puts in danger the future of this area. Due to overfishing, of 500 species of fish existing initially, there are only 300 now. From an original area of 1 million ha of forest there are only 20,000 ha remaining now due to the incontrollable expansion of agricultural area. While the resources are diminishing, the population grows and the need to find alternatives for providing food is only required.

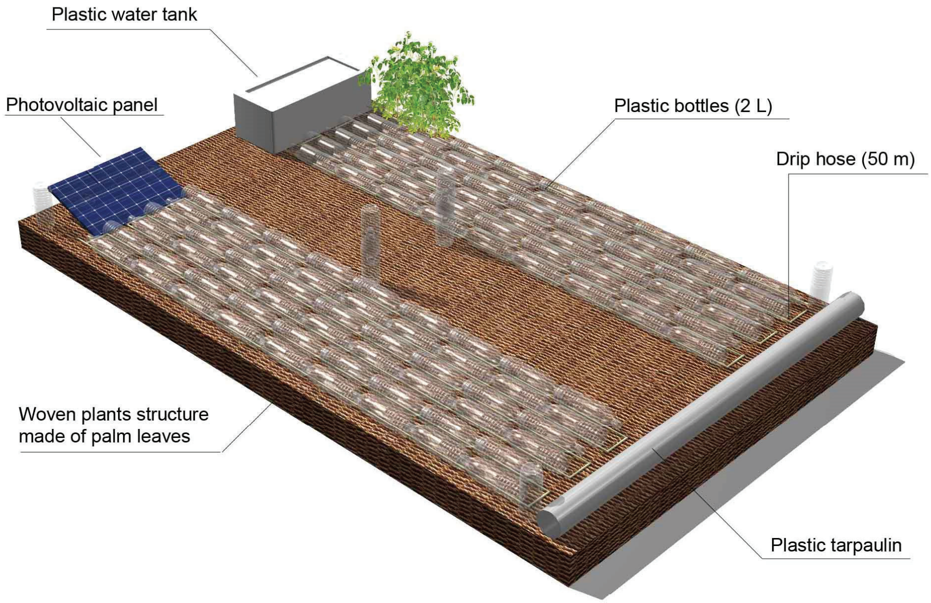

Figure 1. Floating hydroponic garden proposal - 3D image

Through this project we propose the development of low-cost floating gardens with important long-term benefits such as increasing crop areas without deforestation, balancing the population's diet that relies in present almost entirely on rice and fish, protection of the remaining species of fishes. The project aims primarily at people in lacustrine settlements. A floating garden can be attached to each house and it consists of one or more units based on the needs of each family and on the space available for configuration. The assembly can be easily accomplished both in terms of purchase of materials and their cost and the actual process of construction. Using the garden is also easy and can be done by any family member from kids to adults with minimal costs.

The proposed floating crops garden represents a horizontal hydroponic system, adapted to the natural conditions and social needs of Tonle Sap Lake area, Cambodia. So, a large part of the construction materials are of local origin, eco-friendly and recyclable. In order to minimize the costs, to reduce the environmental impact and to benefit from the natural local resources, the floating crops contain a simple self-sustaining solar energy generator. Also, all the materials, excepting the solar pump, doesn`t require major investments. According to organic farming principles, the nutrients used for the crops would be made of local fish emulsion. Thus, the proposed technology is based on sustainability principles in terms of environment, energy, agriculture and economy. The components of the floating

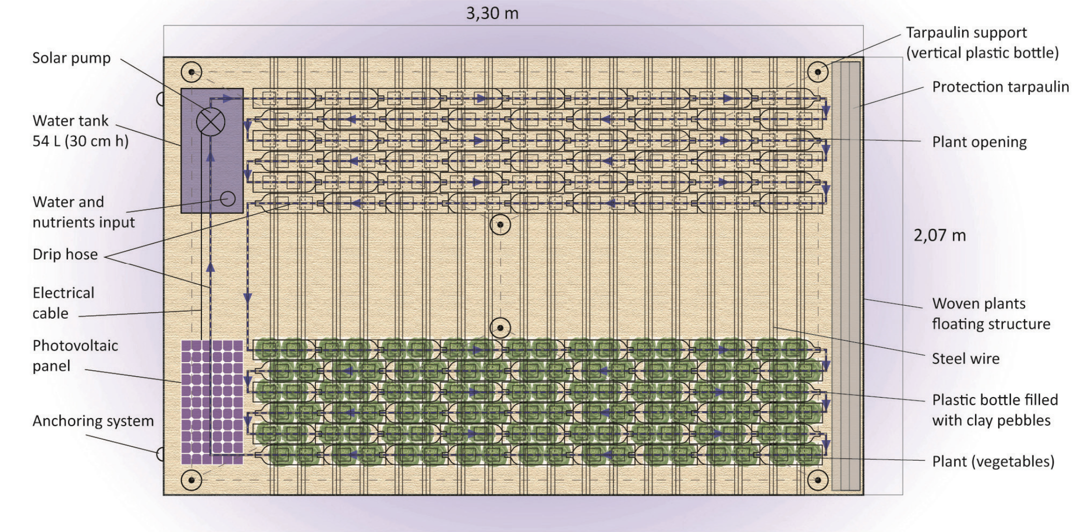

Figure 2. Floating Hydroponic Crops – Plan view

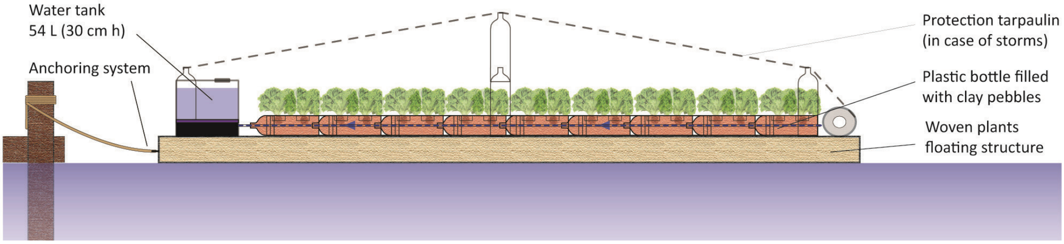

Figure 3. Floating Hydroponic Crops – Longitudinal section view

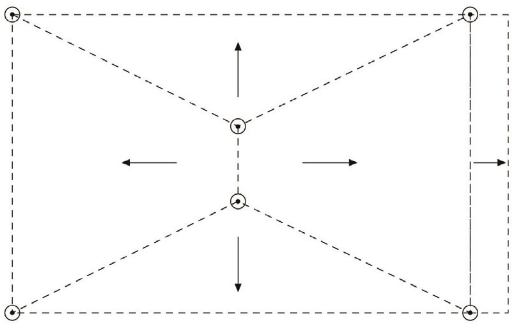

Figure 4. Tarpaulin structure made of vertical plastic bottle

Figure 5 Floating garden proposal for local households

garden include: a simple solar pump (photovoltaic panel included), a drip hose (50 m), a plastic water tank (50 L), steel wire (100 m), a plastic tarpaulin (for protection in case of storms), a rope (15 m), clay pebbles (40 L), nutrients (natural fish emulsion), 50 plastic bottles (2 L), woven plants structure made of palm leaves.

Vegetables species that can be cultivated on the floating garden include: lettuce, Chinese cabbage, tomatoes, basil, spinach, etc. The constructing process of the floating garden is not complicated, so that it can be built without requiring the presence of a specialist. Thus, the plastic bottles filled with clay pebbles are installed on the woven plants structure and fixed on it with steel wire (as shown in the flowing diagram). A solar pump placed inside the water tank activates the irrigation system, by a drip hose which connects all the plastic bottles. Water and nutrients should be inserted manually into the water tank, depending on the needs of the plants. Considering the variable level of Tonle Sap Lake, the floating garden has to be anchored to a close built structure by a rope.

CONCLUSIONS

The functionality of the floating garden relies on sustainable development principles in terms of health, social, economic, financial and environmental issues. The main task of the project was to solve the basic supply problems in Tonle Sap Lake Area.

Thus, the proposed solution would improve the quality of life for the local communities by increasing and diversifying the food supply, in a healthy manner, without any use of harmful substances. The system has a very low impact on the local environment taking into account the use of biological nutrients. Regarding the economic and financial issues, the concept could be developed as a family business or used just for self-consumption. In the second case, it would contribute to local job creation, depending on the size of the business. After the initial investment, the costs would be very low on long term considering that all the resources can be found in the area. These include: fresh water (for irrigation), solar energy (for water recirculation pump), fish emulsion (for nutrients), seeds (from the plants cultivated on the floating gardens).

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THE INFLUENCE OF TEMPORARY PROTECTION ON PRODUCTION PRECOCITY OF EGGPLANTS CULTIVATED ON SANDY SOILS IN SOUTHERN OLTENIA

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Abstract

In order to increase the production precocity of eggplants, different cultivation methods were studied: 1 - unprotected crops; 2 - soil mulching with polyethylene; 3 - protected plants with polyethylene tunnel; 4 - protected plants with polyethylene tunnel and soil mulching with polyethylene; 5 - protected plants with agryl type foil and 6 - protected plants with agryl and soil mulching with polyethylene .The protection system was maintained until around 20 of May, when the air temperature reached the threshold of 30^{0}C. The methods of protection employed created different microclimate conditions that influenced the different processes of growth and development with implications on early yield. Considering the precocity, only the variant protected with agryl type foil and soil mulched with polyethylene was harvested before 15 of June. During 16-30 of June variant protected with agryl and mulched harvested 5 t/ha, while variant only protected with agryl harvested 3.5 t/ha. In the unprotected variants the first harvests were obtained between 1-15 of July. Through the mulching of soil with polyethylene the precocity increases regardless the method of plants protection.

Key words: *eggplants, precocity, protection****,*** *Agryl*

INTRODUCTION

Eggplants grow and develop normally at optimum temperatures of 25-30^{o}C and can withstand temperatures of 45^o C. The grow stops at temperatures below 10^0 C. The plants are destroyed by frost and affected by cold periods without frost (Ceausescu et al., 1980). Air currents are unfavourable for eggplants which require protecting measures of culture. Insufficient light determines stagnation of growth, flowers abortion, and related fruits to remain small. Eggplants are demanding water plants; the optimal level of water supply of the soil is 70% at depth of 50 cm (Marinica Gh., 1989). Getting early yields from eggplant implies using of early varieties (Toma V. et al., 1999) or crops' protection (Voican V., and Lacatus V., 1998). For the greenhouses culture of eggplant it should be used specifically adapted varieties (Pochard E., 1974). To increase precocity in eggplants field crops vegetable seedlings has to be planted much earlier than the optimal time and therefore the young plants are exposed to many risky factors. The normal metabolic processes in plants are performed only if the environmental conditions for each species are assured. Removal or reduction of undesirable effects occurring in the early part of the period from vegetation can be achieved by applying technologies adapted to the cultural area. So far, it have been carried out researches concerning protecting the culture of eggplant on all over the vegetative period.

In this paper temporary protection of eggplant crops in order to avoid unwanted effects occurred in the early part of vegetation period, to improve plants growth and development and, thus, to increase precocity was studied. Plants protection is not necessary during June, July and August, when heat surplus in the studied area is recorded.

Temporary protection of the eggplant culture is an important mean of increasing the precocity, contributing at market fresh products supply and capitalizing the efficiency of specific climate conditions of the sandy soils area of southern Oltenia.

MATERIALS AND METHODS

Research has been carried out during 2011-2013 at CCDCPN Dăbuleni, Southern Oltenia, on a sandy soil with less than 1% humus, 0.06-0.11 ppm total nitrogen, 55-60 ppm potassium and 86-100 ppm phosphorus content. The experiment with eggplant crops had the following variants: V1 - unprotected crops – control; V2 - soil mulching with polyethylene; V3 - protected plants with polyethylene tunnel; V4 - protected plants with polyethylene tunnel and soil mulching with polyethylene; V5 - protected plants with Agryl type foil and V6 - protected plants with Agryl and soil mulching with polyethylene. Experience was located in the experimental field in 4 randomized blocks. *Aragon* F_1 was used in the experiment. Planting in the field was done around 15 of April, with 15 days earlier than the optimal date of eggplant planting in area. The system of plants protection was installed at the same time with planting and maintained until 20 of May, when no longer risk of temperatures below the biologic threshold for eggplant arise.

The height of plants at the time of protection system removal, total yield and yield dynamics were determined. The obtained results were calculated and statistically analysed.

RESULTS AND DISCUSSIONS

Cover the ground with polyethylene mulch determined soil heating, kept soil moisture, and contributed to the increase of plants height with 6 cm. Protecting plants with polyethylene tunnel has accelerated the growth, the plants register 24.3 cm, with 12.1 cm more than in the unprotected plants variant. Plants raised under protection of Agryl measured 24.3 cm height, with 14.3 cm more than the unprotected ones. The resulted microclimate by protecting plants of eggplant has influenced the growth dynamic of plants. At removal time of protection system the plants height was comprised between 10.0 - 29.2 cm (Table 1). The porous nature of Agryl allows the penetration of air inside and create a favourable microclimate for the eggplants. Also, by adding polyethylene mulch plants growth is favoured too. Plants with mulch and tunnel of polyethylene had the height of 25.9 cm, and plants protected with mulch and Agryl recorded the largest increase (29.2 cm).

Table 1. The plant height of eggplant at the protection system removal

Variant	Plants height (cm)	Plants height (%)	Difference (cm)	Significance
Unprotected crops	10.0	100	Mt.	
Soil mulching with polyethylene	16.0	160	+6.0	*
Protected plants with polyethylene tunnel	22.1	221	+12.1	***
Polyethylene tunnel and polyethylene mulch	25.9	259	+15.9	***
Protected plants with Agryl	24.3	243	+14.3	***
Protect with Agryl and polyethylene mulch	29.2	292	+19.2	***

LSD 5% = 5.37 cm
LSD 1% = 7.63 cm
LSD 0.1% = 11.06 cm

The protection of plants has accelerated the growth development and advancing the eggplant production. The first harvest was performed at different data (Table 2). By 15th of June, it have been harvested eggplants only in protective conditions (polyethylene mulch and Agryl). The average production obtained up to this time was of 1 t/ha. During the period 16-30 June, the culture protected with mulch and Agryl has been harvested and it was obtained a production of 5.1 t/ha. Only Agryl protection conduct to an harvested yield of 3.5 t/ha, and for the protected culture with polyethylene tunnel and mulch it has been harvested 1.9 t/ha. The protected culture only with polyethylene tunnel and only with polyethylene mulch the harvest recorded was of 2 t/ha.

First harvest for the unprotected crop have been done during the 16-30 June period, because of the low temperatures which determined extending vegetation period and a delay in obtaining the production. Although the mulch protects only the root of the plant, soil mulching with polyethylene contributed to both yields and precocity increases at eggplant because the mulch heats the ground well, it keeps the moisture in the soil, and minerals substances are not lost through leaching.

Table 2. Dynamics of eggplant yield depending on the method of protection

Variant	The yield of eggplant (t/ha) obtained in the period:							
	Up to 15 of June	16-30 June	1-15 July	16-31 July	1-15 August	16-31 August	1-15 September	16-30 September
V1	-	-	9.0	15.7	11.8	7.2	3.2	1.5
V2	-	2.2	9.5	18.1	10.9	7.4	2.9	1.4
V3	-	2.2	8.7	16.5	11.8	11.1	2.9	1.5
V4	-	3.0	10.0	17.0	14.4	8.6	3.2	1.8
V5	-	3.5	11.6	15.3	12.2	8.5	3.8	1.7
V6	1.0	5.1	12.3	18.2	12.2	9.1	2.8	1.1

It is known that polyethylene let the solar radiation to penetrate easily causing the increase of temperature inside. In the days with high solar radiation temperature inside the shelters may reach high values against the requirements of the plants. Very high temperatures increases the transpiration process and reduced the accumulation of carbohydrates in plant due to a respiration intensification process. This explains the delay of fruits harvested at protected eggplants with polyethylene tunnels. In order to the maintain the high production, the Agryl was kept also in the 1-15 July period. The yields reached to 11.6 t/ha in the variant protected with Agryl and 12.3 t/ha in the variant protected with Agryl and mulched with polyethylene. In the other variants yields varied between 8.7-10 t/ha. During 16-31 of July, the obtained yields varied between 15.3-18.2 t/ha. Up to the end of vegetation period the harvests of eggplants have dropped gradually and the differences were small between variants. Total yield of eggplant was influenced by the method of protection (Table 3).

Table 3. The yield of eggplant depending on the method of protection

Variant	Yield		Difference (t/ha)	Significance
	t/ha	%		
Unprotected crops – control	48.4	100	Mt.	
Soil mulching with polyethylene	52.4	108	+4.0	
Protected plants with polyethylene tunnel	54.7	113	+6.3	*
Polyethylene tunnel and polyethylene mulch	58.0	120	+9.6	**
Protected plants with Agryl	56.6	117	+8.2	*
Protect with Agryl and polyethylene mulch	61.8	128	+13.6	***

LSD 5% = 5.50 t/ha
LSD 1% = 7.82 t/ha
LSD 0.1% = 11.33 t/ha

The smallest eggplant yield (48.4 t/ha) was obtained in unprotected variant. Compared to unprotected variant in all other variants yield increases between 2.5-8.5 t/ha were achieved. In the variant with polyethylene mulched soil the yield was of 52.4 t/ha, recording an increase of 4.0 t/ha compared to the control. Protection with polyethylene tunnel determined a statistically significant yield increase of 6.3 t/ha compared to the control. Adding polyethylene mulch under the polyethylene tunnel increased the eggplant yield at 58.0 t/ha determining a significantly distinct yield difference of 9.6 t/ha compared to the control. By covering plants with Agryl it was obtained a production of 56.6 t/ha, and by adding mulch under Agryl it has been registered an increase the production with 8.2 t/ha.

The highest eggplant yield was obtained in conditions of protection with Agryl and soil mulching with polyethylene. The yield was of 61.8 t/ha, with a very significant increase of 13.6 t/ha compared to the control.

CONCLUSIONS

Microclimate created by protecting crops ensures an increase in plants' height. The largest increase in plants' height was registered in conditions of protecting with mulch and Agryl and it was about 29.2 cm, with 19.2 cm more than in the unprotected plants.

The protection methods used have helped in creating outstanding microclimate conditions with the influence on both precocity and total yield of eggplants.

By protecting the plants with polyethylene mulch and Agryl the first eggplant fruits can be harvested before 15 of June, with 15-20 days earlier than in unprotected conditions.

The biggest yield (61.8 t/ha) was obtained in conditions of protecting plants with Agryl and mulching soil with polyethylene. Adding mulch to protect plants under polyethylene tunnel or Agryl increases the precocity and total yield of eggplants.

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TECHNIQUES OF COMPANION PLANTING FOR IMPROVING FRUIT QUALITY AND THE PROTECTION AGAINST DISEASES AND PESTS IN TOMATO CULTURE

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Abstract:

The crop association in the vegetable gardens should be performed considering the effects they will produce and that some plants deliver higher yields, are tastier and are more resistant to diseases and pests, depending on the plants in their vicinity. Determining the compatibility of the vegetable plants provides a decrease of the diseases attack and a qualitative and more stable production. The association of tomatoes with aromatic herbs, medicinal plants or vegetables pertaining to botanical families indicates the beneficial influence that some species exert in decreasing the pests attack and also the influence on the harvested fruits quality. The attraction of the beneficial fauna due to the presence of flowering plants within the crop and the association with repellent plants may diminish the incidence of pest attack. The presence of aromatic plants like coriander and basil resulted in attracting pollinators and confusing tomato crop specific pests. The presence of peas in catch crop led to a better management of the existing resources in the soil and also provided a production increase of 20%. Use of a large number of vegetables species, aromatic and medicinal flowers created the circumstances of a balanced culture system in which the pests were kept below the harm threshold. This study aims to examine both the inter and intraspecific relations between tomato plants and the associated species, and how they influence the physiological processes and also the yield quality and quantity.

Key words: *companion planting, organic agriculture, tomatoes, vegetable species*

INTRODUCTION

Applied cultivation practices in vegetable farming influences the quality of the resulting products (Stoian L., 2004).
The agricultural pollution is caused by the irrational usage of synthetic fertilizers when applying cultivation practices.
Since the applied practices can play a high antipollution function, the conducted researches aimed to highlight the benefits of some cultivation practices in vegetable farming in the context of organic farming and the impact of the mixed cultivation of species on yield quality and quantity.
The experiments took place between 2012 and 2013.

MATERIALS AND METHODS

The experimental variants were initiated in the specific pedoclimatic conditions of Olt County, on an area of 720 m^2 divided in three unheated polyhouses of 240 m^2 each.
The cultivation practices applied according to the technical specifications were the same for all experimental units as follows:
- Choosing the right crop rotation
- Organic fertilization with green manure
- Ploughing the land at 20 – 30 cm without turning the soil
- Hand hoeing to control weeds

The control of pests and diseases was done using the repellent effect of basil and coriander, or by confusing the pests when planting them with the tomatoes (Bomford M.K., 2004), and by using treatments based on maceration extracts from plants, using Renaud's recipes.

The species used for preparing the treatments were: *Urtica* spp., *Artemisia* spp., *Solanum* spp. in concentration of 12%.
The applied treatments were the same for all experimental units.
The varieties used were:
- Tomatoes: 'Siriana F1', indeterminate growth hybrid made by S.C.D.L. Buzau
- Basil: 'Ruby Red' and 'Aromat de Buzau'
- Coriander: 'Cilandro'
- Parsley: 'Comune 2' and Dill: 'Common'

Variant V1 – Tomato (Control)

The tomato crops were placed on 5 raised beds with the width of 120 cm and 20 cm between the beds serving as space for walking and maintenance. The planting took place on the 10th of April.

Variant V2 – Tomato, Basil, Coriander, Leaf parsley, Peas

Figure 1. Coriander and tomato

The crops were placed on 5 raised beds with the width of 120 cm and 20 cm between the beds serving as space for walking and maintenance, and on the sides of the polyhouse 40 cm were cultivated with leaf parsley.
Planting and sowing took place in two stages:
In the first stage the peas and parsley were sown on the 15th of February and then the seedlings of tomato, coriander and basil were planted on the 10th of April.
The pea plants around the tomato seedlings inserted in the pea crops were rooted out.
The planting took place using the technical specifications of 40/60 cm, and the distance between plants on a row of 35 cm.
The associated planting of the tomato and coriander seedlings took place as follows: on the odd beds, 1, 3 and 5, there were planted five seedlings of tomato and one seedling of coriander; on the even beds, 2 and 4, the basil was used instead of coriander.
There was no treatment applied when the seedlings of tomato and aromatic herbs were cultivated.

Variant V3 - Peas, Tomato, Basil, Dill

Figure 2. Tomato, peas and basil

The crops were placed on 5 raised beds with the width of 120 cm and 20 cm between the beds serving as space for walking and maintenance, and on the sides of the polyhouse 40 cm were cultivated with dill.
Planting and sowing took place in two stages:
In the first stage the peas were sown on the 15th of February and on the 10th of April, the dill was sown and the seedlings of tomato and basil were planted.

In the case of tomato plants that overlapped the pea's culture, the pea plants were pulled out around the tomato plants. The planting took place using the technical specifications of 40/60 cm, and the distance between plants on a row of 35 cm.
The combination of tomato seedlings with basil took place as follows: a 5:1 ratio was respected, planting for five tomatoes seedlings, one basil seedling.
After harvesting the peas, the resulting biomass was used as follows: 40% was buried in the soil, and the remaining part was used for mulching the soil.
There was no treatment applied when the seedlings of tomato and aromatic herbs were cultivated.

RESULTS AND DISCUSSIONS

The results shown in Table 1 refer to the yield harvested per square meter, from the tomato crops.

Table 1. Tomato yield (kg/m^2)

Tomato	V1	V2	V3
'Siriana F1'	6.8	7.37	9.0

Analyzing the obtained data there can be seen the differences between the three applied cultivation practices concerning productivity.
Thus in the variants using crop associations, V2 and V3, were recorded production increases between 8.5% and 20%.
A total of 10 plants randomly chosen from each experimental variant were analyzed to determine the frequency and intensity of tomato blight attack.
There were performed the following examinations to determine the necessary indices for each plant: counting the attacked organs (stem, leaves, flowers, fruits) and the degree of the attack on the organs.
When calculating the intensity of the attack the following formula was used: $I = (i \times f) / n$, the percentage of the results being listed according to the scale of intensity with 6 levels (Calin M., 2005). The results can be seen in table 2.

Table 2. Incidence of diseases and pests per m^2

No..	Crop Variant	Disease	
		Phytophtora infestans	*Trialeurodes vaporariorum*
1.	V1	8%	10%
2.	V2	7%	7.3%
3.	V3	7.8%	8.2%

For determining the organoleptic qualities, a group of ten persons tasted the resulting production from the three experimental units.
The production was sorted out to match in terms of appearance, dimension and degree of maturation.
The test examiners were asked to give a grade from 1 to 5 according to the following three criteria: sugar content, acidity and specific flavor.
As regards the sugar content and specific flavor, the results were insignificant, but 6 out of 10 examiners declared that the tomatoes grown in association with aromatic herbs were less acidic.
On a close inspection of the data from Table 2, there can be seen that the incidence of diseases and pests attack is slightly lower in case of the variants cultivated in association with aromatic herbs and vegetables, in comparison with variant 1 where there was no association of the tomatoes with other plants.

CONCLUSIONS

The recorded data reflects the difference between applied cultivation practices monitoring the production per m^2, the flavor and the incidence of diseases and pests attack on tomato crops.
Due to the presence of pea in V3 two months before planting the tomato seedlings and therefore to the incorporation of the resulting biomass in the soil, a yield increase of almost 20% was recorded in comparison with the other experimental units.
The difference of production and the decrease of diseases and pests attack in V2 could be accounted for the cultivation of the tomato plants in association with coriander, whose repellent smell played a protective part, and thanks to the flowers a role in attracting the pollinators within the polyhouse.

From a quality point of view, the cultivated variety does not show significant differences about its organoleptic attributes.

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THE GRAFTING INFLUENCE ON SOME CHARACTERISTICS AT A ROMANIAN EGGPLANTS COLLECTION CULTIVATED IN GREENHOUSE

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Abstract

The experiment was conducted on a Romanian grafted eggplant collection consisting of two varieties, 'Luiza' and 'Rodica' and one hybrid, 'Andra F_1'. Three rootstocks were used to obtain the grafted seedlings: 'Hikyaku', 'Espina', 'Arazy (500294)'. Visual observations and biometric measurements of the fruits were made during the 2013 growing season. The research was carried out at Horting Institute - Bucharest in the Laboratory of Protected Cultures and it was aimed to evaluate some characteristics of several Romanian grafted eggplants. The results show the characteristics of the eggplants that were influenced by the grafting.

***Key words**: grafted culture, parameters, Solanum melongena*

INTRODUCTION

Grafting imprints resistance to pathogenic agents and soil pests, tolerance to abiotic stress factors, improves water and nutrient absorption and increases the graft vigor (King et al., 2010, Lee J.M., 1994).
Discrepant results concerning fruit quality provided by grafted plants were reported (Davis et al., 2008).
In protected culture, there are only few results concerning the *Solanum* rootstock effect on fruit production, development and quality for uninfected soils (Çürük et al., 2005, Passos et al., 2005) and for *Verticillium* infected soils (Bletsos et al., 2003).
Many researchers reported that the fruit average weight was significantly influenced by the grafting (Çürük et al., 2009).
Pană (2010) has proposed the improvement of field culture technology for eggplants (Luiza variety), by grafting them on tomatoes (Pontica variety). The results showed smaller and more ovoid fruits, similar to tomatoes, the fruit average weight was much lower compared to the ungrafted plant fruits and the productivity of the grafted plants was much lower.

MATERIALS AND METHODS

The biological material used in this research was a collection of Romanian eggplants (grafted and ungrafted plants).
The Romanian eggplants scions consisted of two varieties, 'Luiza' and 'Rodica' and one hybrid, 'Andra F_1'.
The used rootstocks for the above scions were two commercial hybrids, 'Hikyaku F_1', and 'Arazy (500294) F_1' and one variety, 'Espina'.
'Hikyaku F_1' (*Solanum melongela*), 'Arazy (500294) F_1' (*Lycopersicum lycopersicum*) and 'Espina' (special variety of *Solanum torvum*) determine vigor, increase the production and rise the resistance to low temperature, vascular diseases (*Fusarium* spp., *Verticillium* spp. etc.), soil pests and biotrophic parasites (nematodes *Meloidogyne* spp.).
Ungrafted and grafted eggplant seedlings were obtained in a specialized greenhouse for grafted seedling production, in alveolar trays with nutrient substrate (fertilized peat) and vermiculite, according to classical technology (for ungrafted plants) and according to grafting technology (for grafted plants).
The experimental plots were set up in protected space, greenhouse (Figure 1).

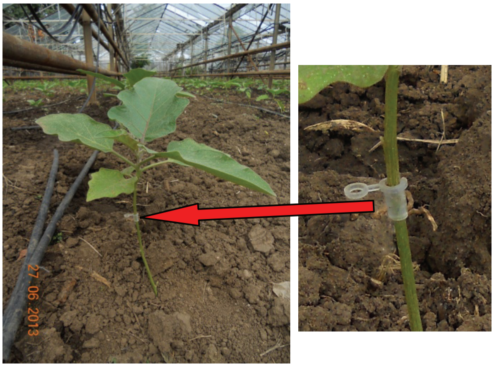

Figure 1. Culture of grafted eggplants - experimental plot

Classical culture technology of the eggplants inside the greenhouse (care works, growth factor guiding, etc.) and a fertilization program was used according to Voican and Lăcătuş (1998) (Table 1).

Table 1. Eggplants culture fertilization (kg/1000 m^2)

Eggplant culture	NH_4NO_3	Complex 16-48-0	K_2SO_4	$MgSO_4$
after 1st harvest	100	-	-	-
after 15 days	150	100	150	100
every 15 days	100	-	150	50

The two-factorial experiment (A-scion and B-rootstock factors), placed upon the randomized block method, in 4 repetitions with the experimental plot surface of 10 m^2, has covered 9 grafted variants (ρ = 18.000 grafted plants / ha): V_1, V_2, V_3, V_4, V_5, V_6, V_7, V_8 and V_9, and 3 ungrafted variants (ρ = 24.000 grafted plants / ha): V_{C1}, V_{C2} and V_{C3} (Table 2).

Table 2. The organizing of the experience

V*	Grafted plants		No. of plants / variant
	Scions	Rootstock	
V_1	'Luiza	Hikyaku	90
V_2	Rodica		90
V_3	Andra		90
V_4	Luiza	Espina,	90
V_5	Rodica		90
V_6	Andra		90
V_7	Luiza	Arazy (500294)	90
V_8	Rodica		90
V_9	Andra		90
Ungrafted plants (control)			
V_{C1}	Luiza		120
V_{C2}	Rodica		120
V_{C3}	Andra		120

*- variants

The visual observations and the biometric determinations of the eggplant parameters were realized in the Laboratory of Protected Cultures of the Horting Institute Bucharest.
The data represent average values of the determinations on grafted and ungrafted fruit variants during 10 harvests.
The Romanian eggplant collection at commercial maturity have ovoid fruits, black main color and shiny epidermis (Figure 2).

'Luiza' 'Rodica' 'Andra'

Figure 2. Romanian eggplants

RESULTS AND DISCUSSIONS

The results concerning the characteristics of the eggplant collection are shown in Table 3.

Table 3. Biometrics data of the eggplant fruits

V*	Weight (g)	Length (cm)	Diameter (cm)		Index of shape
			basic	maximum	
V_1	236	14	5	7	2
V_2	245	18	4.5	5.5	3.27
V_3	335	16	4.5	7.3	2.19
V_4	258	18.5	3.5	5	3.7
V_5	277	13.5	5	7.5	1.8
V_6	320	14	4.2	7	2
V_7	230	14	5	6.9	2.03
V_8	240	17	4.2	5	3.4
V_9	330	15	4.3	7.2	2.08
V_{C1}	171	13	4	6	2.17
V_{C2}	129	14	3.2	5	2.8
V_{C3}	176	16.5	3.5	4.8	3.44

*- variants

The average fruit weight was bigger for the grafted eggplants (236-335 g/fruit) compared with the ungrafted eggplants, control (129-176 g/fruit):

–'Luiza' variety - grafted (V_1, V_4, V_7) had the average weight/fruit of 241.33 g while the ungrafted one (V_{C1}) had only 171 g;
–'Rodica' variety - grafted (V_2, V_5, V_8) had the average weight/fruit of 254 g while the ungrafted sample (V_{C2}) had 129 g;
–'Andra F_1' hybrid - grafted (V_3, V_6, V_9) had the average weight/fruit of 328.33 g while the ungrafted (V_{C3}) had 176 g;

The average length of the fruit has varied but not significant, with small differences between the grafted variants (13.5-18.5 cm) and the ungrafted variants (13-16.5 cm):
–'Luiza' variety had the average length/fruit for grafted (V_1, V_4, V_7) and ungrafted (V_{C1}) of 15.5 cm and 13 cm, respectively;
–'Rodica' variety had the average length/fruit for grafted (V_2, V_5, V_8) and ungrafted (V_{C2}) of 16.17 cm and 14 cm, respectively;
–'Andra F_1' had the average length/fruit for grafted (V_3, V_6, V_9) and ungrafted (V_{C3}) of 15 cm and 16.5 cm, respectively.

The fruits were elongated for all analyzed variants. The grafting has influenced the precocity (Figure 3).

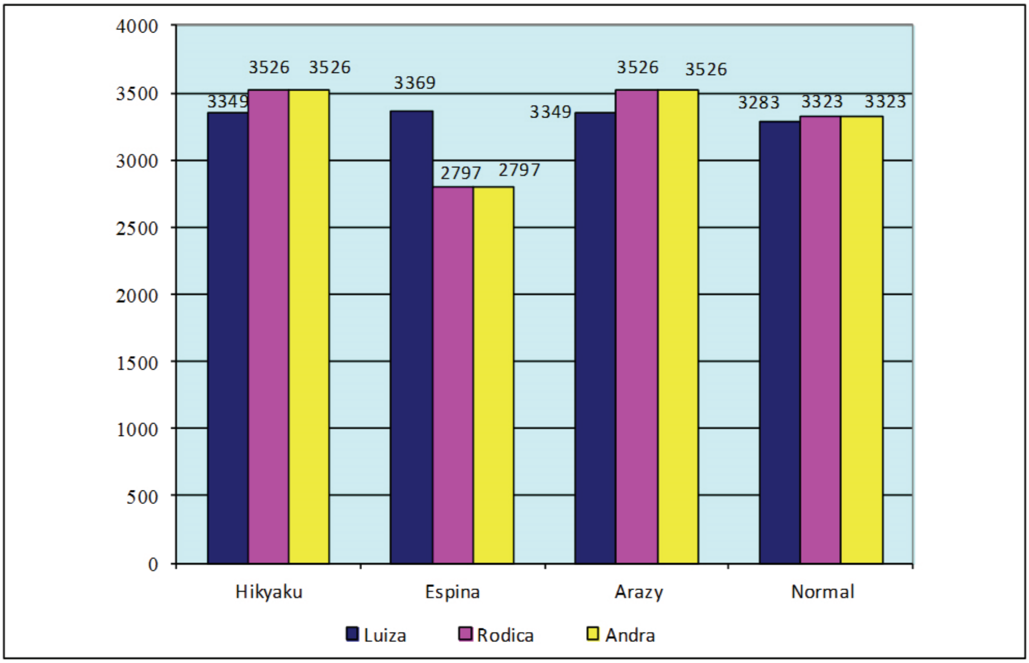

Figure 3. The sum of the degrees of temperature to first harvest at the eggplants collection

It was observed that the rootstocks have induced precocity or tardiness. 'Hikyaku' and 'Arazy (500294)' have determined tardiness while 'Espina' has determined precocity comparative with the ungrafted plants, control.

The grafting has influenced the precocity as follows:
- for 'Luiza' variety grafted on 'Hikyaku' (V_1) and 'Arazy (500294)' (V_7) were more precocious compared with the variant grafted on 'Espina' (V_4). All grafted variants were more tardive compared with ungrafted plants (V_{C1});
- for 'Rodica' variety - the variant grafted on 'Espina' (V_5) was more precocious compared with the variants grafted on 'Hikyaku' (V_2), 'Arazy (500294)' (V_8) and the ungrafted variant (V_{C2});
- for 'Andra F_1' - the variant grafted on 'Espina' (V_6) was more precocious compared with the variants grafted on 'Hikyaku' (V_3), 'Arazy (500294)' (V_9) and the ungrafted variant (V_{C3}).

The influence of grafting on the average production is presented in Figure 4.

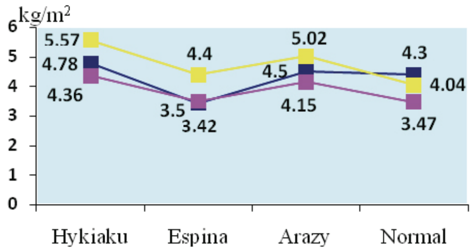

Figure 4. Average production (kg/m^2)

It is observed that the grafted plants had productions of 3.5-5.57 kg/m^2, at a density of 18000 plants/hectare compared with the ungrafted plants that had 3.47-4.38 kg/m^2 productions, at 24000 plants/ha density.
The plants grafted on 'Hikyaku 'rootstock have obtained the highest productions (5.57; 4.78; 4.36 kg/m^2), followed by the plants grafted on 'Arazy (500294)' rootstock (4.4; 3.42; 3.5 kg/m^2) and the plants grafted on 'Espina 'rootstock (5.02; 4.5; 4.15 kg/m^2).
The ungrafted plants (control) have obtained the highest production compared with the plants grafted on 'Espina' rootstock.

CONCLUSIONS

Grafting influenced some characteristics of the eggplants (weight, length, diameter). The grafted plants have mainly registered higher values compared with the ungrafted plants.
The fruits were elongated at all analyzed variants (grafted and ungrafted).
The production precocity of grafted eggplants was different from that of ungrafted eggplants., The rootstocks have induced precocity or tardiness by grafting as follows:
- at 'Luiza' variety, the rootstocks ('Hikyaku', 'Espina', 'Arazy 500294') have induced tardiness;
- at 'Rodica' variety and 'Andra F_1' hybrid, the 'Espina' rootstock has induced precocity and the 'Hikyaku', 'Espina', 'Arazy (500294)' rootstocks have induced tardiness;
- the ungrafted plants were more precocious. One exception was some combinations – 'Rodica' x 'Espina' (V_5), 'Andra' x 'Espina' (V_6) – with an important precocity compared with the ungrafted variants, 'Rodica' (V_{C2}) and 'Andra' (V_{C3}).

The plants grafted on 'Hikyaku' rootstock have obtained the highest production, followed by the plants grafted on 'Arazy (500294)' rootstock and the plants grafted on 'Espina' rootstock.
The average production (kg/m^2) was also influenced by grafting. The grafted plants had higher production compared with ungrafted plants, exception was the grafted variant 'Luiza' x 'Espina' (V_4) that has registered a lower production compared with the other variants (grafted and ungrafted).

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BIOCHEMICAL CHANGES IN GARDEN EGG *(Solanum melongena* L.*)* FRUITS CAUSED BY FUNGI

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Abstract

Garden egg (Solanum melongena L.) fruits are major sources of food in Nigeria, especially during weddings and occasions. This major food crop is constantly being affected by fungi, causing deterioration of the fruit. Biochemical analyses were carried out, according to the methods of the Association of Official Analytical Chemists, to determine the changes that occur in two locally identified varieties of garden egg (Solanum melongena L.) fruits inoculated with Alternaria alternata, Aspergillus flavus, Mucor hiemalis and Rhizopus stolonifer. The two varieties are 'Yallon bello' (YB) and 'Chi da Masoyi' (CM). Significant increases were observed in moisture, protein, crude fat, fibre and ash contents of the two varieties inoculated with the fungi, as compared with uninoculated fruits. In the YB variety, A. alternata, A. flavus, M. hiemalis and R. stolonifer increased the moisture content 2.4%, 4.2%, 7.2%, 6.0%; protein content 2.0%, 2.0%, 3.2%, 2.2%; crude fat content 4.5%, 5.5%, 4.5%, 5.5%; fibre content 2.5%, 2.0%, 2.0%, 2.0%; and ash content 20.0%, 20.0%, 24.3%, 22.9%, respectively. The four fungi caused a significant reduction of 8.0%, 9.4%, 14.0%, 14.0% in carbohydrate, and 1.2%, 1.6%, 1.3%, 1.6% in vitamin contents, respectively. Similarly, in the CM variety, A. alternata, A. flavus, M. hiemalis and R. stolonifer increased the moisture content 3.3%, 3.8%, 4.8%, 3.8%; protein content 4.8%, 3.0%, 4.8%, 4.1%; crude fat content 6.9%, 5.4%, 8.5%, 10.0%; fibre content 3.4%, 2.5%, 4.1%, 3.6%; and ash content 10.4%, 11.6%, 14.0%, 10.5%, respectively. The four fungi caused a significant decrease of 7.0%, 6.2%, 8.5%, 7.7% in carbohydrate, and 1.5%, 1.1%, 1.9%, 2.1% in vitamin contents, respectively. The results of this research showed that fungi are associated with the deterioration of garden egg fruits and alters the nutritional components of the fruit.

Key words: *biochemical changes, fungi, garden egg*

INTRODUCTION

Garden egg (*Solanun melongena* L.) is grown in almost all parts of the world, including Africa, Europe, Asia, South and North America (Aguiar et al., 1998). The plant produces small to moderately white fruits, resembling the chicken egg, hence the name "garden egg" (Granberry, 1990). An official report by the United States Department of Agriculture (USDA, 2005) explains that the fruit is nutritious and contain C vitamin and potassium. It also contain 24 Kcal of energy, 5.7g of carbohydrate, 3.4g of dietary fibre, 1.01g of protein, 4% of vitamin C and 5% of potassium. Fungi have been identified to be associated with garden egg fruit rot. Anthracnose, a common fungal rot caused by the fungus *Colletotrichum coccodes,* was reported by Zitter (1989) as a major fungus associated with garden egg fruit rot. The fungi *A. alternata*, *A. flavus, M. hiemalis* and *R. stolonifer* were identified as the major fungi affecting garden egg (Kuc'mierz and Sumera, 2009). Therefore, it is important to note that post harvest fungal fruit rot is inevitable. The fungi increase the fruits' vulnerability to other pathogens, leading to certain biochemical changes on the fruits (Kuc'mierz and Sumera, 2009). There is no official report documented on the effects of fungi on the nutritional composition of garden egg fruits in Northern Nigeria, and Plateau State in particular. The present study was therefore undertaken to investigate the roles of the fungi (*A. alternata*, *A. flavus, M. hiemalis and R. stolonifer*) on the biochemical changes of the fruits.

MATERIALS AND METHODS

The two garden egg fruit varieties used in this study were obtained in markets within the Jos – Bukuru Nigerian metropolis in Plateau State. The fungi were isolated from naturally infected fruits on Malt Extract Agar (MEA) medium, using the procedure of Clement and Voros (1974).

The fungi were identified under a stereo binocular microscope (6 – 50x) based on their habit characteristics. Slides were made to confirm identification following descriptions by Ellis and Ellis (1987).

Pure, single spore cultures of each fungus were obtained by growing them on MEA. The cultures were grown for 5 – 7 days in complete darkness at 21 ± 2ºC before use as inoculum. Healthy samples of the two varieties of the garden egg fruits were thoroughly cleaned, and surface sterilized with cotton wool, soaked in 1% sodium hypochlorite solution, to remove sand and other contaminants before weighing.

After weighing, a sterilized cork borer (size 3) was used to bore holes in the fruits in Petri dishes. Another cork borer (size 3) was used to bore out a culture disc from 7-day old cultures (inoculum) grown on MEA medium.

Each fungal culture was picked with a different sterile cork borer to avoid contamination. Some of the healthy fruits were left without holes to serve as control. The inoculum discs, of each fungus isolated, were inoculated into the holes made on the fruits. Vaseline cream was used to seal the surface of the fruits, while cotton wool was used to cover the areas where Vaseline cream was applied. This was meant to avoid external contaminants (Cherry and Beuchat, 1975).

The fruits that served as control received the same treatment, but they were not inoculated with any fungus. All the fruits (fungus–inoculated and control) were wrapped in sterile polythene bags containing moistened cotton with distilled water and incubated at 25 ± 2ºC in complete darkness for 7 – 14 days.

After 14 days, fungus–inoculated fruits and control samples were collected and weighed. After weighing, they were sliced differently into small sizes for uniform drying.

The samples were oven–dried at 100ºC for about 24 hours. After drying, they were milled into powdery form. Ten grams (10g) of each sample was used for the biochemical analyses of the various nutrient components (moisture, carbohydrate, protein, crude fat, fibre, ash and vitamins) in both fungus inoculated and uninoculated fruits of the two varieties at various incubation periods (7, 14 days).

The analyses were done following the procedures recommended by the Association of Official Analytical Chemists (AOAC, 1980).

The results, for each food component, were subjected to statistical analyses using the Analysis of Variance (ANOVA).

RESULTS AND DISCUSSIONS

The results of the biochemical analyses for the fungus – inoculated garden egg fruits and uninoculated controls in the two varieties incubated for 7–14 day intervals are presented in Tables 1 and 2, for the YB and CM varieties, respectively. The fungi caused appreciable changes in the food components of the two varieties.

Moisture content, protein content, crude fat content, fibre content and ash content showed significant increases (P = 0.05) in the fruits inoculated with *A. alternata*, *A. flavus,* M. *hiemalis and R. stolonifer,* as compared to the uninoculated fruits (Tables 1 and 2). The carbohydrate and vitamin contents decreased significantly (P = 0.05), as compared to the uninoculated controls.

In the YB variety, fruits inoculated with *M. hiemalis* recorded the highest increase in moisture and ash contents (7.2%, 24.3%), while those inoculated with *A. flavus* and *R. stolonifer* recorded the highest increased in crude fat content (5.5%). Similarly, fruits inoculated with *M. hiemalis* recorded the highest increase in protein content (3.2%).

The lowest decrease in carbohydrate and vitamin contents were recorded in fruits inoculated with *A. alternata* (8.0%, 1.2%).

In the CM variety, fruits inoculated with *M. hiemalis* recorded the highest increase in moisture and ash contents (4.8%, 14.0%). Also, fruits inoculated with *M. hiemalis* recorded the highest decrease in carbohydrate and vitamin contents (8.5%, 1.9%).

Table 1. Changes in the levels of nutrients of the YM variety of garden egg fruits inoculated with fungi and incubated at 25± 2ºC for 7-14 days

Nutritional composition (%W/W)	Control (uninoculated)		*A. flavus*		*A. alternata*		*M. hiemalis*		*R. stolonifer*		**L.S.D**
	7	14	*7*	*14*	*7*	*14*	*7*	*14*	*7*	*14*	
Moisture content	8.3	8.3	8.6	8.7	8.4	8.6	8.8	9.0	8.7	8.9	**0.9**
Carbohydrate content	6.9	6.9	6.3	6.2	6.5	6.2	6.0	5.8	6.1	5.8	**0.7**
Protein Content	20.3	20.3	20.6	20.8	20.6	20.8	20.8	21.1	20.7	20.8	**1.2**
Crude fat content	5.5	5.5	5.7	5.9	5.6	5.9	5.6	5.9	5.7	5.9	**0.7**
Fiber content	20.3	20.3	20.6	20.8	20.7	20.9	20.5	20.9	20.6	20.9	**1.2**
Ash content	3.5	3.5	4.1	4.3	4.1	4.3	4.2	4.5	4.2	4.4	**0.7**
Vitamin content	40.8	40.3	40.3	40.0	40.5	40.1	40.4	40.1	40.3	40.0	**1.8**

*L.S.D. - Least significant difference for comparison of treatment means

Table 2. Changes in the levels of nutrients of the CM variety of garden egg fruits inoculated with fungi and incubated at 25± 2ºC for 7-14 days

Nutritional composition (%W/W)	Control (uninoculated)		*A. flavus*		*A. alternata*		*M. hiemalis*		*R. stolonifer*		**L.S.D**
	7	14	*7*	*14*	*7*	*14*	*7*	*14*	*7*	*14*	
Moisture content	10.5	10.5	10.8	11.0	10.8	10.9	10.8	11.2	10.8	11.0	**0.7**
Carbohydrate content	6.5	6.5	6.2	6.0	6.2	5.9	6.1	5.8	6.1	5.9	**0.8**
Protein Content	22.0	22.0	22.6	22.7	23.0	23.1	22.9	23.2	22.8	23.0	**1.3**
Crude fat content	6.5	6.5	6.8	6.9	6.9	7.0	6.9	7.2	7.0	7.3	**0.9**
Fiber content	22.1	22.1	22.5	22.8	22.8	22.9	22.9	23.1	22.8	23.0	**1.4**
Ash content	4.3	4.3	4.7	4.9	4.7	4.8	4.8	5.0	4.6	4.9	**0.7**
Vitamin content	41.2	41.2	40.9	40.6	40.8	40.4	40.6	40.2	40.6	40.1	**1.8**

*L.S.D. - Least significant difference for comparison of treatment means

Inoculation of garden egg fruits with pure cultures of *A. alternata, A. flavus, M. hiemalis* and *R. stolonifer* and incubated for 7 – 14 days at 25 ± 2ºC resulted in various changes in garden egg fruits' nutrients.

These fungi were identified by Kuc'mierz and Sumera (2009) as the major fungi affecting garden egg seeds.

Increases recorded in the moisture content were probably due to the fungi using some of the components of the garden egg fruits as nutrient, and as a result, producing water in the process. This is in conformity with the results obtained by Ataga and Akueshi (1986a) on sunflower seeds. The inoculated fungi also lead to an increase in crude fat content in the two varieties of the garden egg fruits.

Ward and Diener (1961) obtained similar results on groundnut seeds. They stated that such an increase was due to a decrease in total oil, due to its hydrolysis.

Increase in protein content also conforms to the findings of Ataga and Akueshi (1986b) on sunflower seeds. This may be due to proteinaceous mycelia from the isolated fungi.

A decrease in carbohydrate conforms to the findings of Umechuruba *et al*. (1992) on groundnut seeds. This could be due to the mobilization and utilization of storage polysaccharides by fungi. Increase in ash and fibre contents could be due to the presence of certain mineral elements such as potassium and phosphorus in mycelia of fungi. This was also observed by Ataga and Umechuruba (1998) on the African yam bean seeds.

A decrease in vitamin content could be due to the increase in moisture content, causing the vitamin to dissolve in it, since it is a water soluble vitamin.

CONCLUSIONS

The result of this study shows that the fungi caused deterioration of garden egg fruits and alters the nutritional values of the fruits.

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RESEARCH REGARDING THE INFLUENCE OF SOME ROOTING STIMULATORS ON THE PRODUCTION OF SOLANO-FRUITING VEGETABLE SEEDLINGS

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Abstract

The experiment was conducted within a solarium, in Bucharest, in 2013. It was a two-factor experiment, in which factor A was represented by the species – tomatoes, capsicum and eggplants, while factor B was the stimulating product, Tecamin raiz 0.2% and Humusil 0.2%, applied at an interval of seven days after the pricking out, until planting the seedlings. The purpose of the experiment was to establish the influence of stimulating products on the growth of tomato, capsicum and eggplant seedlings and to evaluate the quality of the seedlings obtained through this experiment. The seedlings were produced on a peat substrate, with a pH value of 5.5-6.5. After processing the data, it could be observed that the stimulating products had a positive influence on the growth of tomato, capsicum and eggplant seedlings in what regards the height of the plant, number of leaves, thickness of the stem, volume of the root system and plant biomass. Strong correlations were established between the main parameters that characterize the quality of the seedling for all studied species, which allow recommending the used products for application in the technology for seedling production.

Key words: *tomato, pepper, eggplant, seedlings, rooting*

INTRODUCTION

Vegetables from the *Solanaceae* family are cultivated through planting the seedling, except for tomatoes that can be cultivated also through sowing directly into the field, for industrialization. An important aspect of the culture technology is represented by the production of seedling, which is used for obtaining qualitative seedling, with a vigorous root system that can sustain a rich aerial part, through increasing the capacity to absorb water and nutrients and through proper plant stability.
Seedling production can be made on various culture substrates, simple or mixtures, with nutrient solutions (Hoza, 1997) for ensuring the necessary nutrition. Substrates can be made of peat, various composts, perlite, vermiculite, vermicompost etc., in different proportions in order to ensure the necessary oxygen, optimum pH level and water retention capacity. The production of tomato and cucumber seedlings had very good results by using compost resulted from mushroom culture mixed with vermiculite in 2:1 ratio, and respectively with perlite in 4:1 ratio (Zhang et al., 2012). Vermicompost mixed with peat (Lazcano et al., 2009) was recommended as alternative for peat, with beneficial influences on the environment and on the quality of the tomato seedlings. Perlite, peat and peat mixed with perlite (Hoza, 1997) were successfully used for tomato, capsicum and eggplant. seedlings.
The quality of the seedling is also influenced by the level of the vegetation factors within the production space (Basoccu et al., 1992). The evaluation of the seedlings quality is performed by evaluating their height, thickness of the stem at package, number of formed leaves, volume of the root system etc. (Hoza, 1997).

MATERIALS AND METHODS

The experiment was conducted in a heated solarium for the production of Solano-fruiting vegetable seedling for field culture, in Bucharest, in 2013, using as biological material tomato, capsicum and eggplant seeds. Two products for stimulating the growth of the root system, namely Tecamin raiz and Humusil, were used,.
The experiment was a two-factor one, in which:

Factor A was represented by tomato, capsicum and eggplant seedlings.

Factor B was represented by rooting stimulators: Tecamin Raiz and Humusil.

The experimental variants were the following:

V_1 – a1b1 – tomato seedling stimulated with Tecamin Raiz;

V_2 – a1b2 – tomato seedling stimulated with Humusil;

V_3 – a1b3 – tomato seedling not stimulated;

V_4 – a2b1 – capsicum seedling stimulated with Tecamin Raiz;

V_5 – a2b2 – capsicum seedling stimulated with Humusil;

V_6 – a2b3 – capsicum seedling not stimulated;

V_7 – a3b1 – eggplant seedling stimulated with Tecamin Raiz;

V_8 – a3b2 – eggplant seedling stimulated with Humusil;

V_9 – a3b3 – eggplant seedling not stimulated.

During the research, observations and measurements were made regarding the following:

- the influence of biostimulators on the germination energy of the seeds;
- the influence of the biostimulators on the germination faculty of the seeds;
- the influence of the biostimulators on the seedling growth as height and number of leaves;
- the influence of the biostimulators on the average height of the seedling at planting, number of leaves, stem diameter at package, volume of the root system, average seedling weight, weight of aerial part, weight of the root system, determining the ratio between the aerial part and root system, determining the growth rate and age of the seedling.

The sowing was performed directly into alveolar pallets with 45 alveoli in peat substrate with pH value of 5.5-6.5.

Tecamin Raiz and Humusil biostimulators as 0.2% concentration were used every seven days after the pricking out until the final planting.

RESULTS AND DISCUSSIONS

The rooting stimulators had a positive influence on the germination energy of the seeds for all studied species (Table 1). In the case of tomatoes, five days after the sowing, the germination energy was different for the stimulated variants compared to the control (not stimulated variant), the highest recorded value being 45% when using Tecamin raiz, 40% with Humusil and 31% for the control. In the case of capsicum and eggplant, it could be observed that, eight days after the sowing, the percentage of the sprung plants was below 40%, the best results being recorded for the variants using Tecamin raiz.

Table 1. The influence of the rooting stimulators on the germination energy of the seeds

Variant	Percentage of sprung plants	Number of days from sowing
V_1	45	5 days
V_2	40	5 days
V_3	31	5 days
V_4	39	8 days
V_5	36	8 days
V_6	22	8 days
V_7	38	8 days
V_8	36	8 days
V_9	23	8 days

Analyzing the influence of the rooting stimulators at the end of the germination period, it was noted that over 90% of the seeds germinated for all variants, the best results however being recorded for the variants with stimulators (Table 2).

Table 2. The influence of the rooting stimulators on the germination faculty of the seeds

Variant	Percentage of sprung plants	Number of days from sowing
V_1	100	10 days
V_2	98	10days
V_3	93	10 days
V_4	95	21 days
V_5	92	21 days
V_6	90	21 days
V_7	96	21 days
V_8	93	21 days
V_9	91	21 days

Analyzing the seedling at final planting it can be noted that the stimulators influenced the growth of both root system and aerial part (Table 3).

The seedling height was great for all used species, having values of 20.1 cm for tomatoes, 17.9 cm for capsicum and 17.4 cm for eggplants.

Humusil also produced good results, the values being slightly lower than for Tecamin raiz. The number of leaves was correlated with seedling height, with values between 5.2 and 11.2 for the studied species. Using stimulators had a positive effect also on the growth in thickness of the stem.

Tecamin raiz had the best results for all species, the diameter in package being 6.8 mm for tomatoes, 6 mm for capsicum and 5.6 mm for eggplants. The growth of the root system was also influenced by the stimulators, the most obvious effect being recorded for the variants stimulated with Tecamin raiz, followed by Humusil, compared to the control variant for which the root system was poorly developed. For the tomatoes, the root system recorded 6.5 cm^3, for capsicum 5.9 cm^3 and for eggplants 4.5 cm^3. Analyzing the obtained results, it could be observed that the differences between the variants were statistically ensured (Table 3).

Table 3. Biometric characteristics of the seedling at optimum planting age

Variant	Seedling height (cm)	Number of leaves piece	Stem diameter at package (mm)	Volume of root system (cm^3)
Tomatoes				
V_1	20.1***	6.7*	6.8*	6.5*
V_2	18.2**	6.1N	6,3*	5.5*
V_3	16.2Ct	5.8Ct	5.2Ct	4.5Ct
DL 5%	0.35 cm	0.60 piece.	0.92 mm	0.92 cm^3
DL 1%	0.75 cm	1.31 piece.	2.01 mm	2.01 cm^3
DL 0.1%	2.57 cm	4.46 piece.	6.82 mm	6.82 cm^3
Pepper				
V_4	17.9 **	11.2**	6.0*	5.9**
V_5	16.4*	10.6**	5.7*	5.5**
V_6	15.8Ct	9.8Ct	5.0Ct	4.2Ct
DL 5%	0.34 cm	0.35 piece.	0.60 mm	0.35 cm^3
DL 1%	0.75 cm	0.76 piece.	1.31 mm	0.76 cm^3
DL 0.1%	2.54 cm	2.59 piece.	4.46 mm	2.58 cm^3
Eggplants				
V_7	17.4 **	6.4 N	5.6*	4.5**
V_8	16.0**	5.8N	5.3N	4.0*
V_9	14.5Ct	5.2Ct	4.8Ct	3.6Ct
DL 5%	0.61 cm	1.26 piece.	0.60 mm	0.35 cm^3
DL 1%	1.31 cm	2.74 piece.	1.31 mm	0.76 cm^3
DL 0.1%	4.49 cm	9.30 piece.	4.46 mm	2.58 cm^3

Calculating the correlation coefficient r^2, it was noted that positive correlations were recorded between the main parameters for seedling quality analysis, the value of the coefficients being higher than 0.78, highlighting strong correlations between the analyzed parameters, for all studied species (Figures 1, 2 and 3).

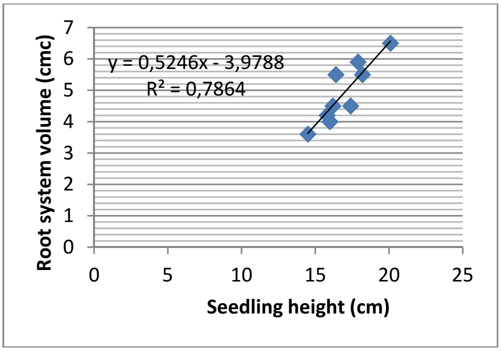

Figure 1. Correlation between seedling height and root system volume

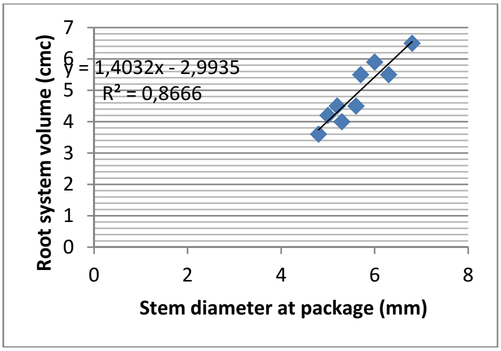

Figure 2. Correlation between root system volume and stem diameter at package

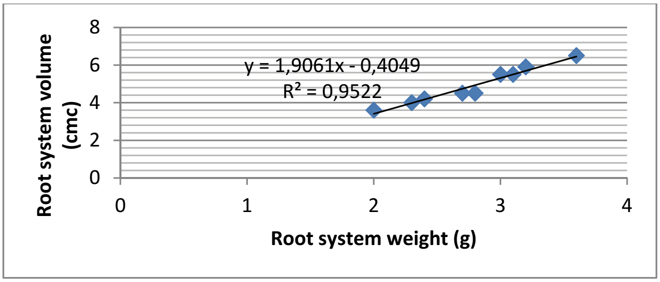

Figure 3. Correlation between root system volume and root system weight

From the point of view of the obtained biomass (Table 4), a growth of both aerial part and root system was observed.
The best results were obtained for the variants with Tecamin raiz, for all studied species.

Moreover, it was noted that for the variants for which the root system had a sharp growth the aerial part was also more vigorous, measured through its weight (Table 4).

Table 4. The biomass content of seedling at planting age

Variant	Seedling average weight (g)	Root system weight (g)	Aerial part weight (g)
V_1	16.0	3.6	12.4
V_2	13.7	3.1	10.6
V_3	11.5	2.8	8.7
V_4	11.7	3.2	8.5
V_5	10.2	3.0	7.2
V_6	8.9	2.4	6.5
V_7	12.3	2.7	9.6
V_8	10.5	2.3	8.2
V_9	8.5	2.0	6.5

The stimulators influenced the seedling growth, so that they reached the optimum parameters for planting – tomatoes after 40 days, capsicum and eggplants after 50 days.

The seedling produced with stimulators recorded a higher growth rate compared to the control, not stimulated variant (Figure 4), the highest values being recorded for the variants with Tecamin raiz, followed by the ones with Humusil, for all species.

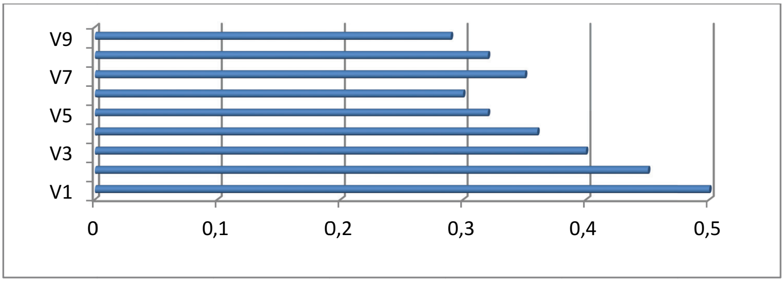

Figure 4. Growth rate of seedlings influenced by rooting stimulators

CONCLUSIONS

From the conducted research, the following conclusions can be drawn:

- Using peat as substrate can be indicated for producing tomato, capsicum and eggplant seedlings because it ensures an optimum air-water regime at root level, with positive effects on root growth and absorption capacity;
- Using root stimulators can be recommended, especially Tecamin raiz 0.2%, which stimulates both the growth of root system and aerial part thus contributing to the obtaining of seedling at planting parameters within a shorter period than not stimulated variants;
- It is recommended to ensure optimum vegetation conditions in strong correlation with the requirements of the vegetable species and growth phase.

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EFFECT OF FOLIAR FERTILIZERS SPRAY, BORON AND THEIR INTERACTION ON BROAD BEAN (VICIA FABA L.) YIELD

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Abstract

A factorial experiment was conducted to study the effect of six spray treatments of foliar fertilizer (neutral, high-phosphorus, high potassium, urea, extracts of seaweeds, in addition to control) with two levels of boron (spray and without spray) and its interaction on dry seeds yield of broad bean and some of its characteristics. The experiment was carried out in the farm Guidelines during the agricultural season 2012/2013 as factorial experiment according to complete randomized block design with three replications. The most important results of the experiment summarized as follows: all fertilizers treatments led to increase pod dry weight, number of seeds / pod, 100 seed weight, seeds yield/ plant, seeds yield per unit area and protein in the seeds significantly compared to control treatment. Boron treatment led to increase all the traits significantly except (100 seeds weight) compared to control. The interaction between the factors had a significant effect on pod dry weight, number of seeds / pod, weight of 100 seeds, seed yield/plant and unit area and protein in seeds. The interaction of urea with boron was superior on all other interactions with a percentage increases of (51.84 %, 56.9 %, 30.8 %, 10.88 %, 10.89 %, 64.1 %), respectively, compared to control.

Key words: *foliar fertilizers, boron, broad bean, seaweed*

INTRODUCTION

Faba bean (*Vicia faba* L.) is one the main leguminous crops grown in winter season in different of Iraq soils. Also, it is considered as one of the basic sources of protein for human consumption. It is nutritionally important vegetable, containing 20-36% protein for human and animal consumption. In addition, broad bean plants improve soil fertility by providing a substantial input of N fixation. So it enters in crop rotation to improve soil conditions (Carmen et al., 2005). Although introduced the cultivation and production of broad bean in Iraq, but its cultivation is still experiencing a lot of problems. The nutritional status of the plant and the lack of availability of some nutrients in the soil, especially during the period of flowering and the contract of the reasons that reduced crop yield by losing flowers and failed in pollination. South Iraq soil (pH 7.5-8.2) leads to a lack of elements availability and its absorption, which reflects negatively on the quantity and quality of the yield. Recently, using macro and micro nutrients through foliar fertilization is preferable to avoid not only nutrients fixation in the soil, but also leaching during irrigation. Foliar fertilizers can compensate for the constraining effects on nutrient availability and uptake (El-Habbasha et al., 2012). Many workers reported that spraying the plants with foliar fertilizers significantly improved the growth, yield and pods quality of legumes crops (El Fouly et al. 2010, Bozorgi et al. 2011 and El-Habbasha et al 2012).

Ayten (2011) found that foliar fertilization is an excellent way and help in giving the best yield of maize whether to add major or minor nutrients. Kocon (2010) found that broad bean yield increased by 14-15% when spraying with urea, compared to 2.4% at ground fertilization. Sharaf and others, (2009) found that foliar fertilization with boron led to increase the number of pods and plant seeds. Based on the foregoing, the study was conducted to know the effect of some foliar fertilizer alone or interaction with boron on dry seed yield of broad bean.

MATERIAL AND METHODS

A factorial field experiment was conducted at winter season 2012-2013 in the farm of experiments extension (Al-mhannawia, 8 km northwest of Hilla, Babylon) in sandy-clay loam soil (Table 1) shows some of its characteristics) to study the effect of some foliar fertilizers and its interactions with boron on broad beans (*Vicia faba* L.) plants. The experiment was conducted according to the randomized complete block design (RCBD) with three replications, each replicates contained 12 experimental unit (each of it contained four ridges (3 m length and 75 cm width). The experiment included six foliar fertilizers (neutral fertilizer 2g/l, high phosphorus 2g/l, high potassium 2g/l, urea 1g/l, seaweed extract 1 ml/l, in addition to the control treatment) with or without boron sprayed at 25 mg/l.

Broad bean seeds of spanish *var*. were soaked in water for 24 hours and planted after calibration on one side of the ridges at 20 cm apart on 11 October 2012. After a month of germination, foliar fertilizers was done and after one month later the second spray was done. In the interactions, boron spray was done after one week later. The other service process to plant and soil was done as the same as it was recommended. After giving the pods and arrival to mature, dry pods of plants in two internal ridges were harvested, and from it, number of pods per plant, dry seeds per plant and unit area, the proportion of protein in the dry seeds, were calculated. Statistical analysis was performed according to the program Gen. Stat (Edition 3), the averages were compared according to Least (LS $D_{0.05}$).

Table 1. Some physical and chemical characters of field soil on (0-0.40 m) depth*

Property	unit	value
Soil texture	Sandy clay loam	----
Bulk Density	$ugm.m^{3}$	1.24
Organic matter	$gm.kgm^{-1}$	4.60
Available nitrogen	$mg.kgm^{-1}$	73.20
Available phosphorus	$mg.kgm^{-1}$	12.80
Available potassium	$mg.kgm^{-1}$	276
Available boron	$mg.kgm^{-1}$	1.04
Ec	$dc.m^{-1}$	3.00
pH	----	7.14

* Central Laboratory of Soil dept. Agric. Coll. Baghdad Univ.

RESULTS AND DISCUSSIONS

Table 2 shows the superiority of all fertilizers treatment significantly in increasing dry pod weight compared to control except for the treatment of (high phosphorus), also urea treatment was superior upon most of other treatments which reached 9.18 g with a percentage increase of 18.9% compared to control. The reason is attributed to the nitrogen that plants need at all stages of their life cycle is vital to the growth of plants and increase production (Chatzopoulou, 2006).

The increase derived from the increase in the number and size of endosperm cells in the seed in the first days after fertilization and thus get the efficiently accommodate a larger amount of food and the weight rises accordingly. This is consistent with the results of Odeleye et al., (2007) who found that foliar nitrogen fertilizer on bean plant led to an increase in the weight of pod. Also may be due to the important role of phosphorus in seed production (Ramadan and Adam, 2007), and the role of potassium in enhanced the photosynthetic activity and in translocation of photosynthesis and its ability to develop bold seeds (Ali et al, 2007).

Boron spray treatment achieved a significant increase in pod dry weight which reaching 8.89 g with a percentage increase of 7.75% compared to control. This is due to the role of boron to encourage vegetative growth and increase the rate of photosynthesis and gathering plant dry matter (Zahoor et al., 2011). The interaction had a significant effect and superior significantly compared to control. The interaction between urea and boron was superior on all other transactions (except high potash and neutral fertilizers with boron) with a percent increase of (51.84%) compared to control.

Table 2. Effect of some foliar fertilizers and its interactions with boron on pod dry weight(g)

Fertilizers Boron	Control	Neutral	High P	High K	Urea	Seaweed extract	Mean of B
B0	6.50	8.73	8.23	8.57	8.50	8.65	8.25
B1	8.93	8.90	8.53	8.77	9.87	8.33	8.89
Fert. mean	7.72	8.82	8.38	8.67	9.18	8.65	
L.S.D. $_{0.05}$	Boron=0.48 Fertilizers=0.83 interaction=1.17						

Table 3 illustrates the superiority of all treatments significantly in increasing the number of seeds per pod compared the control(4.7), also the treatment of urea was superior upon most other fertilizers, which gave 5.8 seeds per pod with a percentage increase of (25.03%) compared to control. The reason is attributed to the nitrogen that led to an increase in plant height, number of branches and leaf area exposed to light and increase the efficiency of photosynthesis, which pushed for increased material representation and thus provided an opportunity to reduce the flower abortion as a result of reducing the state competition, including the food product during the growth and development of these flower, and nitrogen was needed by plants in all stages of their lives because of its vital to the growth of plants and increase production (Chatzopoulou, 2006). This was consistent with the results of Ayed (2012) on the beans. And it is attributed to potassium which enhanced the photosynthates activity which resulted in more number of seeds per pod (Ali et al, 2007).

Boron treatment achieved a significant increase in the number of seeds reaching 5.65 with a percentage increase of 10% compared to control. This is due to the positive role of boron in the transport of carbohydrate materials from the source to the sink, which gave a greater chance to reduce competition, (Barker and Pilbeam, 2006). This was consistent with Sharaf et al (2009) on broad beans when sprayed boron. The interaction between treatments had a significant effect, and all interactions were superior significantly compared to control. The interaction between urea and boron was superior on all others with a percentage increase of 56.9% compared to control. This was consistent with Shaaban et al (2006) that interaction of boron (25-50 mg/l) with nitrogen led to an increase in the number of seeds.

Table 3. Effect of some foliar fertilizers and boron on seeds no. per pod

Fertilizers Boron	Control	Neutral	High P	High K	Urea	Seaweed extract	Mean of B
B0	4.250	5.417	5.500	5.667	5.000	5.000	5.139
B1	5.083	5.333	5.750	5.833	6.667	5.250	5.653
Fert. mean	4.667	5.375	5.625	5.750	5.835	5.125	
L.S.D.$_{0.05}$	Boron= 0.196 Fertilizers= 0.341 interaction=0.482						

Table 4 showed the superiority of all treatments, and caused a significant increase in weight of 100 seeds compared to the control (121.3 gm) except seaweed extract and neutral fertilizer, urea treatment was superior upon most other transactions and reached 151.0 gm , with a percentage increase of (24.5%) compared to control. The reason is attributed to the role of nitrogen in vegetative growth and delay aging, or that the increase derived from the increase in the number and size of endosperm cells in the seed in the first days after fertilization and thus get a sink efficiently accommodate a larger amount of food and the weight rises accordingly. This is consistent with the results of Ayed (2012) on broad bean. It is attributed to P as a constituent of nucleic acids, phospholipids, coenzymes and the high emerge phosphate compounds, these aspects encourage dry matter accumulation and dry weight of seeds (El-Habbasha et al 2007). Also attributed to potassium in motivate and activate

plant enzymes, which led to an increase in the rate of 100 seed weight, or due to role of potash in translocation of photosynthates and its ability to develop bold seeds and this is consistent with Ali et al (2007).
Boron treatment had no significant effect in the 100-seed weight, it was consistent with Nel, (2001). The interaction had a significant effect compared to control (except the interaction of high-potash, neutral and seaweed extract with boron). The interaction between urea and boron was superior on all others (except high-phosphorus with boron) which gave a percentage increase of (30.8%) compared to control.

Table 4. Effect of some foliar fertilizers and boron in 100-seed weight (g)

Fertilizers Boron	**Control**	**Neutral**	**High P**	**High K**	**Urea**	**Seaweed extract**	**Mean of B**
B0	119.3	133.7	137.7	135.5	146.0	127.3	133.2
B1	123.3	135.3	139.3	136.7	156.0	130.7	136.9
Fert. mean	121.3	134.5	138.5	136.0	151.0	129.0	
L.S.D.$_{0.05}$	**Boron**= N.S. **Fertilizers**= 13.49 **interaction**= 19.08						

Tables (5-6) showed that all treatments caused a significant increase in dry seed yield compared to control (except seaweed extract) in dry seed yield of plant and unit area. The treatment of urea was superior upon most of other treatments with a percentage increase of (37.85%, 37.80%), respectively, compared to control. It may be due to the role of nitrogen in improving vegetative growth and increase the number of branches and encourage the emergence of floral buds represented by increasing the number of inflorescences floral, or to influence the improvement of physiological plants operations such as photosynthesis and thus led to full of seeds fully and increase the yield (Kocon, 2010), and the transition from the vegetative stage to the reproductive stage requires the transfer of materials manufacturer in leafs to seeds formed and this process needs phosphorus (Ping and Li, 2005). These results are consistent with Ehsanipour et al, (2012). And also may be due the impact of phosphate fertilizer for its role in the improvement of plant growth and yield in broad bean (Abdalla, 2002), and in beans (Kandil et al, 2013), as well as spraying potassium on the vegetative works to increase vegetative growth and thereby increase yield. The table showed that boron treatment gave a significant increase in dry seeds of plant and unit area with a percentage increase of (10.88%, 10.89%), respectively, compared to control. The reason to outweigh the spray treatment boron in plant holds the dry seeds to its superiority in the average number of pods which constitutes a major component of winning (Sharaf et al, 2009). The reason of boron superior in yield due to its superiority in the average number of pods and seed per pod, which is a key component of yield (Sharaf et al, 2009). And also may be due to the positive role of boron in transport carbohydrate material from the source to the sink. These results are consistent with Zahoor et al, (2011). The interaction had a significant effect and all interactions were superior upon control (except seaweed extract with boron).

Table 5. Effect of some foliar fertilizers spray and boron in plant dry seeds yield (g)

Fertilizers Boron	Control	Neutral	High P	High K	Urea	Seaweed extract	Mean of B
B0	33.97	46.67	46.30	46.67	50.20	45.83	44.94
B1	43.33	51.63	52.70	49.50	56.33	45.50	49.83
Fert. mean	38.65	49.15	49.50	48.09	53.28	45.67	
L.S.D.$_{0.05}$	Boron= 4.78 Fertilizers= 8.28 interaction= 11.71						

Table 6. Effect of some foliar fertilizers spray and boron in dry seeds yield (t/h)

Fertilizers Boron	Control	Neutral	High P	High K	Urea	Seaweed extract	Mean of B
B0	2.263	3.108	3.082	3.108	3.343	3.052	2.993
B1	2.886	3.438	3.509	3.296	3.751	3.030	3.319
Fert. mean	2.574	3.273	3.296	3.202	3.547	3.041	
L.S.D.$_{0.05}$	**Boron**= 0.318 **Fertilizers**=0.551 **interaction**= 0.779						

Table 7 shows the superiority of all treatments significantly in increasing the proportion of protein in seeds compared to control. Urea treatment was superior upon all other treatments, which reached (26.88) with a percentage increase of (49%) compared to control. The reason is attributed to the role of nitrogen in the formation of nucleic acids needed to build proteins in plant and foliar fertilizer helped in processing plant directly with nitrogen to form amino acids, and this is consistent with Daur et al, (2008). It may also be due to the role of phosphorus in the composition of nucleic acids (RNA)which is important in the composition process of proteins (Gad, 2012), it consistent with Shafeek et al (2004). It also contributes to potassium in the process of photosynthesis and sugars transmission from the source to the sink and plays an important role in the formation of the protein, which was confirmed by the Radulov et al, (2010).

Boron treatment gave a significant increase in the proportion of protein (23.47) with a percentage increase of (7.37%) compared to control. This may be attributed to the role of boron in the process of protein synthesis through its importance in nitrogen fixation vital air as well as through its influence in the process of formation of RNA (Mahler, 2004) and this is consistent with Ziolek and Ziolek (1988). The interaction had a significant effect and all interactions were superior upon control. Urea and boron was superior compared with other interactions with a percentage increase of (64.1%) compared to control. These results are consistent with Gabal et al, (2005) when spraying nutrients (K and B) on leaves led to a significant increase in the percentage of protein compared to untreated plants.

Table 7. Effect of some foliar fertilizers spray and boron on seed protein percentage

Fertilizers Boron	Control	Neutral	High P	High K	Urea	Seaweed extract	Mean of B
B0	16.74	24.15	21.93	21.41	26.29	20.66	21.86
B1	19.34	25.40	23.38	23.36	27.47	21.88	23.47
Fert. mean	18.04	24.775	22.655	22.385	26.88	21.27	
L.S.D.$_{0.05}$	**Boron**= 0.832 **Fertilizers**=1.441 **interaction**=2.038						

CONCLUSIONS

Foliar fertilizers of macro nutrients and urea alone or with boron caused an enhance in plant growth and increase seed yield of broad bean.

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LOCAL POPULATION OF LONG HOT PEPPER, 'CORNUL CAPREI' IMPROVED AT V.R.D.S. BUZAU

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Abstract

In order to prevent biological degradation and impurification over time of local population 'Cornul Caprei', was included in the preservation and valorization program of autochthonous genetic patrimony initiated in 1996 at V.R.D.S. Buzau. In the vegetable basin Buzau, this cultivar has a tradition regarding presence in culture and application. It is known for over 200 years by traditional vegetable growers. This paper presents the genealogy of the local population, quality characteristics and productivity potential resistance to pathogens and vegetation period. This local population has attracted particular attention by its uniqueness, cultivar with long, large fruits, similar to those of long pepper but, at technical and physiological maturity are colored in yellow-orange and are hot. After a detailed evaluation of the main characteristics it was found that in this local population are found several biotypes and 3 of them have distinct expressivity. Were retained the lines L16, L17A, L17 B and the intermediary form were eliminated. The improvement methods used were the ones particular to the species, especially intraspecific hybridization between valuable lines. Resulting hybrid populations were subjected to repeated individual selection for 6 generations. The main objective of this paper was achieved. The line L17 A, being representative for this population, was obtained in 2012 and submitted at ISTIS for homologation and patenting. For this improved cultivar was kept the original consecrated name, 'Cornul Caprei'. The initial material is a valuable reservoir of genes for obtaining new creations.

Key words: *cultivar, local population, selection, genotype, 'Cornul Caprei'*

INTRODUCTION

Pepper crop in Romania occupies an important place, ranking the fourth place after tomatoes, cabbage and onion. Because of the quality of its fruits, and also by the great diversity of varieties grown, the pepper has a great importance, both for fresh consumption and processed form. High food value of pepper fruit is given by the high content of sugars and vitamins, ascorbic acid is present in large amounts in fruits of pepper, content that varies depending on the kind and condition of the fruit ripening, being between 100 mg% fresh substance at fruits that reached technological maturity and 200-300 mg% fresh substance at fruits that reached physiological maturity.

Besides vitamin C content, fruits of pepper also contain vitamins B1 and B2, vitamin PP, vitamin E and provitamin A (Pintilie, 1996).

For pepper, the goal is to achieve high productivity varieties and hybrids, with high content of dry substance and ascorbic acid, multiple resistance to diseases and adverse environmental conditions, with large fruit, dark green to yellow white at technological maturity and red or yellow orange at physiological maturity (Sutea, 1983).

The vegetable basin Buzau accounted for SCDL Buzau the main genetic resource for improvement. Traditional local growers carefully preserved some local populations, old, valuable, which in time were made known and appreciated on the market. At the base of the main creations homologated by SCDL Buzau are local populations e.g.: onion 'Aurie de Buzau', 'Diamant', 'Rubiniu', variety of cabbage 'Buzau', 'Magura', bell pepper 'Arum' etc. These cultivars, although they were genetically well sanded and improved by researchers, we encounter today on their raw form, unimproved in the holdings of traditional vegetable growers. Besides these cultivars which were studied and have generated valuable varieties are also some populations that have been neglected. Of these, particularly drew the attention the population of long pepper, 'Cornul Caprei'. In the vegetable basin Buzau, this cultivar has tradition regarding it in culture and cultivation, being known for over 200 years by traditional farmers. This paper presents the genealogy of this local population, the quality characteristics and yield potential, resistance to pathogens and the vegetation period. The cultivar shows uniqueness thru its long fruit, large, similar to those of Kapia pepper, but at technical and physiological maturity are colored in yellow-orange and are hot.

MATERIALS AND METHODS

The researches were performed at S.C.D.L. Buzau in the Laboratory of Plant Breeding. Since 1996, the laboratory has placed great emphasis on the conservation of biodiversity and also had campaigned for the protection of indigenous genetically patrimony. On this occasion a local population of long pepper was discovered, 'Cornul Caprei'. It has been brought long ago in the vegetable basin Buzau by Bulgarian immigrants who settled here. Due to imports of seeds and the introduction in culture of many new biological creations, the local population was in danger of genetic depreciation. The basic genetic material (seeds) were purchased from local traditional growers. Works for improvement have started with a careful assessment of the main characters and their purity in lineage transmission. After the evaluation were identified three distinct genotypes coded as follows: L 16 L17 A, L 17 B were found in crop with the following percentages: L16-12% L 17 A - 62% L17 B - 8% and 18% were eliminated.

After identifying and establishing the main characteristics of distinctively, three genotypes were separated:

- L 16 presents long dark red and hot fruit.
- L 17 A presents long dark green fruit and it colors orange at physiological maturity
- L 17 B presents long light green fruit that can be consumed at this stage, and at physiological maturity turns in yellow orange.

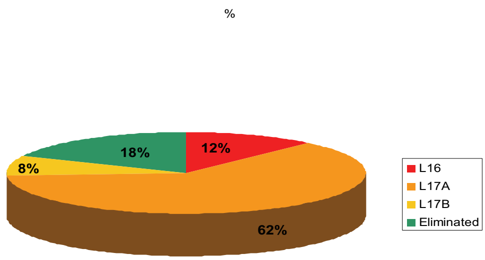

Figure 1. The genotypes percentages

Figure 2. L 16, L 17 A and L 17 B

The line L 17 A is representative for the pepper population 'Cornul Caprei' and resembles very much with the basic genotype.

The other lines appeared in time, due to contamination with other cultivars. In order to obtain these lines, repeated mass selection was done annually in valuable local populations and intraspecific hybridization between valuable lines, followed by genealogical selection until generation 7, continued by annual repeated mass selection in advanced generations.

Way of placing the variants was in randomized blocks in four repetitions.

During the growing season, observations and measurements were made according to the objectives of the proposed improvement.
To maintain the genetic identity of the line L 17 A, 'Cornul Caprei', it was introduced in the maintenance program using the following methods: individual selection of typical plants in the choice field, selection on lines in the field of study of descendants and individual selection after negative characters in pre-basic and basic fields.
Statistics indices for each character were: the average ($\bar{x}$),standard deviation (s), coefficient of variation (CV%), limits of variability ($\bar{x}$±S). The number of individuals in which biometric determinations were made is 100 and at sequence variation preparation were used 8 classes. Based on the study variability of coefficients and limits of variation calculated for each quantitative character, in the links from the selection scheme. The choice of biological material was made every year in order to maintain variety in the range of specificity and authenticity.

RESULTS AND DISCUSSIONS

Researches completed until now with the obtainment of 3 new lines of hot pepper. In this experience was not used any witness variety because we do not have inscribed in the National Catalogue any Romanian creation from this group. Lines obtained meet the condition of variety, have strong distinct characters, are uniform and they transmit unaltered characteristics in descendants.

Table 1. The main characteristics of plants (average values) 2010-2013

Character	Lines		
	L16	L17A	L17B
Pant height (cm)	44,2	48,8	48,1
Sten height (cm)	16	18	17
Bush diameter	40,7	43,2	41,5
Fruit position on the plant	Pendulum	Pendulum	Pendulum
Immature fruit color	Dark green	Dark green	Light green
Mature fruit color	Red	Orange	Orange
Fruit weight per plant (g)	989	1108	868

Figure 3. L 16, L 17 B and L 17 A

We consider that the main objective of the researches was reached. Line 17A has been inscribed at ISTIS Bucharest under the name of 'Cornul Caprei', thus preserving its traditional name. It is in the second year of testing and the results obtained so far are very good.

Table 2. The main characteristics of fruits (average values) 2010-2013

Studied character		Lines		
		L16	L17A	L17B
Weight (gr)		52,6	63,3	47,8
Receptacle weight (gr)		7,8	7,3	6,9
Seeds weight (gr)		2,1	1,4	1,9
Seed (no./fruit)		162	101	153
Receptacle rosette diameter (cm)		2,4	2,3	2,1
Tail length (cm)		3,6	4,1	4,2
pulp thickness (cm)		0,3	0,4	0,4
Length (cm)		15	18	21
Fruit diameter (cm)	Base	3,6	3,9	3,1
	Middle	2,9	3,3	2,6
	Apex	1,2	0,9	0,8

The lines obtained at S.C.D.L. Buzau have a high content of capsaicin which concentration grows significantly with fruit maturation.

Figure 4. L 16, L 17 B and L 17 A, detail for immature and mature fruit

Variability of the main characters at L 17 are shown in Table 3.

Table 3. Variability of the main characters at the variety of long pepper 'Cornul Caprei' (17A)

Character	Statistical indices calculated (average on 4 years)			
	$\bar{X}$	S	CV%	$\bar{X}\pm S$
Plants height-cm	48,8	3,0	6,14	45,8-51,8
Fruits length-cm	18,0	2,0	11,1	16,2-20,2
Fruits weight-gr	63,3	4,0	6,3	59,3-67,2
Production of fruits / plant -gr	1108	120	10,8	988-1228

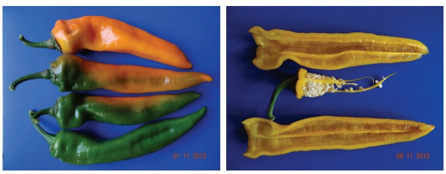

Figure 5. L 17 A, fruit evolution and fruit longitudinal section

Statistical indices characterizing samples collected for the characters studied at the variety 'Caprei Cornul' is as follows: plant height(cm), overall general average of the experimental year 2010- 2013 was of 48,8 cm with a small coefficient of variability registering a value of 6,14%. Length of fruit - coefficient of variability calculated for the entire period of experimentation recorded somewhat higher values of 11,1%, which proves that this character has a greater margin of variability.

Regarding the weight of the fruit, the limits of variability were between 59,3 and 67,2 and the coefficient of variability was 6,3, These values recorded demonstrate that the variety shows uniform fruit size, also presenting a pleasant commercial aspect.

The production of fruits harvested from a plant varied within the limits 988 - 1228 gr, recording an average of 1108 gr and the coefficient of variability being 10,8 %.

Due to seniority in culture and natural selection has made this line to manifest the phenomenon of ecological plasticity. Also in terms of resistance to diverse pathogens, it was observed that all three lines possess resistance genes for specific diseases of this species.

New variety obtained opens a new direction of use for this species, being the only long hot pepper that can be preserved by pickling, both alone and combined with other vegetables, doesn't depreciating it's firmness during conservation.

CONCLUSIONS

The researches were finalised with reaching the main objectives proposed:

- saving the local population 'Cornul Caprei' which was in danger to extinct;
- obtaining 3 distinct valuable genotypes: L 16 with hot red fruit, L 17 A-typical 'Cornul Caprei', with hot orange and L 17 B immature green fruit and mature yellow fruit;
- patenting the line L 17 A under the name 'Cornul Caprei' represents the major success of the research undertaken until now;

Improved genetic material in the research is a valuable genetic resource for obtaining new biological creations.

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RESEARCH CONCERNING THE ACCLIMATIZATION AND BREEDING OF *MOMORDICA COCHINCHINENSIS*, A CUCURBITACEAE SPECIES

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Abstract

V.R.D.S. Buzau was interested in the acclimatization, breeding and crop production of rare plants since the station foundation in 1957. Rare Plants Laboratory was founded in 1962 and was led by the well known prof. Oros Victor. Here were cultivated for the first time in Romania species as asparagus, rhubarb, fennel, Luffa cilindrica. The Laboratory activity ceased and it was resumed after 1990 to continue valuable creations acclimatizations which were patented and crop production: Cichorium crispum, Cichorium latifolium, Cucumis metuliferus, Momordica charantia. Starting 2010, research were focused on acclimatization and breeding of new species like Momordica cochinchinensis, obtaining the first pot plants. This species shows major interest for prestigious world institues due to their valuable fruits with high content in lycopen, carotenoids and antioxidants. The basic genetical material (seeds) used comes from Vietnam, Nepal and India. The first fruits were obtained in 2012 and in 2013 the yield increased meanwhile it was elaborated the crop technology. Two of the three studied biotypes adapted to climatic conditions of our country. To specify that this new acclimated species can be cultivated only in high protected spaces and pallisate system in our country . After acclimatization phase, research focuses on breeding this species to obtain new distinct varieties adapted to climatic and soil conditions of our country.

Key words: *acclimatization, breeding, biotype, dioecious, Momordica cochinchinensis*

INTRODUCTION

Plant species that we have in the culture in our country, have an area of origin in a particular geographical area, very few of them having origins in our country or in nearby areas. There are few species cultivated in our culture that can fit in endemic populations or species. The introduction of new species or populations of culture in our country was made gradually, in the course of history. For very many species the exact period of introduction in the country and their authors is unknown. After 1990, when circulation outside the country was possible more easily, the laboratory of Genetics and Improvement of the unit aimed to resume the work of acclimatization and introduction to the culture of new species suitable for vegetable growing. Since 1962, this activity has been developed by the laboratory of Rare plants, although it has had outstanding results, in time it was disbanded. Founding members of this laboratory promoted species and varieties of fennel, rhubarb, asparagus, Lufa cilindrica, etc. At present, the laboratory of Genetics and Improvement was able to improve, acclimate and to introduce the cultivation of valuable species: *Momordica charantia, Cucumis metuliferus, Cichorium crispum, Cichorium latifolium, Ocimum basilicum* etc, species of which have been obtained and approved new valuable varieties. In addition, special attention was paid to the species *Momordica cochinchinensis*, which is the subject of this work. This species has not been cultivated and studied so far in Romania. Research has actually started in 2010 by getting the first plants in pots. Although it is a cucurbits and pedoclimatic features of our country are favourable to this family, this species has presented many unpredictable unknown acclimatization program but that could pass. This species caught the attention especially for its chemical content of the fruit. Phytochemical studies revealed that seed membrane and seed oil are the excellent source of bio-available Beta-carotenes and Lycopene (Voung, 2005) which promotes healthy vision, and inhibit the proliferation of cancer cells. Lycopene levels in fruit are higher than those in any of the plant sources reported a good source of vitamin-E alpha-tocopherol and contain antimicrobial and anti-diabetic properties (Debnath B., Sinha S., Sinha R.K., 2013).

MATERIALS AND METHODS

The studies and research carried out on this species were conducted after a thoroughly prepared program developed in several stages. The first phase was that of documentaries and the genetic material of the seeds, respectively. The seeds were obtained with great difficulty, sources from areas with tradition in the cultivation of this species. (Figure 1 and Figure 2) Were purchased and used to experience three genotypes called L1, L2 and L3 are the following background: L1 India, L2-Vietnam, L3-Nepal. The main characteristics of seeds for the three studied genotypes are shown in Table 1.

Figure 1. L1, L2, L3 seeds

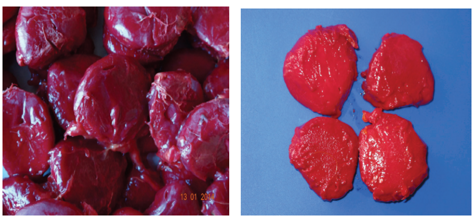

Figure 2. Aryl coated seeds

Table 1 . The main characteristics of seeds

Features / Genotypes		L1	L2	L3
The weight of the seed after extraction (g)		4,5	4,6	4,4
Seed weight after drying (g)		4	3,2	3,2
The weight of the seed/fruit after harvest (g)		85	73,6	83,6
Seed/fruit weight after drying (g)		66	51,2	60,8
Aryl thickness (cm)	Mantle	1,5	1,6	1,4
	Pulp	2	2,1	2,2
Aryl weight (g)		3,6	3,7	3,5
No.seeds / fruit		18	16	19
Seed length (cm)		3,1	3,3	2,8
Width seed (cm)		2,9	2,8	2,6
Seed thickness (cm)		0,7	0,5	0,8

The second stage concerned the acclimatization of species to the pedoclimatic of our country. This stage has embedded the largest volume of work assigned to this programme. There have been observations and detailed measurements starting from seed, continuing throughout the entire vegetation period up to fruition and harvest. The first plants were obtained in potting mixes in the laboratory and then were transferred to the cold greenhouse, without heating, and in the field. This stage was faced with most novelty items which were finally overcome.

The third stage aimed at elaboration of the specific culture. At this stage, were studied several options and technological links such as crop establishment by planting and direct seeding studies on seed germination, seedling production, genotypes growing in pots vegetation in protected areas in the field, planting distances specific to each cultivar, special care and domestic work, monitoring pest and disease occurrence etc.

After purchasing seeds, they were sown in pots filled with peat on 15.02.2009. The first seeds germinate after about 6 months after July 15. In order to solve this situation, research has been directed towards this objective to find a viable solution to reduce germination period. After careful consideration of the seeds was observed that the skin is thick and extremely tough. In this situation it were created several experimental variants of germination: normal seed sown directly in pots and in the field, seed scarified, pre germinated seeds in water, seed with fissured skin and seed despoiled skin. Following this research concluded that the most effective method is the removal of the skin covering of the seed itself. This version recorded at time of germination in pots 20-30 days after sowing (Table 2).

Table 2. The main phenological data of plant *Momordica cochinchinensis*

Phenological data / Genotypes	L1	L2	L3
Sowing	20.03	20.03	20.03
Rising	16.04	20.04	13.04
Planted	7.05	7.05	7.05
Male plants begin of blooming	20.07	25.07	15.07
Female plants begin of blooming	15.08	8.08	28.07
Fructification	22.08	17.08	12.08
Reached technological maturity	27.10	22.10	15.10

RESULTS AND DISCUSSIONS

Culture can be established in protected areas both through seedling and direct seeding. The best results were obtained in culture through seedling establishment. After emergence of seeds, the plants have a slow progress, until the first normal leaves appear. Then the growth rate accelerates, gaining lush plant appearance. In terms of culture from sowing until planting, seedlings occurs in about 65-75 days (Figure 3). The plant has the appearance of liana, with a bright, lush vegetative growth, can surpass 15 m tall, with a number of extremely high lateral shoots. In protected spaces behave as a biennial herb, on the understanding that in year 2, vegetative development is much stronger, with more abundant fructification and something more (Figure 4).

Figure 3. Seedling, year 1 and year 2

Figure 4. Beginning of vegetation, year 2

In terms of adaptability and expressiveness of the studied genotypes there have not been recorded significant differences between the characteristics of fruit and plant genotype, but L3 proved to be superior in terms of adaptability to the other two. This genotype showed a greater ability of binding and fructification, its seeds were the first to reach physical maturity.
Technology culture and strict observance of technological flux in this species is the main key to success culture.
Regarding culture technology were established major technological milestones for the cultivation of this species. In protected areas concluded that the most important technological component is the planting (seed culture) and density. We studied several variants with different densities, but since it was a massive lush plant growth it was determined that the distance between plants in the row must be at least 3 m and minimum 10 m distance between rows. Shortly after planting, plants must have a trellis system, well supported and provided space for developing optimal central axis and lateral shoots. For the trellis system it is recommended not to tie the string at the base of the plant, but at 3-4 leaves above, because the stem at the base, gaining a bulb elongated layout, develop powerful herb that can strangle. The eye of the string should be broad and carefully watched the entire vegetation period. We recommend the using of the trellis system for the lateral shoots. At the top of the greenhouse, to more than 2 m height must be held out pick-up wires from a distance of 30-40 cm between them, because plant creates a powerful vault.
A special attention must be paid to fruit sustaining because the shoots that feed the fruits could be affected. It is recommended to

use support nets similar for melons or to tie the fruits stalk by the support wire. Culture does not require any special snubbing, pinches but only the routing of shoots in vertical and horizontal, at the level of the support system. Water supply is rhythmically during summer when temperatures are high, and twice a week in the spring and once a week in autumn. In terms of disease and pests was found that the species shows genetic resistance against them was not reported until now their attack without having to counter chemical treatments during the growing season. The plant shows a strong well-developed root system consisting of a main root, pivoting and a large number of secondary roots, strongly developed horizontally on the fertile layer from the surface (Figure 5).

Figure 5. Root aspect

The stem is strong, powerful developed and lignified at the base with a high growth rate, up to 2.5 -3 m, then shoots coming from this, take over the mission of forward travel. Plant has a large number of shoots branching off from main which many secondary shoots. The number of primary and secondary shoots is significantly higher in the second year. Main shoots have the appearance of rope vines, slightly lignified, with gray-brown shell with small roughness and the secondary are herbaceous, green. Main shoots have a round shape at the base and top are slightly grooved and the secondary shoots show the entire surface slightly grooved.
Lobed palmed leaf is composed of five lobes, presenting a large number of plant leaves, on the tips of shoots of growth shoots they are small and would submit on the rise they shall increase. The leaves are dark green and have at the base 2-4 glands that secrete a sweet substance similar to bees preferred nectar. The leaves are in the lower layers of the vault, due to lack of light, turn yellow and dry (Figure 6).
The leafs presents a long petiole and after dry does not come loose from the sprout, becoming fibrous. From the same plant shall meet leaves with 2 and 4 glands, rarely 3. The main characteristics of leaves are shown in Table 3.

Table 3. The main characteristics of the Momordica cochinchinensis leaves

Characteristics	Small leaf	Medium leaf	Large leaf
Length (cm)	12	17	19
Width (cm)	12	16	16,5
Petiole length (cm)	5,5	6	5,5
Number of lobes	3	5	5
Number of glands	2	2-4	2-4

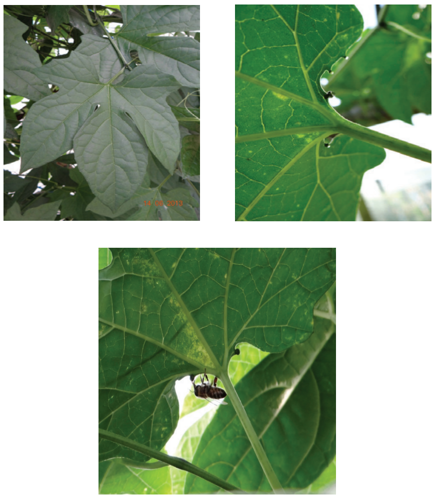

Figure 6. Leaf appearance; leaf with 4 and 2 glands

Shoots presents down of the leaves from place to place tendrils of two types: the ones at the top shoot are in the form of the snail's horns and shall have the task of exploring and search for support (Figure 7) .
They, in time rigidify and shall be fixed in the form of an arc of the support point, appearing in their place other explorer tendrils, helping plant to submit on the rise. If the explorer tendrils do not meet a support point, they wilt, dry and that sprout is commanded to stop

growth, plant directing its energy other shoots. But there are situations when the shoots do not meet support point and then they self support each other very often occurring the phenomenon of self strangulation (Figure 8).

Figure 7. Agressive tendrils

Figure 8. Self strangulated shoot

The plant pollination is entomophilies, being made by insects. In our country conditions, pollination is poor. Under normal conditions, very little flowers seem to be pollinated and to seize. Therefore, in order to increase the number of flowers it is recommended the manual pollination. Recognition male and female plants shall be assessed at the time of flowering. Investigations so far did not reveal any other indication in respect of the recognition sex before the appearance of flowers. Both male flowers and female remain open only one day. After flowering, the next day, the flower tightens, shrivels and then falls (Figure 9).

Figure 9. ♀ Floral buds without bracts; ♀ Floral buds with bracts; ♂Floral bud

Male plants are the first to bloom 3-4 weeks before the female plants. Shows large flowers special form of cups, yellow-cream, and three petals at the base shows a dark brown to black stain. Flower buds are located at the armpit leaves protected by a green bracts as the bud grows, allowing the flower to open out and open. Male flower shows larger than the female (Figure 10 and 11).

Figure 10. Male flower Male flower petals and calyx

Figure 11. The evolution of male flowers

The female plants flower shows distinct size, slightly smaller than the male ones, based on corolla miniature fruit. Flower petals are yellow-based without stains unlike the male ones. The unfertilized and fertilized partially wilting shortly after starting to turn yellow flower stem gradually dries completely (Figure 12 and 13). The same plant can meet two types of flowers with bracts at the base or without bracts. The main features of the male flowers and female are presented in Table 4 (Table 4).

Table 4. The main characteristics of male and female flowers at *Momordica cochinchinensis*

Floral characteristics	♂ flower	♀ flower
Floral bud length (cm)	8,5	4,5
Length of sepals (cm)	1	0,8
Calyx diameter (cm)	3,8	1,2
Number petal corolla	5	5
Petal length (cm)	10	6,5
Number of stamens	6	-
Stamens length (cm)	1,5	-
Flower weight (g)	6	6,4
Pedicel length (cm)	10,5	7
Fruit length after binding (cm)	-	2
Flowers diameter (cm)	11,8	10,4
Lifetime of open flower (days)	1-3	1-3

Figure 12. Evolution of the fertilized flower and unfertilized flower

Figure 13. Unfertilized fruit and manually pollinated flower

After fertilization, the fruit is growing quickly in size, reaching up to 1-1.5 weight kg, featuring green color on the exterior to beautifully ornate physiological maturity with spikes easy aggressive 2 mm high (Figure 14). Round shape fruit has lengthened slightly and fasten with a long peduncle of 12,5 cm rough and fibrous. The main characteristics of the fruit are presented in table 5.

Table 5.The main characteristics of the fruit *Momordica cochinchinensis*

Characteristics	
Weight of the fruit (kg)	1,1
Diameter of the fruit (cm)	13
Height of the fruit (cm)	14,5
Length of peduncle (cm)	12,5
Peduncle base diameter (cm)	2
Peduncle diameter at grip (cm)	0,7
Peduncle diameter in the middle (cm)	0,6
Height ornamental spines (mm)	2
Blossom end diameter (mm)	14
Blossom end form	Star
shape of the fruit	Round high
Pericarp thick (mm)	1
Thickness pulp (mm)	15

Figure 14. Fruit evolution

Mature plant withstand low temperatures close to the freezing threshold, in protected spaces, the pace of growth slows but the fruit never depreciates. The fruit has a rate of growth accelerated after pollination but travels a long period of time from the phenophase green color to orange (Figure 15, 16 and 17).
In Table 6 is presented the variability of the main characters at L1. In terms of the height of the plant, it proves to be stable, with a coefficient of variability low, below 10% (7,35%).
In terms of the size of the fruit are a little larger variability, represented the fruit of 900 g - 1.3 kg.

Table 6. The variability of the main characters at L1

Character	Statistical indices (3 years average)			
	$\bar{X}$	S	CV %	$\bar{X}\pm S$
Plant height (m)	13,6	1	7,35	12,6-14,8
Fruit weight (kg)	1,1	0,2	18,1	0.9-1,3
Fruit lenght (cm)	14,5	2	13,7	12,5-16,5
Fruit diameter (cm)	13	2	15,3	11-15
Seeds (no./fruit)	18	2	11,1	16-20
Seeds weight/fruit (g)	60	5	8,3	55-65

Figure. 15. Fruit evolution

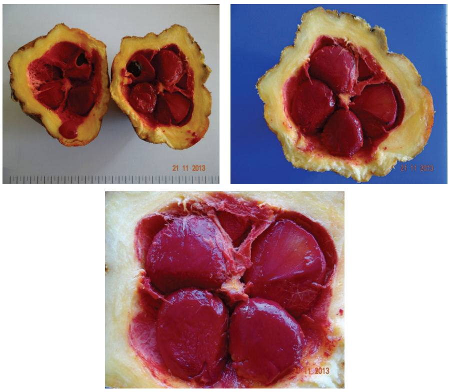

Figure. 16. Mature fruit sections

In the table no.7 the variability of the main characters is presented for L 2. The values are close to those of L2 to L1, variability is higher by registering to the weight of the fruit on the plant with a coefficient of 21.4%. And in terms of seeds number per fruit was recorded 12,5% variability.

Table 7. The variability of the main characters at L2

Character	Statistical indices (3 years average)			
	$\bar{X}$	S	CV%	$\bar{X}$±S
Plant height (m)	15.4	1.2	7,7	14.2-16.6
Fruit weight (kg)	1,4	0,3	21.4	1.1-1.7
Fruit lenght (cm)	14,6	2.2	15	12,4-16.8
Fruit diameter (cm)	13.8	1.2	8.6	12.6-15
Seeds (no./fruit)	16	2	12.5	14-18
Seeds weight/fruit (g)	51.2	3.6	7	47.6-54.8

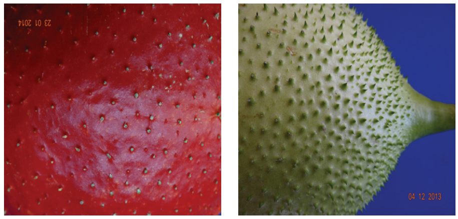

Figure 17. Fruit spines detail

Table 8. The variability of the main characters at L3

Character	Statistical indices (3 years average)			
	$\bar{X}$	S	CV %	$\bar{X}$±S
Plant height (m)	12.6	1.2	9.5	11.4-13.8
Fruit weight (kg)	1,75	0,10	5.7	1.65-1.85
Fruit lenght (cm)	16.4	1.2	7.3	15,2-17.6
Fruit diameter (cm)	12.8	0.8	6.2	12-13.6
Seeds (no./fruit)	19	3	15.7	16-22
Seeds weight/fruit (g)	60.8	5.4	8.8	55.4-66.2

For all the studied characters, L3 has demonstrated reduced varibilitate coefficient of less than 10%, except the number of seeds in the fruit that has a higher coefficient of variability, 15.7%. recorded values and the results obtained demonstrate that this line is genetically superior and at the same time registering the highest production/plant.

CONCLUSIONS

Research undertaken for acclimatization and placing in the culture of the species *Momordica cochinchinensis* till now have been completed with a great success.

All three genotypes showed a great capacity of adaptation to the pedoclimatic features of our country. This feature has been observed better at L3 genotype.

Were obtained for the first time harvestable fruits with germinable seeds in protected unheated spaces. Currently we are working on improvement of the studied genotypes for stabilization of the main characters and the distinctively and uniformity (DUS) with the purpose of reaching of new varieties.

Specific technology has been developed for growing species in protected areas.

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THE IMPACT OF BIOFERTILISERS ON THE QUALITY PARAMETERS OF THE PEPPER FRUIT (*CAPSICUM ANNUUM* L.) IN ORGANIC AGRICULTURE CONDITIONS

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Abstract

Organic agriculture, as a way of thinking and practice, originated in the first years of the XX century upon the application of various alternative methods of agricultural production. This experiment was carried out in 2009- 2011 on the fields of the Agroecological Centre at the Agricultural University - Plovdiv, situated on the territory of the certified ecological farm. The research included pepper variety 'Sofiiska Kapiya' cultivated as mid- early field production according to the principles of organic agriculture. The following biofertilisers were tested: Seasol (Earthcare), applied on two basic fertilisations, namely: Boneprot and Lumbrical. The objectives of the study were to establish the impact of biofertilisers on the quality parameters of the pepper fruits (dry matter, total sugars and vitamin C). The dry matter content of pepper fruits showed the highest values in 2009 and 2011, when the average for the period was 8,87 % and was shown by the variant fed with the biofertiliser Seasol on basic fertilisation Lumbrical. The total sugar content in the pepper fruits showed a maximum value of 7,22 % as the average for the study period and after application of the biofertiliser Seasol on the basic fertilisation Lumbrical. The combined application of the biofertiliser Seasol on the two basic fertilisations showed higher values of the total sugars in comparison with the single application of the basic fertilisations in optimum concentrations. The average for the period vitamin C content in fruits showed highest value upon application of the biofertiliser Seasol on the basic fertilisation Boneprot, i.e. 159,2 mg%, thus determining this combination as useful for vegetative feeding. The values of all treatments exceeded the control, thus confirming the beneficial impact of biofertilisers with respect to the improvement of pepper quality under organic management.

Key words: *biochemical parameters, biofertilisers, Capsicum annuum* L., *organic agriculture, quality*

INTRODUCTION

Nowadays, organic agriculture is the synonym of a modern and contemporary production system, and biological products are the standard for healthy and quality food (Semos, 2002). Organic agriculture approach aims at establishing an integrated, ecological and economically sustainable system for the production of agricultural products (Stacey, 2003). The first documented use of the term “organic farming” was by Lord Northbourne in his London-published 1940 book *Look to the Land* (Paull, 2008).

In recent years there has been a significant increase in the demand for high quality fruit and vegetables (Vlahova, 2006). The great importance and distribution of pepper are due to its high biological value and the possibilities for its various use, both fresh and processed (Cholakov, 2009). Red pepper (*Capsicum annuum* L.) is among the products with quality dependency on drying conditions (Alves-Filho et al., 2007). The total sugars contained in the long fleshy red pepper are an element of the nutritional value of fruits and affect their gustatory properties (Pevicharova et al., 2007). Pepper fruits obtained from organic cultivation system comprised higher amount of vitamin C, beta-carotene and total flavonoids (Szafirowska and Elkner, 2008). Vlahova et al., (2011) show that according to Salami (2002) and Narayan et al. (2009) pepper contains a number of nutritionally-important compounds such as vitamin C and other mineral nutrients.

The research on the impact of biofertilisers on the quality of the pepper fruits cultivated in a ecological farm are relatively limited in

Bulgaria. However, such results are needed for the popularization of the advantages of organic pepper production, in view of ensuring quality and underlining the advantages of organic agricultural system.
The objectives of the study were to identify the impact of biofertilisers on the quality parameters (i.e. dry matter, total sugars and vitamin C) of pepper cultivate under organic farming.

MATERIALS AND METHODS

This experiment was carried out in 2009-2011 on the experimental fields of the Agroecological Centre at the Agricultural University - Plovdiv (Bulgaria), situated on the territory of the certified ecological farm.
Pepper is an annual crop and belongs to the Genus *Capsicum* of Family *Solanaceae.* The research included pepper of the variety 'Sofiiska Kapiya' used for average early and late field production (Panayotov, 2000).
Treatments:
1. Control (non-fertilised)
2. Basic fertilisation with Boneprot (optimum)
3. Basic fertilisation with Boneprot (50 %) + Seasol
4. Basic fertilisation with Lumbrical (optimum)
5. Basic fertilisation with Lumbrical (50 %) + Seasol

Two basic fertilizations were used, namely Boneprot and Lumbrical, applied into the soil through incorporation prior to planting of the seedlings on the field. The biofertilisers were applied in two concentrations - optimum (corresponded to 70 kg/da for the basic fertilization Boneprot and 400 L/da for the basic fertilization Lumbrical) and reduced by 50 %.
Liquid biofertiliser Seasol was introduced as a soil amendment in concentration 1:500- 0.3-0.4 L/da during the vegetation at the plant growing stage ‘flower-bud’ and ‘mass fruitfulness’.
The seedlings were planted on a permanent place during the third decade of May, on a high levelled seed-bed, according to the scheme 120+60x15 cm.
The experiment was worked out according to the method of long plots, into four replications, with a size of the test plot of 9.6 m^2.
Characteristics of the biofertilisers included into the study. This study includes the following biofertilisers: Boneprot, Lumbrical and Seasol (Earthcare), which are included in the list of the biofertilisers according to Regulation (EC) No. 889/2008 (Enclosure No. 1).
Boneprot (Arkobaleno, Italy) it is pellet organic fertilisers, and has the composition (organic nitrogen (N) - 4.5 %; phosphorus anhydride (P_2O_5) total - 3.5 %; potassium (K_2O) - 3.5 %; calcium (CaO) - 5-8 *%;* Magnesium (MgO) - 0.8-1 %; Organic carbon (C) of biological origin - 30 %; Humification rate (HR) - 10-13 %; Humidity-13-15%; pH in water- 6-8. Boneprot is an entirely organic fertiliser consisting exclusively of Cattle manure.
Lumbrical (Plovdiv, Bulgaria) is a product obtained from the processing of natural fertilizer and other organic waste of the Californian red worms (*Lumbricus rubellus* and *Eisenia foetida*) and consists of their excrements. The commercial product has humidity of 45-55% and organic substance content of 45-50%. Ammonium nitrogen (NH_4N)-33.0 ppm; nitrate nitrogen (NO_3N)-30.5 ppm; P_2O_5 and K_2O-respectively 1410 ppm and 1910 ppm, MgO- 1.8%.It contains useful microflora $2x10^{12}$ pce/g, humic and fulvic acids, nutritional substances. The product has activity of 6.5- 7.0 (pH in H_2O).
Seasol (Earthcare) Seasol International Pty Ltd. (Australia) – an extract of brown algae *Durvillaea potatorum*. Seasol is a 100 % liquid natural seaweed extract. It contains 60 % of alginic acids. The commercial product contains as follows: raw protein (2.5 ± 0.1 % w/w); alginates (6 ± 2 % w/w); total solidity (10.0 ± 0.5 % w/w), and pH (10.5 ± 0.5% w/w), and has a variety of mineral elements and traces of Ca (0.05 ± 0.03 % w/w), N (0.10 ± 0.05 % w/w), P (0.05 ± 0.02 % w/w), К (2.0 ± 0.5 % w/w), Cu (0.3 ± 0.2 % w/w), and cytokines.

Study of parameters

Production quality

Biochemical analysis was carried out on an average sample of 20 fruits from each treatment. Following parameters were observed: dry matter (refractometrically- %), vitamin C (acc. to Tilman's reaction - mg %) and total sugars (according to Schoorl-Regenbogen) (Genadiev et al., 1969).

Statistical Analysis

All data were analyzed by using Duncan's multiple range test (Duncan, 1955) at the P<0.05 level. Differences between mean values were evaluated by a one-way analysis of variance (ANOVA) (SPSS treatment 7.5).

RESULTS AND DISCUSSION

Quality of organic pepper production

The data on the biochemical content of pepper fruits of the variety of 'Sofiiska Kapiya' are presented in Figure 1, 2 and 3 below.

In 2009, the dry matter content of the fruits from the variants cultivated on the basic fertilisation Lumbrical is higher, compared to the other background. The maximum value of the dry matter content in pepper fruits was shown by the variant fed with the biofertiliser Seasol on the basic fertilization Lumbrical (Figure 1).

The highest value of the total sugar content in 2009 (i.e. 6.45%) was reported after the optimum concentration of the basic fertilization Boneprot (Figure 1). It was found that the pepper plants cultivated on the basic fertilisation Lumbrical, topped up by liquid biofertiliser Seasol, had a higher content of total sugars in comparison with those after a single application of the biofertiliser Lumbrical in an optimum concentration.

The vitamin C content in pepper fruit in 2009 (i.e. 123.3 mg%) showed a maximum value after a single application of the biofertiliser Lumbrical in its optimum concentration. In comparison with the results of both combined variant, it was found that the value of vitamin C content in fruits was higher after application of biofertiliser Boneprot, which was due to the content of the two biofetilisers (Figure 1).

In the experimental year of 2010 the dry matter content of pepper fruits showed highest values after basic fertilisation with Boneprot in optimum concentration (8.80%). Upon comparison of the effect of the combined variants on both basic fertilisations, higher results were detected upon the application of the biofertiliser Seasol on the basic fertilization Boneprot (Figure 2).

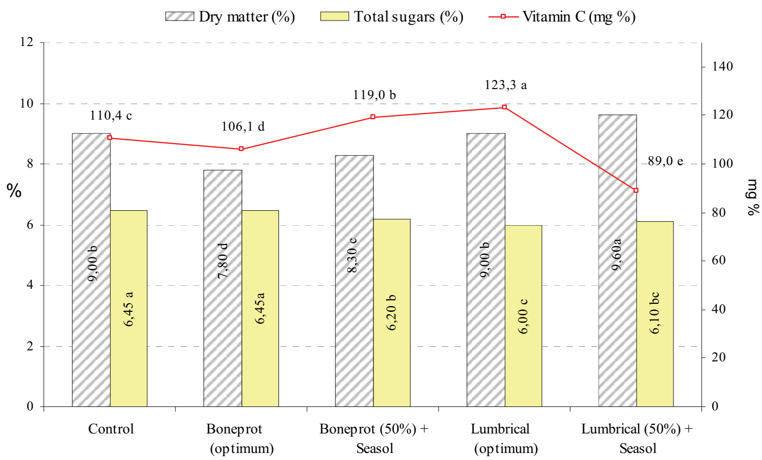

Figure 1. Content of dry matter, total sugars and vitamin C in the pepper fruits-2009

The maximum value of the total sugars content in 2010 was reported for the variant with the applied biofertiliser Seasol on the basic fertilization Lumbrical (8.25%). The results regarding the combined application of a biofertiliser with basic fertilization, in comparison with the detached application of a biofertiliser as basic fertilization, in optimum concentration showed better effect on the total sugar content when a combined fertilizer treatments. The results showed that application of the liquid biofertiliser Seasol on the basic fertilization Lumbrical in 2010 had a maximum value (i.e. 209.4 mg%). The values of vitamin C content in the pepper fruits were reported higher in combined treatments in comparison with control, thus determing the positive effect of the application of the biofertilisers.

The results concerning the dry matter content in pepper fruits in 2011 showed highest values on the basic fertilization Lumbrical (i.e. 8.60 %), thus determine stimulating impact on the biofertilisers (Figure 2). The content of the total sugars in the pepper fruits for the vegetation 2011 year showed that the maximum values by the variant with the biofertiliser Seasol of the basic fertilization Boneprot (i.e. 7.40%). The higher values of the total sugars content was reported in the fruits after the combined application of biofertilisers (i.e. basic fertilization topped up by vegetative feeding), which confirmed the findings in 2010.

Upon application of basic fertilization Boneporot in 2011, highest values of vitamin C content in the pepper fruits (i.e. 181.3 mg%) were shown. There was a better effect on the vitamin C content (i.e. 177.3 mg%) after application of biofertiliser Seasol on the basic fertilization Boneprot, in comparison with the results shown after basic fertilization in optimum concentration.

The results described above are in conjunction with Vlahova and Popov (2013), who showed that the application of the biofertiliser Seasol on both basic fertilisations (i.e. Boneprot and Lumbrical) had a positive effect on the vitamin C content and total sugars, in comparison with single application of basic fertilisations in an optimum concentration in the pepper fruits of variety Kurtovka Kapiya 1619.

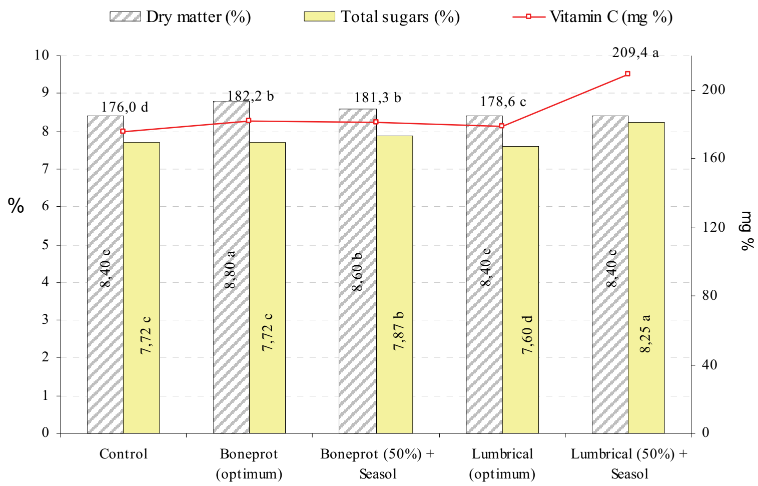

Figure 2. Content of dry matter, total sugars and vitamin C in the pepper fruits-2010

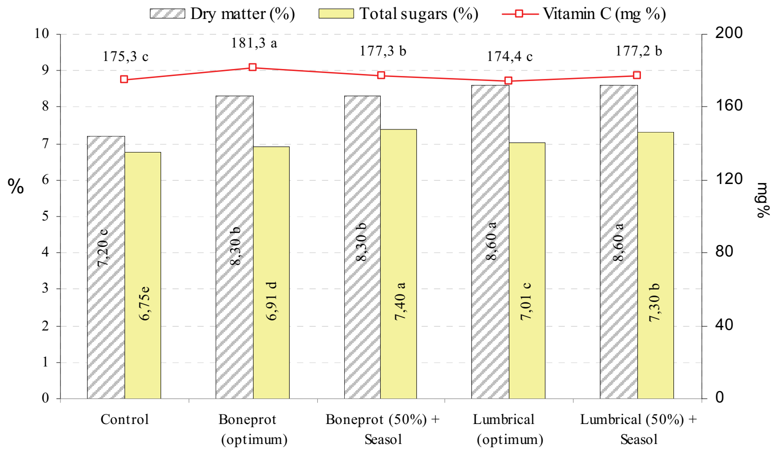

Figure 3. Content of dry matter, total sugars and vitamin C in the pepper fruits – 2011

CONCLUSIONS

It was found that the dry matter content in pepper fruits is highest after application of biofertilisers Seasol on basic fertilisation Lumbrical (2009, 2011) with an average value for the period of 8.87%. The positive impact of the combination of the biofertilisers, i.e. Seasol and Lumbrical, defines the usefulness of the application, which might be applied for other varieties of pepper as well.

It was found that the content of total sugars in pepper fruits during the experimental period varied, as the maximum values were reported for different variants. However, on the average, the higher values of total sugars were shown after the combined application of Seasol on both basic fertilisations in comparison with the single application of the basic fertilisation Lumbrical (i.e. in 2009, 2010, 2011), and on the basic fertilisation Boneprot (i.e. in 2010, 2011). The better combination was the biofertiliser Seasol on the basic fertilisation Lumbrical, which has shown an average of 7.22 % for the period. The positive impact of this combination can be attributed to the nature of these biofertilisers, i.e. enriching the soil with the necessary nutrients and gradually ensuring their release during the growing stages of the pepper. It reflected in better quality of pepper fruits shown by higher content of dry matter and total sugars.

There was no unidirectional tendency towards a maximum value of Vitamin C content shown after application of one type of treatment with biofertilisers. The higher values were reported upon application of the combined variant of Seasol on the basic fertilisation Lumbrical (i.e. in 2010, 2011). The higher attention is paid to Vitamin C content it is perceived as a significant indicator for the quality of pepper fruits.

The results of the study shown by the three indicators for quality of the pepper production are a sufficient evidence for the applicability of biofertilisers in vegetable growing. Besides the improved quality, it is a guarantee for a low risk for the human health.

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FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

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GREEN INFRASTRUCTURES FROM THE PERSPECTIVE OF EUROPEAN INSTITUTIONS

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Abstract

The paper presents a research about the approach of green infrastructure (GI) in recent official documents elaborated by European Institutions including the European Commission (EC), the European Environmental Bureau (EEB) and the European Environment Agency (EEA). Green infrastructure is an emerging concept which has developed in the last decades being used by professionals from different areas of study such as landscape architecture, ecology, environmental engineering, forestry, agriculture, geography, spatial planning and regional development. The study is concerned on the understanding of the complex and multiple roles of green infrastructures for the future of environment and human society in the 21st century. The aim of the research is to emphasize significant issues regarding green infrastructures, such as terminology, benefits, opportunities for integrated development strategies and specific policies, in order to synthesize the current situation of green infrastructure management in Europe. Analyzed materials for the study include recent reports and communications of European institutions. Among these are Green Infrastructure (GI) — Enhancing Europe's Natural Capital (EC, 2013), The Multifunctionality of Green Infrastructure (EC, 2012), Green Infrastructure – Natural Resource Care Areas. Opportunities and benefits (EEB, 2011), Building Green Infrastructure for Europe (EEB, 2008) and Green Infrastructure and Territorial Cohesion (EEA, 2011), the last being one of the most comprehensive official document in this direction. The conclusions of the study highlight the importance of integrating green infrastructures among European policies and among the opportunities to implement an EU Green Infrastructure strategy in order to enhance and conserve the natural capital in a sustainable manner.

Key words: *Green infrastructure, Landscape planning and management, Sustainable development, European institutions, Conservation and development policies*

INTRODUCTION

Green Infrastructure is a recent and emerging concept which has been developed mostly in the last twenty years by landscape architects, ecologists, environmental engineers, urban planners and geographers. In the last years, the importance given to green infrastructure by the EU has increased significantly. The concept has been introduced into several European Documents concerning environmental, economic and social issues.

Multiple benefits of green infrastructure such as food resources, clean water, clean air, climate balance, flood prevention and recreation are an essential condition for a high level of human security at global, regional, national and urban scale. In Europe, the continuous and insufficient controlled development of the built environment – known as gray infrastructure – represents a significant threat for the future of green infrastructure. Thus, several measures should be taken to manage urban and regional development in order to generate a sustainable, smart and inclusive growth, which is a major priority within the EU agenda for 2020 (EC, 2013). As well, according to the European Biodiversity Strategy, a GI (Green Infrastructure) strategy will be developed till 2020 (EUEC, 2011).

MATERIALS AND METHODS

The main task of the research consists in evaluating the current situation and perspectives of EU regarding Green Infrastructures by analysing the most comprehensive European documents published between 2008 and 2013. The aim of the analysis was to identify terminology aspects, benefits, major risks, policies and opportunities regarding green areas in order to determine the development premises of a green infrastructure integrated strategy at European level.

The examined documents are concerned mostly on theoretical aspects, containing also a number of particular case studies. Thus, analyzed issues within the documents are approached both at quantitative level, as in the case of ecological or economic benefits, and at qualitative level, like in the case of terminology, social benefits or proposed policies.

The conclusions of the study conclude the important role of Green Infrastructure for the sustainable development of EU which strongly depends on the implementation of a future GI European Strategy.

RESULTS AND DISCUSSIONS

Terminology. Even if many definitions of Green Infrastructure (GI) concept have been developed during the last years it is hard to cover all aspects within a short paragraph (EEA, 2011). According to EC, “GI is a successfully tested tool for providing ecological, economic and social benefits through natural solutions. It helps us to understand the value of the benefits that nature provides to human society and to mobilize investments to sustain and enhance them” (EC, 2013). Pursuant to EEB, “Green infrastructure areas take the form of a sustainable coherent interconnected network of regional characteristic landscape elements, natural areas and open spaces in the land which brings a wide range of ecological benefits” (EEB, 2011). Thus, by protecting and enhancing green areas, human communities can get benefits from nature. GI can and should be integrated it into urban, landscape and regional planning and development, in order to became complementary to the built (grey) infrastructure solutions (Figure 1).

GI Benefits and EU Policies. According to Cohesion Fund and the European Regional Development Fund (ERDF), Green Infrastructure represents one of the investment priorities within the regional policies. GI is considered as a contributor to “regional policy and sustainable growth in Europe, facilitating smart and sustainable growth through smart specialization” (EC, 2013). The GI policies and solutions are considered to be important mostly in urban areas, in which over 60% of EU citizens live. GI plays an important role in urban environments because of its benefits which ensure a high level of human security. This implies health related aspects such as improving air and water quality and reduction of diseases, contribution to the cultural identity of local communities and helping to combat social isolation and exclusion. Green areas contribute to pleasant landscapes which could be used for tourism and recreational activities. GI offers also individual benefits at physical, psychological and emotional level.

An important opportunity offered by GI in the field of landscape planning is making connections between urban, suburban and rural areas, providing pleasant places to live and work in (EC, 2013).

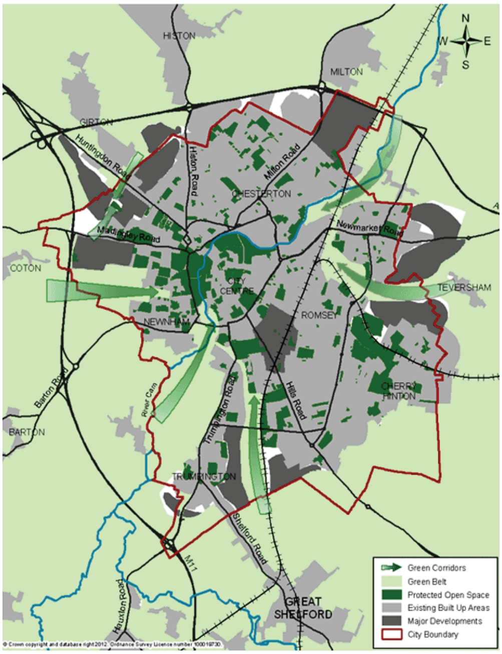

Figure 1. Green Infrastructure in Cambridge
(source: Cambridge City Council)

GI is also a contributor to food security and to the education process within local communities through urban food production.

GI plays an in important role in EU policies focused on climate change and disaster risk. Biodiversity and ecosystem services are part of an adaptation strategy to combat the adverse effects of climate changes. For example, initiatives in agriculture and forestry with a positive impact on greenhouse gas balances and

CO_2 storing are considered to be included into EU climate policies (EC, 2013). Future GI policies of EU should be concerned on reducing the carbon footprint, mitigation the negative effects of land fragmentation and integrating better land use and ecological issues into spatial planning. The purpose of these aspects refers to increase the GI benefits as water retention, air purification and biodiversity enrichment.

GI solutions, like flood plains, riparian woodlands, wetlands or protection plantations, could be also integrated into EU policies in order to combat extreme weather events and natural disasters (floods, landslides, avalanches, forest fires, storms), reducing vulnerability to risks for human settlements (EC, 2013).

Another significant role of GI in consists in the protection, conservation and enhancement of the EU's natural capital, according the commission's recent proposal for an Environmental Action Programme to 2020 (EC, 2013).

Towards an EU Strategy for GI. As presented in above, GI can make an important contribution to achieve several key objectives of EU policies.

Over the last 20 years, more and more GI sustainable projects have been carried out on a local, regional, national or trans-boundary scale. A condition to optimize and maximize the functionality and benefits of GI lies in interconnecting projects at different scales to achieve consistency and coherence at EU level.

GI must become a "standard part of spatial planning and territorial development that is fully integrated into the implementation of these policies" (EC, 2013).

Funding mechanisms, such as Common Agricultural Policy, the Cohesion Fund, the European Regional Development Fund, Horizon 2020, the Connecting Europe Facility Fund and the Financial Instrument for the Environment, should facilitate funding for GI projects during 2014-2020 budgetary envelope. Decisions regarding GI projects should be taken both at local, national at regional scales with a view to be assessed in a coherent and coordinated way across the EU (Figure 2).

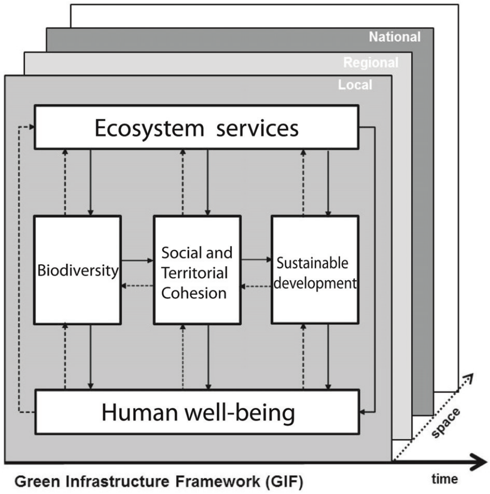

Figure 2. Green Infrastructure Framework (Lafortezza, R., Davies, C., Sanesi, G., Konijnendijk, C.C., 2013)

Development and protection of GI should be strengthened by expanding research regarding GI benefits and associated technologies and processes. For example, in urban environments efficient buildings, incorporating green features such as green roofs and walls can deliver ecological, social and health benefits (Figure 3).

Figure 3. Green wall on a building in Paris (source: www.livegreenbegreen.com)

Many natural areas such as mountain ranges (the Alps, the Carpathians), river basins (the Rhine, the Danube) and forests (the Fennoscandinavian Forests) belong to EU's natural and cultural heritage and identity, requiring a coordinated pan-European vision, which can be implemented through macro-regional strategies and through European territorial cooperation programs (EEA, 2011). An example for a macro-regional strategy is the European Green Belt Initiative. It comprises an ecological network running from the Barents Sea to the Black Sea which connects national parks, natural parks, biosphere reserves, protected areas and other natural areas along or across borders, conserving and protecting some of most impressive and fragile European landscapes.

The further strategy should enable a framework in order to provide a combination of policies and technical or scientific actions. Currently, it thinks that the strategy can be implemented within the context of existing legislation, policy instruments and funding mechanisms including the following elements (EC, 2013):

- Promoting GI in the main policy areas (climate change and environmental policies, disaster risk management, health, etc.)
- Improving and strengthening the knowledge base and promoting innovation
- Improving access to finance
- GI projects at EU level (by the end of 2015)

CONCLUSIONS

Green Infrastructure can contribute significantly to achieving many of the EU policy objectives. The EU can promote the development of GI by generating a framework to encourage and facilitate GI projects within existing laws, policies and funding mechanisms.

The future GI Strategy should be a driver for a sustainable development of EU states, regions and cities. It should coordinate planning and management strategies at national, regional and urban level in order to create coherent and cohesive green infrastructure networks over the entire EU. The GI Strategy will require an interdisciplinary approach with the involvement of many European, national, regional and local institutions as well as a wide range of specialists with different professional backgrounds.

Thus, reconciliation between green and grey infrastructures, by enhancing GI benefits to the human society and protecting the wildlife, will be a difficult task that will need to involve all the local communities from EU.

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HOW TO REGENERATE PLANTLETS FROM PROTOPLASTS OF FRITILLARIA IMPERIALIS

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Abstract

There is no special method recommended for protoplast isolation and regeneration from Fritillaria imperialis L. The present study reports the isolation and regeneration of protoplasts from callus of Fritillaria imperialis L. A range of parameters which influence the isolation and regeneration of F. imperialis protoplasts were investigated. From the results obtained, callus fresh weight (FW) of 0.4 g produced the highest number of viable protoplasts, which was 1.12 × 10^5 protoplasts/g FW. The best treatment for isolation of Fritillaria imperialis protoplast (7.8 × 10^5 protoplasts/g FW) was 2% cellulase and 0.1% pectinase with 9% manitol for 8 h. For enhancement of the protoplasts division and the percentage of colony formation, different concentrations from casein hydrolysate, 2,4-D and BA were used. The results revealed that cell wall and colony formation were better in a liquid medium than those on a semi-solid medium. The highest plating efficiency (1.26×10^6 per gr FW) and highest callus formation was obtained by using a medium containing 0.5 mg l^{-1} 2,4-D,1 mg l^{1} BA and 200 mg l^{-1} casein hydrolysate. Micro calli were formed after one month of culture. Many plantlets were formed on the calli after transfer of the proliferated calli to regeneration medium. The highest plantlet regeneration (100%) was obtained by using a medium containing 0.5 mg l^{-1} NAA, 1.5 mg l^{-1} BA.

Key words: *Callus formation, Fritillaria imperialis L, Plant regeneration, Protoplast culture*

INTRODUCTION

Crown imperial (*Fritillaria imperialis* L.) or ‘‘*Tears of Mary*’’ (because of great drops of nectar at the petal base) is a perennial plant with high medicinal and ornamental importance. Approximately, *Fritillaria* genus includes 100 species which 14 important species are native to Iran (De Hertogh and LeNard, 1993). In Iran, wild populations of important species, like *F. imperialis* and *F. persica*, are at the risk of rapid eradication, because of irregular grazing of *Fritillaria* stands, lack of protecting rules, changing the pastures to dry farmlands, and pest overflow (Ebrahimie et al., 2006a). Wild populations of *F. imperialis* are mostly found in high altitudes (>2,000 m) of western parts of Iran, particularly in two provinces, Chahar Mahal-va-Bakhtiari and Kohkyluyeh-va-Bouyrahmad. The species of the genus *Fritillaria* were first described in 1753, as *F. imperialis* L., *F. persica* L., *F. pyrenaica* L., and *F. meleagris* L. (Linnaeus, 1753). *Fritillaria* is represented worldwide by 7 subgenera, 2 sections, and 165 taxa (Rix, 2001). As the production of a better adapted *Fritillaria imperialis* hybrid through conventional plant breeding techniques is difficult and time consuming. Hence, biotechnology strategies particularly the somatic hybridization could provide a promising alternative. The development of protoplast systems has enlarged the flexibility of plants in biochemical and genetic research (Rao and Prakash, 1995) as well as provides a great prospect in genetic improvement of medicinal plants (Azad et al., 2006). The development of protoplast technology and regeneration procedures played an increasingly significant role in the plant improvement through somatic hybridization and protoplast transformation (Umate et al., 2005). However, a step towards the plant genetic manipulation and integrated approach of breeding programs is primarily laid on an efficient protocol in protoplast isolation, culture and regeneration (Duquenne et al., 2007). Cells derived from protoplasts subsequently undergo sustained division and gave rise to visible colonies within 3 weeks. Shoots formation was induced in the colonies by transferring them to MS-differentiation medium (Murashige and Skoog,

1962) containing NAA and BA at 4 mg l^{-1} and Kin at 2.56 mg l^{-1}, respectively. Shoots were transferred to White's basal medium to induce root formation. Protoplasts have been isolated from various genotypes of *Petunia hybrid* (Izhar and Power, 1977), as well as from *P. inflata, P. violocea* and *P.axillaris* (Dulieu et al., 1983). On the other hand, Arnalte et al. (1991) reported the procedure for enzymatic isolation of protoplasts from *Digitalis obscura,* it was developed from pollen of this medicinal plant as a tool of genetic improvement of the species. There are no published reports on the isolation, culturing and regeneration of protoplasts from the *Fritillaria imperialis* L. Therefore, the objective of this study was to find out a proper protocol for isolation and culturing of protoplasts from *Fritillaria imperialis* L. and regeneration of plantlets from such protoplasts. *Fritillaria imperialis* L. is considered an important source of pharmaceuticals. It is one of the native Iran medicinal plants, and was also very popular for its supposed magical properties.

MATERIALS AND METHODS

Culture of protoplasts

Protoplasts were cultured at a density of 1×10^5 protoplasts/ml. protoplasts were suspended in 4 ml of liquid media (MS without agar, with 9% mannitol), in small Petri dishes (5.5 cm diameter). 5 days after protoplast culture, the cells were transferred to Erlenmeyer flasks containing MS liquid medium and incubated at 120 rpm on a rotary shaker in the darkness at 25 ± 2°C. After 10 days, every time, 5 ml of fresh medium was added to the culture medium. Star shaped microcalli developed within 15 days of culture. After the development of microcalli visible by naked eye, the cultures were transferred to the light. The plating efficiency defined and measured as the ratio of cell number undergoing division to the total cultured protoplast number. After one month when the calli attained sizes of 0.5–1.0 mm in diameter, they were transferred to the semi-solidified MS medium at 23°C under fluorescent light (40 μmol per m^2/s) in a 16/8 h of day/night regime in the culture cabinets.

Experimental designs, data collection and analysis

In this study three separate experiments were done and each experiment was repeated twice. In first experiment, in order to optimize the medium for protoplast growth and cell proliferation, the effect of various plant growth regulator combinations in MS medium (0, 100, 150, 200 and 250 mg l^{-1} casein hydrolysate (Cas), 0, 0.5,1 and 1.5 mg l^{-1} 2,4-D, 0.2 and 0,0.5,1 and 1.5 mg l^{-1} BA) were tested as a suspension culture based on completely randomized design with factorial arrangement and three replications.

In second experiment, to determine the growth possibility of protoplast-derived cells on the semi-solid medium, all of cells proliferated in suspension culture were sub-cultured on semi-solidified MS medium supplemented with various combinations of 2,4-D (0, 0.5, 1, 1.5 mg l^{-1} and BA(0, 0.5, 1, 1.5 mg l^{-1}) and casein hydrolysate (Cas) (0, 100, 150, 200 and 250 mg l^{-1}). After callus formation, callus mass were counted. In third experiment, after 26 days of callus proliferation, the developed calli in suspension culture were transferred to regeneration medium consisting of semi-solidified MS medium supplemented with NAA (0, 0.5,1 and 0.5 mg l^{-1}), BA (0, 0.5, 1 and 1.5 mg l^{-1}) based on completely randomized design with factorial arrangement with three replications. The cultures were kept in light conditions of l6 hrs/day at 25°C. Cell density was estimated with a Nageotte hematocytometer. Results were expressed as yield per gr FW for leaves or calli. Callus mass was evaluated by naked eye. Data analyses were performed using SPSS (SPSS Inc. Version 19.0) software and MSTATC. Mean comparisons were done using Duncan's multiple range test (DMRT) at a probability level of 0.05.

RESULTS AND DISCUSSION

Effect of different hormones on cell growth and deviation

The results of ANOVA showed that different concentrations of 2,4-D and BA significantly ($P\leq0.01$) affected proliferation of protoplast derived cells. Significant ($P\leq0.01$) interaction effects of 2,4-D×BA, casein hydrolysate×BA, casein hydrolysate×2,4-D and casein hydrolysate ×2,4-D × BA were found on cell proliferation.

Means comparison by DMRT showed that the highest and lowest cell proliferation were produced in MS suspension medium containing 0.5 mg l^{-1} 2,4-D, 1 mg l^{-1} BA and 200 mg l^{-1} casein hydrolysate (1.26×10^6 cell/g FW), and 0 mg l^{-1} 2,4-D and 0 mg/lit BA (8.2×10^5 cell/gr FW), respectively (Tab 1). However, other MS suspension media containing 0.5 mg l^{-1} 2,4-D , 1.5 mg l^{-1} BA and 200 mg l^{-1} casein hydrolysate, 1 mg l^{-1} 2,4-D , 1.5 mg l^{-1} BA and 150 mg l^{-1} casein hydrolysate, 0.5 mg l^{-1} 2,4-D , 1 mg/l BA and 150 mg l^{-1}casein hydrolysate and as well as 0.5 mg l^{-1} 2,4-D, 1 mg l^{-1} BA and 100 mg l^{-1}casein hydrolysate produced significantly highest density of cells. Hence, the latest mentioned media did not use in next experiments.

Thus, the best treatment for proliferation and growth of *F. imperialis* cells was MS medium supplemented with 0.5 mg l^{-1} 2,4-D, 1 mg l^{-1} BA and 200 mg l^{-1} casein hydrolysate. The first cell divisions were observed 48 hours after protoplast culture. Cell density was measured every 5 days and the first density measurement was done 15 days after protoplast culture.

Callus mass formation from plating of cell suspension on solid MS medium

The results of ANOVA showed that growth of plated cells and formation of calli (detectable by naked eye) on semi-solidified medium were significantly (P≤0.01) influenced by different combinations of plant hormones and casein hydrolysate. Means comparison revealed that the highest and lowest callus induction from plated cell on semi-solidified MS medium were produced on media containing 0.5 mg l^{-1} 2,4-D and 1 mg l^{-1} BA with 200 mg l^{-1} casein hydrolysate (35.33) and 0 mg l^{-1} 2,4-D and 0 mg/lit BA and 0 mg l^{-1} casein hydrolysate (0) respectively (Table 2).

Table 1. The mean effect of different combinations of hormones on density of cells in *F. imperialis.*

2,4-D (mg l^{-1})	BA (mg l^{-1})	casein hydrolysate (mg l^{-1})	Dencity of cells (in 1ml)
0	0	0	8.2×10^5z
0	0	100	8.42×10^5z
0	0	150	8.49×10^5z
0	0	200	8.59×10^5z
0	0	250	8.50×10^5z
0	0.5	0	9.49×10^5hijklmnop
0	0.5	100	9.53×10^5hijklmnopqr
0	0.5	150	9.41×10^5ijklmnopqrstu
0	0.5	200	9.57×10^5hijklmnop
0	0.5	250	9.66×10^5ghi
0	1	0	9.48×10^5hijklmnopqrst
0	1	100	9.58×10^5hijklmnopqrst
0	1	150	9.48×10^5ijklmnopqrst
0	1	200	9.56×10^5hijklmnopq
0	1	250	9.53×10^5hijklmnopqrst
0	1.5	0	9.36×10^5hijklmnopqrst
0	1.5	100	9.44×10^5hijklmnopqrst
0	1.5	150	9.60×10^5hijklmnopqr
0	1.5	200	9.49×10^5hijklmnopqr
0	1.5	250	9.7×10^5ghij
0.5	0	0	9.2×10^5tuvw
0.5	0	100	9.3×10^5 rstuvw
0.5	0	150	9.55×10^5hijklmnopq
0.5	0	200	9.36×10^5klmnopqrstu
0.5	0	250	9.62×10^5hijklmnopqrs
0.5	0.5	0	9.84×10^5hijk
0.5	0.5	100	1.02×10^6 fg
0.5	0.5	150	1.03×10^6 f
0.5	0.5	200	1.05×10^6 ef
0.5	0.5	250	1.04×10^6f

0.5	1	0	9.81×10^5 hijkl
0.5	1	100	1.04×10^6de
0.5	1	150	1.05×10^6d
0.5	1	200	1.26×10^6 a
0.5	1	250	1.04×10^6de
0.5	1.5	0	9.7×10^5hijk
0.5	1.5	100	1.05×10^6f
0.5	1.5	150	1.00×10^6gh
0.5	1.5	200	1.18×10^6b
0.5	1.5	250	9.97×10^5 hijklmno
1	0	0	8.98×10^5uvw
1	0	100	9.38×10^5nopqrstuv
1	0	150	9.34×10^5ijklmnopqrst
1	0	200	9.41×10^5ijklmnopqrst
1	0	250	9.47×10^5 hijklmnopq
1	0.5	0	9.16×10^5qrstuvw
1	0.5	100	9.26×10^5opqrstuv
1	0.5	150	9.38×10^5ijklmnopqrstu
1	0.5	200	9.41×10^5jklmnopqrstu
1	0.5	250	9.54×10^5hijklmnopqrst
1	1	0	9.16×10^5 vwx
1	1	100	9.25×10^5 mnopqrstuv
1	1	150	9.45×10^5hijklmnop
1	1	200	9.26×10^5 nopqrstuv
1	1	250	9.57×10^5pqrstuv
1	1.5	0	8.97×10^5xyz
1	1.5	100	9.95×10^5ghi
1	1.5	150	1.12×10^6c
1	1.5	200	9.95×10^5ghi
1	1.5	250	9.21×10^5 stuvw
1.5	0	0	8.93×10^5z
1.5	0	100	9.52×10^5ijklmnopqrst
1.5	0	150	9.50×10^5 hijklmnopqrst
1.5	0	200	9.41×10^5ijklmnopqrstu
1.5	0	250	9.51×10^5hijklmnopqrst
1.5	0.5	0	9.06×10^5 wxy
1.5	0.5	100	9.57×10^5hijklmn
1.5	0.5	150	9.59×10^5 hijklmnop
1.5	0.5	200	9.64×10^5hijklm
1.5	0.5	250	9.33×10^5klmnopqrstu
1.5	1	0	8.90×10^5xyz
1.5	1	100	9.33×10^5 klmnopqrstu
1.5	1	150	9.40×10^5 hijklmnopqrst
1.5	1	200	9.44×10^5 jklmnopqrstu
1.5	1	250	9.31×10^5lmnopqrstuv
1.5	1.5	0	8.87×10^5yz
1.5	1.5	100	9.36×10^5 opqrstuv
1.5	1.5	150	9.35×10^5 klmnopqrstu
1.5	1.5	200	9.43×10^5hijklmnopqrst
1.5	1.5	250	9.38×10^5jklmnopqrstu

Means followed by different letters in each column are significantly different at $P \leq 0.05$.

Table 2. The effects of different treatment on callus formation from plated cells of *F. imperialis*.

2,4-D (mg l^{-1})	BA (mg l^{-1})	casein hydrolysate (mg l^{-1})	Number of callus mass formed in each petridish
0	0	0	0^{i}
0.5	0.5	100	1.66^{ghi}
0.5	0.5	150	2^{ghi}
0.5	0.5	200	3.33^{gf}
0.5	0.5	250	1^{hi}
0.5	1	100	10.33^{e}
0.5	1	150	17.66^{d}
0.5	1	200	35.33^{a}
0.5	1	250	5^{f}
0.5	1.5	100	3^{fgh}
0.5	1.5	150	4.33^{f}
0.5	1.5	200	24.33^{b}
0.5	1.5	250	1.66^{ghi}
1	1.5	150	20^{c}

Means followed by different letters in each column are significantly different at $P \leq 0.05$.

Plant regeneration

The results of ANOVA showed that different concentrations of NAA and BA significantly ($P\leq0.01$) affected plant regeneration of *Fritillaria imperialis* L. Significant ($P\leq0.01$) interaction effects of NAA× BA were found on regeneration.

Means comparison by DMRT showed that the highest and lowest regeneration were produced in MS medium containing 0.5 mg l^{-1} NAA, 1.5 mg l^{-1} BA (%100), and 0 mg l^{-1} NAA and 0 mg/lit BA (0), respectively (Fig. 1**)**. However, other media containing 0.5 mg l^{-1} NAA and 1 mg l^{-1} BA (%66.66), 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA (%55.55), 1 mg l^{-1} NAA and 1.5 mg l^{-1} BA (%33.33) and as well as 1 mg l^{-1} NAA , 1 mg l^{-1} BA (%22.22) produced significantly highest regeneration. (Figure1). Thus, the best treatment for growth and regeneration of *F. imperialis* was MS medium supplemented with 0.5 mg l^{-1} NAA and 1.5 mg l^{-1} BA (Figure 1).

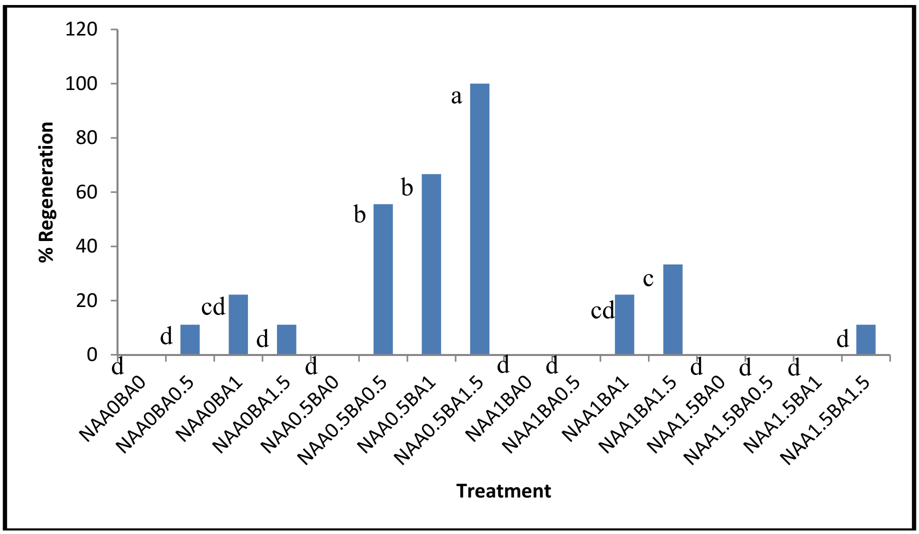

Figure 1. The effect of different treatments on plant regeneration in *F. imperialis*.

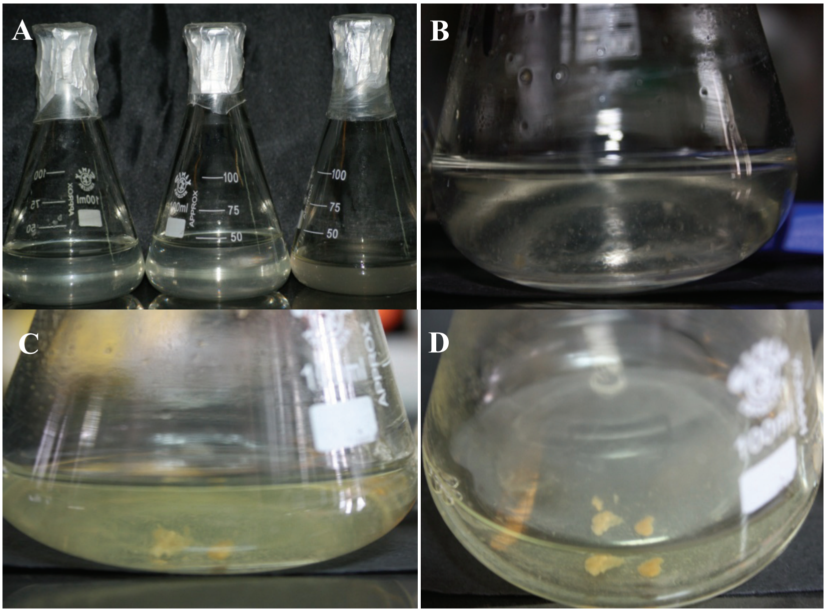

Figure 2. Developmental stage of protoplast in culture suspension A) culture suspension contain release protoplast B) cell proliferation and growth after two days and turbid suspension medium C) Formation of cell masses After 14 days D) Cell mass enlargement and callus formation after 20 days of culture.

In this study, plants were isolated and regenerated from *Fritillaria imperialis* L. protoplasts. Likewise, 0.2 gr of friable and yellow embryogenic suspension cell cultures was chosen to be used in the protoplast isolation of *Cinnamomum camphora* L. (Du and Bao, 2005). In fact, this study was directly concerned with the enzyme-substrate relationship (Bodansky, 1954).

This result indicated that combination of BA and 2,4-D in high concentration inhibited protoplast division.

This result was consistent with earlier findings that the combined optimal auxin and cytokinin were relatively effective for cell division in petal protoplast of *Petunia hybrid* (Oh and Kim, 1994), and in cell suspension protoplast of *Allium cepa* (Karim and Adachi, 1997). Another important factor for protoplast culture is the culture system.

In these experiments protoplasts were cultured either in liquid and solid MS medium comprising 1×10^5 and 1×10^5 protoplasts/ml. Division of protoplasts obtained in liquid MS medium at optimal density was 1.26×10^6 protoplasts/ml. The density of protoplasts influenced the initiation of cell divisions, as has been reported in oat by Hahne *et al*. (1990). The suspension- derived protoplasts of vetiver did not divide in gelrite.

In contrast to published data (Kisaka *et al*., 1998) the same gelrite was successfully used for protoplast culture. There were some reports that agarose and phytagel have been used to improve protoplast culture in *Medicago sp*. and *Garcinia atroviridis* Griff., respectively (Te-chato, 1997)

During the present study, cell-wall regeneration, cell division, and callus formation were obtained. Among the plant growth regulators we tested, only the combination of 2,4-D and BA induced cell division. In earlier studies on rose mesophyll protoplasts, NAA and BA were the most efficient growth regulators for the regeneration of microcalli (Marchant et al., 1997).

In lily protoplasts, the addition of picloram to the culture medium was critical of development of microcalli (Horita et al., 2002).

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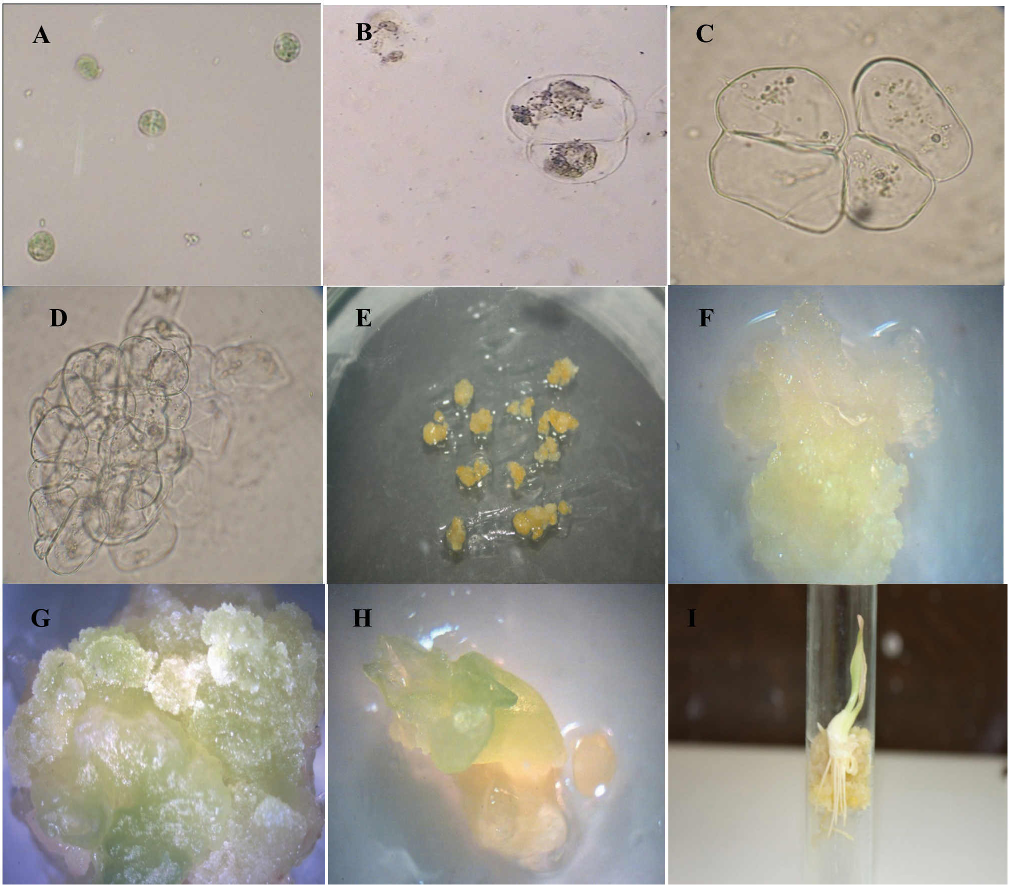

Figure 3. General overview of protoplast culture and regeneration procedure developed for *F. imperialis.* A) Isolated protoplasts from callus. B) First division after 48 hr of culture C) second division after 4 days of culture D, E) colony formation after 3 weeks of culture F) Plate of cell suspension and callus formation can be detected with the naked eye after 25 days G, H) Callus regenerated I) regenerated plants from protoplasts.

The number of microcalli we obtained was close to those obtained in earlier studies in banana (Assani et al., 2001). However, the obtained calli did not develop into plants in our study. Auxin is involved in cell division and callus formation. The high concentration of auxin, does not make root formation but makes callus formation (Pierik, 1998). Shoot organogenesis depends on many parameters, including the genotype, protoplast-derived material, plant growth regulators, culture system, and exposition time of protoplasts on nurse cells (Chabane et al., 2007). Previous investigations showed the impact of genotype on plant regeneration from protoplasts in apple and banana (Assani et al., 2002). Chang (1999) reported the optimum callus formation from inflorescence explants of *lilium* was obtained in medium containing 3 mg l^{-1} 2,4-D and 0.25 mg l^{-1} BA. In another experiment, Naik and Nayak (2005) reported callus induction in scale explants of *Ornithogalum virens* was obtained in medium containing 1-4 mg l^{-1} 2,4-D and 2 mg l^{-1} BA. Chen (2005) also stated that, the highest percentage of callus induction from another culture of Narcissus was obtained in medium containing 1 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BA. The main plant growth regulators such as auxin and cytokinin, alone or in combination, are generally essential for efficient protoplast division in plant systems (Davey et al., 2005). Plant growth regulator concentrations and combinations need to be optimized for each protoplast development step. The following plant growth regulators were tested in our preliminary experiments: 2,4-D, BA, NAA and casein hydrolysate. Only the combination of 2,4-D and BA induced sustained cell divisions and callus formation. None of the plant growth regulators induced plant regeneration, which may be related to the negative interaction between those plant growth regulators and some metabolites produced by callus tissues. Nagata and Takede (1984) succeeded in isolating of protoplasts from *Nicotiana tabacun* L. leaves using enzyme solution. They isolated 10^7 protoplasts from 1 gr fresh weight of tobacco leaves. After 3 weeks, shoots were induced in the colonies by transferring them into differentiation medium containing NAA and BA at 4 mg l^{-1} and Kin at 2.56 mg l^{-1}. Shoots were transferred to hormone free MS-medium to induce root formation. Concentrations of 0.2 mg l^{-1} 2,4-D, l mg l^{-1} NAA and 0.5 mg l^{-1} Zeatin, was produced the highest protoplast regeneration and cell division (Pongchawee et al., 2006). According to Tamura *et al*. (1992) report, high concentration of glucose (0.5M) is followed the best outcome for protoplast culture. They also proved that, addition of Zeatin (1 mM) and NAA (10 mM) gives the normal size of the colonies formed. Changed protoplast culture medium to 5.4 mM NAA and 2.3 mM Zeatin was suitable for protoplast regeneration. So, that was the appropriate density of cells in the medium (Tian *et al*., 1999). Also, cultured of protoplasts onto 1/2 strength MS-medium containing 0.01 mg l^{-1} NAA , 0.5 mg l^{-1} BA had a high plant regeneration (Saker et al., 1999).

CONCLUSIONS

The best treatment for isolation of protoplast, growth, division cells, cells suspension culture, callus mass formation from plating of cell suspension on solid MS medium and plant regeneration. This is, to our knowledge, the first report of plant regeneration from protoplasts of *Fritillaria imperialis* species. We hope the protocol can be applied to the regeneration of protoplasts from other plant species as well.

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CONTRIBUTIONS TO THE DEVELOPMENT OF ALTERNATIVE FORMS OF ACTIVE RECREATION - DIRT PARK

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Abstract

If at the beginning of the last century the landscape was perceived mostly as artistical, functional, ambiental, economical, social, at the beginning of this millennium it gains in addition a new quality, given by cultural weight and the mark it leaves by the utility and the ''consumption'' of the landscape. Cultural and daily landscape falls within the line of sight of urban and landscape architects, sociologist, geographers, anthropologists , and in all domains of activity that are linked to landscape, requiring special measures for its protection and conservation (European Convention of Landscape – Florence 2000). Representing an important component of ecological, social, and cultural domain, the landscape is also a resources that contributes to improving the quality of human lives, and it could generate economical activities in the context of maintaining the harmonious balance between social, economical and environmental needs. In terms of the super- technologization of most activities, outdoor recreation becomes a physiological need for people in general and teenagers in particular. Social studies show that, in the urban environment, time spent outdoors is greater at the end of the week than during it, and the social groups who frequent these urban and suburban landscapes are mostly teenagers and children (Iliescu, 2003). These social groups (in personal development) need special attractions, capable to spark their interest and to compensate for their need of adrenaline. In this context, our study succeeded in answering social challenges to create in a small place, but sufficiently big enough to be declared the biggest in Romania, the first landscaped Dirt Park that's made by the technical and constructive requirements of spaces for extreme sports on bikes, but also for spending time outside. It's about a landscaping project for a surface of 5000s.m., designed to attract teens from the northern part of the capital, passionate bikers, and thought as a challenge by the fact that we wanted to implement it in a public-private partnership. The landscaping project of the first Dirt Park of this size in Romania was carried out by its construction on the field, so in two months after the theme debut, it was finalized and officially opened.

Key words: *landscape theme, extreme sport, partnerships*

INTRODUCTION

In this scientific paper a common issue in the contemporary city is addressed: the more and more pronounced gap between the need to diversify forms of recreation in public space and limited supply that can benefit (Oswalt, 2005).
I also watched both the harmful effects of this phenomenon, and (especially) effective ways to counter this situation.
In a context in which most cities do not provide even a minimum of quality regarding public space to the population, and also, their peripheries know a large development period, it is to be observed only paradoxically that an upward evolution in residential segment is followed closely by that of services.
These new territorial expansions in the city are at best only small local centers of social focus.
Even if developers provide these new areas, ensuring an optimal level of functional features designed to head off as many of the needs of the community as possible, the intended effect is far from achieved.

It is frequently found that these extremely important issues are neglected or omitted: encouraging social cohesion, encouraging space engagement, developing a local sense of belonging, *creating a unique center of attraction which is the local identity of the*

territory and could generate an attractive pole of interest city-wide (Kevin Lynch, 1960).
In this context of new regional development authorities looms opportunity to create various objectives in these areas, unique to urban: green feathers, sports and theme parks.
For example, an entire city sports complex offering the benefit of a set that includes a wide range of special facilities long expected by athletes and by users of urban recreational space was founded in the peripheral area of the city of Copenhagen 2009 (Figure 1-4).
The project's title is "Plug and Play", it has an area of about 25 000 square meters and it is the newest and most attractive socio- economic center of the city.
Residential areas surrounding the sports complex have known a flourishing period, and residents on the outskirts are involved in the most active form of social interaction. (http:/www.landezine.com).

Figure 1. Design proposal

Figure 2.The site, before and after

Figure 3. Day of opening

Figure 4. Dirt Park

Starting from the assumptions stated above we propose a scientific approach whose purpose is to track the impact of a similar resolution such as "Plug and Play" in Copenhagen being applied in the context of Romanian urban civilization.
To clearly underline what is the impact of a project such as this one on the evolution of social life in public space, and the urban space, first a series of specific landscape analysis aimed to identify areas and activities appropriate to the scientific approach were undertaken, then a project that is suitable for the function space and chosen was executed, and finally, the implementation and execution of a landscaping project with special character – a Dirt Park.

MATHERIALS AND METHODS

The main issues pursued in the analysis were: delimitation of the administrative area of interest, mainly monofunctional spaces (especially residential neighborhoods), accessibility by residents of that area and by all other citizens of the city, openness of the local community the initiatives of this kind, degree of involvement and the level of interest of the locals on the quality of life in public space. (LaGro, James, 2001)
A number of sociological surveys conducted among young people (the main segment that generates the need to diversify forms of recreation and public space typologies of consumption) highlighted the growing interest in alternative transportation, mainly by bicycle. This further advocates the need for establishing a comprehensive system, consistent and safe bicycle lanes and simultaneously, for special areas that provide entertainment and attractiveness of this trend affiliates.

On the other hand general urban plans were studied, zoning and detailed areas in the city, from its periphery or within the perimeter of the Bucharest metropolitan area in order to identify a suitable area in all respects to highlight the impact of implementing such a program.

The summary of the analysis indicated the point of the site, the north of Bucharest metropolitan area, with a very dense residential sector lacking alternative recreation among youth segments. Very suitable in terms of territory and the distance from downtown (38 km - the distance that can be covered easily by bike), the location - Corbeanca residential neighborhood – was designated.

The program was implemented as an interpretation of the results of sociological analysis conducted among young people in the neighborhood and the surrounding areas, who mentioned the Dirt Park as the most suitable theme.

The entire analytical process was completed by developing professional landscape designs, studied both from a technical standpoint (ramps and Dirt Park's trails) and the aesthetic. With the goal of creating a coherent whole, which integrated a landscape solution that is to be modern, multifunctional, versatile and meet all the necessities and the specific standards of any dirt park, the final project was selected from three analysed alternatives. These projects were also presented to representatives of the local community, which in turn, reviewed proposed designs and decided, along with the people involved voluntarily in this endeavor, the winning option to be implemented. (Figure 5-8)

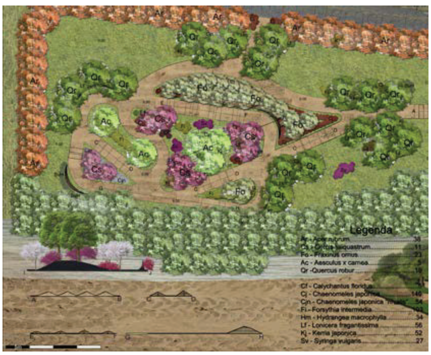

Figure 5. Design proposal. Var. 1

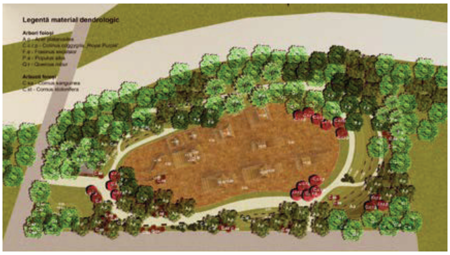

Figure 6. Design proposal. Var. 2

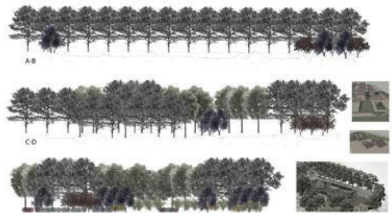

Figure 7. Design proposal. Var. 3

Figure 8. Design proposal. Var. 4

RESULTS AND DISCUSSIONS

The execution of this project involved successive stages of each landscaping project, debuting with site organization, procurement of raw materials and equipment necessary for the execution of terrain and specific buildings, ending with lawn works and finishing touches to the whole ensemble.

The step of site organization and the training of persons who volunteered in this initiative was also a highly significant benefit to the community because of the fact that there was an exchange of experience and information between residents and experts in various fields such as engineering construction, landscaping, horticulture, PR, arts, etc. (Figure 9-11).

The next step was to picket and field tracing of the elements that will constitute the "matter" of the future design. In this stage descriptive geometry and topography knowledge were used in order to make precise measurement of these elements in the field.

Ramps necessary for the execution of varying degrees in difficulty path have been executed both by mechanical means, the main embankment, and hand finishing, the level of detail.
One very interesting aspect of this project was that although ramp parameters, technically calculated in terms of difficulty level of medium and small, meeting all the needs expressed by users of the park, they had to recalculated and adjusted, along with future beneficiaries of this extreme park to meet an emphasized off-road character.
Circulations and plant components were organized in a natural manner so they meet simultaneously strictly functional purposes while decorative aspect and
ambient were also key elements of the project.

Figure 9. Work site management

Figure 10. Beginning the work site

Figure 11. Volunteer helping the construction of pathways

Constructive wooden elements are part of the design, providing a wide range of functions, from the starting ramp of the Dirt Park to the mini stage or exhibition facility.
The official opening day of Corbeanca's Dirt Park took place on May 25, 2013.
Project execution took about 30 days and involved local authorities, various sponsors, local community, local materials and manpower, and not least the work of volunteers and enthusiasts.
The Dirt Park opening event, the largest park of this kind in the country, at the time, was well publicized, both on the internet (websites, blogs, and social networks) and on television stations. This has led to a large number of visitors attracted directly by the new facility that has been made available, but also generally curious people.
Residential District incorporated their new territory. Young people, especially, but also the elderly, were present. They were excited by what they've seen and also thrilled about the new space that is intended for them. (Figure 12-20)
The Dirt Park site is considered by locals, a legible, attractive, open, secure, interactive and versatile space, these being some of the main qualities that public space, or space in general should possess (Lawson, 2001).

Figure 12. Easy wood structures

Figure 13. Planting trees

Figure 14. Mechanized execution ramps

Figure 15. Design in middle stages

Figure 16. The ramps

Figure 17. Media involvement

Figure 18. First starters

Figure 19. Alternative uses of space
(music and dance)

Figure 20. Alternative uses of space
(rest)

CONCLUSIONS

With the establishment of the Dirt Park, the local community was strongly encouraged to be involved in such approaches currently scrolling down a number of other projects generated by its own initiators.
These actions are aimed at increasing community improvement issues related to quality of life, and to solve certain problems faced at the local level.
The most relevant aspect of this research is the fact that the local community has understood and learned the benefit of active and voluntary involvement in identifying and fulfilling the interests of the common law.
People have realized that an overview is generated through dialogue and improvement of community life is determined by personal involvement in view of achieving that vision.

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MICROPROPAGATION OF OF *ORCHIS CATASETUM* - A RARE AND ENDANGERED ORCHID

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Abstract

The application of modern biotechnology for mass propagation of endangered species needs to develop a proper in vitro protocol. Here, a protocol was developed for high frequency in vitro multiplication of an endangered orchid, Orchis catasetum. Protocorms, as explants were cultured on Murashige and Skoog (MS) medium fortified with different concentrations of N^6-benzyladenine (BA), α-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) either individually or in combination. A combination of 0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA was found to be suitable for maximum protocorm-like bodies (PLBs) regeneration (20.40/plantlet). The maximum number of root (7.16/plantlet) and leaf (10.10/plantlet), also the highest plant height (114.20 mm/plantlet) and root length (193.40 mm/plantlet) were obtained on MS medium supplemented with 0.5 mg l^{-1} BA a long with 0.5 mg l^{-1} NAA. Plantlets with well-developed leaves and roots were transplanted to pots filled with perlite, wood pieces, ionolite and mineral cartridge shell (1:1:1:1), also perlite individually and transferred to the greenhouse. Upon ex vitro transfer, 100% of plants survived.

Key words: *Auxins, Cytokinins, Endangered species, In vitro propagation, Protocorm like bodies (PLBs), Tissue culture*

INTRODUCTION

Orchids are one of the most diverse of the flowering plant families, containing 800 genera and 25000 species (Chugh et al., 2009). Orchids have beautiful flowers and exhibit an incredible range of diversity in size, shape and colour. Orchids are grown as ornamentals and are valued as cut flowers not only because of their exotic beauty but also for their long shelf life (Chugh et al., 2009). Large-scale multiplication of orchids, especially rare hybrids and endangered species using tissue culture techniques has helped orchids occupy a position as one of the top ten cut flowers (Chugh et al., 2009). Orchid propagation by seed caused to the production of heterozygous plants. Thus, *in vitro* proliferation is a suitable alternative procedure for propagation of orchids. Different protocols have been established for micropropagation of orchids species through *in vitro* culture of various parts consisting shoot tips, root tips, stems, leaf, nodes, buds, inflorescence and rhizome, as well somatic embryos, callus and thin cell layer (Seeni and Latha, 2000; Geetha and Shetty, 2000; Park et al., 2002b, 2003; Teixeira da Silva, 2003; Bhadra and Hossain, 2003; Wang et al., 2004; Wu et al., 2004; Ket et al., 2004; Sheela et al., 2004; Kuo et al., 2005; Kalimuthu et al., 2006; Sinha et al., 2007; Janarthanam and Seshadri, 2008). In comparison to plantlet development from seeds or adventitious shoots, the micropropagation through PLBs is more efficient because PLBs can be rapidly proliferated on solid or in liquid culture medium, and a large number of PLBs can be provided in a short period (Luo et al., 2003a). Many studies have revealed that the optimization of medium composition was an important approach to improve the micropropagation process of orchids by culturing PLBs that is species-specific (Shimura and Koda, 2004; Luo et al., 2009). In order to stimulate efficient micropropagation PLB, much effort has been done to modify the culture media, mainly by inclusion of plant growth regulators (Nayak et al., 2002; Nge et al., 2006) such as BA, thidiazuron (TDZ), N^6-benzylaminopurine (BAP), NAA, 3-indoleacetic acid (IAA) and gibberellic acid (GA_3) (Prakash et al., 1996; Roy and Banerjee, 2003; Subramanium and Taha, 2003; Saiprasad et al., 2004; Malabadi et al., 2005; Roy et al., 2011). Cytokinins are the most important factors to improve the plant regeneration from PLBs (Nayak et al., 2002; Nasiruddin et al., 2003; Luo et al., 2009). Many orchid species such as *Orchis catasetum* are threatened with the danger of extinction. In the work presented here, PLB multiplication and growth of *Orchis catasetum* have been studied under the controlled conditions of tissue culture in the absence and presence of BA, IBA and NAA.

MATERIALS AND METHODS

Explant collection and surface sterilization

Healthy and sterilized protocorms of *Orchis catasetum* was prepared from a plant tissue culture, Mahmoudabad, Iran, as a source of explants.

Culture media and conditions for protocorm germination

Once the micropropagation system had been established, protocorms were cultured in MS (1962) medium supplemented with 3% (w/v) sucrose and 0.8% agar-agar. All media were adjusted to a pH of 5.7 ± 0.02 with HCl and NaOH prior to autoclaving at 121°C and 105 kg cm^{-2} for 20 min. All the cultures were incubated at 24 ± 2°C under cool white fluorescent light (56 µmol m^{-2} s^{-1}) with a 16-h photoperiod.

Plant growth regulators and protocorm multiplication

The effect of plant growth regulators added to MS medium on protocorm multiplication and subsequent plantlets growth and development was evaluated. The protocorms were cultured in MS medium containing BA (0.0, 0.2, 0.5, 1.0, 1.5 and 3.0 mg l^{-1}), IBA (0.0 and 0.5 mg l^{-1}) and NAA (0.0 and 0.5 mg l^{-1}). Each treatment consisted of three Petri dishes and in each Petri dishes four protocorms were inoculated. Explants secrete phenolic compounds into the media, therefore, 0.5 mg l^{-1} activated charcoal was added to the media. Activated charcoal absorbs phenolic compound. Observations on propocorms regeneration, number of root, number of leaf, plant height and root length were recorded 60 days after the culture initiation.

Statistical analysis

The experimental units were setup in a completely randomized block design. Each experiment was carried out in three replicates and each replicate includes four specimens (totally; 12 specimens for each treatment). The data were analyzed by analysis of variance (ANOVA) by using MSTAT-C software and the mean values were compared using Duncan multiple range test (DMRT) at P = 0.05.

RESULTS AND DISCUSSION

The results of this research present a simple and reliable protocol for rapid micropropagation of *Orchis catasetum*, an endangered orchid species. This method may be applied to produce large number of plantlets during a short time.

Influence of BA and IBA or NAA on protocorm regeneration

Protocorm-like bodies (PLBs) number is affected by the presence of BA, IBA and NAA in MS medium. The effect of BA, IBA and NAA, individually or in combination with each other on protocorm regeneration and growth are shown in Table 1. A combination of 0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA induced maximum PLBs regeneration (20.40/plantlet). Among all treatments of BA, highest PLBs regeneration (12.30/plantlet) was obtained in medium containing 0.2 mg l^{-1} BA. Higher concentrations of BA did not yielded more PLBs. Minimum PLBs number was observed in media supplemented with 1.5 mg l^{-1} BA along with 0.5 mg l^{-1} NAA (4.30/plantlet) and 3.0 mg l^{-1} BA along with 0.5 mg l^{-1} NAA (4.50/plantlet). There is no positive correlation between the increases of BA concentration and enhance of PLBs number (Table 1). DMRT showed significant differences among different concentrations of BA, also reciprocal effect of BA and IBA or NAA for PLBs number ($p \leq 0.01$). DMRT showed that the effect of IBA and NAA was no significant on PLBs number.

Influence of BA and IBA or NAA on plant height

The effects of auxins and cytokinin on the plant height were significant. Among different concentrations of BA, 0.2 mg l^{-1} was found to be the most effective on enhancing the plant height (100.10 mm/ plantlet) (Table 1). Maximum plant height (114.20 mm/ plantlet) was obtained on medium enriched with 0.5 mg l^{-1} BA along with 0.5 mg l^{-1} NAA (Table 1).

0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA were not proper for inducing the plant height, lonely, because those stimulated only 40.00 and 49.00 mm long per plant (Table 1). Minimum plant height (39.97 mm/plantlet) was recorded on medium supplemented with 0.2 mg l^{-1} BA along with 0.5 mg l^{-1} IBA.

Influence of BA and IBA or NAA on leaf number

Explants cultured in the presence of 0.5 mg l^{-1} BA along with 0.5 mg l^{-1} NAA contained the largest number of leaf (10.10/plant) being more than 2.50-fold higher than that found in explants grown in the medium containing 0.5 mg l^{-1} BA (4.00/ plantlet) (Table 1). Among all treatments of BA, largest number of leaf (9.10/ plantlet) was obtained in medium supplemented with 0.2 mg l^{-1}. DMRT showed significant differences among different concentrations of BA ($p \leq 0.01$), NAA and IBA ($p \leq 0.05$) also reciprocal effect of BA and IBA or NAA ($p \leq 0.01$) for leaf number.

Influence of BA and IBA or NAA on root number and length

Root number and root length are affected by the presence of BA, IBA and NAA in MS medium. The effect of BA, IBA and NAA, individually or in combination with each other on root number and root length are shown in Table 1. A combination of 0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA provoked the largest number of root (7.16/plantlet) and the highest length of root (193.40 mm/ plantlet). A combination of 1.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA was a suitable treatment for induction of root number (6.60/ plantlet) and root length (135.10/ plantlet). Among all treatments of BA, largest number of root (5.50/plantlet) and the highest length of root (106.30 mm/plantlet) were calculated in MS medium containing 0.2 mg l-1 (Table 1). Higher concentrations of BA did not produced more root number and length. Minimum root number (2.20/plantlet) was observed in medium supplemented with 0.5 mg l^{-1} BA. Also, the least root length (47.50/plantlet) was calculated in the medium enriched with 1.5 mg l^{-1} BA along with 0.5 mg l^{-1} IBA (Table 1). There is no positive correlation between the increases of BA concentration and enhance of root number and length (Table 1). Analysis of variance showed significant differences among different concentrations of BA along with IBA or NAA for production of root ($p \leq 0.01$).

Table 1. Interaction effect of BA and IBA or NAA on protocorms multiplication and growth of *Orchis catasetum*

Phytohormones (mg l^{-1})			PLBs number	Plant height (mm)	Leaf number	Root	
BA	IBA	NAA				Number	Length (mm)
0.0	0.0	0.0	11.60^{bcd}	76.50^{bcd}	7.60^{bcd}	4.50^{cdef}	82.08^{defg}
0.2	0.0	0.0	12.30^{cde}	100.10^{a}	9.10^{ab}	5.50^{bcd}	106.30^{bcd}
0.5	0.0	0.0	10.80^{cde}	40.00^{f}	4.00^{i}	2.20^{i}	50.13^{fg}
1.0	0.0	0.0	8.10^{efg}	64.50^{cdef}	5.60^{defghi}	4.20^{defg}	90.50^{defg}
1.5	0.0	0.0	$9.20d^{efg}$	46.00^{ef}	7.00^{bcdef}	5.10^{cde}	60.50^{efg}
3.0	0.0	0.0	10.10^{de}	47.37^{ef}	$5.70^{cdefghi}$	4.70^{cdef}	101.40^{bcde}
0.0	0.5	0.0	11.00^{cde}	42.00^{f}	6.80^{ab}	4.30^{defg}	88.20^{defg}
0.2	0.5	0.0	12.20^{bcd}	39.97^{f}	4.40^{ghi}	3.80^{efgh}	74.35^{defg}
0.5	0.5	0.0	14.50^{bc}	66.95^{cdef}	8.08^{abc}	5.60^{bc}	93.12^{cdef}
1.0	0.5	0.0	9.00^{defg}	49.25^{def}	4.70^{fghi}	2.90^{ghi}	48.92^{fg}
1.5	0.5	0.0	9.60^{defg}	41.00^{f}	4.12^{i}	2.60^{hi}	47.50^{g}
3.0	0.5	0.0	10.10^{cde}	57.75^{cdef}	5.20^{efghi}	4.00^{efgh}	73.67^{defg}
0.0	0.0	0.5	12.50^{bcd}	49.00^{def}	6.88^{ab}	4.50^{defg}	90.20^{defg}
0.2	0.0	0.5	6.00^{gh}	51.58^{def}	4.40^{hi}	3.08^{ghi}	56.58^{fg}
0.5	0.0	0.5	20.40^{a}	114.20^{a}	10.10^{a}	7.16^{a}	193.40^{a}
1.0	0.0	0.5	15.60^{b}	84.13^{bc}	8.02^{abcd}	6.60^{ab}	135.10^{bc}
1.5	0.0	0.5	4.30^{h}	64.73^{cdef}	7.20^{bcde}	3.90^{efgh}	58.67^{bc}
3.0	0.0	0.5	4.50^{h}	53.92^{def}	5.60^{defghi}	3.50^{fghi}	64.67^{defg}

*Mean values followed by the same letters are not significantly different at 0.05 levels (DMRT)

Protocorm-like bodies (PLBs) can be safely used for rapid propagation of orchids. Addition of low concentrations of BA and NAA promoted protocorms multiplication and growth of *Orchis catasetum* plantlets. The combined use of BA and NAA is proposed in micropropagation of *Orchis catasetum*, an endangered orchid. However, this paper can be introduced 0.2 mg l^{-1} of BA as an individual plant growth regulator to produce proper shoot formation and root induction. Kalimuthu et al. (2007) obtained the results same as our findings, but with BAP on *Oncidium* sp. These researchers showed that the maximum PLBs formation, number of shoots and roots were observed in MS medium supplemented with 2.0 mg l^{-1} BAP. BAP individually was better than in combination with NAA. Of course, 2.0 mg l^{-1} BAP individually or in combination with 1.5 mg l^{-1} NAA induced the same roots on shoots (100%).

Since the seeds of orchid are without endosperm hence it needs specific nutritional and environmental conditions (Arditti et al., 1990). Protocorm is a rudimentary organ that differentiate to a new shoot.

Cells of protocorms are highly meristematic, thus can be applied to enhance proliferation and simultaneous production of orchid plantlets (Teixeira da Silva et al., 2005).

Protocorms are being applied by many researchers as explants for micropropagation of many rare and endangered orchid species (Seeni and Latha, 2000; Sheelavantmath et al., 2000; Nagaraju and Mani, 2005; Dev and Temjensangba, 2006; Teixeira da Silva et al., 2006; Hossain et al., 2010; Roy et al., 2011).

Orchids need auxins and cytokinins for plantlets development (Roy et al., 2011). The type and concentrations of plant growth regulators plays an important role during micropropagation of many orchids (Arditti and Ernst, 1993). Our study showed positive effect of BA for maximum protocorm multiplication. BA acts more efficiently when used in combination with NAA. This finding is in agreement with some other findings obtained in micropropagation of orchids (Seeni and Latha, 2000; Roy et al., 2011).

Study of Luo et al. (2009) on micropropagation of *Dendrobium huoshanense* showed that a high frequency of shoot formation was recorded on medium with 5-15 μM 2-iP, when compared to growth regulator-free medium. Several studies demonstrated the positive effect of BAP, NAA, TDZ and KIN for plantlet regeneration from PLBs (Nasiruddin et al., 2003; Luo et al., 2008; Chugh et al., 2009). BAP and NAA are most applicable plant growth regulators for micropropagation of most orchids (Chugh et al., 2009).

Luo et al. (2008) showed that 5.0 mg l^{-1} BAP was the best for induction of PLBs (15/explant) per stem segment within 6 weeks. 0.5 mg l^{-1} KIN was also good for PLB formation. BAP in combination with NAA had been suggested by some studies to obtain the maximum number of PLBs (Kim, 2003; Puchooa, 2004). Our finding is in consistent with these findings. However, Luo et al. (2008) showed that NAA added to the medium containing optimal BAP did not significantly improve response of explants in *Dendrobium densiflorum* and even decreased production of PLBs at concentration of more than 1.0 mg l^{-1}. In some orchids IBA induced rooting (Nayak et al., 2002).

In the present study, NAA was found more effective than IBA for micropropagation of *Orchis catasetum*. Study of Roy et al. (2011) on *Vanda coerulea*, an endangered orchid, showed that a synergistic combination of NAA (5.36 μM) and BAP (3.80 μM) led to maximum protocorm proliferation.

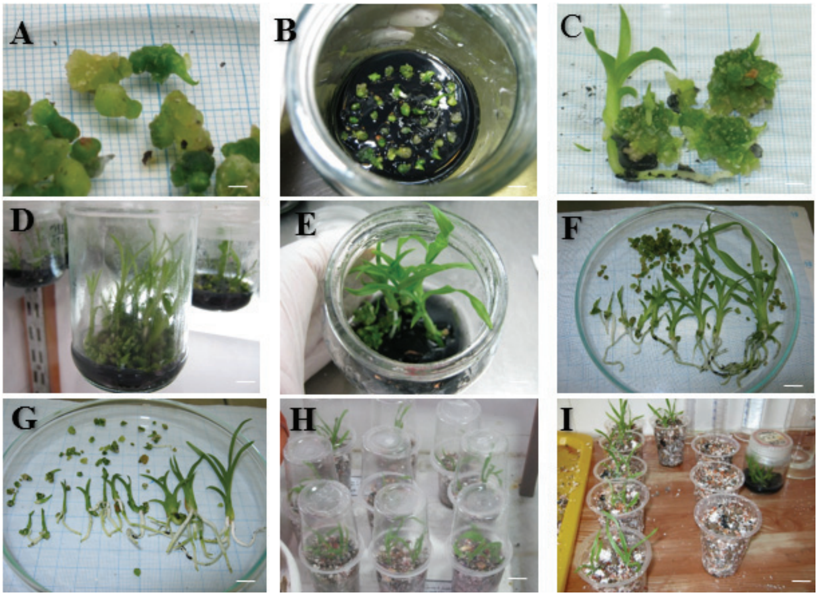

Figure 1. Micropropagation of *Orchis catasetum* through protocorms-like bodies (PLBs). (A) PLBs formed from seeds used as explants (Bar = 1 cm). (B) PLBs cultured on MS medium containing plant growth regulators (Bar = 2 cm). (C) A developing PLBs (Bar = 1 cm). (D) Micropropagated shoots from PLBs on medium containing 0.5 mg l^{-1} BA a long with 0.5 mg l^{-1} NAA (Bar = 1 cm). (E) Well-developed plantlets derived from PLBs (Bar = 2 cm). (F and G) Plantlets obtained from media enriched with different concentrations of growth regulators (Bar = 2 cm). (H and I) The process of plantlets acclimatization (Bar = 2 cm).

CONCLUSIONS

Micropropagation of rare and endangered orchids in large scale have to be developed further because of commercial value and conserve them.

A proper ratio of auxins and cytokinins is required for optimal protocorm multiplication. The efficiency of type and concentration of plant growth regulators varied with kind of species or varieties.

BA and NAA at a particular concentration are suitable for protocorms-like bodies (PLBs) multiplication and growth of *Orchis catasetum*.

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CRAMBIDAE ASSOCIATED WITH PARKS AND ORNAMENTAL GARDENS OF BUCHAREST

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Abstract

The study presents two species of defoliating insects found in parks and ornamental gardens. These species are less known in our country and belong to Crambidae family. The study shows: the mode of attack, the unaesthetic appearance of the attacked plants in the landscape, the possibilities and difficulties to control these species of insects. The larvae of Cydalima perspectalis Walker are voracious and consume the leaves of Buxus plants. The larvae of Palpita vitrealis Rossi attack in groups Ligustrum plants until they defoliate them. Both species affect the decorative value of the plants up to their elimination from the landscape.

Key words: *defoliating insects, parks, ornamental gardens, attack, control*

INTRODUCTION

Trees, flowers and plants' pests produce considerable damages by decreasing the plants' ornamental value and also by changing the urban areas' microclimate. The climate changes throughout the last years, excessive drought and also heavy rain, the great amount of imported plants and flowers as a result of the urban areas surfaces' growth favored pests from parks and ornamental gardens and their aggressiveness' growth by enlarging the host plants' circle.

The present project's purpose is to analyze the impact of two defoliating *Crambidae* species (less known in our country) upon some plants' species frequently encountered in parks and ornamental gardens from urban areas and these pests' destruction methods starting from pest control difficulties.

MATERIALS AND METHODS

Following visual observation done in parks and green areas throughout Bucharest, during 2010-2013 two species of lepidopters were spotted. They are less known in our country: plants' moth of *Buxus (Cydalima perspectalis* Walker) on buxus plants *Buxus sempervirens* and green olives' moth (*Palpita vitrealis* Rossi) on dog wood plants (*Lygustrum vulgare*). Samples were taken from the attacked plants and Lepidoptera species' larvae were also taken, in order to grow them in laboratory conditions. On species' level, the laboratory obtained adults were identified using the morphological criteria (wing, genital part). Also, in the case of the two Lepidoptera species, photos were taken concerning their attack method and concerning their development stages.

RESULTS AND DISCUSSIONS

Cydalima **(*Diaphania*)** ***perspectalis*** Walker (1859) is a Lepidoptera species belonging to the *Crambidae* family, native from East Asia (Korycinska and Eyre, 2011). In Europe, moth was for the first time signaled in Germany in 2007. In 2010 it was present in Switzerland, Austria, Great Britain and Liechtenstein. In 2011 it was also signaled in Belgium and Hungary (Szlekely et al., 2011). In Bucharest in 2010 it was signaled by Maria Iamandei, and in 2011 by Levente Szekely and his colleagues signal it in the north-eastern part of Bucharest.

It has been observed by us on *Buxus sempervirens* plants, in the north of Bucharest, in a private garden in the summer of 2010. In the spring of 2012 (april - may) young larvae were collected and bred in captivity, obtaining determined adults. In 2013, this moth was seen on a buxus green fence from Herăstrău park, near a children's playground zone (Figure 1).

Figure 1. *Buxus* green fence attacked by *Cydalima perspectalis*, situated near children playground area

Data regarding this species' biology are not entirely clear for our country. Based on their laboratory growth and development, we present a few morphological features for the following stages: larva, youngster and adult. On apparition, the larva has a yellow-greenish color with a black head. The mature larvae (Figure 2) it has a green body, with white and black stripes and big black dots all along its body. Its body length is approximately 4 cm.

Figure 2. *Cydalima perspectalis* – larvae

The pupae (Figure 3) has a 1,5 – 2 cm length. On apparition it is green, with black stripes on the back side, and when it becomes an adult it is brownish, hidden inside a white cocoon, among leaves and young branches.

Figure 3. *Cydalima perspectalis* – pupae

The adult (Figure 4) is medium-sized, with its wings of 4 – 4,5 cm. The body is white with a black head and the lower belly part is brown. The wings have brown edges and a large white surface. The front wings have a white extension in the brown edge, situated in the middle. The brown areas have golden reflexes, and the white ones violet reflexes.

Figure 4. *Cydalima perspectalis* – adult

Larves are greedy and consume the *Buxus* plant's leaves. On apparition they chew the lower part of the plant's skin and the middle part, leaving the upper part of the leaf untouched. Later on, the plants are completely stripped, while among the young plants numerous silk threads and green-black excrements can be seen. On the soil, under the plants, small remains of plants and green excrements can be noticed (Figure 5). The plants are damaged and their ornamental value is totally compromised (Figure 6). Also, the plants' growth and development processes are slowed down, especially since it is a known fact that *Buxus* species has a relatively slow growth.

An important aspect which needs to be given special attention is the fact that this moth can become an invasive species in parks and ornamental gardens from urban areas (decorated in a landscape type in green fences or isolated plants with *Buxus* genre species. At the same time, it can become a serious enemy for seed beds.

Figure 5. Chopped leaves and excrements under attacked plants

Pest control regarding this particular species is less known. Professional literature indicates laboratory testing with biological products such as: the baculovirus *Anagrapha falcifera* (Rose et al., 2013), entomopathogen bacteria *Bacillus thuringiensis* var. *kurstaki* (www.neozoen.ch) or *Steinernema carpo-capsae*.

Figure 6. Green fence of *Buxus* attacked in different stages

For seed beds piretroid products, based on deltametrin or cipermetrin are recommended; also, it is a good idea to use metamorphosis inhibitors such as diflubenzuron, but we have to mention it must be applied to young larves, before the silky threads appear.

In private gardens and on smaller isolated plants a mechanic combat can be done, by gathering and destroying the larvae or physically, by cutting and burning the ones which have been severely damaged. Also, home and gardening chemical products can be used.

Defeating this pest is tougher as the import and export of *Buxus* plants is free, favoring the spread and establishing of this species in new zones. On top of this the lack of natural enemies is added, such as: birds. The host plant has a high level of toxicity, and host plants can be encountered near playgrounds etc.

Palpita vitrealis Rossi is a *Crambidae* family moth, spread all over the world. It was signaled in south Europe, but it was also seen in north. In our country it was spotted in Banat, Crişana, Transilvania, Moldova and Dobrogea (Rákosy et al., 2003) under the *Palpita unionalis* name (Hübner, 1796) without notations regarding host plant or attack method. In 2011 Levente Szekely mentions it to be more frequent in southern Romania. The observations elaborated by us show it present in Bucharest in 2013 on a green fence of *Lygustrum vulgare*. The images regarding this moth attack method lead us to more than its simple signaling in Bucharest. The larva is light-green, a little bit yellowish throughout the first stages, afterwards green with a yellow-brown head (Figure 7).

Figure 7. Older larve

On apparition they feed on the lower skin and on the middle part (Figure 8), and later on they chew the highest plant part (Figure 9). The attack is manifested in such a way that they plants are totally stripped (Figure 10).

Figure 8. The larvae attack in the first stages

Figure 9. Total and irregular chew of the leaf

Figure 10. Larvae attack group

They create something similar to a spider web before starting to develop themselves on the host plant. On the plants and at their base we can notice black excrements.

The attacked plants are damaged and they dry off (Figure 11).

Figure 11. Dried branches after attack

The pupae pest is at first green, then it becomes brown (Figure 12). The development is done in silky threads, among the attacked leaves. The adult (Figure 13) has shiny transparent wings. The front wings have a brown-red edge and two black dots in the middle. The wings' size is of 27 – 31 mm. Standing still, they have a triangle shape.

Figure 12. *Palpita vitrealis* – pupae

Figure 13. *Palpita vitrealis* – adult

Defeating ornamental plants' pests from urban areas has a specific domain, because there are difficulties regarding: host plants' place (parks, public zones, playgrounds, rest places, isolated plants, private gardens), lack of reduced toxicity products or lack of biological products and lack of chemical products for parks and ornamental gardens.

CONCLUSIONS

The two *Crambidae* species present in parks from Bucharest can generate major damages to host plants signaled, the number of *Buxus* and *Lygustrum* in landscape areas being quite large. These species can become invasive in parks and ornamental gardens from Bucharest.
At the present time there are no appropriate plant protection products for pest control from parks and public ornamental gardens.
In private gardens chemical control measures can be taken.
In public areas (parks, playgrounds) chemical pest control cannot be applied.

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IDENTIFICATION OF MALE AND FEMALE *Cycas revoluta* Thunb. BEFORE MATURITY USING MORPHOLOGICAL AND ANATOMICAL FEATURES

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Abstract

This paper reports sex determination of cycad (Cycas revoluta Thunb.) before maturity and cone formation. Sex determination in cycad is possible only after maturity and cone formation. In ideal conditions, it takes at least 10 to 12 years for cycad to reach maturity. In this research, two-years-old male and female off-shoots of cycad were separated from the adult male and female plants and were treated in natural conditions, uniformly. Morphological characteristics of male and female plants were visually compared. Anatomical features were studied on the sections prepared from primary leaf rachis, leaflet and root using light microscope. According to the Discriminate Analysis (DA) method, differences between morphological characteristics of male and female plants such as leaf rachis diameter, leaflets width, leaflets distance on the rachis, leaflets angle on the rachis, and roots diameter and length, were significant. The arrangement of leaves in one third of middle part was bipinnate with opposite leaflets and bipinnate with alternate leaflets in male and female plants, respectively (new leaflets initiate forms before and after complete formation of previous leaflets in female and male plants, respectively). Anatomical studies showed that the difference between the lengths of lower stomata in male and female leaflets were significant. Stoma type in cycad leaf is actinocytic.

Key words: *Cycad, Ornamental plants, Sex determination*

INTRODUCTION

Cycas is the only genus of the Cycadaceae family and includes about a hundred species, with the most prominent specie *Cycas revoluta*. *Cycas revoluta* is the second species in the genus *Cycas* and it is the only species present in Asia (Liu et al., 1991; Donaldson, 2003; Hill, 2004). This plant has a long history and is popular because of beautiful pinnate and evergreen leaves. *Cycas revoluta* is used as an ornamental pot plant, and in landscape and environmental design. The habitat of *Cycas revoluta* is southern Japan at 31′ north latitude including Ryukyu, Mitsuhama and Satsuma islands in the Southern island of Kyosho (Stevenson, 1990). *Cycas revoluta* is one of the most widely grown cycads in subtropical regions or in greenhouses in colder areas (Donaldson, 2003). Cycads propagate by seeds, off-shoots, and stem cuttings and in tissue culture conditions (Jones, 1993; Rainald, 1998).

Cycad is a dioecious plant and its sex determination is possible only after maturity and cone formation. In ideal conditions, it takes at least 10 to 12 years for cycad to reach maturity (Raju and Jonathan, 2010). Thus, appearance and morphological identification of *Cycas revoluta* male and female plants is not possible until maturity. Therefore, sex determination takes place only after maturity and formation of flowers and cones. Assessment of male and female plants before maturity is necessary for breeding, reliable care and production planning. According to the literature, no study has been performed in terms of the identification of male and female trunks of *Cycas revoluta* before maturity. In various sources announced that the identification of male and female trunks of *Cycas* before maturity and cone formation is not possible (Raju and Henry, 2010). Thus, this study was performed to achieve valid and reliable diagnostic methods to identify *Cycas revoluta* male and female plants before sexual maturity.

To achieve this goal, anatomical and morphological features of *Cycas revoluta* in the vegetative growth period were evaluated.

MATERIALS AND METHODS

For morphological studies, off-shoot of matured *Cycas revoluta* which were characterized in terms of sexual and were grown under outdoor conditions. The samples were taken from Cycads breeding centers around the city of Rasht, Iran in early April, 2011. The two-year off-shoots in the physiological age were uniform and containing leaves but without roots. Separated shoots from the parental stock were disinfected with a fungicide. Leaves were cut from shoots to reduce surface evaporation and transpiration and then were planted in isolated pots with uniform soil composition containing a mixture of peat moss, sand and garden soil (1:1:1). To implement the plan, six pots from each male and female trunk were selected and randomly named. Then, the pots were randomly assigned into the 3 columns and 4 rows and were kept under suitable conditions. Plants were irrigated twice a week in summer and once a week in the fall and winter. Shoots produced roots during the spring and new leaves during August. Morphological studies were carried out in May 2012.

Measured morphological traits of male and female trunks of *Cycas revoluta* were: leaf rachis diameter, leaflet length, leaflets thickness, leaflet width, petioles diameter, distance between the leaflets on the leaf rachis, angle between leaflets and leaf rachis, shoots diameter, root diameter, root length, chlorophyll content, leaflet color, leaflets arrangement on leaf rachis, way to start up and formation of leaflets at the end of leaf rachis, the position of leaflet blades compared to the leaf rachis and the shape and color of the roots. To measure quantitative traits, digital caliper, ruler and chlorophyll content, manually chlorophyll meter (SPAD-502) was used. Qualitative study was performed as visual observation by the manual lens and light microscope.

The diameter of leaf rachis was calculated between leaflets 29 and 30 using a digital caliper. The length of the leaflets was calculated by a digital caliper on leaflets number 30. The thickness and width of leaflets were measured in the middle of leaflet number 30 using a digital caliper. The diameter of the petiole was obtained in the connection place of leaflets to rachis in leaflet number 30 using a digital caliper. Distance between leaflets in the interval of leaflets number 29 and 30 in leaf rachis was measured using a digital caliper. Measurement of the angle between leaflets (number 30) and leaf rachis was done using conveyor by hand after removing the other leaflets from leaf rachis. Diameter of the shoots in the place of largest diameter was obtained by a digital caliper. To measure the diameter of roots, secondary roots were used. Thus, diameter of secondary roots was measured at 3 cm from the terminal of the root. For determination of the amount of chlorophyll, leaflets were selected. For this purpose, the hand-held chlorophyll meter SPAD-502 was used. Shape, the position of blade leaflets in relation to the leaf rachis and leaflet arrangement was evaluated by direct observation (naked eye). To investigate initiation and formation of leaflet at the ends of leaf rachis, apical meristem of leaf rachis were observed continuously over several days. To assess the shape and color of the roots, they were washed with water and assessed by direct observations on male and female plants.

Anatomical studies on leaf rachis, leaflets and root was performed. The anatomical traits of leaf rachis of the male and female trunks of *Cycas revoluta* were the number of layers of the parenchyma cortex in rachis, stele diameter, the number of procambium layers, and number of vascular bundles, pericycle layer thickness, density and type of trichoms. Anatomical characteristics of leaflets rachis of male and female trunks were the number of upper and lower layers of parenchyma, type of stoma, upper and lower stomatal density, height of the upper and lower stoma, types of hairs and hairs density in upper and lower layers. Anatomical features of roots of male and female trunks were the number of cortex parenchyma layers, number of cambium layers, stele diameter, pericycle layer thickness and the number of vascular bundles.

For study anatomical characteristics of *Cycas revoluta*, at first the various organs were sampled. Plant samples were fixed in a solution containing 10% formalin + 85% alcohol + 5% acetic acid. The transverse sections of leaf rachis, petiole and root were performed manually using a sharp blade. Samples were cleared by sodium hypochlorite and acetic acid. Samples were stained with Congo red and methyl blue. To study hairs in the leaf rachis and the stomata and hairs in the leaflets, living specimens without fixation was used. By removing the epidermis, the hairs on rachis and stomata hairs in the leaflets were examined. The prepared samples were photographed by Olympus camera DP71 attached to the light microscope. Identify the cells and different tissues on the taken images were performed. To evaluate the anatomical features of leaf rachis, one of the newly produced leaf rachis containing soft and young tissues was randomly selected and cut from the interval of leaflets number 15 and 16. To determine the presence or absence of the hairs in the rachis, epidermis was separated from the leaf rachis and the interval of leaf number 15 and 16 was selected and observed under a light microscope. Number of hair in one square millimeter from the rachis was counted. The leaflet number 30 was randomly assigned to evaluate its anatomy. To determine the type and density of stomata on leaflet, the upper and lower epidermis was separated from the selected leaflet number 30 and was studied by light microscopy. Type and density of stomata per square millimeter of a leaflet and length of stomata per micrometer was determined and photographs were taken from the stomata. Young roots were randomly selected to evaluate their anatomy.

RESULTS AND DISCUSSIONS

In this study, various morphological characteristics of male and female trunks of *Cycas revoluta* have been studied. The results have been showed in Tables 1 and 2. According to Tables 1 and 2, the difference between the diameter of leaf rachis in both male and female trunks of *Cycas revoluta* before the maturity is significant and indicated that the diameter of leaf rachis in male and female trunks is different. Contrary to our results, Stevenson (1993) reported that the leaf rachis in *Cycas* is convex-shaped and there is no any difference between the male and female trunks. Results showed that the difference in leaflets height between male and female trunks is not significant (Tables 1 and 2). Studies of Jones (1993) confirm our results. According to Tables 1 and 2, the difference between leaflet thickness in male and female trunks is not significant. Studies of Hill (2004) revealed that the thickness of leaflets at the trunks of the male and female in *Cycas* is the same and is variable from 0.3 mm in young leaves to 0.8 mm in older leaves.

Current investigation demonstrated that there is noticeable difference between leaflet widths at the mid-point of 30 leaflet trunks. Hill (2004) showed that leaflet width in male and female trunks of *Cycas revoluta* is equal and varies between 0.6 to 1.5 cm. Jones (1993) has reported that the diameter of node of leaflet varies from 2 to 4 mm (Jones, 1993).

Table 1 shows that there is no significant difference between petiole diameter in male and female trunks.

Distance between leaflets on the leaf rachis in the interval of leaflet numbers 29 and 30 on the trunks of *Cycas revoluta* were significant. Stevenson (1993) states that cycas leaf length in the trunks of male and female is alike and up to 200 cm. Also, the number of leaflets per leaf reaches 250.

The results of current study indicated that angle between leaflets and leaf rachis was up to 53 and up to 48 in female and male trunks, respectively. Study of Stevenson (1990) showed that male and female leaflets of *Cycas revoluta*, with an angle of about 45; are located on the rachis. The results obtained in our report are not consistent with those of Stevenson (1990).

According to Tables 1 and 2, the difference in shoot diameter is not significant.

Our findings is in accordance with study of Tang (1991). Study of these researchers on off-shoots removed from the lower parts of male and female stems showed that there is not any difference between the place of shoot formation, number and size.

Also, data obtained from the current study showed that the differences in the root diameter and size are significant. Hill (2012) reported that size, diameter and volume of root were variable in cycads, ranging from very small branches to large branches and sub-branches. Our reports are consistent with the results of Hill (2012).

Difference in root length was significant. Root length was longer for female trunk than that of male trunk (Tables 1 and 2).

Table 1. Discriminate Analysis (DA) of means of groups for morphological characteristics of male and female trunks of *Cycas revoluta*.

Source of variances	Vilex ƛ	F	df_1	df_2	Sig.
Main axils diameter of leaf (X_1)	0.459	11.765	1	10	0.006^{**}
Length of leaflet (X_2)	0.832	2.017	1	10	0.186^{ns}
Leaflet diameter (X_3)	0.953	0.494	1	10	0.498^{ns}
Leaflet width (X_4)	0.111	80.112	1	10	0.000^{**}
Petiole diameter (X_5)	0.898	1.137	1	10	0.311^{ns}
Distance of leaflets (X_6)	0.147	58.209	1	10	0.000^{**}
Angle between leaflets (X_7)	0.314	21.797	1	10	0.001^{**}
Off-shoots diameter (X_8)	0.903	1.079	1	10	0.323^{ns}
Roots diameter (X_9)	0.177	46.593	1	10	0.000^{**}
Length of roots (X_{10})	0.240	31.743	1	10	0.000^{**}
Chlorophyll content in leaflets (X_{11})	0.995	0.054	1	10	0.822^{ns}

**: Significant at $\alpha = 1\%$, ^{ns}=Not significant

Table 2. Quantitative data of morphological characters in male and female trunks of *Cycas revoluta*

Morphological characters	Female	Male
Main axils diameter of leaf (mm)	3.21-3.51 ± 0.3 (3.81)	3.02-3.41 ± 0.39 (3.8)
Length of leaflet (mm)	73.98-85.05 ± 11.07 (79.515)	81.8-85.2 ± 3.4 (83.5)
Leaflet diameter (mm)	0.32-0.58 ± 0.26(0.45)	0.35-0.47 ± 0.12 (0.41)
Leaflet width (mm)	4.51-4.98 ± 0.47 (4.745)	3.77-4.04 ± 0.27 (3.905)
Petiole diameter (mm)	2.42-2.63 ± 0.21 (2.525)	2.25-2.63 ± 0.38 (2.44)
Distance of leaflets (mm)	5.33-6.41 ± 1.08 (5.87)	3.13-4.39 ± 1.26 (3.76)
Angle between leaflets (°)	47-53 ± 6 (50)	42-48 ± 6 (45)
Off-shoots diameter (cm)	5.05-6.15 ± 1.1 (5.6)	5.09-5.85 ± 0.76 (5.47)
Roots diameter (mm)	2.07-2.63 ± 0.56 (2.35)	3.08-3.92 ± 0.84 (3.5)
Length of roots (cm)	21.2-24.2 ± 0.3 (22.7)	15.5-20.4 ± 2.45 (17.95)
Chlorophyll content in leaflets	47.7-52.1 ±4.4 (49.9)	43.9-54.6 ± 10.7 (49.25)

Jones (1993) reported that the roots of cycads often are short and fleshy and do not tend to move into deep soil.

Young leaves in *Cycas* are soft and light green whilst older leaves are glossy, leathery and dark green (Stevenson, 1993). There is no significant difference in chlorophyll content of male and female trunk leaflets in *Cycas revoluta*.

Leaflet shape on male and female trunks was examined, observationally. Leaflets in both trunks are spear-shaped. Jones (1993) has reported that the leaflets in *Cycas revoluta* are numerous, sharp and spear. Leaflets have longitudinal veins and lack secondary and lateral veins. Cycas leaf is bi-pinnate with complete parts and edges (Ghahraman, 2003).

In our researches, leaflet arrangement on the rachis is divided into three parts. Leaflets are located in the both sides of rachis as alternate and opposite with no particular order.

In the middle third of the leaf, leaflet arrangement plays an important role in identifying male and female trunks of *Cycas revoluta* and is considered as one of the morphological differences between male and female trunks; so that leaflets are opposite in a male trunk and are located across from each other in two sides of the leaf rachis, whereas, leaflets in the female trunk are alternate on the leaf rachis (Figure 1).

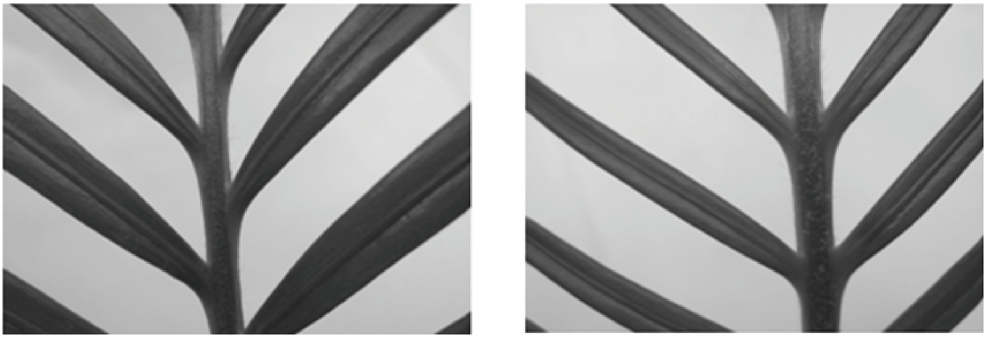

Figure 1. Arrangement of alternate in leaflets of female trunk (left), and opposite in leaflets of male trunk (right) of *Cycas revoluta*.

In the final one third of the leaf rachis, leaflets are compact and close together where the arrangement of the leaflets is irregular.

Results obtained in our work suggest that forming new leaflets at the male trunk starts after differentiation of previous leaflet; so that the leaf apex of leaf has differentiated and individual leaflets (Figure 2).

On the female trunks, initiation and formation of the new leaflets occurs before full differentiation of earlier leaflets, while the prior leaflets are not reach to full development and separate from it. Consequently, the apical leaf rachis in the female trunks of *Cycas revoluta* have two leaflets with one being more differentiated than another (Figure 2).

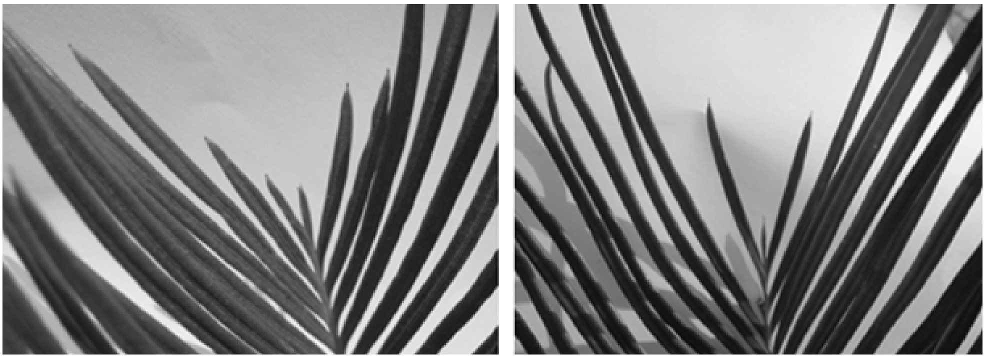

Figure 2. Formation of new leaflets in female trunk (left), and male trunk (right) of *Cycas revoluta*.

The position of the leaflet blades relative to the rachis occurs in three forms;

1. At the beginning of rachis and near the junction of off-shoots, leaflet blades are perpendicular to the leaf rachis and are almost horizontal to the ground surface (Figure 3).
2. In the middle part of the leaf, leaflet blades as the first case was not perpendicular to the rachis and located in angle of about 30° with respect to the leaf rachis and is not horizontal with respect to the earth's surface (Figure 3) and
3. In the terminal part of leaf rachis, the area of leaflet blades is aligned with the rachis and is at the same level (Figure 3).

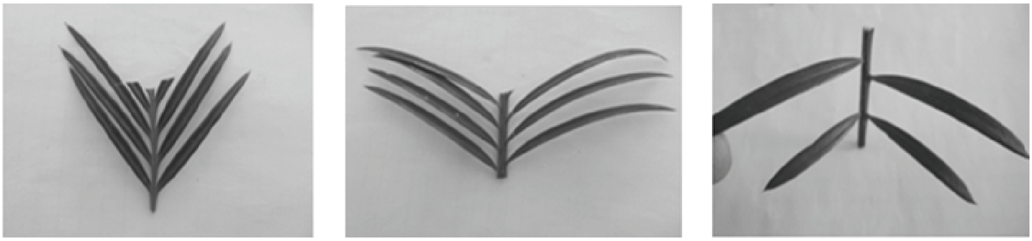

Figure 3. Arrangement of leaflets in base (right), center (middle) and top (left) of leaf in *Cycas revoluta*.

Roots in *Cycas revoluta* are white, creamy white, fleshy and dichotomous with primary and secondary roots which do not tend to move into deep soil (Stevenson, 1990). Size, diameter and root volume were very variable in *Cycas* and vary from very little branches and fleshy to lots of branches and subsidiary (Hill, 2012).
The study of morphological characteristics of male and female trunks of *Cycas revoluta* in the present study showed some differences.
In the female trunks, root volume is high and root has high subsidiary branches and its color is light brown (Figure 4).
In the male trunk, root volume compared to the female trunk is low with fewer branches, however is fleshy and has a tendency to bifurcate. The root color in male cones is cream and has a lighter color than the female trunk (Figure 4).

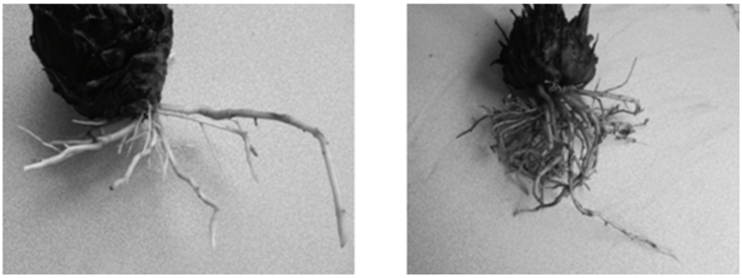

Figure 4. Root in male trunk (left), and female trunk (right) of *Cycas revoluta*

Quantitative data and analysis of variance for anatomical characteristics obtained from male and female trunks of *Cycas revoluta* that are shown in Tables 3, 4. Anatomical comparison of the leaf rachis showed that the number of cortex parenchyma layers is 6-10 and 7-9 in the male and female trunks, respectively (Table 5).
The lowest and highest layers were observed in male trunk. Stele diameter in male and female trunks is variable from 17.00 to 21.50 and 15.00 to 26.00 micrometers, respectively. Stele was not integrated and each strip is called a vascular bundle.
The leaf rachis of male and female trunks of *Cycas revoluta* has 1 to 2 procambium layers located between the central vascular bundles. In this taxon, the first procambium creates primary xylem and phloem and then produces the vascular cambium (Ladder, 1995).
The rachis has collenchyma bundles under the epidermis that offer sufficient solidity; so that the leaf rachis is like the semi-woody stems of other plants. Mesophyll contains parenchyma cells with spongy and palisade arrangement and has intercellular spaces.
This finding is consistent with Ladder (1995). Vascular bundles in the male and female trunks are equal (three bundles) and central. The diameter of pericycle in the male and female trunks are 3-5 and 3-4.5 micrometers, respectively. Pericycle surrounds the vascular bundles (Table 5).

Table 4. Discriminate Analysis (DA) of means of groups for anatomical characteristics of male and female trunks of *Cycas revoluta*.

Source of variances	Vilex ƛ	F	df_1	df_2	Sig.
No. of parenchyma layers in main axils of leaf (X_1)	0.97	0.21	1	10	0.65^{ns}
Stele diameter in main axils of leaf (X_2)	0.80	2.39	1	10	0.15^{ns}
No. of procambium layers in main axils of leaf (X_3)	1.00	0.00	1	10	0.10^{ns}
No. of vascular bundle in main axils of leaf (X_4)	a	-	1	10	-
Pericycle diameter in main axils of leaf (X_5)	0.87	1.81	1	10	0.20^{ns}
No. of trichoms in main axils of leaf (X_6)	0.98	0.17	1	10	0.68^{ns}
No. of upper parenchyma layers in leaflet (X_7)	0.89	1.21	1	10	0.29^{ns}
No. of lower parenchyma layers in leaflet (X_8)	0.96	0.38	1	10	0.54^{ns}
No. of stomata in upper surfaces of leaflet (X_9)	0.81	2.28	1	10	0.16^{ns}
No. of stomata in lower surfaces of leaflet (X_{10})	0.92	0.78	1	10	0.39^{ns}
Length of upper stomata in leaflet (X_{11})	0.92	0.86	1	10	0.37^{ns}
Length of lower stomata in leaflet (X_{12})	0.64	5.41	1	10	0.04^{*}
No. of trichoms in upper surface of leaflet (X_{13})	0.71	4.06	1	10	0.07^{ns}
No. of trichoms in upper surface of leaflet (X_{14})	0.95	0.46	1	10	0.51^{ns}
No. of cortex parenchyma layers in root (X_{15})	0.88	1.28	1	10	0.28^{ns}
No. of cambium layers in root (X_{16})	0.97	0.29	1	10	0.59^{ns}
Stele diameter in root (X_{17})	0.96	0.41	1	10	0.53^{ns}
Pericycle diameter in root (X_{18})	0.97	0.22	1	10	0.64^{ns}
No. of vascular bundle (X_{19})	-	-	1	10	-

*Significant at $\alpha = 5\%$, ^{ns}=Not significant

Table 5. Anatomical characters in main axis of leaf of male and female trunks of *Cycas revoluta*

Trunk / Anatomical characters	Female	Male
No. of cortex parenchyma layers	7-9 ± 2 (8)	6-10 ± 4 (8)
Stele diameter (µM)	15-26 ± 11 (20.5)	17-21.5 ± 5.4 (19.25)
No. of procambium layers	1-2 ± 1 (1.5)	1-2 ± 1 (1.5)
No. of vascular bundle	3	3
Pericycle diameter (µM)	3-4.5 ± 1.5 (3.75)	3-5 ± 2 (4)
No. of trichoms/mm^2	10-13 ± 3 (11.5)	8-15 ± 7 (11.5)
Type of trichoms	Protective	Protective

Leaf rachis epidermis in the male and female trunks of *Cycas revoluta* is covered with trichoms. Trichoms are protective, needle-shaped and multi-cellular and similar in the male and female trunks (Figure 5).

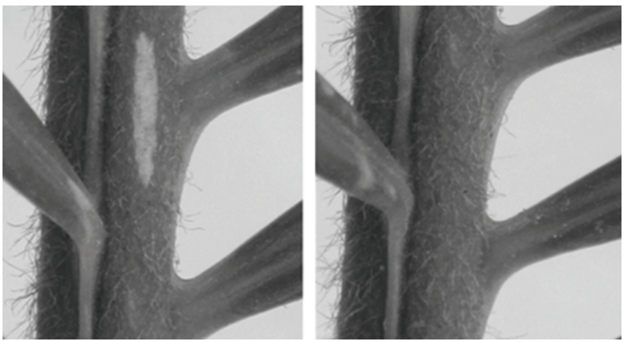

Figure 5. Trichoms on main axils of leaf in female trunk (left), and male trunk (right) of *Cycas revoluta*

The number of hairs in the rachis of the male and female trunks was counted as 8-15 and 10-13/mm^2, respectively. The presence of trichoms in the leaf rachis represents the adjustment of *Cycas* with the present climatic condition (Fan, 1990).

The anatomical study of leaflet in male and female trunks shows similarities and differences (Table 6).

Table 6. Anatomical characters in leaflets of male and female trunks of *Cycas revoluta*

Trunk / Anatomical characters	Female	Male
No. of upper parenchyma layers	8-9 ± 1 (8.5)	8-9 ± 1 (8.5)
No. of lower parenchyma layers	7-8 ± 1 (7.5)	7-8 ± 1 (7.5)
Stoma composition	Projecting	Projecting
Type of stoma	Actinocytic	Actinocytic
No. of upper stomata/mm^2	25-27 ± 2 (26)	24-30 ± 6 (27)
No. of lower stomata/mm^2	29-35 ± 6 (32)	26-37 ± 11 (31.5)
Length of upper stomata (μM)	5.5-8 ± 2.5 (6.75)	5-9.5 ± 4.5 (7.25)
Length of lower stomata (μM)	7-9.5 ± 2.5 (8)	6.5-8.5 ± 2 (7.5)
Type of trichoms	Protective and simple	Protective and simple
No. of trichoms in upper surface/mm^2	3-5 ± 2 (4)	3-8 ± 5 (5.5)
No. of trichoms in lower surface	5-10 ± 5 (7.5)	7-9 ± 2 (8)

Upper parenchyma in the male and female trunks have 7-9 and 8-9 layers, respectively. Parenchyma cells can be seen as a void and polygons. The number of lower parenchyma in the male and female trunks is similar and variable from 7 to 9 layers. Like angiosperms, mesophyll of *Cycas* leaflet includes palisade and spongy parenchyma. Primary xylem in middle veins is a primitive type. Secondary xylem is created from the cambium that is located between the two types of vascular tissue. The middle vein is surrounded by endodermis. Stoma type in the male and female trunks is alike. That is projecting and actinocytic; so that, with a cycle of subsidiary cell has been isolated from the epidermal cells. Subsidiary cells surround stoma by circular (Figure 6 and 7). *Cycas* leaflet is leathery and hard and epidermal cells have thickness wall and cuticle, and stomata in the lower leaf surface is depth (Ladder, 1995).

Upper stomata density of leaflet in female and male trunks ranges between 25 and 28, also 24 and 30/mm^2, respectively. Lower stomata density of leaflet in female and male trunks ranges between 29 and 35, as well 26 and 37/mm^2, respectively (Figure 6).

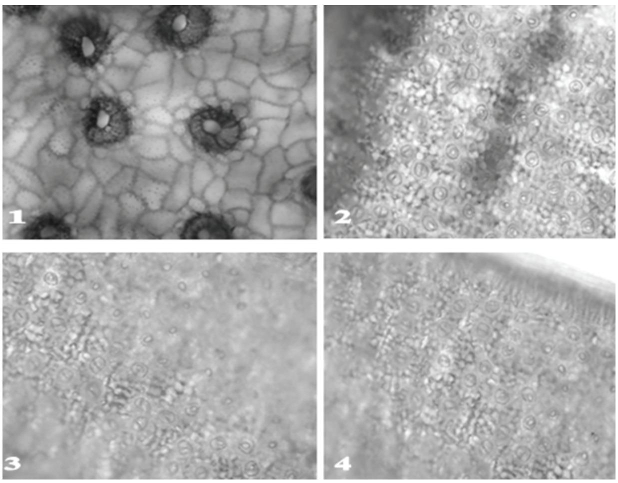

Figure 6. Stomata in epidermis of leaflets of *Cycas revoluta*, 1. Lower epidermis of female trunk, 2. Lower epidermis of male trunk, 3. Upper epidermis of female trunk, and 4. Upper epidermis of male trunk

Upper stomata length in female and male trunks is variable from 5.5 to 8.0 and 5.0 to 9.5 µM. Also, lower stomata length in female and male trunks is variable from 7.0 to 9.5 and 6.5 to 8.5 µM. Stomata density on each plant species was almost constant and its change is limited. Hair density in the lower surface of leaflets is higher than the upper surface; so that in the male and female trunks ranges between 5 and 10, also 7 and 9/mm^2 (Figure 7).

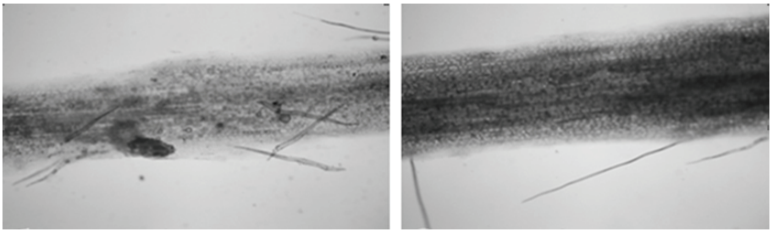

Figure 7. Trichoms on epidermis surface of female leaflet (left) and male leaflet (right) of *Cycas revoluta*

The study on anatomical characteristics of the root showed similarities and differences. Root parenchyma layers at male and female trunks are equal and including 8-9 layers. In transverse section of root, the number of cambium layers was 2-3 and in male and female is equal. The stele diameter in the female and male trunks ranges from 25 to 30 and 25 to 28 µM, respectively.

Table 7. Anatomical features of male and female roots in *Cycas revoluta*

Trunk	No. of cortex parenchyma layers	No. of cambium layers	Stele diameter (µM)	Pericycle diameter (µM)	No. of vascular bundles
Female	8-9 ± 1 (8.5)	2-3 ± 1 (1.5)	25-30 ± 5 (27.5)	0.50-0.55 ± 0.05 (0.525)	1
Male	8-9 ± 1 (8.5)	2-3 ± 1 (1.5)	25-28 ± 3 (26.5)	0.45-0.58 ± 0.13 (0.515)	1

Pericycle layer thickness in female and male trunks ranges from 0.50 to 0.55 and 0.45 to 0.58 µM, respectively. Current study revealed that the vascular bundles in male and female trunks are equal. The studies on cycads root showed that there are four types of root as follows; primary root, secondary or lateral root, swollen roots and coralloid root (Stevenson, 1990). The results of the present study are consistent with these results.

CONCLUSIONS

Sex determination of cycad (*Cycas revoluta* Thunb.) before maturity reports here.

Anatomical and morphological studies showed that there are some differences between male and female trunks.

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THE EFFECT OF IRON NANO-CHELATE AND CYCOCEL ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS, PROLIFERATION AND ENHANCING THE QUALITY OF *EUPHORBIA PULCHERRIMA* WILLD

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Abstract

The successful application of various Nano-platforms in medicine under in vitro conditions has generated some interest in agro-nanotechnology. This technology holds the promise of controlled release of agrochemicals and site targeted delivery to improve efficient nutrient utilization and enhanced plant growth. Nano-encapsulation shows the benefit of more efficient use and safer handling of pesticides with less exposure to the environment. Thus, nano-fertilizers can be substituted for conventional fertilizers. Growth reduction in some ornamental plants enhance their overall quality. Cycocel is one of the most important growth retardants which inhibits gibberellin. These experiments were carried out based on a randomized complete block design in three replications to evaluate the effect of various levels of nano iron chelated fertilizer and cycocel on growth parameters of Euphorbia pulcherrima. Treatments were Fe chelated fertilizer (0, 0.9, 1.8, 3.6 and 4.5 g l^{-1}) and Cycocel (0, 500, 1000, 1500 and 3000 mg l^{-1}). Plant height, internode length, shoot number, node number, root length, root number, the length of longest root, the length of shortest root, root volume, fresh and dry weight of root and shoot, leaf number, leaf surface, leaf chlorophyll content, measurement of iron in leaf and the number and permanent of bracts were evaluated. The least plant height and the most shoot, leaf number, root length, root volume, the number and permanent of color bracts were obtained in treatments of 1.8 g l^{-1} iron nano-fertilizer without or with the concentration of 1000 mg l^{-1} cycocel. The most leaf surface, which is a good trait, was calculated in plants treated with 1.8 g l^{-1} iron nano-fertilizer without Cycocel. In some traits such as root volume and chlorophyll content, the minimum amount was seen in the maximum of iron nano-fertilizer and cycocel concentrations. Suitable root characters were severely reduced under the effect of 3000 mg l^{-1} cycocel. Overall, the most suitable treatment, especially for reduction of plant height and enhancing some vegetative traits (such as leaf number) and flowering (such as permanent of bract) is introduced in treatment of 1.8 g l^{-1} iron nano-fertilizer along with 1000 mg l^{-1} Cycocel.

Key words: *Plant growth regulators, Flowering, Soilless culture, Fertilization*

INTRODUCTION

Poinsettia plant of the euphorbia family (*Euphorbiaceae*) as potted and cut flower has great importance in ornamental plant industry. *Euphorbia* family plants have specific primary inflorescence called cyathium. Each cyathium inflorescence usually is surrounded by involucres consists of five continuous leaf origin bracts and the structure of flower is simple (Ghahraman, 1993).

One of the factors affecting the quality of potted and cut flowers is plant nutrition management.

Access to availability, cost and efficiency domestic fertilizer resources using new technology which while more economical to produce, leading to reduce loss of fertilizer and reduce environmental pollution can be very important.

Nanotechnology is opened new opportunities to enhance nutrient use efficiency and minimize the cost of environmental protection (Naderi and Danesh S., 2011). Also with the help of this technology, it is possible to enable the production of higher value-added products and remove environmental toxicity (Garda-Torredey et al., 2002).

The benefits of nano-fertilizers and their superiority over conventional fertilizers can include: increased nutrient uptake with the properties to slow release of the elements; cost reduction; prevent accumulation of high concentrations of soluble salts in the root environment and prevent damage to the plant; reduce the convert of the usable form to unwanted or unusable items in response to usual reaction in the soil; reduce losses due to leaching of nutrients in the root environment and subsequently reduce environmental pollution.

Iron role in the activity of some enzymes such as catalase, peroxidase and cytochrome oxidase has been shown (Blakrishman, 2000). Iron as a cofactor involved in the structure of many antioxidant enzymes and results indicate that in the lack of micro-nutrients elements, antioxidant enzyme activity decreased and therefore the sensitivity of plants to environmental stresses will increase.

The use of chelated iron compounds is the best solution to remove this problem. Iron chelated nano-fertilizer is a unique complex which has 9% of the water-soluble iron.

Some regulators such as cycocel, paclobutrazol, bayleton and daminozide reduce the growth.

Today, a variety of organic and chemical compounds that are artificially made and delay plant growth are used in agriculture (particularly in horticulture and ornamentals industry).

Some ornamental plants, if have less height, are looking more appealing and its transportation is easy.

The effect of plant growth retardants depend on the time and method of application, concentration and species and varieties type, type of target organ and environmental and physiological conditions (James et al., 1999; Latimer et al., 2001).

Plant growth retardants delay cell division and elongation of shoot and restricting the construction of gibberellin reduces internodes length and vegetative growth (Magnitskiy et al., 2006).

Plant retardants inhibit gibberellin biosynthesis. Among these compounds, the most used are mercury chloride and cycocel.

Plant growth retardants, especially cycocel reduced the height of some ornamental plants and increase their quantity and quality characteristics have been used by some researchers (Garner, 2004; Karlovic et al., 2004; Rossini Pinto et al., 2005; Leclerc et al., 2006; Hashemabadi and Zarchini, 2010; Gholampour et al., 2012; Fathi et al., 2012).

Poinsettia plants are including ornamental plants that studies on the effect of nano-fertilizers have been not done on it.

Therefore, the aim of the present study was to increase the quantity and quality of ornamental poinsettia plant using iron nano-chelated and cycocel. If proves appropriate, they can replace with conventional fertilizers and can be reduced contamination of soil, water and environment.

MATERIALS AND METHODS

Cuttings with a height of 15 to 20 cm, each with 3 nodes were prepared from mother plant of poinsettia.

Cuttings due to latex content were placed in water for 24 hours. After this period, cuttings were planted in perlite for rooting. After rooting (60-65 days), cuttings were transferred into substrates.

A number of pots were prepared and cocopeat, municipal compost and soil (1:1:1) were added insides them.

Dimensions of pots were 12 × 12 × 10 cm and medium size was 2 kg. Poinsettia cuttings as the plant samples were grown in pots. Different ratios of iron nano-chelated fertilizers as well as the first experiment factor and different concentrations of cycocel as the second factor were prepared.

EDTA-based iron nano-chelate intake as spray on plants at the beginning of the trial and 30 days later, also using cycocel 30 days after the starting experiment was conducted as spraying.

Levels of experimental factors were: concentrations of 0, 500, 1000, 1500 and 3000 mg/l cycocel and concentrations of 0, 0.9, 1.8, 3.6 and 4.5 g/l of Fe nano-chelated fertilizers (Table 1).

Studies were conducted in a factorial experiment based on randomized complete block design (RCBD) in three replications. Experiment had 75 blocks and three pots were used at each block and in total, 225 pots were used.

Plots were pots containing iron nano-chelated fertilizers and cycocel. Plants were kept in the greenhouse with a temperature of 22-24° C, humidity 85% and were grown in natural light.

Plant height, internode length, shoot number, node number, root length, root number, the length of longest root, the length of shortest root, root volume, fresh and dry weight of root and shoot, leaf number, leaf surface, leaf chlorophyll content, measurement of iron in leaf and the number and permanent of bracts were evaluated.

To measure the length of internodes, in each sample, the length of the lower third of the internodes were calculated.

Shortest and longest root was reported in two ways: among all plants (all observations), shortest and longest root length was measured; the shortest and longest root of three replicates per treatment were measured and divided by 3.

To measure the root volume, at first, the roots were removed from soil and were washed thoroughly under running water.

In a graduated cylinder, poured some water in a particular volume and plant roots to the collar was placed inside the container.

Difference between the measured volumes of water represents the root volume.

In order to measure the dry and fresh weight of plant samples, they quickly were weighed after harvest on a digital scale (fresh weight). Then, the samples were dried in an oven with the temperature of 103°C for 24 h and reweighed (dry weight).

Graph paper was used to measure leaf area. Each leaf was placed on a sheet of paper and it's around was marked with a pen. Area of a square was measured in paper and was multiplied by the number of squares occupied by leaves. The resulting number is the leaf area. Leaf area of each iteration was obtained from the average area of 10 leaves.

For the measurement of chlorophyll a, chlorophyll b and total chlorophyll, 0.5 g leaves was placed in a mortar and after adding 20 ml of acetone 80%, it was absolutely beaten. Green extract was filtered with a funnel and filter paper into 50 ml graduated cylinder. The remaining residue on the funnel was returned to the mortar and 15 ml of acetone 80% were added and beaten. The obtained extract was filtered again. And the remaining residue twice with 5 and 10 ml of acetone 80%, were beaten and filtered, respectively. Finally, acetone 80% extract was brought to a volume of 50 ml. Solutions obtained by spectrophotometer at wavelength (A) 660 and 642.5 nm were read.

Pigments concentration were calculated in ml/g fresh weight.

To measure iron, 0.3 g of dried herb in a temperature of 75°C for 24 h were spilled in 3 ml of mixed acid (100 ml sulfuric acid + 6 g of salicylic acid and 18 ml distilled water) and digestion was carried out on the heater. After bleaching the samples, samples obtained using filter paper was filtered and the volume was brought to 50 ml with distilled water.

Iron content of the samples was obtained using atomic absorption device. Measurements were performed at 120 days after treatment.

Data analysis was carried out using MSTAT-C software and EXCEL software was used to draw graphs.

The mean comparison was carried out using LSD test.

RESULTS AND DISCUSSIONS

The minimum plant height (29.80 cm) and the most shoot (6.00), leaf number (49.23), root length (23.57 cm), root volume (2.23 ml), the number (9.15) and permanent of color bracts (62.33 days) were obtained in treatments of 1.8 g l^{-1} iron nano-fertilizer without or with the concentration of 1000 mg l^{-1} cycocel.

Maximum plant height (49.46 cm) and internode length (4.33 cm) were calculated in plants treated with 4.5 g l^{-1} iron nano-fertilizer without cycocel.

There was no any positive effect between increasing internode length and increasing iron nano-fertilizer concentration.

The minimum root volume (1.26 ml) and chlorophyll content (2.19 mg g^{-1} F.W.) were obtained in plants treated with maximum concentrations of iron nano-fertilizer and cycocel (4.5 g l^{-1} and 3000 mg l^{-1}).

Maximum chlorophyll content (6.61 mg g^{-1} F.W.) was obtained in plants treated with 3.6 g l^{-1} iron nano-fertilizer with the concentration of 500 mg l^{-1} cycocel.

Less shoot number (2.66) was obtained in plants treated with 4.5 g l^{-1} iron nano-fertilizer without cycocel.

Maximum (30.30) and minimum (18.30) node number were seen in plants treated with control and 1.8 g l^{-1} iron nano-fertilizer along with the concentration of 500 mg l^{-1} cycocel.

The largest (9.46) and smallest (6.63) number of root were observed in plants treated with 3.6 g l^{-1} iron nano-fertilizer without cycocel and 0.9 g l^{-1} iron nano-fertilizer along with the concentration of 3000 mg l^{-1} cycocel, respectively.

The biggest leaf surface (19.55 cm^2), which is a good trait, was calculated in plants treated with 1.8 g l^{-1} iron nano-fertilizer without cycocel.

The least leaf surface (9.79 cm^2) was calculated in plants treated with 4.5 g l^{-1} iron nano-fertilizer along with 1500 mg l^{-1} cycocel.

In some traits such as root volume and chlorophyll content, the minimum amount was seen in the maximum of iron nano-fertilizer and cycocel concentrations. S

uitable root characters were severely reduced under the effect of 3000 mg l^{-1} cycocel.

Maximum (112.40 mg/1000 ml) and minimum (41.50 mg/1000 ml) concentration of iron were seen in plants treated with 500 mg l^{-1} cycocel without iron nano-fertilizer and 0.9 g l^{-1} iron nano-fertilizer along with 3000 mg l^{-1} cycocel, respectively.

Overall, the most suitable treatment, especially for reduction of plant height and enhancing some vegetative traits (such as leaf number) and flowering (such as permanent of bract) is introduced in treatment of 1.8 g l^{-1} iron nano-fertilizer along with 1000 mg l^{-1} cycocel.

The results of this study indicated the role of iron nano-chelated fertilizer and cycocel to change some vegetative growth, flowering and physiological indexes of poinsettia plant, so that the change in the indexes of plant height, internode length, number and area of leaves, number and length of roots, dry/fresh weight of foliage shoot and root, number of bracts and iron and chlorophyll contents were significant.

Given the new nanotechnology and the growing trend of studies in the technology, there are not many reports about the effect of the fertilizer on plant growth and development.

Similar results were obtained in several plants (Abbas et al., 2009; Sheykhbaglou et al, 2010).

Peivandi et al. (2011) studies on comparing the effect of iron nano-chelated fertilizer on *Ocimum basilicum* showed that replacing iron fertilizer produced with nanotechnology compared with conventional iron fertilizer at the appropriate concentration or less increase the growing quantity and quality of this species.

These researches showed that differences in the mean of shoots fresh weight, shoot dry weight, dry/fresh weight of leaves and roots in the different treatments was significant.

The highest mean shoot fresh weight (5.84 g), root fresh weight (0.56g), fresh weight of leaf (2.70 g), shoots dry weight (0.71 g), root dry weight (0.13 g), leaf dry weight (0.3 g) and root length (14.41 cm) was observed in the treatment of 1 kg/h of iron nano-chelate.

In the present research, maximum root length obtained at a concentration of 1.8 g l^{-1} that confirms the results obtained by the Peivandi et al. (2011).

There are similar results in relation to the fresh/dry weight of vegetative organs, so that fresh/dry weight of shoots and roots in treatment 4.5 g l^{-1} were maximized in iron nano-chelate.

With increasing concentration of iron nano-chelated fertilizer, the content of chlorophyll a, b and a + b increased compared to control.

The highest chlorophyll content was observed in Fe nano-fertilizer with higher concentrations (5 kg/ha).

The highest amount of leaves chlorophyll in our study was not found in the highest concentration of iron nano-chelate (4.5 g l^{-1}), but was obtained at a concentration of 3.6 g l^{-1}.

Increased iron may be associated with a decrease in chlorophyll content and photosynthetic rate and may lead to reduced growth (Chatterjee et al., 2006).

Cycocel is one of the plant growth regulators in euphorbia (such as poinsettia) and Chrysanthemum (Fathi et al., 2012). Gholampour et al. (2012) study on ornamental cabbage (*Brassica oleracea*) showed that with increasing concentration of cycocel, plant height was reduced. All plants treated with cycocel were shorter than control plants.

These researches showed that 1500 mg l^{-1} cycocel were produced shortest plants (10.79 cm) that was shorter compared to the control plants (15.20 cm).

With increasing cycocel concentration, stem length also declined. Similar results were observed in the present study, so that the maximum height of the stem in the plant was observed and with increased cycocel to 1000 ml l^{-1}, stem length was reduced.

Although the stem height in the plants treated with 1500 and 3000 mg l^{-1} cycocel was a little more than stem height in plants treated with 1000 mg l^{-1} cycocel, was much less the height of the control plants.

Reduced plant height by cycocel application has been observed in many plant species (Oliveira and Browning, 1993; Garner, 2004; Karlovic et al., 2004; Rossini Pinto et al., 2005; Hashemabadi and Zarchini, 2010). Karlovic et al (2004) reported on reduced height in chrysanthemum (*Chrysanthemum* L. cv. Revert) after application of 2000, 3000 and 4000 mg l^{-1} cycocel. Hashemabady and Zarchini (2010) showed that minimum plant height (29.93 cm) obtained in rose using 1500 mg l^{-1} cycocel. Shoot length in control plants was 35.70 cm. The researchers found a significant reduction in the length of stem after application of cycocel.

This study confirms other research studies. The present study shows that cycocel play effective role on changing fresh/dry weight on the ground and underground part, as well as leave iron and chlorophyll. Gholampour et al. (2012) Study on ornamental cabbage (*Brassica oleracea*) showed that the maximum chlorophyll index with 1000 mg l^{-1} cycocel were obtained after 60 days (18.50) and at a concentration of 1500 mg l^{-1} after 90 days (20.14).

Plant growth retardants increase the content of cytokinin which leads to increased levels of leaf chlorophyll (Rossini Pinto et al., 2005). Our study revealed that the highest and lowest chlorophyll in the leaves, was in the plants treated with the lowest (500 mg l^{-1}) and highest (3000 mg l^{-1}) concentrations of cycocel, respectively.

CONCLUSIONS

The most suitable treatment, especially for reduction of plant height and enhancing some vegetative traits (such as leaf number) and flowering (such as permanent of bract) is introduced in treatment of 1.8 g l^{-1} iron nano-fertilizer along with 1000 mg l^{-1} cycocel.

Maximum root length was obtained at the same concentration of 1.8 g l^{-1}.

Cycocel play effective role on changing fresh/dry weight on the ground and underground part, as well as leave iron and chlorophyll.

The highest chlorophyll content was observed in Fe nano-fertilizer with higher concentrations (5 kg/ha). The highest and lowest chlorophyll in the leaves, was in the plants treated with the lowest (500 mg l^{-1}) and highest (3000 mg l^{-1}) concentrations of cycocel, respectively.

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THE GARDENS OF THE CENTRAL SCHOOL IN BUCHAREST - DISPLAY OF NATIONAL HISTORY AND OUTDOOR CULTURAL SPACES

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Abstract

A garden is more than vegetation; it is art, cultural memory, national patrimony and it is the sentimental expression of one's identity, capable to becoming a mnemonic for future generations to discover. The Central School in Bucharest, one charming piece of the national patrimony, was recently restored, but its gardens were ignored. The current state of the gardens alters the image of the entire ensemble and contradicts the original architectural style and composition of the monument. The study focuses on recovering both the image and the spirit of the school's remaining gardens, offering the necessary details for recomposing the images the school was identified throughout its history. To this end, our research was conducted on mineral and vegetal compositions and pattern displays, successive style transformations and historical layouts, text descriptions and also on "in situ" observations. The aim of the present study is thus, to demonstrate that the gardens are part of the Central School's history and identity and that they hold all the necessary attributes to becoming themselves recognized as landscape monuments. This must lead in the end to defying the Central School as a historical ensemble consisting in a mixture of both architectural and landscape creations alike.

Key words: *restoration, garden, monument, patrimony*

INTRODUCTION

The aim of the present study is to emphasize the importance of the Central School's gardens in what concerns the restoration programme of an A class monument in Bucharest – The "Central School for Young Women".
Built in 1890, the school is a historical and architectural ensemble composed by a series of historical classified buildings and of a series of geometrical gardens, components yet unclassified as monuments and as part of the historical ensemble.
Inspired by the Italian Certosa di Pavia monastery, Romanian architect Ion Mincu designed the school as a rectangular building surrounding a "*chiostro*" type of garden, described as "a enchanting interior garden, prisoner in between Byzantine-style corridors with stone arcades; a kind of a green-flowery *chiostro*" (Radulescu-Pogoneanu, 1953). Moreover, the school was integrated into a green layout composed by two more gardens and green corridors. Thus the Central School ensemble is made up by both its buildings and its gardens: the front garden, the interior or central garden and the large garden or the backyard.

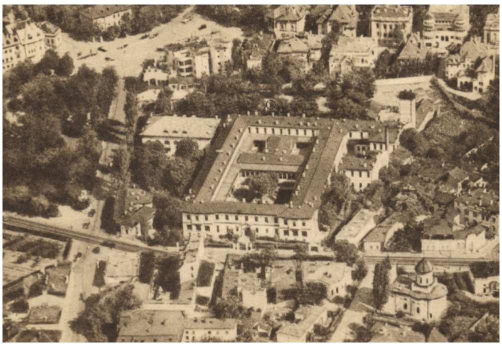

Figure 1. The School ensemble and the neighbourhoods, 1935

Figure 2. Certosa di Pavia Monastery's chiostro
www.tuttocollezioni.it

Figure 3. The Central School's interior garden
Photo: Simion Luana Andreea
archive of a former student – Margareta Dan

MATHERIALS AND METHODS

Our historical research is based on both text and imagistic archives and also on *in situ* research.

The Central School's gardens – short history

Archive documents have shown that the front and the interior gardens were designed along with the school's buildings, while the large garden was designed just after the school bought two nearby tangent opened fields in 1933-1935; "two tennis fields were placed in the back of the new garden, while nets tied between old chestnut trees invited the students to play ball games during their recreations."(Radulescu-Pogoneanu, 1953).

The large garden was the only garden in which the students were allowed to relax and play, and, this is the reason why this garden is the only one to which they were attached to - "I cannot see the blossom of the chestnuts without thinking of the large garden, in which we went up studying and playing throughout the evenings" (Demetreius, 1852-1877 Central School monography).

Due to ageing, improper maintenance and the appearance of new buildings and sports fields within the large garden, it is no longer possible to restore the backyard to its original image.

However, although the front and the central garden faced numerous transformations throughout the years, they still maintain much of their original components and composition, making restoration not only possible but necessary as well.

The central garden

The present image of the garden is the result of numerous transformations that brought significant changes throughout the years. Archive documents reveal that the garden went through four major stages of transformation:

A. The first stage- 1890-1901

The first description of the central garden is represented by a photography taken in 1901 revealing an Italian influenced geometrical garden design, similar to the 15^{th} century *chiostro* in Certosa di Pavia Monastery.

Figure 4. The central garden, 1901
The Central School's archive

Straight alleys, geometrical pruned vegetation, main and secondary axes represent components to be found in both gardens. The main difference between the two is the central element: a large water feature in the Italian *chiostro* and an ash tree in the Central School's garden centre.

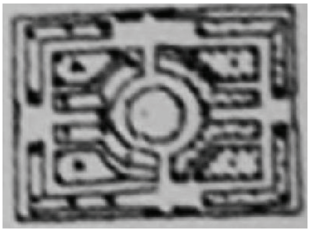

Figure 5.The interior garden of Central School
www.googleimages.com

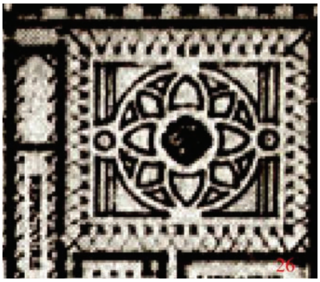

Figure 6. The *chiostro* of Certosa di Pavia Monastery
www.courses.cit.cornell.edu/lanar5240/MedievalImages

B. The second stage - 1901-1935

Best described by all archive documents, this stage brings minor changes in what concerns the garden's style. However, newly added plants represent positive new features brought to the general image of the garden. A main difference between this period and the pre 1901's one is the form and height of the ash tree, a topic later to be discussed.

Figure 7. The central garden, 1935
former student personal archive – Margareta Dan

C. The third stage - 1936-1940

This period is described by public and private initiatives to transform the central garden into a small botanical garden. For this reason, the garden went through significant changes regarding both the alteration of the geometrical layout of the alleys and of the vegetal composition as well.

Figure 8. The central garden, 1940
www.arhitectura-1906.ro

D. The fourth stage - 1940-2013/14

This period is characterised by the lack of written or graphic information. *In situ* research shows that the general layout of the garden hasn't changed much since 1940. However, the vegetal component is seriously altered and completely different from the one of the mid 20th century as vegetation is nowadays composed by ornamental and invasive species of plants alike. The current composition is therefore no longer in accordance with the rigors of the initial geometrical style imposed in 1890-1901 and 1901-1935.

Figure 9. The central garden in 2013
Photo: Simion Luana Andreea

A mention must be made regarding the use of the garden. Thereby, research revealed that throughout its history, the interior garden was opened to students just for the end of the year group photos, and thus, the garden represents a frame for the school's architecture, physically inaccessible to the students.

The front garden

The front garden is represented by a green stripe placed in front of the school's main entrance.
Lack of information ended up in speculating that the front garden was designed in an Italian influenced geometrical manner alike the interior garden. Moreover, significant similarities stand up between the Central School's front garden and Certosa di Pavia Monastery's front garden, emphasizing that the Italian geometrical style was adapted to the central garden as well as to the front garden of the school.

Figure 10. Certosa di Pavia Monastery's front garden www.tuttocollezioni.it

Figure 11. The Cetral School's front garden in 1935 former student personal archive – Margareta Dan

This garden also went through a series of transformations that ended up in the loss and alteration of the initial geometrical style. To this end, major changes took place in what concerns the vegetal composition and the form and height of the garden's plants.
Hardly remembered by former students, the front garden represented only a frame for the main entrance of the school. Margareta Dan, 1949 graduate: "I cannot remember much of this garden. I only recall the tall poplars that reminded me of all the strict rules of the school in that period of time. I also somehow recall seeing pruned hedges and red roses."

RESULTS AND DISCUSSIONS

This chapter aims to analyse the role and the current state of preservation of all the components of the Central School's gardens. To this end, each architectural and vegetal component was carefully measured and analysed.

I. **The central garden**

A. Architectural components

A.1. Alleys

Initially composed by a series of symmetrical, concentric, radial or straight alleys leading up to creating main and secondary perspective axes, the layout of the alleys in the garden was designed in accordance with the principles of the Italian geometrical style.
The alleys are geometrically subordinated, so that the main alley connects the two entrances to the institution, marking the main perspective while the side alleys keep the outline of the building creating perspectives perpendicular on the main axis.
Besides the transit function, they were designed to lead the passers-by to a centre of interest from which multiple possibilities of perceiving the garden were created.
Due to the 1940's interventions to transform the central garden into a botanical garden it is possible that some of the alleys were eliminated in order to make room for more plants.

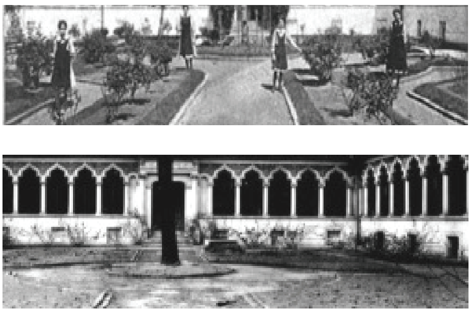

Figure 12. Alley comparison (1935-1940)
Above – 1935
Bellow - 1940
former student personal archive – Margareta Dan

A.2. Pavement

According to the geometrical style principles, the alleys were paved with sand and gravel. However, innovations were made as the contour alleys were paved with Klinker bricks, making it easier to explore the garden.
Nowadays, both the brick and the gravel paved alleys are in an advanced state of deterioration due to ageing, improper maintenance and to recent engineering.

A.3. Bordures

Initially designed with stone bordure alleys doubled by green curbs, during the 1936-1940 period, the green belt was eliminated in favour of doubling the stone bordure.
However, the circular curb, surrounding the ash tree, was dismantled and reconfigured in a more restrained form.

Figure 13. The ash's circular bordures
Photo: Simion Luana Andreea

Historical image comparison and *in situ* research revealed remains of the original belt, showing that the new circle bordure has a smaller diameter compared with the initial one.

A.4. Furniture - benches

First appeared in the 1935's illustrations, the benches were made up by a cast iron frame, partially covered with wooden planks as it can be seen below. The images show the presence of four benches placed around the central ash tree, and two more placed on the left and right side of the garden.
Though the present furniture seems completely different from the original one, detail image comparison and *in situ* research revealed that the cast iron frame is highly similar to the 1935 one and that only the wooden covers differ. Our hypothesis is that only the original wooden planks were dissembled and reassembled afterwards in a completely different new way on the original frame.
Nowadays, all benches are highly deteriorated and in need of urgent repair.

Figure 14. Composition and benches in the 1930's
former student personal archive – Margareta Dan

Figure 15. Present image of the central area
Photo: Simion Luana Andreea

A.5. Sculptures

A series of sculptures, especially busts are now present in the garden while no related information about them can be found in any of the studied documents. They are randomly placed around the garden, partially covered by invasive and spontaneous vegetation.

A.6. Corridor

Though part of the building's architecture, the arcade corridor is also a part of the garden's components. Similar to the Certosa di Pavia Monastery accolade arcade corridor, the school's corridor is in fact representative for the Romanian National architectural style.
The corridor is the only architectural component of the garden that has yet been restored. The image of the restored corridor is not a topic to be discussed in this study.

Figure 16. Certosa di Pavia arcade corridor
www.tuttocollezioni.it

Figure 17. The Central School's arcade corridor Photo: Simion Luana Andreea

B. Vegetation

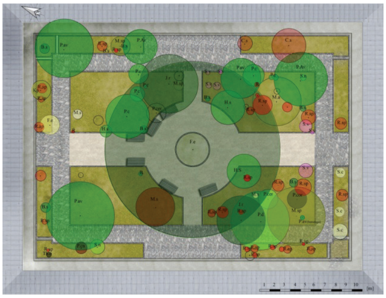

Figure 18. The interior garden
Vegetation surveying

B.1. The ash tree

The central ash tree represents the only vegetal component of the garden that survived all the dramatic changes that the garden has suffered.
According to 'legends' and to the old local customs, it seems that the ash tree was planted during the school's opening ceremony.
However, the fact that the 1901's tree seems to be a pendulum cultivar, different from the present one it is possible for it to be either replaced with a basic ash tree in between 1901-1935, or for the rootstock to suppress the graft and so that the ash continued its growth according to the basic specie characteristics.

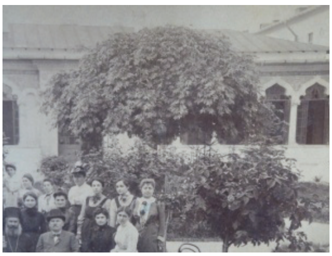

Figure 19. The ash tree in 1901-left
Central School's archive

Figure 20. The ash tree in 1935-right
former student personal archive – Margareta Dan

Nowadays, the health status of the ash is precarious due to lack of proper maintenance and to a recently suffered trauma caused by a defoliator or an external agent as it can be seen in the sprout's growth. This may consist in the recent engineering, application of snow clearing toxic substances etc.
The initial impact the ash had had in the general image of the garden is seriously diminished by randomly planted vegetation. The newly added plants compete with the ash both in term of size and of habitus.
The ash tree is not only the main component of the garden, but also represent a symbol and mnemonic to all the former students.

B.2. The ornamental cherry trees

Four ornamental cherry trees, from which only two are left nowadays, were planted in the corners of the garden recently after 1940, probably as to enrich the vegetal composition of the new botanical garden.
Alike the ash tree, the cherry trees represent symbols and mnemonics for the students, as some text documents reveal: "the thick shadow of the Japanese cherry trees, snowing spring after spring in the interior garden of the school, creating piles and piles of pink satin petals" (www.confluente.ro).

B.3. The roses

Roses also represented symbols of the garden as they are most remembered for "blossoming before the end of the school year final exams." (Demetreius, 1852-1877 Central School monography)
Initially placed along the alleys, they are nowadays placed singular or in groups randomly around the garden.

B.4. Other plants

In the 1935 illustrations, few coniferous species were placed besides one of the garden's entrance. In the 1940's plans and images, they are no longer present.

Another vegetal component that is no longer present in the garden is the green bordure which was probably made up by *Pennisetum* or *Mischantus* species, as we were able to figure out from the archive images and text descriptions. However, the 1901's green bordure seemed to be doubled by different kinds of flowers such as *Hemerocallis fulva lilies*, which are now spread around the garden and form compact carpets along with invasive species such as *Convallaria majalis*, *Hedera helix*, *Lonicera japonica* and *Parthenocisus quinquefolia*.

Over time, new ornamental plants were added, leading to an overcrowded vegetal composition and to a loss of the original image and atmosphere of the garden.

The new vegetal composition of the garden consists both in the presence of the ash, cherry trees, roses and of the ornamental or invasive species such as *Lonicera japonica, Parthnocissus quinquefolia, Convallaria majalis, Hemerocalis fulva, Hedera helix, Hibiscus syriacus, Syringa vulgaris, Magnolia soulangiana, Morus alba, Malus domestica, Prunus cerasifera, Prunus domestica, Prunus avium, Prunus cerasus, Juglans regia* etc. alike.

Thus, the compositional confusion took over the rigorous design of the 19th and 20th century composition.

I. The front garden

A. Architectural components

A.1. Fences

Made of wrought iron bars on concrete foundations, the fence represents both a physical limit and an aesthetic and artistic component of the front garden. The wrought iron fence is the only architectural component of the garden which is nowadays in a good state of preservation.

The fence's design is similar to that of the Italian Certosa di Pavia Monastery, alike other features.

Besides the wrought iron fence, a new one, improper wire mesh fence surrounds the green areas of the garden.

A.2. Pavement

The garden's pavement is made up of concrete slabs with small aggregates, with shaded beiges colours. The pavement is in a good condition of preservation, and from the artistic and historical point of view, it largely resembles the stabilized gravel pavement that seems to have been used in the past. The pavement currently supports a practical purpose, but does not neglect the overall landscape design.

B. Vegetation

According to the historical illustrations, the main vegetal components of the front gardens are represented by poplars (*Populus nigra 'Italica'*). In a undated pre 1935 illustration the front garden contains an alignment of poplars and only a group of two poplars placed in front of the main entrance in both a 1935 illustration and in a 1989 photography.

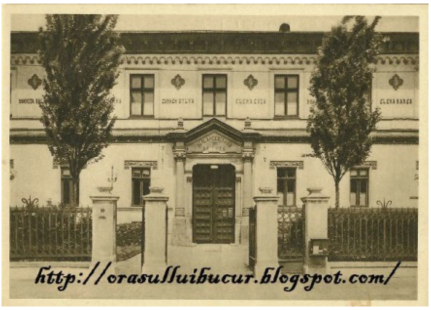

Figure 21. The main entrance and the poplars
www.orasulluibucur.ro

The two front poplars have disappeared and another two, planted at each end of the garden took their place and nowadays stand in between mulberry or fig trees.

The green *buxus* hedges can be seen in both the 1935 illustration and the 1989 photography but, due to lack of pruning and proper maintenance the plants overgrew and now stand as tall stems, partially leafless shrubs, being unsightly and uneven.

Other hedges made up by *Hibiscus syriacus* and *Spiraea x van Houttei* plants present in the garden along the wrought iron fence seem to be parts of the vegetal composition of the garden since 1935, as archive images and plans suggest. Also a series of roses planted in between hedges also seem to be part of the original layout of the garden, as documentation and questionnaires reveals.

Besides the species mentioned above, other species of plants are present in the garden and are altering its original image (*Ficus caria, Ulmus carpinifolia, Morus alba*, different fruit trees etc.).

CONCLUSIONS

The research revealed the way the Central School's gardens were first designed and transformed in time. As recently mentioned, both gardens went through a series of four major stages of transformation. Due to the fact that the 1901-1935 stage is both best described by historical documentation and it is the stage in which both gardens represent a homogeneous composition, designed in accordance to the principles of the Italian geometrical garden, we considered this period of time determinant in what concerns a rehabilitation and restoration of the frontal and central gardens alike.
The restoration proposal is based on the 1935's design and composition of the gardens, but, according to Article 16, in the Florence Charta "restoration work must respect the successive stages of evolution of the garden concerned" (Florence Charta, 1982) the project also integrates mineral and/or vegetal components, symbolic and relevant for each of the four described stages.

The interior garden restoration proposal

Based on the 1935's images and plans, the garden is proposed to be rehabilitated in accordance to the rigours of its original geometrical style, redesigned as a coherent and homogeneous ensemble, and as a sum of its past and present most important features.
Firstly, the original design of the alleys is to be remade, thus creating a series of both curve and straight axes concentrated around the central ash tree. The contour alley's pavement is to be reconditioned, while the central one's are to be repaved with stabilized gravel. A single, brick-type bordure is to cover de margins of the alleys, while in the case of the circular bordure surrounding the ash tree; only the recent, smaller bordure is to be preserved.
All of the benches are proposed to be rehabilitated, restored and re-planked with wooden planks according to their original 1901-1935 design. Four benches are to be placed around the ash tree, while the other two are to be placed in between the secondary entrance's double doors, as shown in the plan below.

Figure 22. Restoration plan

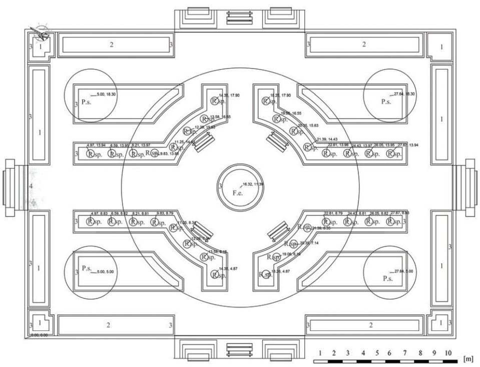

Figure 23. Restoration plan -technical details

Figure 24. Restoration proposal section

Figure 25. Restoration proposal overall image

Concerning the vegetation, the proposal is based on a series of decision making factors such as each stage of the landscape design, collective memory, plant's age and planting norms etc.

The restoration project proposes the use of a handful of plants, iconic for all historical layouts of the garden. Thus, the central ash tree is proposed to be properly pruned and carefully maintained, while four young Japanese cherry trees (*Prunus serrulata*) are to be placed in all the four corners of the garden. The central alleys are to be double-bordered by red, pink and orange roses (*Rosa polyantha hybrida*) and by flower bands (*Zinnia sp., Aster sp., Chrisanthemum sp., Rudbekia sp., Festuca sp., Mischantum sp., Pennisetum sp.*) as shown in the plan above. Finally, a *Lonicera japonica* arcade is to be placed at the entrance to the amphitheatre.

The front garden restoration proposal

Alike the central garden, the front garden restoration proposal project is based on the 1935' images and plans, and integrates components from all its historical layouts.

Planimetric and volumetric restructuration of the garden will create a buffer space between the street and the building. Plant composition will comply with the rigors of the geometrical style and it will be characterized by a series of hedgerows and cover plate with red roses, according to many of the brief historical descriptions.

The mineral components are generally well preserved or restored while significant changes are to be made concerning the vegetal components.

Thus, two oak trees - *Quercus petraea 'columna'* - replacing the original poplars, are to be planted symmetrical to the main entrance. Columnar oaks were preferred to poplars because they have relatively the same height and habitus but they are stronger and outlive poplar trees.

Two hedgerows made up by *Spiraea x van Houttei* one side and pruned Buxus sempervirens on the other will be flanking a band of red *Rosa Polyantha hybrida roses.*

Figure 26. The front garden –restoration plan

Figure 27. Restoration proposal overall image – left
Figure 27. Restoration proposal section - right

In conclusion, though the buildings of the Central School themselves were restored, the Central School ensemble is not yet entirely restored. To this end, its gardens represent a vital component in absence of which the overall image of the historical ensemble is yet to be rehabilitated - "whether or not it is associated with a building in which case it is an inseparable complement, the historic garden cannot be isolated from its own particular environment, whether urban or rural, artificial or natural." (Art. 7, Florence Charta, 1982).

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USING THE TECHNIQUE OF VEGETAL ENDOTERAPY AGAINST THE HORSE CHESTNUT'S LEAF MINER (LEPIDOPTERA: CAMERARIA OHRIDELLA DESCHKA & DIMIE)

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Abstract

Described for first in 1986 near Ohrid lake from Macedonia, by Deschka & Dimie, the horse chestnuts leaf miner (Cameraria ohridella) a highly invasive moth, in just two decades has spread all over the continent, and caused considerable damages in horse chestnuts populations across Europe. In Romania has been described in1993. In our climatic conditions develops 3-4 generations per year. Adult moths are transported by wind, anthropogenic transport by vehicles in infested leaf fragments or infested nursery stocks. The most important damage is esthetical, because of fallen foliage but also ecological do to the effect of the reduced active surface of photosynthesis, and progressive decline of trees. Also causes economic losses due to the costs of protect or heal the affected trees. As a consequence different techniques have been adopted in the recent years to control the moth like collection and destruction of fallen leaves where the pupae over-winter or spray of systemic insecticides. They native natural enemies are insufficiently adapted and not able to regulate the leaf miner's population density, even after two decades of moth infestation typical parasitism rates are merely around 0–10%.The chemical control is difficult especially through the application of insecticides; the aerial spraying to treat trees in urban areas requires specialized expensive equipment. Moreover the systemic insecticides may cause side effects on non-target species. The endoterapy technique by introducing pesticides directly inside the trees can be the best solution. Microinjection is a type of trunk injection where small amounts of therapeutic chemicals are introduced directly into the trees body without any contact with the environment. During our experiment a total number of 84 chestnut threes where identified from which 38 were treated with two different active chemical agents: abamectin and imidacloprid. Treatment applications were made in May 2011. A plot of 46 threes where used as control without treatment. The analysis of the collected results shows, that the treated trees of up to 90% are protected with endoterapy against horse chestnuts leaf miner (Cameraria ohridella).

Key words: *vegetal endoterapy, pest management, invasive species, horse chestnuts leaf miner*

INTRODUCTION

Belonging to the Gracillariidae family, the horse chestnut leaf miner *Cameraria ohridella* was observed and described for the first in 1984, Macedonia, near lake Ohrid (Deschka and Dimic´, 1986). In 1986 it was observed only in a few places in the southern Balkans, but in a few years later it spread throughout Western Europe causing major damage to the horse chestnut tree populations (Pschorn-Walcher, 1997).

In 2000 appeared in Central Europe, Austria and France, in 2003 was reported from Spain and Denmark while in 2004 from England, Ukraine, Sweden, and Lithuania, and was expected its spread to the border of the east coast of the Scandinavian countries, where the climatic factors provide favorable conditions for the development of at least one generation per year (Augustin et al., 2004). Natural spreading speed of the species is high , at least 11.8 km per year , but can reach even 37.9 kilometers per year (Augustin and Denux, 2009). In just two decades it was present all around Europe, approaching the necessary living conditions in northern limit. Knowledge of the mechanism of spreading is very important considering the high ability of infestation (Baraniak et al., 2005).

Adult moths are transported by wind, anthropogenic transport by vehicles. Transport of infested leaf fragments or infested nursery stocks can occur frequently.

Adult females attract males with sexual pheromones. In 2002 a Czech team identified

the composition of the leaf miner pheromone as (8E, 10Z) -8,10 - tetradecadienal (8E10Z - 14Al), and first produced a synthetic sample (Franke et al., 2002).
The electron microscopic histological studies identified and described the female moth pheromone glands internal and external morphology. The gland is a modified epidermal cell line, on the membrane surface between the eighth and ninth abdominal sections, without pores. In retracted condition disappears into the body cavity between the two sections.
With the elongation of the abdominal part reveals and facilitate the evaporation of the pheromone molecules (Raspotnig et al., 2003).
Considering the number of generations per year it was observed that under Mediterranean climatic conditions develops more generations than in colder climates. Swarming can be observed almost the entire summer with first peaks in May.
The second generation appears in July; with significantly increase of the number of individuals. The third generation occurs in August-September (Czencz and Burges, 1996).
The horse chestnuts leaf miner is a daily active, morning and early afternoon flying moth, most active from 20 to 24 ° C (Syeryebryennikov, 2008). A female lays 20-80 eggs hatching depending on season and the climate after 4 to 21 days.
The emerging little larva is a flat, white legless larva, which penetrate inside of the leaf blade. The whole developmental period is 20 - 40 days depending on the weather conditions, during this time the larvae lives inside the mine, feed with the leaf blade palisade parenchyma tissue (Sefrova, 2001). The fourth larval stage, cylindrical -bodied larvae pupate in the original mine, that expands along the main veins, up to 4-7 cm under the development.
The final, third, or fourth generation pupa overwinters in fallen leaves.
The population densities annually changes and the annual mortality of larvae beside of temperature is also affected by the intraspecific competition of the last generation (Girardoz et al., 2007a), (Girardoz et al., 2007b).
The horse chestnuts leaf miner damage first visible effect is aesthetic. The park trees ornamental value is seriously reduced because of countless mines. The trees lose a significant part of leaves reducing the efficiency of internal water balance.
Beside of reduction of the decorative value, the trees are not able to perform its function with the reduced foliage, trees growth slows down the weight and number of fruits decreases gradually. The photosynthetic activity decrease, and is negatively affected their air cleaning ability (Binimelis et al., 2007). The weakened trees die after a few years.
The continuous infection decrease up to 30% of tree life (Salleo et al., 2003).To avoid this, each control option must be used in order to preserve and ensure the survival of the trees, especially in urban areas (Straw and Tilbury, 2006).
Economic impact is also not negligible, the damage reduction requires immediate attention, but the treatment of infected trees is difficult, time - consuming and expensive.
The defense is complicated due the large damaged surface, the great number of mines, where other agents can penetrate easily, so, the leafs becomes over infected with guignardia leaf spot (*Guignardia aesculi*) (Gherghel and Batin, 2008).
Complete removal of leaf litter, in which pupae hibernate, is one effective measure to reduce the damage.
The mechanical control by collecting and destruction of litter, in the autumn, must be supplemented by other measures during all growing seasons.
One of these methods is chemical control, especially in forest stands, where the mechanical method is inefficient, or difficult to be applied (Fora, 2011).
Aerial spraying with systemic insecticide is efficient, but is difficult in urban areas, beside may cause side effects on non-target species.
Over 60 generalist parasitoids have been recorded, however, for biological control a highly specialist parasitoid still needs to be found (Grabenweger et al., 2005a).
A number of natural predators of the larval stages of *C. ohridella* have been recorded. Observations have shown that blue tits *(Parus caeruleus),* great tits *(Parus major)* and marsh tits *(Parus palustris)* feed on the larvae, but they can reduce the number of the larva between 2 and 4% (Grabenweger et al., 2005b).
The endoterapy technique by introducing

pesticides directly inside the trees can be the best solution.
Microinjection is a type of trunk injection where small amounts of therapeutic chemicals are introduced directly into the trees body without any contact with the environment.

MATERIALS AND METHODS

During our experiment a total number of 84 chestnut threes where identified from which 38 were treated with two different active chemical agents: abamectin and imidacloprid.
Treatment applications were made in May 2011. A plot of 46 threes where used as control without treatment.
The six groups of trees were selected from different places in Târgu Mureş town, four groups in University of Medicine park each spaced about 300 meters, and other two groups, in the city public area on a 2000 m distance. Some over 100 years old, their trunk circumference in many cases was up to 238 cm. According to their height and trunk girth, during the treatment we bored 4-5 cavities in each trees trunk.
The 3-5 cm deep holes were prepared, taking into account the vascular bundles progress. In the prepared cavity was introduced the applicator, and then injected the chemical product into the trunk of tree (Figure 1). After injecting 10 ml of active ingredients in each hole, with the removal of the injector, the applicator closes hermetically, preventing the escape of pesticides in the environment.

Figure 1. The hole on horst-chestnut trunk.

Two different insecticides were used, Vertimec 1.8 EC and KOHINOOR200SL. The EC Vertimec 1.8 is an acropetal effect systemic insecticide, acaricide, nematocide, digestive poison, the active ingredient is abamectin ($C_{48}H_{72}O_{14}$, avermectin B1, major component , and $C_{47}H_{70}O_{14}$, avermectin B1b, minor component), is a colourless or pale yellow water soluble and organic solvents-soluble crystal, sensitive to strong acids and bases, and by exposure to UV radiation can be easily decomposed.
The KOHINOOR 200 SL, acropetal effect systemic insecticide, digestive poison, the active ingredient is imidacloprid ($C_9H_{10}ClN_5O_2$), a colourless, mild, characteristic odour, water-soluble crystal. Treatment applications were made in May 2011.
Data Analyses
Altogether a number of 5376 pictures were made to screen the density of the mines on leaves in both treated and untreated trees. All photography where taken weekly from 4 sides of each tree in the next 4 months after the treatment.
The number of mines was counted from pictures by selecting randomly five leaves by picture.
When the mine numbers increased and overlapped the surface covered by mines were estimated by using codifications Codes 0, for 0 – 10%, 1, for 11 –20%, 2 for 21 – 40%, 3 for 41 – 60%, 4 for 61 – 80%, and 5, for 81 – 100% damages. All counted data were registered in counter pages.
Data where analyzed with the Kruskall-Wallis test from the PAST statistical suite, supplemented with the Mann-Whitney test.

RESULTS AND DISCUSSION

According to endoterapy treatments all trees became highly protected against horse chestnut's leaf miner and very low or no damages have been observed on trees.
In contrast on control trees the first and second generations' damage was high and were observed all over the year.
The statistical data analysis shows that a significant difference can be detected between the treated and control trees.
The effect of the only one treatment in May was significant and conferred whole protection during the vegetation season even for plants in close proximity to control, untreated plants (medical University park) (Figure 2a, b, c, d).

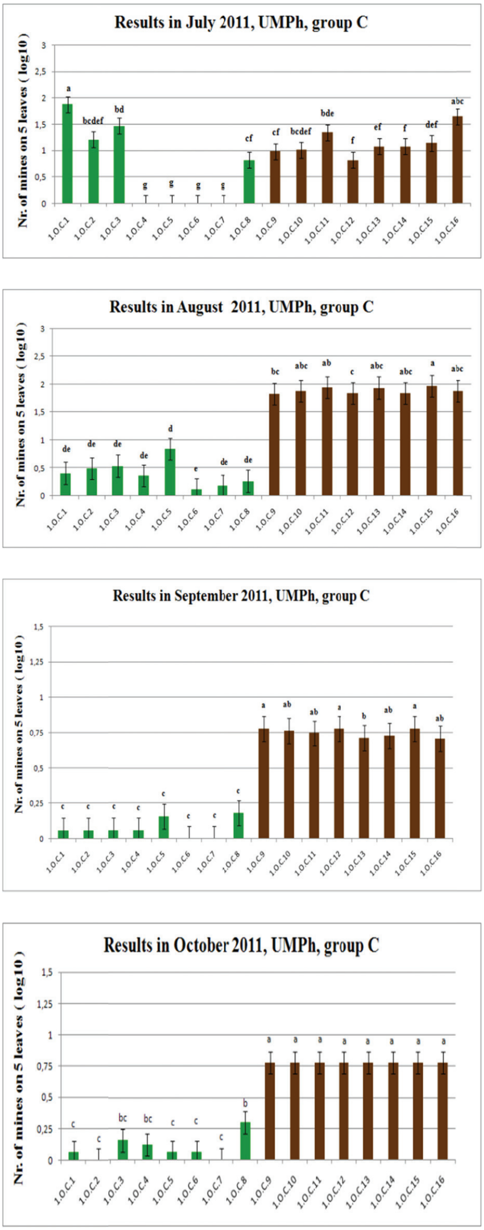

Figure 2. The effect of endoterapy by counting the number of mines on treated (green bars) and control (brown bars) trees located together in park. Different letters represents statistical significant differences (Mann-Whitney test).

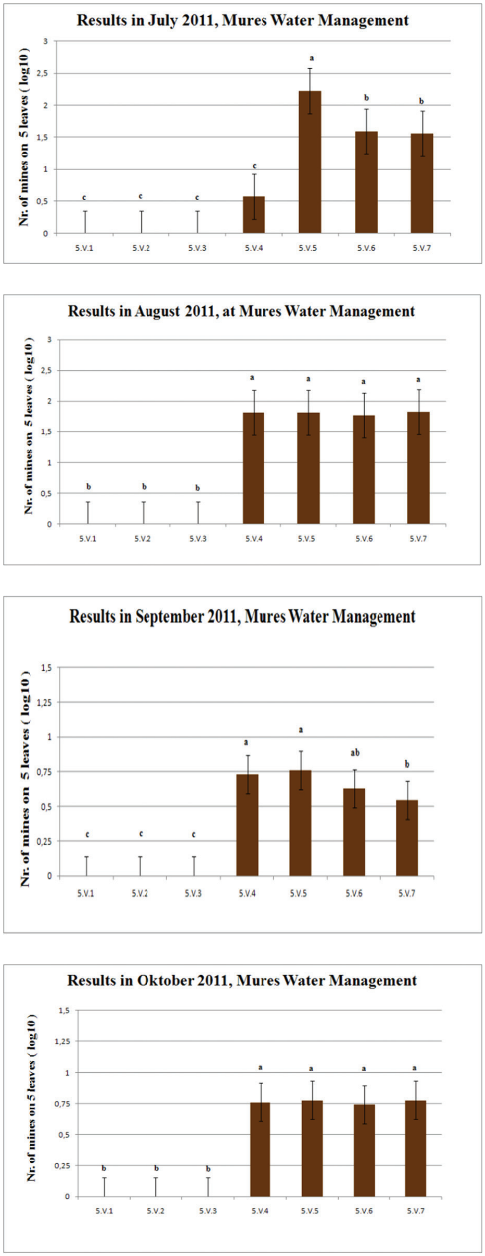

Figure 3. The effect of endoterapy by counting the number of mines on treated (green bars) and control (brown bars) solitary trees. Different letters represents statistical significant differences (Mann-Whitney test).

The endoterapy conferred even higher protection for solitary plants all over in the town, with highly significant differences between treated and untreated trees (Figure 3a, b, c, d).

The use of endotherapy method on horse chestnuts trees proved to be effective, the treated trees we reached to keep the pests damage below 10% (Figure 4).

Figure 4. Treated tree in summer.

The control trees in late August and early September have lost their foliage, and blossomed again at the end of September (Figure 5).

Figure 5. Untreated tree in autumn

CONCLUSIONS

From our perspective, this eco-friendly pest control method provides sufficient internal defense, and is absolute safe for other living beings and environment. The treatment does not depend on weather, can be carried out under any circumstances. This method can be used to protect any high tree that can't be protected with conventional spraying.

The results proved to be satisfactory with both of the used active agents. The effects of plant protection with insecticide containing the active ingredient abamectin (1.8 Vertimec EC) on the basis of subsequent observations is longer, stretches of the year following treatment. The therapy containing imidacloprid like active ingredient (KOHINOOR 200 SL) was less durable in the next year. Evaluating data of the experiment can be said that the suppression of a dangerous and highly invasive pest with using this technology, this new eco-friendly approach, we have achieved remarkable results. The vegetal endotherapy can be used not only to reduce the horse chestnuts leaf miner damage, can be successfully used for any ornamental or farm trees against pests, fungal diseases, or treat nutrient deficiency.

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MILITANT EPHEMERAL IN MOGOSOAIA ENSEMBLE

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Abstract

Landscape is ever-changing. The malleability of the landscape is closely related to the concept of transience. Mogosoaia assembly seems to evade the passage of time, lasting for over than three centuries, apparently unchanged. Its inclusion in the list of heritage monuments, ensure a comfortable status for the mapped elements, a guarantee of the preservation of its integrity. But what happens with its context, with the landscape of Mogosoaia? The loggia of the palace was designed to provide an outlook to the gardens and the lake. The terraced gardens offer a gradual transition to the water and landscape. Recent decades have altered radically the landscape and its physiognomy. On the lake's banks occurs a "colonization" with villas. How can be negotiated the vicinity between a residential area and a monument of such importance? There have been studied documents, text and images, from the archives, similar cases and references to Mogosoaia ensemble. The mapping of the lakeshore garden was elaborated. The perspectives from the palace's loggia and from the terraced gardens were analyzed. In order to elaborate a comparative analysis for the opposite bank to the ensemble, pictures from the archives and up-to-date images were confronted. In order to draw attention to this situation which, in fact, is far from being singular and it becomes increasingly worrisome because it affects valuable components of the national and universal heritage, the idea for the development of a project was born. The project addresses both regular visitors and officials with decisional power in the heritage conservation field and aims to attract their attention to this degradation process and to the need for the implementation of a highly severe legislation for the preservation of historical monuments. The project consists of a series of shocking events both in a visual and in a conceptual sense, achieved by means of landscape design. It is a powerful militant attitude achieved through ephemeral interventions in the landscape, attractive for young people especially.

***Key words**: cultural landscape, happening, historical monument*

INTRODUCTION

With justified concern, we are looking at the aggression upon the monuments in our country and not only. The causes are various. In communist Romania, the churches were demolished, lift and moved and 'well' hidden behind some megalomaniac blocks.

In our days, the greatest danger is the housing pressure. New buildings invaded a lot of protected areas while historic monuments are allowed to decay for getting out off the List of historical monuments or to be demolished. This study is related to the violation of the protected area of Brancoveanu-Bibescu ensemble in Mogosoaia, one of the most flagship national, historical and cultural, Romanian ensembles. Mogosoaia ensemble was founded by the rich and scholar Prince Brancoveanu for Stefan, one of his four sons. This ensemble had a very troubled and tumultuous history, with some brilliant periods and with periods of serious decline too. In 1702, the palace was inaugurated. In 1714, after the beheading of the Prince and of his four sons, the palace was devastated searching for 'hidden treasures'. In 1911, after a long period of decay, Mogosoaia Domain became the property of Martha and George Valentin Bibescu and has gained a new life. Princess Bibescu restored the Palace and the gardens over 17 years. In 1925, the palace was inaugurated after the 'restoration' made by the Italian architect Domenico Rupolo.

The great Romanian architect G. M. Cantacuzino is responsible for the interior decoration carried out between 1930 and 1935.

In 1940, legionaries confiscated a lot of Martha's documents, personal letters and manuscripts. In the communist era, the ensemble became museum and guest house for writers and artists. This ensemble, iconic for the Rumanian patrimony and history, is part of the List of the historical monuments in Romania, as an ensemble, including the palace, the church and other buildings, the English park, the Italian gardens and 'Mogosoaia Lake', a fragment of the Colentina River. The most important feature of this palace is the *loggia*, opened onto the landscape of Colentina River. In 1945, due to the high importance of this ensemble, a large protection area was instituted, extended on the other side of the river, on its highest altitude (Royal Decree. 1222, 1945, Minutes of the Commission for Historical Monuments, 1945 and 1952). This was requested because of the importance of the view.
But the presence of the palace attracts like a magnet. In our days, Brancoveanu's residence is reduced to a background image. A 'colonisation' with villas occurs on the lake's banks: pergolas, decks, lounges. All the eyes watch the palace. But what happens on the other side of the bank? How can be negotiated the vicinity between an ordinary and illegal residential area and a monument of such importance?

MATERIALS AND METHODS

Documentary material on the assembly and its context, historic and urban, is huge. It consists of: a lot of archives historical documents, written and imagistic documents, and *in situ* studies. The written documentary is extremely necessary to know the atmosphere of the Domain and of its surroundings. We are fortunate to have the testimonials of one of the most important architects and scholars of the 20th century, the architect G.M. Cantacuzino. The literary quality of his texts is well known and is very helpful for our work.

Figure 1. Martha Bibesu and her daughter Valentina in a boat on "Mogosoaia lake", around 1925

Figure 2. The palace reflected in the "lake", around 1905

Figures 3 and 4. The banks of Colentina River around 1965

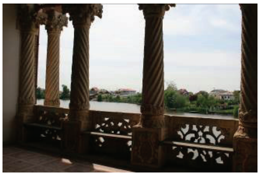
To compare the old images on the one side with the actual ones on the other side and to

emphasize the evolution of the buildings illegally raised in the protected area of the ensemble was the method we used.
The evaluation of the actual situation follows from the analyses of these transformations, arising from the violation of the protected area or *non aedificandi* area (Minutes of the Commission for Historical Monuments, 1945).
This study began in 2000 and continues.

RESULTS AND DISCUSSIONS

The parallels between images of the early 20th century and the beginning of the 21st century reveal that protecting the monument itself is not sufficient. In order to keep intact the charm and historic character, it is imperative to protect the surroundings of the monument too.
First of all, there were analysed the old images of the palace, from the 20th century, in its context, on the left side of the river and images of the opposite side of the river. Images 1 and 2 reveal a quasi natural landscape on the both banks of Colentina River, which is currently named 'Mogosoaia Lake': water, water lilies, reeds, forest. This is a romantic landscape having a little palace as central point of view. This landscape is the reason for creating a specific element for the Romanian architectural stile, Brancovan style: the *loggia*. This feature is not an ordinary Venetian *loggia*, but a very large space opened onto the landscape. It was designed to provide an outlook to the gardens and the 'lake', to the landscape. The *loggia* is an element that mediates the transition between indoor and outdoor space. The location of the *loggia* is not accidental.
G.M. Cantacuzino gives us a suggestive description of the palace and the lake: "As much as the façade facing the entrance is austere and almost closed, so that towards west is smiling and bright. In front of it, Colentina runs its course which has there the exact width of the Grand Canal in Venice. And the façade facing the lake has, at first glance, something festive, like the palaces of Venice. A central loggia with light arcades and a rope molding carved colonnade, with a railing where dolphins play, in which the vegetal vigor of the park retrieves in the capitals, in the decorated grooves and in all this sculptural liveliness in which the verve of the detail never contradicts the line. While the Venetian houses get out of the shining waters without any transition, Mogosoaia Palace stands withdrawn from Colentina's mirror, putting a distance between the world of swans and its pinkish walls. [...] When the sun begins to drop, the royal palace receives full light and seems to emanate its own glow. [...] While the park closes its horizon onto Bucharest, the view stretches and fades slowly in the light, rarely interrupted by a forest or a group of trees. The entire length of the scenery takes part in the general harmony, as everything is in line with the central theme and is organized according to this theme: 'the palace'." (Cantacuzino, 1977).
Narcis Dorin Ion, in turn, describes the same *loggia*, without underlining the link with the landscape: "At the *piano nobile* (*bel étage* or noble level), is the most exquisite architectural element: the splendid *loggia* of the façade to the lake, inspired by Venetian models. […] This *loggia*, with its six stone columns supporting five accolade arcades, is framed by two arbours easily unhooked, added during the renovation between 1860 and 1880 […]. (Ion, 2002, p.26)
Here are Argentoianu's thoughts about the atmosphere in Mogosoaia and especially the *loggia* of the palace, an open space linking architecture and nature: "This terrace, enclosed by a colonnade of carved stone, in the purest Venetian style, plays the role of a salon during the summer and autumn; there, the mistress of the house spends late nights, among flowers and books, lounging on exotic furs and wrapped in silks; there she receives guests who indulge themselves on the wings of dreams, in moonshine evenings, listening charmed the Mermaid's stories, the watchful birdsong, the frogs' anthem that rises from the reeds or the crickets' chink_- as the sign of the days and of the soul [...]" (Argentioanu, 1997)
The wilderness of the landscape surrounding the ensemble was present until the end of 20th century (images 3 and 4).

In our days, from the level of the *loggia*, to be found at 4.00 m. above the highest terrace of the Italian garden, the landscape is overwhelming in magnitude but also by volumetric, stylistic, chromatic and compositional incoherence of the buildings erected on the opposite bank, since the last decade of the last century.
The protection area was instituted for preserving the image of the banks of the river, not to affect the atmosphere of the loggia, the main feature of the palace. It is undeniable that severely alteration of the outdoor landscape, affects the quality of the historical monument (images 5, 6 and 7).
There is not an absurd claim of some dusty old people. It is the respect for our own history, our ancestors and their creations.

Mogosoaia Museum Archive 1965 Photo: Ioana Streza 2010

Photo: Ioana Streza 2010

Figures 5, 6 and 7. The landscape viewed from the *loggia*

The formal Italian gardens with a savant *buxus* design, underlines the axis of the west façade of the palace, that with the generous *loggia*, that place of leisure, delight and magnificence, linking indoor space with the outdoor landscape.
The terraced gardens offer a gradual transition to the water and landscape. The Italian gardens were well preserved and carefully restored but the opposite bank of the river, the protected area of the ensemble, was invaded by illegal constructions.

Source: *The Romanian National Institute of Historical Monuments* Cliché 6429 around 1935

Photo: Oana Baloi 2010

Figures 8 and 9. Images from the *loggia* to South

around 1938
Source: *The Romanian National Institute of Historical Monuments*

Photo: Ioana Streza 2010
Figures 10 and 11. The Italian Gardens in 1938 and 2010

Images from the *loggia* to South (images 8 and 9), and images from the Italian garden to North (10 and 11) are the evidence.

The *loggia* and the Italian gardens were places for delight and enjoyment of the landscape, especially the beautiful sunsets reflected in the river water. From the three terraces of the Italian gardens, we can see a shocking image because we can see the details: lack of coherence, a lot of elements and shapes.

In *non-aedificandi* area, the first buildings were erected in the last decade of the 20th century.


Photo: Sorin Ciorapciu 2002


Photo: Oana Baloi 2010


Photo: Ioana Streza 2010

Figures 12, 13 and 14. End of perspective from the Italian terraces – top and from the *loggia* - bottom

Old documents are important testimony. G.M. Cantacuzino is perhaps the one who understood best the importance and the beauty of such ensembles. "Mogosoaia is nothing but the free expression of dignity made of introspection and serenity. […] Mogosoaia lived its history not without drama, not seamless, but still on the line of refinement. Mogosoaia is not only the appearance of the past, but just as well the expression of a lively present, testimony of a becoming. That is why Mogosoaia has a special place among the historical monuments of Romania." (Cantacuzino, G. M., 1977, p. 411)

About the 'testimony of a becoming' we can say that characteristic of the landscape is its ever-changing. The malleability of the landscape is closely related to the concept of transience. Mogosoaia assembly seems to evade the passage of time, lasting for over than three centuries, apparently unchanged. Its inclusion in the list of heritage monuments, ensure a comfortable status for the mapped elements, a guarantee of the preservation of its integrity. But what happens with its context, with the landscape of Mogosoaia? Recent decades have altered the landscape and its physiognomy radically.

Image 15 is an eloquent testimony of the 'evolution' of the aggression onto the *non-edificandi* or the protection area. The Brancoveanu-Bibescu ensemble in Mogosoaia was ranked historical monument on 19th of April, 1945 by a royal decree. In this decree it is noted that this status was given to the palace and outbuildings together with "the park, terraces, gardens and orchards" (Royal Decree. 1222, 1945).

In a report of the Commission for Historical Monuments drawn on 7th of April, 1945 are listed in detail the buildings ranked as historical monuments and the limits of the protected area including the portion of lake adjacent to the domain and the portion of land located across the lake bank from the royal porch: "the royal palace, the chapel court, vaulted kitchen with its natural context that includes the park, terraces, gardens and orchards, bounded to the east by the outer enclosure wall, to the northeast by the robinia curtain, until the municipal road; Colentina's water and the right bank, on the planted portion, from the existing dam, up to the north boundary of the property, as well as the non aedificandi area in front of the royal porch, west of the waterfront and up to Viei road, included in the general inventory of historical monuments of Romania." (Minutes of the Comission for Historical Monuments 7th of April, 1945).

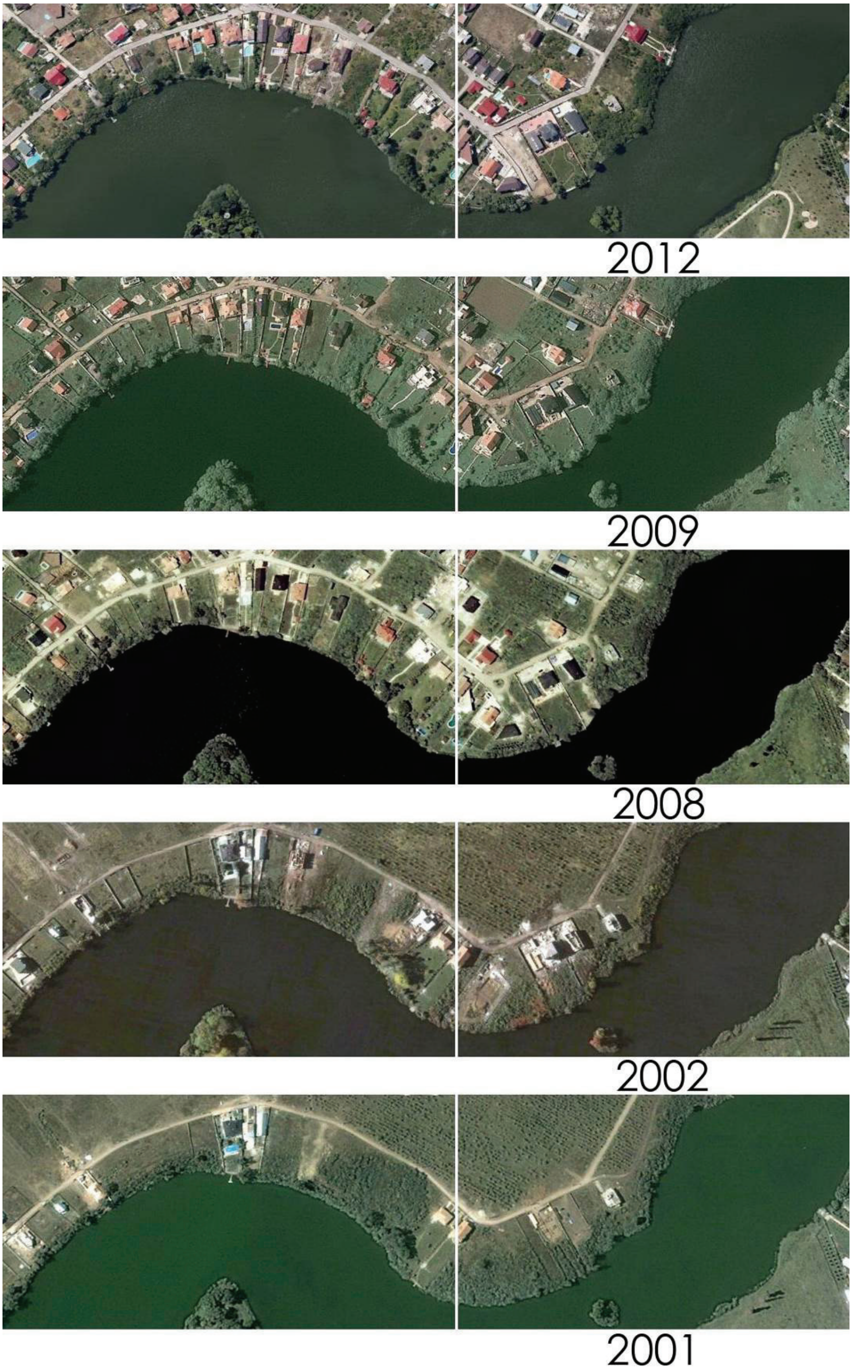


Figure 15. The evolution of the aggression onto the protection area of the Mogosoaia ensemble
Source: Google Earth

Photo: Sorin Ciorapciu 2003

Photo: Tiberiu Adrian Galan 2009

Photo: Iulia Luca 2010

Figures 16, 17 and 18. The evolution of the aggression onto the protection area of the Mogosoaia ensemble

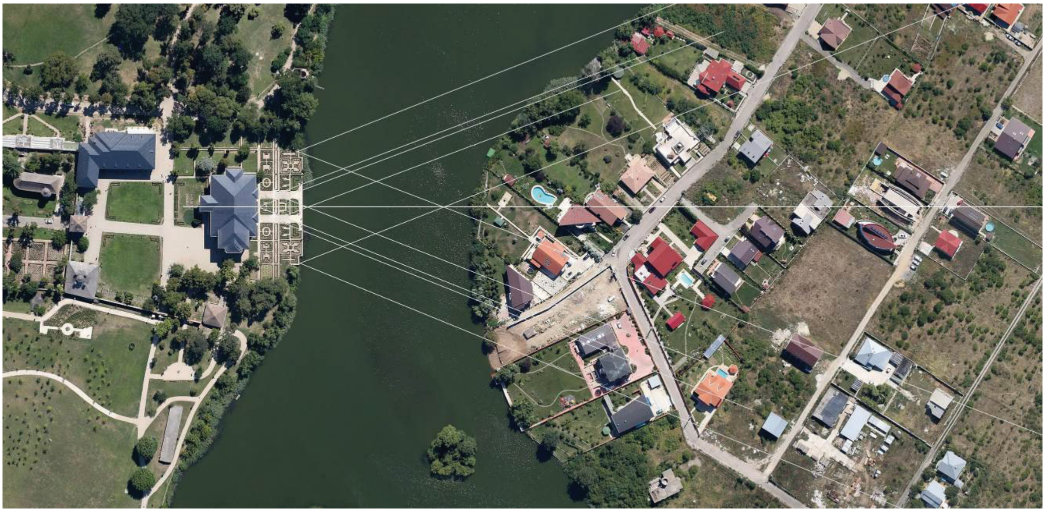


Figure 19. The axis of the loggia and the Italian gardens on the left side of the lake
and the opposite bank of the lake in 2014

A few years later, in 1952, the protection area was reconfirmed. Mogosoaia ensemble, comprising the portion of lake adjacent to the domain, is mentioned as well in a decree from 31 of October, 1952: "Mogosoaia Palace, buildings, […] together with the park, the lake and the other annexes of the Palace, has passed from the usage of the Ministry of Public Education and the Ministry of the People's Council of Bucharest and the Armed Forces, to the usage of the Committee for the Arts, the Writers' Union, the Artists' Union.

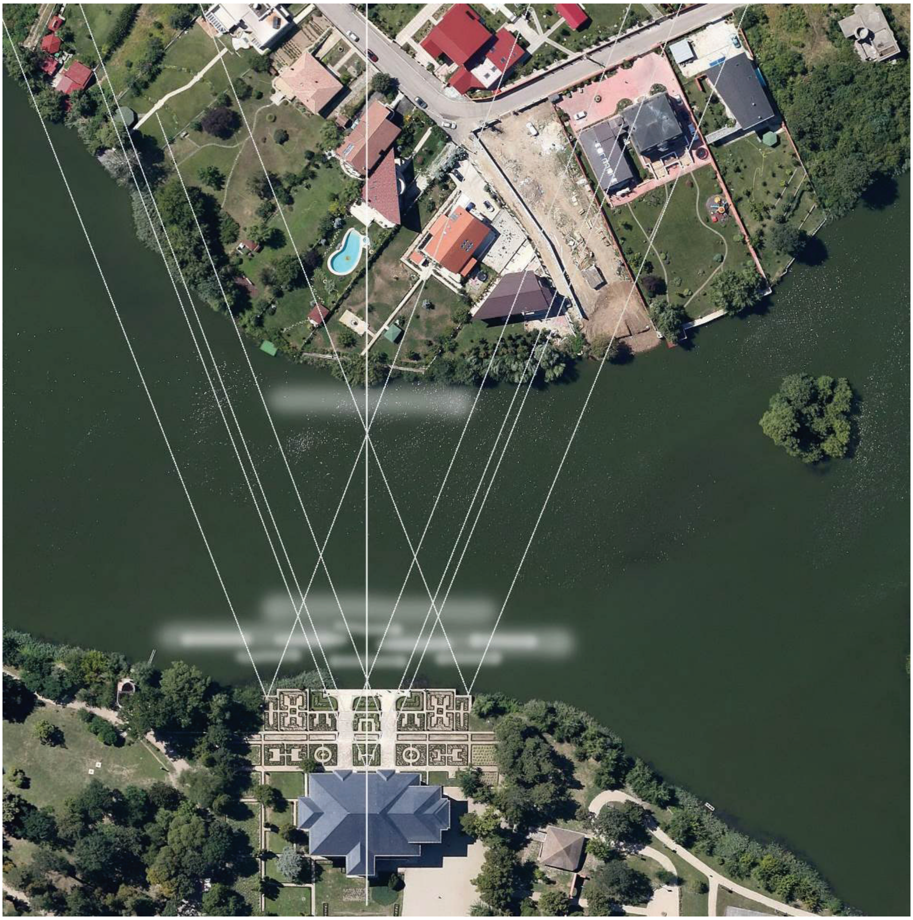


Figure 20. The proposal

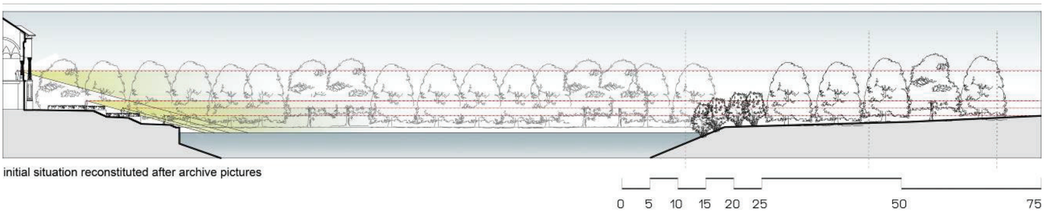


Figure 21. Section - initial state

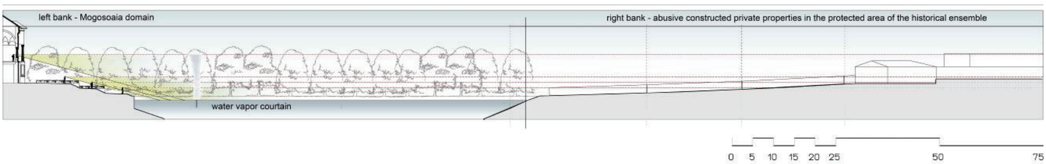


Figure 22. Section - the state after the edification of numerous villas in the protection area of Mogosoaia ensemble on the opposite bank of the lake in 2014

A first approach would be denial, shielding by (re) planting the banks of the river with woody species. Testimony of a wild shore is a series of photos, the last of them made in the 60s, revealing reed and weeping willow. Returning to this picture, however, is unlikely; there is no physical space for these plantations, private properties stretch to the water surface. Moreover such an initiative, which aims to return in time, tends to contradict the spirit of ensemble - oriented to reinterpretation, filtering and adaptation to the present (for example if we think at the creative process of restoration).

Although not a defining element of Italian gardens, water is an essential tool, generating movement and sound. Water appears in various forms giving life to the garden. The vertical presence of water makes it entertaining and able to create diversions, in so-called *Giochi d'aqua*. Focusing on the water surface and on water as a tool can be an interesting exercise.

We propose a succession of curtains of artificial mist, which will rise from the lake. It is about a composition of linear submerged structures giving birth to the mist.

Unlike a screen, these mist curtains will be in constant motion, responding to the stimuli given by the wind, rain and light. The sun, reflected in the water particles, may create rainbows.

Besides the sensory effects created by this veils of mist, it can also serve as a means of expressing direct messages. Its surface can become support for projections of image-manifestos that suggest the former landscape of Mogosoaia.

CONCLUSIONS

The lakeside space and the Italian gardens could become playful and at the same time a cultural catalyst, generating events and social occasions. At the end of the 'show', the wind will fall apart this wonderful mirage and the harsh reality will appear again.

We do not want to hide the reality. We want to show it to the people. We want to arouse debate and a current of opinion against breaching the legislation in the field of preserving the historical monuments.

Our concept, *Militant Ephemeral* or *l'Ephémère Militant*, is suppose to show the reason for respecting the laws concerning historical monuments, to attract the interest of the common people but also of those responsible for the national heritage, on local and central level.

This is a proposal which can be realised. Such an installation was realised in France, in Bordeaux. It is about *La place de la Bource* and the project '*Water Miror*' or '*Quai miroire*'.

Figure 23. '*Water Miror*', *La place de la Bource, Bordeaux, France*
Source : http://www.bordeaux.fr/l10812

The '*Water Miror*' runs daily from 10.00 to 22.00, according to the following cycle: 3 minutes filling 15 minutes of mirror, 5 minutes drain and 3 minutes of fog. This is a great success. The inhabitants and the tourists too are grateful to participate. It is one of the busiest places in Bordeaux. The fountain-maker Jean-Max Llorca and the landscape architect Michel Corajoud are the creators of this unique public open space. Creativity has to support the patrimony, appealing to young people and making them part of the event.

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This research work was carried out with the support of Doina Mândru, cultural manager of the 'Brancoveanu Palaces Cultural Centre', the director of the archives of the National Institute of Patrimony and some enthusiastic young people: Oana Baloi, Iulia Luca and Christian Voinescu, now graduates of Landscape Architecture Department in the University of Agronomical Sciences and Veterinary Medicine in Bucharest and with the support of ACCR - The European Network of Cultural Centres - Historic Monuments (L'Association des Centres Culturels de Rencontre). We are grateful to Richard Edwards, Director de l'Association Atlante and the initiator of the workshop 'Vers un atelier européen d'Architecture n°2' with the theme '*The invention of landscape, ruptures and*

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THE IMPLEMENTATION OF FOOD SAFETY MANAGEMENT SYSTEM IN THE HORTICULTURAL PRODUCTS PROCESSING COMPANY

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Abstract

HACCP is the abbreviation for the English phrase "Hazard Analysis Critical Control Points". In order to obtain high – quality and safe products, capable of meeting the consumer's demands and complying with the food safety standards, certain risk-prevention and control methods should be applied (Bonsi R.,2001). In the horticultural products' processing company, the application of a HACCP system allows the identification of the key-elements of the technological process. The system analyses the hazard related to the product and the process, indicating the critical control points to the hygienic quality of the product.. Starting with the fact that the raw materials come from the farms, there are major concerns regarding the microbiological aspects, the level of pesticides and other chemical contaminants, the maintenance of hygiene during harvesting, the handling, the processing, the storage, and commercialisation (Aversano, 2006). In order to prevent or reduce the above-mentioned hazards, the big specialised companies and small producers must apply HACCP prevention methods, not methods based on the final product control (which may affect consumer's health and may lead to important economic loss). Therefore, this paper contains a HACCP study, characteristic for horticultural processed products and explains the main steps in order to prevent or control the significant hazards for food safety, as presented in the HACCP plan.

Key words: *CCPs, food safety, HACCP, standard*

INTRODUCTION

On the producer-user line (from manipulation to processing) there are a high number of factors that can affect fruits quality (Bonsi, 2001). Considering these products as primary product for the fruit canned products or as finite product in the case of their fresh consume, the major preoccupations are in relation with pesticides level and others chemical contaminants (fertilisers), as well as to preserve the hygiene during harvesting, manipulation, processing and storage. To reduce these risks, it is necessary that the small producers, as well as the high-specialised companies, to apply prevented methods as HACCP type and not those based on the end control of products (that can affect the consumer healthy) and can induce significantly economical losses (Aversano, 2006).

MATERIALS AND METHODS

The fruit canned products has been obtained by an automated technological flux, with the aid of the Materazzi equipment in the SC Contec Food Company Tecuci, Galati county, using fresh fruits (apples from cultivars: 'Jonathan' and 'Golden Delicious'), having as destination, the processing.

A HACCP study was perform, based on the following working stages:

1. the presentation of the specifications about product;
2. the production technological flow description;
3. the potential risk identification and evaluation;
4. the critical control points (CCP) determination;
5. establish the critical limits;
6. the monitoring of the CCP parameters;

7. corrective actions, implemented if the critical limits in CCP have been excelled.

The laborious study was finished by elaboration of the HACCP Plan, a base document, which represents a guide to follow, with a view to maintain under control the relevant risks, that could affect the safety of fruits canned product.

RESULTS AND DISCUSSIONS

Risk identified during the processing of fruit canned products is concerned especially to: pesticides residue provided from the fruits, as a consequence of the chemical treatments, nitrates provided by the excessive fertilization and micro-organisms (yeast, moulds) presented on the fruits or on the technological equipment, because of the inadequate hygiene (Table 1).

As a consequence of this study, there were identified two Critical Control Points:

- Primary matter reception, for the risks generated by the pesticides and nitrates;
- Sterilization of the product, for the risks generated by yeast and moulds;

Data presented in Table 2, emphasis that for these risks, there were established the critical limits and the specifically parameters (product quantities, temperature or NTG) were controlled.

HACCP system, predicts also the critical limits surpass situation, therefore, there were predicted the corrective actions too, to determine the effect removing and the elimination of the causes which generated the manifested risk.

To assure the product traceability on all the production and selling process, it acts to register in specifically forms, which are useful as well to HACCP system revision.

To apply the HACCP Plan, as it was realized, determines to maintain under control the relevant risks, for the food safety of the fruit canned products and to grant an adequate product for the people consume.

Table 1. Hazard analysis

Processing step	Fruit canned products				Preventive / Control measures
	HAZARD				
	KIND OF HAZARD	G	P	RC	
Fruit reception	B) Clostridium sp.	high	low	3	- Training of the workers - Supplier assessment - Analytical analysis
	B) E. Coli	medium	low	2	
	B) Aspergillus flavus	medium	low	2	
	C) pesticides residue	high	low	3	
	C) heavy metal	medium	low	2	
	C) nitrit	medium	low	2	
Sterilization	B) Salmonella sp.	high	low	3	- Training of the workers - Analytical analysis - Process monitoring
	B) Clostridium	high	low	3	
	B) E. Coli	medium	low	2	
	B) Aspergillus flavus	medium	low	2	
	B) Bacillus sp	medium	low	2	
	B) Staphyloccocus	high	low	3	
	B) E. Coli	medium	low	2	
	B) Aspergillus flavus	medium	low	2	

B = biological C = chemical F = physical G = gravity
P = probability RC = risk class

Table 2. HACCP Plan

Nr crt	Process step	Relevant hazard	Charac-teristics	Critical control point	Critical limits	Monitoring			Correction/ Corective actions	Records
						Responsa-bility	Freq-uency	Method		
1	Raw material reception	Pesticide residues Nitrate	-lindan -diazinon -diclorvos -etion -paration $-NO_3$	CCP 1	< 1 mg/kg < 0,3 mg/kg <0,1 mg/kg < 0,5 mg/kg < 0,5 mg/kg < 60 mg/kg	Laboratory technician	2 week before provide	Cromato-graphic	Fruits rejection Supplier selection Personnel training	Test report
2	Sterili – zation	Yeast Bacteria Mould	Sterili-zation schedule	CCP 2	T=120C degree t=10-30min	Sterili-zation operator	Conti-nously	Sterili-zation diagram	Product rejection Process resume Personnel training	Sterilization report

CONCLUSIONS

On the fruits canned products technology, there have been identified two Critical Control Points: at primary raw material reception and at the sterilization step.

The established monitoring system allows maintaining the relevant risks under control, for the hygienically quality of the analyzed product.

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